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Judul artikel : Hedonic Property Value of Water Service in Tidal Lowland Agriculture

Seminar : 2013 Internasional Conference on Agricultural Scince and Environment Engineering

Universitas Sriwijaya

Penulis : Muhammad Yazid

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ABSTRACT

Despite its important role in agricultural water management in tidal lowlands, water service has not yet been considered as an input in crop production. The cost of water service is still born to the government budget such that budget cut will consequently results in abandoning of operation and maintenance of water infrastructures. This paper examines whether water service is a determinant of tidal farmland value. A survey involving 500 farmers was conducted in Telang, a rice production center in tidal lowland area of South Sumatra Province, Indonesia. Hedonic Property Value analysis was employed to provide answer to this examination. Analysis revealed that the value of tidal farmland with water service was significantly higher than that without water service. The value of tidal farmland was determined by soil fertility, distance to the local markets, the availability of water service, and farmland productivity. Being one of the significant determinants of tidal farmland value, imposing fee upon water service will expectedly be followed by farmers' willingness to pay for the fee.

Keywords: water service, tidal lowland, agriculture

INTRODUCTION

Even though self-financing and cost recovery of operation and maintenance (OM) costs have been issues in water management for quite long, water charging has just appeared to be a policy issue since 1992 after the International Conference on Water and the Environment in Dublin. Water charging is part of the efforts to achieve efficient water management through improvement of water distribution and limitation of water use. Water charging is implemented in various different ways, but in general there are four bases for water charging, which are area-based, crop-based, volumetric, and tradable water rights [1].

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Water charging as a policy issue has been adopted in Indonesia through the issuance of The Water Resource Law in 2004 (UU No. 7/2004). However, the term water charging is only implicitly stated in a phrase of "financial responsibility". Farmers (water users) financial responsibility according to the law includes construction, operation and maintenance of water infrastructures at tertiary level. These responsibilities in practice are shared among farmers within a tertiary block, which include routine gate operation (opening or closing of gates according to crop water needs) and tertiary canal and gates maintenance. In tidal lowland area of Telang, one tertiary block consists of 16 ha farmland served by one tertiary canal, 8 ha at each side. Two tertiary gates are installed, one at each end of the tertiary canal. Normally, these 16 ha farmland is owned by 8 farmers or 2 ha farmland in the average. Since water management actions directly affect the 16 ha farmland, the operation and maintenance of tertiary water infrastructures are collectively planned and implemented by the farmers, following the planned cropping patterns in the area.

In spite of being mandatory in the Water Resource Law (UU No. 7/2004), water charge has not yet been applied in tidal lowland agriculture in Indonesia. In lieu of charging, water users are obliged for a payment as members of water user association (WUA), not for the water they use for cultivation. The term water charge is new and might not be acceptable to them. Therefore, the term water service fee is considered appropriate, emphasizing that the charge is made for the service of making the water available according to the crop needs, not for the water itself.

The value of farmland is determined by physical, spatial, and economic characteristics of the farmland such as its fertility, relative distance to the market, and the existence of facilities to support cultivation [2] [3] [4]. The existence of water infrastructures and proper operation and maintenance of gates and canals contribute to the farm output. Therefore, it is researchable to value water service as a characteristic of tidal farmland. The objective of this study was to determine the value of water service on tidal farmland. It was expected from this study that the value of water service could be considered as basis for imposing water service fee for which water users (farmers) were financially responsible.

METHODS

Hedonic pricing (HP) and travel cost method (TCM) are two most commonly used of revealed preference methods. Both TCM and HP have advantages of observing actual behavior. While TCM has particularly been used in estimating non-market value of ecotourism and recreational sites [5], HP was frequently employed in valuing property with regard to the environmental characteristics and changes. It particularly extracts effect of environmental factors on price of goods that include those factors [6] [7] [8]. The hedonic property method has been used to value characteristics of goods that are not formally traded in the market [9]. This method has also been used to estimate the value of open space proximity [10] [11], the improvement in air and water quality [12] [13] and the evaluation of scenic views [14] [15].

In water management research, hedonic method has been used in various studies with regard to water resource as a single resource or water in attachment to land resources. Butsic and Netusil (2007) valued water (rights) by using the hedonic method to estimate the minimum payment an owner (of water right) would be willing

to accept for the sale or lease of a water right in Douglas County, Oregon, USA. On the side of a buyer, it is the maximum price a buyer would be willing to pay for a water right [16]. In the study, Butsic and Netusil employ several parameters of water as characteristics of agriculture land property. Using the hedonic method, this study has successfully put a monetary value on irrigation water ($261 per acre-foot irrigation water) which is consistent with other studies and actual transaction in the study area. Similar to irrigation water, water service can be considered as an attribute of agricultural farmland which benefit can be valued similarly. Therefore, HP was considered appropriate in this study to value water service as an environmental characteristic that determine the price of tidal farmland as a property.

Hedonic property value of farmland is referred to as the market price of farmland. Its value has been studied based on several characteristics, including soil fertility [4], productivity and spatial aspects [2], and availability of water service [3]. In this study, the value of water service reflects the marginal willingness to pay for discrete change in water service, which is the change from without water service to with water service. Assuming two pieces of farmland that are identical except that one is with water service and the other is without, the difference in market price between the farmlands is attributed to the value of water service for which the farmers are willing to pay.

In this research, the hedonic function for tidal farmland market price was stated as follow:

$$P_i = f(Q_S, Q_P, Q_{WS}) \quad (1)$$

where P_i = market price of tidal farmland
Q_S = the vector representing farmland quality
Q_P = the vector representing proximity of the farmland
Q_{WS} = the vector representing facility on the farmland

The quality of tidal farmland was measured in three indicator variables, namely farmland fertility, productivity, and income obtained from crop production. The proximity of the farmland was defined and measured as the distance of the farmland from the local market. The facility on the farmland was represented by the availability of water service on the farmland and the maintenance of gates and canal connected to the farmland.

Based on the above description, the following regression equation was proposed in order to estimate the function:

$$P_i = \beta_0 + \beta_1 FERT + \beta_2 PRO + \beta_3 INC + \beta_4 DIST + \beta_5 D_{WS} + \beta_6 MAINT \quad (2)$$

where P_i = the market price of tidal farmland per hectare
FERT = fertility level of farmland
PRO = farmland productivity
INC = income from crop production
DIST = distance of farmland to the local market
D_{WS} = dummy variables water service
MAINT = canal and gates maintenance

The above regression equation was predicted using ordinary least square (OLS) method to yield the predicted market price of farmland based on its affecting factors. Subsequently, some statistics were employed to examine the goodness-of-fit

of the overall model and the significance of each of the affecting factors. In addition, interpretations on the significant factors were made in term of direction and magnitude of their effects on the market price of tidal farmland [17]. For the dummy variable water service, assuming significant, the interpretation was made as suggested by Gujarati [18]. Supposed, the model contained only the intercept and the dummy variable water service as the following:

$$P_i = \beta_0 + \beta_1 D_{WS} + \varepsilon_i \quad (3)$$

where D_{ws} = 1 for farm with water service and 0 otherwise
The market price of farmland without water service was estimated as:

$$\mathrm{E}(P_i | D_{WS} = 0) = \beta_0 \quad (4)$$

The market price of farmland with water service was estimated as:

$$\mathrm{E}(P_i | D_{WS} = 1) = \beta_0 + \beta_1 \quad (5)$$

Therefore, the intercept β_0 was the mean estimated market price of farmland and the slope coefficient (β_1) was the difference in mean estimated market price between farmland with water service and without.

This study was designed as a survey, conducted in the deltaic area of Telang, South Sumatra, Indonesia. Telang, a reclaimed tidal lowland area for agriculture, is located in the lower reaches of Musi River. Research sample of 500 farmers were drawn using random sampling from some 10,000 farmers, covering 12 secondary blocks (approximately 3,072 ha). Data were collected through field observation and structured interview with the sampled farmers.

RESULTS AND DISCUSSION

As discussed in the methods, tidal farmland value is determined by soil fertility, productivity, income obtained from farming, distance of the farmland from the local market, the availability of water service, and the maintenance of gates and canal connected to the farmland. The mean value of farmland as measured in farmland market price is Rp 52,610,650,00 (± Rp 10,188,170,00). The farmland market price varies from as low as Rp 25,000,000,00 to as high as Rp 100,000,000,00 per ha. The mean market price of farmland with water service is Rp 56,123,640,00 per ha, whereas without water service is Rp 48,075,120,00 per ha. The mean market price of farmland with water service is significantly higher than that without water service (t=9.399, sig. t=0.000).

Table 1 presents the regression coefficients with the t statistics and significant level of each of the independent variables assumed to determine the farmland market price. Out of 6 independent variables assumed to have an effect on the farmland market price, only one variable that is significant at 90 percent confidence interval, which is farmland fertility. Others are proved to have statistically significant effect on the farmland market price at 99 percent confidence interval, which are: (1) productivity; (2) income; (3) distance of farmland from the local market, (4) the availability of water service, and (5) maintenance of canal and gate connected to the farmland. In Table 1 both direction and magnitude of the effect of these independent variables on the dependent variable are shown.

The statistical tests of the regression on farmland market price and its affecting factors indicate that the overall model is statistically significant. However, the variation in farmland market price explained by its determinants is approximately 26

percent. There is no collinearity detected in this analysis since the collinearity statistics coefficient of tolerance of each of the independent variables is far from 0 (0 indicates perfect collinearity).

TABLE I. REGRESSION COEFFICIENTS AND THE VALUE OF STATISTICS

Variables	B	Std. Error	t	Sig.
(Constant)	33132758.632	3997802.536	8.288	.000
Fertility	2500777.738	1309442.781	1.910	.057*
Productivity (tons/ha)	1531738.264	497256.682	3.080	.002***
Income ($Rp10^6$)	178810.108	57019.518	3.136	.002***
Distance from the market	-1909067.120	358702.668	-5.322	.000***
Water Service (Dummy)	4058815.800	1256751.765	3.230	.001***
Canal & gate maintenance	3671982.726	1250081.538	2.937	.003***

R^2 = 0.258; F = 28.580; Sig of F-test = 0.000.
*Significant at 10%; **Significant at 5%; ***Significant at 1%

Farmland fertility is a statistically significance determinant of farmland price (p=0.057). Its coefficient indicates that farmland fertility is positively related to its market price as expected. Fertility is an important attribute of farmland. Farmland fertility is a result of interaction between physical conditions of the farmland and its accessibility to water infrastructures. The higher the fertility the higher the farmland price would be. Measured in ordinal level, one level increase in fertility would increase its price by about Rp 2,500,000,00.

Productivity which is measured by the yield (tons of rice) per hectare farmland has a positive coefficient as hypothesized. Its effect on the farmland market price is significant. Its coefficient tells that every ton increase in productivity will be followed by an increase in the farmland market price of about Rp 1,531,738. Productivity remains an important target in tidal lowland agriculture, especially rice production. With current productivity level of about 5.35 tons per hectare, it can potentially be increased to 10 tons per hectare as has been achieved by few farmers in the study area. Higher productivity will consequently result in higher farmland market price of the farmland.

Income from farming contributes significantly to the price of the farmland. Its coefficient indicates that every million Rupiah increase in total income will be followed by an increase of farmland market price of Rp 178,810. While farmland productivity seems to directly affect the farmland market price, income affects the farmland market price indirectly through the perception regarding the value of property. Farmers with higher farm income tend to perceive higher value of their property than those with lower income.

The distance of farmland from the local market has a negative sign for its coefficient as expected. This indicates that the further the farmland from the local market, the lower the price of the farmland. For every km increase in the distance of the farmland from the local market, the price of the farmland decreases by Rp 1,900,000.

Maintenance of canals and gates as hypothesized has also a positive effect on the market price of the farmland. This variable was measured as a dummy variable which was coded 1 for maintained canals and gates, 0 otherwise. Farmland with

maintained canals and gates connected to it has higher market price than that without canals and gates maintenance. Farmland which is connected to the maintained canals and gates has market price which is Rp 3,671,983 higher than that which is connected to un-maintained canals and gates. Maintained canals and gates enables farmers to properly irrigate, retain and drainage water from the field according to crop water needs. This condition would ensure the optimum crop growth and maximum yield obtained from the farmland.

The availability of water service significantly affects the market price of farmland. The positive value of its coefficient indicates that farmland with water service has higher price than that without water service. The difference in mean estimated market price between farmland with and without water service is indicated by the value of its coefficient (β_1). This means that the price of farmland with water service is Rp 4,058,816 higher than that without water service. This result is in accordance with the hypothesized relationship between the availability of water service with the market price of farmland, proving that water service is an important attribute of tidal farmland.

CONCLUSION

The result of hedonic property analysis indicates that, as a property, the mean price of farmland with water service is significantly higher than that without water service. The value of farmland is significantly affected by the availability of water service beside other characteristics of the farmland (farmland fertility, productivity, distance from the local market, and infrastructure maintenance) and farmer's income. The availability of water service accounts for approximately one half of the difference in the farmland price. Based on the findings of this study, the value of water service can be considered as basis for imposing water service fee for which farmers are financially responsible to contribute in the operation and maintenance of tidal water infrastructures at tertiary level.

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ICASEE 2013 received over 350 submissions which were all reviewed by at least two reviewers. As a result of our highly selective review process about 130 papers have been retained for inclusion in the ICASEE 2013 proceedings, less than 40% of the submitted papers. The program of ICASEE 2013 consists of invited sessions, and technical workshops and discussions covering a wide range of topics. This rich program provides all attendees with the opportunities to meet and interact with one another. We hope your experience is a fruitful and long lasting one. With your support and participation, the conference will continue its success for a long time.

The conference is supported by many universities and research institutes. Many professors play an important role in the successful holding of the conference, so we would like to take this opportunity to express our sincere gratitude and highest respects to them. They have worked very hard in reviewing papers and making valuable suggestions for the authors to improve their work. We also would like to express our gratitude to the external reviewers, for providing extra help in the review process, and to the authors for contributing their research result to the conference. Special thanks go to our publisher DEStech Publications. At the same time, we also express our sincere thanks for the understanding and support of every author. Owing to time constraints, imperfection is inevitable, and any constructive criticism is welcome.

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We wish all attendees an enjoyable scientific gathering in Beijing, China. We look forward to seeing all of you next year at the conference.

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Cover page

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ABSTRACT

Despite its important role in agricultural water management in tidal lowlands, water service has not yet been considered as an input in crop production. The cost of water service is still born to the government budget such that budget cut will consequently results in abandoning of operation and maintenance of water infrastructures. This paper examines whether water service is a determinant of tidal farmland value. A survey involving 500 farmers was conducted in Telang, a rice production center in tidal lowland area of South Sumatra Province, Indonesia. Hedonic Property Value analysis was employed to provide answer to this examination. Analysis revealed that the value of tidal farmland with water service was significantly higher than that without water service. The value of tidal farmland was determined by soil fertility, distance to the local markets, the availability of water service, and farmland productivity. Being one of the significant determinants of tidal farmland value, imposing fee upon water service will expectedly be followed by farmers' willingness to pay for the fee.

Keywords: water service, tidal lowland, agriculture.

INTRODUCTION

Water charging as a policy issue has been adopted in Indonesia through the issuance of The Water Resource Law in 2004 (UU No. 7/2004). However, the term water charging is only implicitly stated in a phrase of "financial responsibility". Farmers (water users) financial responsibility according to the law includes construction, operation and maintenance of water infrastructures at tertiary level. These responsibilities in practice are shared among farmers within a tertiary block, which include routine gate operation (opening or closing of gates according to crop water needs) and tertiary canal and gates maintenance.

Water charging policy has not yet been implemented in tidal lowland irrigation eventhough it has been mandated in the Water Resource Law. Instead of charging, a payment is applied to water user for its membership in water user association (WUA), not for the water used for cultivation. Water charge remains unknown and un

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acceptable to water users. Therefore, water service fee is considered more appropriate term to emphasize that the charge is not for the water itself, but for the service of delivering water down to the tertiary canals to fulfill crop water needs,.

In tidal lowland area of Telang, one tertiary canal serves 16 ha farmland, 8 ha at each side. Two tertiary gates are installed, one at each end of the tertiary canal. Normally, these 16 ha farmland is owned by 8 farmers or 2 ha farmland in the average. Since water management actions directly affect the 16 ha farmland, the operation and maintenance of tertiary water infrastructures are collectively planned and implemented by the farmers, following the planned cropping patterns in the area.

The existence of water service and proper operation and maintenance of water infrastructures contribute to the farm output as well as the value of farmland. The value of farmland is also determined by farmland characteristics [1] such as fertility, relative distance to the market [2], and the existence of facilities to support cultivation [3]. Therefore, the objective of this study was to determine the value of water service and physical, spatial, and economic characteristics of tidal farmland. It was expected that the value of water service could be considered as basis for imposing water service fee for which water users (farmers) were financially responsible.

METHODS

Hedonic pricing (HP) and travel cost method (TCM) are two most commonly used of revealed preference methods. Both TCM and HP have advantages of observing actual behavior. While TCM has particularly been used in estimating non-market value of ecotourism and recreational sites [4], HP was frequently employed in valuing property with regard to the environmental characteristics and changes. It particularly extracts effect of environmental factors on price of goods that include those factors [5] [6] [7]. The hedonic property method has been used to value characteristics of goods that are not formally traded in the market [8]. This method has also been used to estimate the value of open space proximity [9], the improvement in air and water quality [10] and the evaluation of scenic views [11].

In water management research, hedonic method has been used in various studies with regard to water resource as a single resource or water in attachment to land resources. Hedonic method has been used to estimate the minimum payment an owner (of water right) would be willing to accept for the sale or lease of a water right in Douglas County, Oregon, USA [12]. Using the hedonic method, this study has successfully put a monetary value on irrigation water ($261 per acre-foot irrigation water) which is consistent with other studies and actual transaction in the study area. Similar to irrigation water, water service can be considered as an attribute of agricultural farmland which benefit can be valued similarly. Therefore, HP was considered appropriate in this study to value water service as an environmental characteristic that determine the price of tidal farmland as a property.

Hedonic property value of farmland is referred to as the market price of farmland. Its value has been studied based on several characteristics, including soil fertility [1], productivity and spatial aspects [2], and availability of water service [3]. In this study, the value of water service reflects the marginal willingness to pay for discrete change in water service, which is the change from without water service to with water service. Assuming two pieces of farmland that are identical except that one is with

water service and the other is without, the difference in market price between the farmlands is attributed to the value of water service for which the farmers are willing to pay.

In this research, the hedonic function for tidal farmland market price was stated as follow:

$$P_i = f\left(Q_S, Q_P, Q_{WS}\right) \tag{1}$$

where P_i = market price of tidal farmland
Q_S = the vector representing farmland quality
Q_P = the vector representing proximity of the farmland
Q_{WS} = the vector representing facility on the farmland

The quality of tidal farmland was measured in three indicator variables, namely farmland fertility, productivity, and income obtained from crop production. The proximity of the farmland was defined and measured as the distance of the farmland from the local market. The facility on the farmland was represented by the availability of water service on the farmland and the maintenance of gates and canal connected to the farmland.

Based on the above description, the following regression equation was proposed in order to estimate the function:

$$P_i = \beta_0 + \beta_1 FERT + \beta_2 PRO + \beta_3 INC + \beta_4 DIST + \beta_5 D_{WS} + \beta_6 MAINT \tag{2}$$

where P_i = the market price of tidal farmland per hectare
FERT = fertility level of farmland
PRO = farmland productivity
INC = income from crop production
DIST = distance of farmland to the local market
D_{WS} = dummy variables water service
MAINT = canal and gates maintenance

The above regression equation was predicted using ordinary least square (OLS) method to yield the predicted market price of farmland based on its affecting factors. Subsequently, some statistics were employed to examine the goodness-of-fit of the overall model and the significance of each of the affecting factors. In addition, interpretations on the significant factors were made in term of direction and magnitude of their effects on the market price of tidal farmland [13]. Supposed, the model contained only the intercept and the dummy variable water service, the interpretation was as the following [14]:

$$P_i = \beta_0 + \beta_1 D_{WS} + \varepsilon_i \tag{3}$$

where D_{ws} = 1 for farm with water service and 0 otherwise
The market price of farmland without water service was estimated as:

$$\mathrm{E}\left(P_i | D_{WS} = 0\right) = \beta_0 \tag{4}$$

The market price of farmland with water service was estimated as:

$$E(P_i | D_{WS} = 1) = \beta_0 + \beta_1 \quad (5)$$

Therefore, the intercept β_0 was the mean estimated market price of farmland and the slope coefficient (β_1) was the difference in mean estimated market price between farmland with water service and without.

This study was designed as a survey, conducted in the deltaic area of Telang, South Sumatra, Indonesia. Telang, a reclaimed tidal lowland area for agriculture, is located in the lower reaches of Musi River. Research sample of 500 farmers were drawn using random sampling from some 10,000 farmers, covering 12 secondary blocks (approximately 3,072 ha). Data were collected through field observation and structured interview with the sampled farmers.

RESULTS AND DISCUSSION

As discussed in the methods, tidal farmland value is determined by soil fertility, productivity, income obtained from farming, distance of the farmland from the local market, the availability of water service, and the maintenance of gates and canal connected to the farmland. The mean value of farmland as measured in farmland market price is US$5,261.07 (± 1,018.82). The farmland market price varies from as low as US$2,500.00 to as high as US$10,000.00 per ha. The mean market price of farmland with water service is US$5,612.36 per ha, whereas without water service is US$4,807.51 per ha. The mean market price of farmland with water service is significantly higher than that without water service (t=9.399, sig. t=0.000).

Table 1 presents the regression coefficients with the t statistics and significant level of each of the independent variables assumed to determine the farmland market price. Out of 6 independent variables assumed to have an effect on the farmland market price, only one variable that is significant at 90 percent confidence interval, which is farmland fertility. Others are proved to have statistically significant effect on the farmland market price at 99 percent confidence interval, which are: (1) productivity; (2) income; (3) distance of farmland from the local market, (4) the availability of water service, and (5) maintenance of canal and gate connected to the farmland. In Table 1 both direction and magnitude of the effect of these independent variables on the dependent variable are shown. The statistical tests of the regression indicate that the overall model is statistically significant. However, the variation in farmland market price explained by its determinants is approximately 26 percent. There is no collinearity detected in this analysis.

TABLE I. REGRESSION COEFFICIENTS AND THE VALUE OF STATISTICS

Variables	B	Std. Error	t	Sig.
(Constant)	33132758.632	3997802.536	8.288	.000
Fertility	2500777.738	1309442.781	1.910	.057*
Productivity (tons/ha)	1531738.264	497256.682	3.080	.002***
Income ($Rp10^6$)	178810.108	57019.518	3.136	.002***
Distance from the market	-1909067.120	358702.668	-5.322	.000***
Water Service (Dummy)	4058815.800	1256751.765	3.230	.001***
Canal & gate maintenance	3671982.726	1250081.538	2.937	.003***

*Significant at 10%; **Significant at 5%; ***Significant at 1%

Farmland fertility is a statistically significance determinant of farmland price (p=0.057). Its coefficient indicates that farmland fertility is positively related to its market price as expected. Fertility is an important attribute of farmland. Farmland fertility is a result of interaction between physical conditions of the farmland and its accessibility to water infrastructures. The higher the fertility the higher the farmland price would be. Measured in ordinal level, one level increase in fertility would increase its price by about US$250.08.

Productivity which is measured by the yield (tons of rice) per hectare farmland has a positive coefficient as hypothesized. Its effect on the farmland market price is significant. Its coefficient tells that every ton increase in productivity will be followed by an increase in the farmland market price of about US$153.17. Productivity remains an important target in tidal lowland agriculture, especially rice production. With current productivity level of about 5.35 tons per hectare, it can potentially be increased to 10 tons per hectare as has been achieved by few farmers in the study area. Higher productivity will consequently result in higher farmland market price of the farmland.

Income from farming contributes significantly to the price of the farmland. Its coefficient indicates that every million Rupiah increase in total income will be followed by an increase of farmland market price of US$17.88. While farmland productivity seems to directly affect the farmland market price, income affects the farmland market price indirectly through the perception regarding the value of property. Farmers with higher farm income tend to perceive higher value of their property than those with lower income.

The distance of farmland from the local market has a negative sign for its coefficient as expected. This indicates that the further the farmland from the local market, the lower the price of the farmland. For every km increase in the distance of the farmland from the local market, the price of the farmland decreases by US$190.00.

Maintenance of canals and gates as hypothesized has also a positive effect on the market price of the farmland. This variable was measured as a dummy variable which was coded 1 for maintained canals and gates, 0 otherwise. Farmland with maintained canals and gates connected to it has higher market price than that without canals and gates maintenance. Farmland which is connected to the maintained canals and gates has market price which is US$367.20 higher than that which is connected to un-maintained canals and gates. Maintained canals and gates enables farmers to properly irrigate, retain and drainage water from the field according to crop water needs. This condition would ensure the optimum crop growth and maximum yield obtained from the farmland.

The availability of water service significantly affects the market price of farmland. The positive value of its coefficient indicates that farmland with water service has higher price than that without water service. The difference in mean estimated market price between farmland with and without water service is indicated by the value of its coefficient (β_1). This means that the price of farmland with water service is US$405.88 higher than that without water service. This result is in accordance with the hypothesized relationship between the availability of water service with the market price of farmland, proving that water service is an important attribute of tidal farmland.

CONCLUSION

The result of hedonic property analysis indicates that, as a property, the mean price of farmland with water service is significantly higher than that without water service. The value of farmland is significantly affected by the availability of water service beside other characteristics of the farmland (farmland fertility, productivity, distance from the local market, and infrastructure maintenance) and farmer's income. The availability of water service accounts for approximately one half of the difference in the farmland price. Based on the findings of this study, the value of water service can be considered as basis for imposing water service fee for which farmers are financially responsible to contribute in the operation and maintenance of tidal water infrastructures at tertiary level.

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Proceeding

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Preface

We cordially invite you to attend 2013 International Conference Agriculture Science and Environment Engineering (ICASEE 2013), Beijing, China during December 19-20, 2013. The main objective of ICASEE 2013 is to provide a platform for researchers, engineers, academicians as well as industrial professionals from all over the world to present their research results and development activities in Agriculture Science, Environment Engineering and Engineering Technology. This conference provides opportunities for the delegates to exchange new ideas and experiences face to face, to establish business or research relations and to find global partnersfor future collaboration.

ICASEE 2013 received over 350 submissions which were all reviewed by at least two reviewers. As a result of our highly selective review process about 130 papers have been retained for inclusion in the ICASEE 2013 proceedings, less than 40% of the submitted papers. The program of ICASEE 2013 consists of invited sessions, and technical workshops and discussions covering a wide range of topics. This rich program provides all attendees with the opportunities to meet and interact with one another. We hope your experience is a fruitful and long lasting one. With your support and participation, the conference will continue its success for a long time.

The conference is supported by many universities and research institutes. Many professors play an important role in the successful holding of the conference, so we would like to take this opportunity to express our sincere gratitude and highest respects to them. They have worked very hard in reviewing papers and making valuable suggestions for the authors to improve their work. We also would like to express our gratitude to the external reviewers, for providing extra help in the review process, and to the authors for contributing their research result to the conference. Special thanks go to our publisher DEStech Publications. At the same time, we also express our sincere thanks for the understanding and support of every author. Owing to time constraints, imperfection is inevitable, and any constructive criticism is welcome.

We hope you will have a technically rewarding experience, and use this occasion to meet old friends and make many new ones. Do not miss the opportunity to explore in Beijing, China. And do not forget to take a sample of the many and diverse attractions in the rest of the China.

We wish all attendees an enjoyable scientific gathering in Beijing, China. We look forward to seeing all of you next year at the conference.

The Conference Organizing Committees
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Cover page

Title: *The Effect of Freezing on Onion Cell Structure.*

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ABSTRACT

To study the effects of different freezing rate on the cells structures of the onion. The onion dices were froze at 1℃/min, 3℃/min, 6℃/min, 9℃/min, 12℃/min, 15℃/min, 25℃/min, 50℃/min, 80℃/min, respectively, to study the intracellular freezing temperatures at different freezing rates and the change of cell structures and the cell internal pressure variation and computing the cell membrane permeability. The results show that, crystallization temperature decrease as freezing rate increase; cell volume decreases as the intracellular pressure increases and increases and then decreases as the temperature decreases; the permeability increase as the temperature decreases and increase as the freezing rate increases; The freezing temperature of the cell increases as the cell membrane permeability increases and decreases as the permeability decreases.

INTRODUCTION

Frozen is one of the good way to save fruits and vegetables, it can maximize to keep fruit's and vegetable's color, flavor and nutritional value. Therefore, the frozen has become the most promising technologies to save fruit and vegetable in the world[1~3]. A large number of academics have been study about frozen, Prestamo and Fuchigamit found that freezing process affect carrot's cell structure and texture[4,5], Momnzo Hung and Anzalda found that freezing process affect pecan's and apple's cell structure[6,7], Zhenjiang Fan and Shaoqing Yan and Jilei Li study the freezing temperature and blanching affect kiwi fruit's and potato's and chestnut's cell structure and texture[8,9,10], Fergal Tansey study different freezing rate and blanching affect carrot's cell structure[11], Rui Wang study vacuum freeze affect potato tissue structure[12]. These studies proposed frozen temperature, rate and blanching have macroscopic effects on the cell structure, but the changes of cell size and the factors did not further study. In this paper select the onion as the research object, studying different freezing rate's effect on the cell structure.

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MATERIAL AND EQUIPMENT

Material

The onions, purchased on the market. Select onions which are maturity consistent, uniform size, no diseases, no damage.

Equipment

Cryogenic stage (BCS196/Linkam), Microscopy (BX51/Olympas), Slicing machine (VT1000S), Image-Pro Plus software.

Experimental procedure as follows:

(1) Selecting onions which are appearance intact and freshness, cleaning and cutting it into 1cm^3 cube as samples.

(2) Cutting samples into 60μm slice with slicing machine.

(3) Observing the process of frozen onions cell with microscopy on cryogenic stage.

(4) Using Image-Pro Plus software to analyze and record the time, temperature and size of the onion cells.

RESULTS

Microscopic Structure of the Cell

In the experiment observing the process of frozen onions cell of different freezing rate, obtaining onion cell crystallization temperature, time, etc. Figure 1 is microscopic images of onion cell and the frozen rate is 12℃/min.

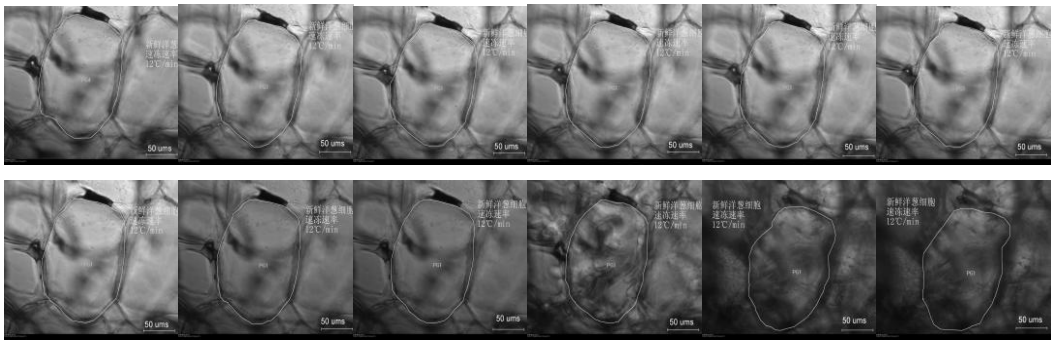

Figure 1. Onion cell 12℃/min freezing process..

Crystallization Temperature

Figure 2 is crystallization temperature curve of different freezing rate.

As shown in Figure 2，When freezing rate lower than 20℃/min, crystallization temperature is instability, but on the whole, the onion cell crystallization temperature decrease as freezing rate increase. This is because that the free water in intracellular of vegetables is first frozen when freezing rate is lower, so between intracellular and extracellular exist vapor pressure difference, resulting in the intracellular moisture move to the ice, and turn into larger ice crystals, the latent heat is released when the

free water turn into ice, resulting in the frozen temperature slowly decline or increase, so the crystallization temperature because higher. When freezing rate is higher, the speed of crystallization is fast, the intracellular moisture don't flow to the extracellular and can't form larger ice, small ice crystals uniformly distribute in cells, the intracellular solute concentration increase and the crystallization temperature decrease.

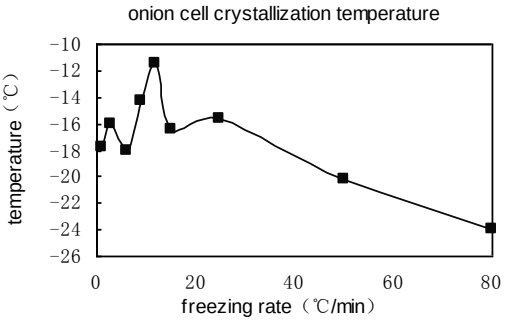

Figure 2. onion cell crystallization temperature.

Change of Cell Internal Pressure and Volume

Obtained the perimeter of the cells with Image-Pro Plus software. Regard the cell as circle in order to calculate sample.

Through calculating obtain the relationship between onion cell internal pressure and volume. Figure 3 represent freezing rate is 6℃/min, 9℃/min, 12℃/min, 15℃/min.

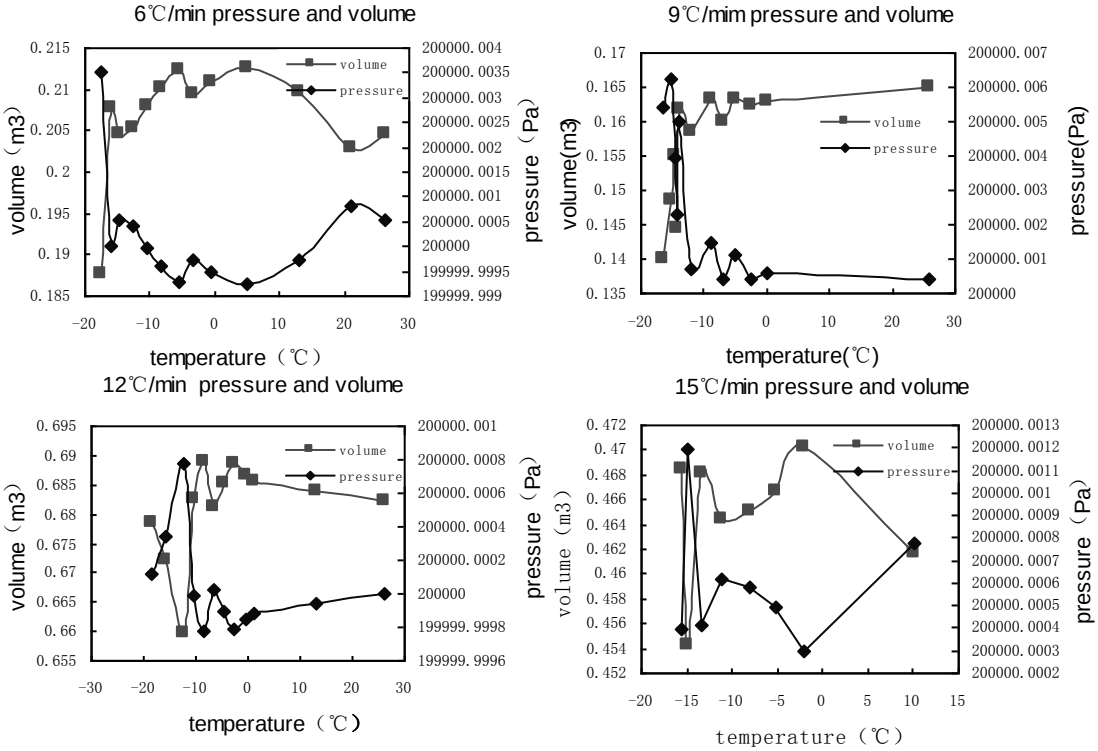

Figure 3. internal pressure and volume.

According to the formula of the pressure in the cell[13] calculate the amount of change of the internal pressure in the cells, as follows:

$$p = \frac{hE\Delta L}{R(1-\gamma^2)2\pi r} \tag{1}$$

γ —— poisson's ratio of the cell wall;

E—— elastic modulus of the cell wall,N/m^2;

L—— cell perimeter, m;

h—— thickness of the cell wall, m;

r—— cell radius, m;

R—— cell perimeter after deformation, m;

Where, $E=2.67\times10^7 N/m^2$; =0.33; $h=0.125\times10^{-5}$m[14];

As shown in Figure 3, the volume of onion cell increase firstly and then decreases as the temperature decrease, the maximum value of volume appear at about 0℃; the change of the pressure in the cell contrary to the volume of onion cell, the volume of cell decrease as the pressure increases. This is because that the density of water reach maximum at 4℃, when the temperature decrease and lower then 4℃, the volume of free water in intracellular increase, the pressure decreases, so the cell volume increases; With the extension of frozen time, the extracellular moisture turn into ice, the cell internal pressure increase, so that the intracellular moisture flow to the intercellular, leading to intercellular ice crystals increase and then press cell, the cell volume decrease.

Permeability of The Cell Wall

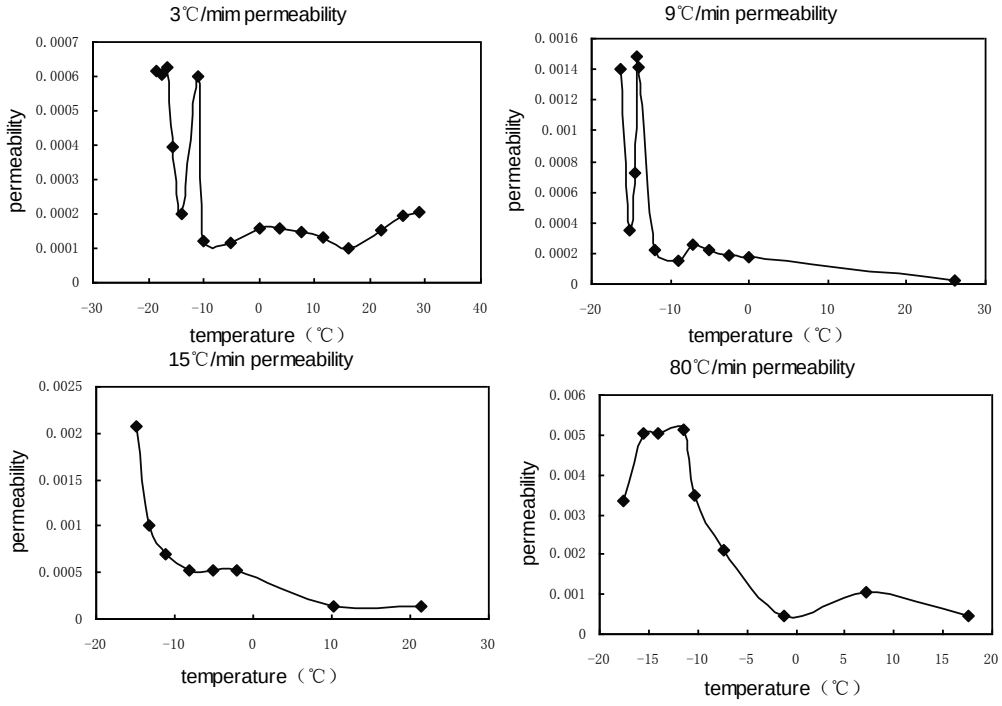

Figure 4. cell membrane penetration

According to the formula to calculate the permeability of the cell wall[15], as follows:

$$L_p = -\frac{1}{A(t)[P(t)-P_i]}\frac{\Delta V(t)}{\Delta t} \tag{2}$$

In the formula: A(t)——cell infiltration area, m^2;

P(t)——internal pressure at time t, Pa;

Pi——the initial internal pressure, Pi =2×10^5 Pa[14];

Figure 4 represent onion cell permeability trends, freezing rate is 3℃/min, 9℃/min, 15℃/min, 80℃/min.

As shown in Figure 4, the permeability increase as the temperature decreases, When the temperature t> -10℃, the amount of change of the permeability is very small; When the temperature t <-10 ℃, permeability suddenly increases. In addition, the permeability increase as the freezing rate increases.

Crystallization Temperature and Permeability

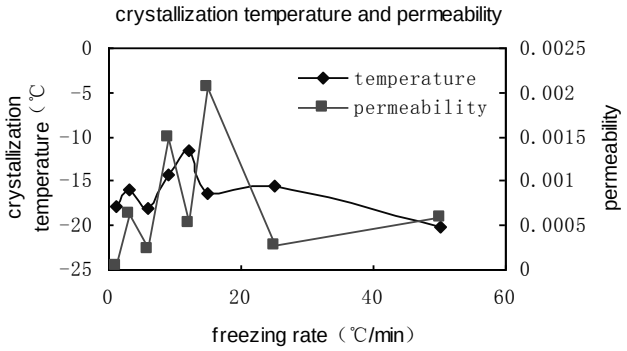

Figure 5. crystallization temperature and permeability

As shown in Figure 5, The onion cell crystallization temperature is different under different freezing rate, the crystallization temperature increases as the cell membrane permeability increases and decreases as the permeability decreases. This is because that crystallization process of frozen onion cell is different under different freezing rate; when freezing rate is lower, the extracellular moisture will prior to freezing than the intracellular moisture, the extracellular pressure drop and lower than the pressure in intracellular, the permeability decrease, so the intracellular moisture outflow and the intracellular solute concentration increase, thereby reducing intracellular freezing point. when freezing rate is higher, extracellular moisture and intracellular moisture freezing at the same time, onion cell solute concentration increase and crystallization temperature decrease.

CONCLUSION

(1) The crystallization temperature decrease as the freezing rate increase.

(2) Onion cell volume decreases as the intracellular pressure increases and increases and then decreases as the temperature decreases.

(3) The permeability increase as the temperature decreases and increase as the freezing rate increases; when the temperature t>-10℃, the change of the permeability is very small; when the temperature t<-10℃, permeability suddenly increase.

(4) The freezing temperature of the cell increases as the cell membrane permeability increases and decreases as the permeability decreases.

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Cover page

Title: *Synthesis and Electrocatalytic Performance of MWCNT-Supported Cu@Pt Core-Shell Nanoparticles for PEMFC*

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ABSTRACT

Cu@Pt core-shell nanoparticles with different Cu/Pt ratios were supported on multi-walled carbon nanotubes (MWCNTs), and used as electrocatalysts for PEMFC. The morphology of the electrocatalysts samples were characterized by XRD and HRTEM. It was found that the Cu@Pt/MWCNTs catalyst exhibits a core-shell nanostructure. The CV and LSV results demonstrated that such core-shell materials exhibited attractive electrocatalytic activity. The specific electrochemically active surface area (A_{EL}) of the Cu@Pt/MWCNTs catalyst was 66.5$m^2 \bullet g^{-1}$ which is higher than the reported values in the literature.

1. INTRODUCTION

Nowadays, proton exchange membrane fuel cells (PEMFCs) as environmental friendly power energy have attracted much attention of manufacturers for transportation and electronic devices [1-3]. Pt electrocatalyst is regarded as the most important electrocatalyst material due to its high activity [4, 5]. However, how to reduce platinum use in the catalyst and improve its activity have thus become the critical issues in the research on fuel cells [6-7].

To this end, the inter-metallic platinum electrocatalysts with cheap transition metal cores, which exhibit enhanced electrocatalytic performance of PEMFC, have been used. Moreno et al [8] prepared a novel trimetallic alloy (Pt-Ru-Co) with layered structures and large specific surface area (102$m^2 \bullet g^{-1}$), and this inter-metallic electrocatalysts exhibited a good performance in reducing oxygen [9]. Recently, for example, Wang et al. [10] synthesized Ni@Pt core–shell nanoparticles with 16.7 wt Pt% which had much higher catalytic activity in oxygen reduction than that of

commercial BASF (Badische Anilin-& Soda-Fabrik, Co., Ltd.,) 20 wt Pt% catalyst. Core-shell structure catalyst can solve the issue of high electrochemical polarization in oxygen reduction, which leads to great reduction in battery efficiency and output voltage, thus improving catalytic activity.Zhu et al [11] synthesized Cu@Pt/C catalyst by a two-step reduction method using Vulcan XC-72R as the supporting material, and the catalyst can improve the ECSA(Electrochemical active surface area) of Pt to

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$53.81 m^2 g^{-1}$. Multi-walled carbon nanotubes with its large surface area and good electrical conductivity can be good support for electrochemical catalysts [12]. Yang et al. [13] studied hydrogenation properties of chloronitrobenzene with Pt/CNT and PtM/CNT catalyst (M=La, Ce, Pr, Nd and Sm). The surface area of CNT is $110 m^2/g$, and 0.37 s^{-1} average pore diameters is 22nm, and the turn over frequency of PtSm/CNT (TOF) is which showed the best catalytic activity.

In this study, nanostructured Cu@Pt core-shell nanoparticles with improved Pt to Cu mass ratios were supported on multi-walled carbon nanotubes (MWCNTs).
Copper can form nanoparticles easily, and its lattice spacing is closer to platinum which helps platinum to grow on the core metal [14]. The formation of core-shell structure is suggested by HRTEM, XRD, and electrochemical techniques. Improved Pt utilization and the best mass ratio of Pt and Cu are also demonstrated.

2. EXPERIMENTAL

A Cu@Pt/MWCNTs (Cu@Pt: C=20:80) electrocatalyst was synthesized by a two-step chemical reduction method with ethylene glycol as reducing agent. Firstly, solid citric acid, MWCNTs and copper sulfate pentahydrate were dissolved in ethylene glycol (EG). The pH of the system was adjusted to 10 with 5wt% KOH/EG solution. After heating at 170°C for 4 h, Cu/MWCNTs electrocatalyst was collected. Secondly, the obtained Cu/MWCNTs powder, H_2PtCl_6 $6H_2O$ was dispersed in ethylene glycol (EG). The pH of the system was adjusted to 7-8 with 5wt% KOH/EG solution. After the mixture was stirred for 4 h at 90°C, the Cu@Pt/MWCNTs electrocatalyst was collected. Cu@Pt/MWCNTs (Cu@Pt:C=20:80) electrocatalysts with different mass ratios of Cu to Pt were synthesized according to the procedure described above, For comparison, Pt/MWCNTs catalyst with the same Pt content was synthesized by using the method described above.

3. RESULTES AND DISCUSSION

The XRD patterns for Cu/MWCNTs and Pt/MWCNTs catalysts prepared by a similar procedure are shown in Fig.1 (a). The peak located at about 2θ=24° in all the XRD patterns is associated with the multi-walled carbon nanotubes support. In curve a and curve b, the other four peaks at 2θ values of 39°, 46°, 67°and 81° are characteristic of the face-centered cubic (fcc) crystalline alloys of Cu and Pt (PDF card 04-0802). A small positive shift in the peak for Pt in Cu@Pt/MWCNTs indicates that the Pt lattice is contracted after covering on the Cu core. It is understandable since the lattice constant of fcc Cu is smaller than that for Pt [15]. Curve c in Fig.1(a) shows that the three peaks at 2θ values of 43°, 50°, and 74° are characteristic of the face-centered cubic (fcc) crystalline Cu. The absence of Cu peaks in curve a implies that the Cu surface in Cu@Pt is mostly wrapped by Pt. The mean particle size of Pt nanoparticles is estimated by the Scherrer formula to be 8.3 nm. The XRD results of Cu@Pt/MWCNTs catalysts with different mass ratios of Cu to Pt shown in Fig.1 (b) demonstrates that, with the decrease of Pt and increase of Cu, the extent of positive shift in the peak for Pt in Cu@Pt/MWCNTs became larger gradually, the inserted

figure of enlargement for characteristic diffraction peaks of CuPt (111) crystal surface can show the positive shift clearly. [16].

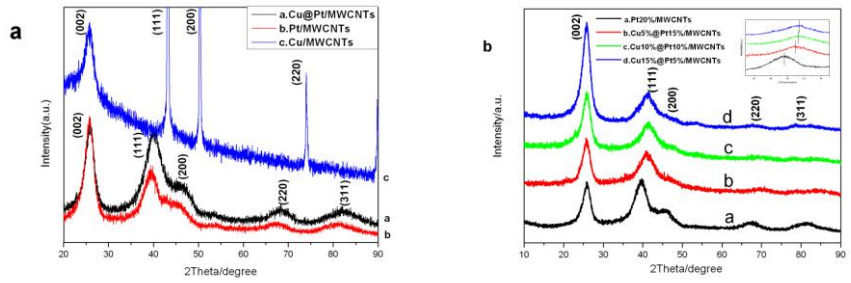

Fig.1 (a) XRD patterns of Cu/MWCNTs, Pt/MWCNTs, and Cu@Pt/MWCNTs catalysts. (b) XRD patterns of Cu@Pt/MWCNTs catalysts with different Cu/Pt mass ratios. Insert figure: enlargement for characteristic diffraction peaks of CuPt (111) crystal surface

The further evidence of core-shell structure is provided by HRTEM image. Fig.2 (a) is the HRTEM image of MWCNTs before loading metals. Fig.2 (b) shows that the core-shell Cu@Pt particles with diameters from 6 to 8 nm are uniformly dispersed in the MWCNTs support. The lattice spacing of Cu crystalline (111) plane is 0.208 nm, while the lattice spacing of Pt crystalline (111) plane is 0.228nm. As shown in Fig.2 (c), the lattice spacing of the center of nanoparticle is 0.222 nm, which is between the lattice spacing of Pt and Cu, implying that the core-shell structure reduces the lattice spacing of Pt [17]. And the lattice spacing of the edge of nanoparticle is 0.228nm which is as same as the lattice spacing of Pt crystalline (111) plane. Therefore, the Cu-core and Pt-shell nanoparticle is synthesized.

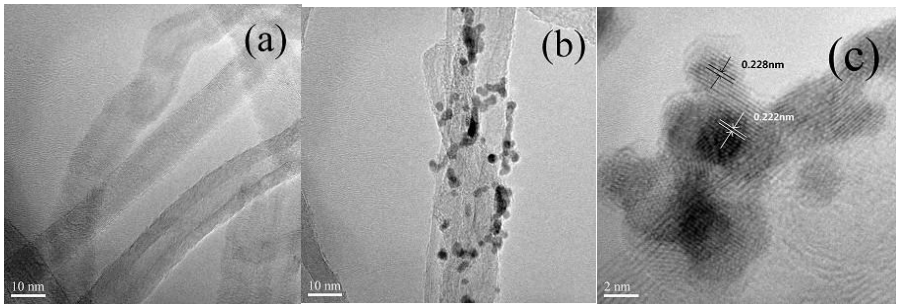

Fig.2 (a) HRTEM image of MWCNTs before loading catalyst, (b) HRTEM image after loading Cu@Pt catalyst, and (c) HRTEM image of Cu@Pt catalyst.

The electrocatalytic activity of the Cu@Pt/MWCNTs catalysts is characterized by CV. The Fig.3 (a) shows Cu@Pt/MWCNTs and Pt/MWCNTs catalysts with 10wt% Pt loading. The catalysts exhibit H_2 adsorption/desorption peaks in the potential range -0.2 to 0 V (vs. SCE). The region 0 V<E<0.35 V is associated with H_{upd} adsorption/desorption ($H^+ + e = H_{upd}$), and the region beyond 0.60 V involves the formation of an OH_{ad} layer ($2H_2O = OH_{ad} + H_3O^+ + e$)[18]. Obviously, Fig.3 (a) shows the larger of the H_2 adsorption/desorption peaks for Cu@Pt/MWCNTs catalysts than for Pt/MWCNTs catalysts, indicating that the catalytic activity and utilization of Pt are better for core-shell nanostructure catalysts than for pure Pt catalysts. The cyclic voltammograms of Cu@Pt/MWCNTs core-shell electrocatalysts with different mass

ratios of Cu to Pt are shown in Fig.3 (b). The electrochemical active surface area (A_{EL}) of the catalysts is calculated according to Eq. (1) [19]:

$$A_{EL}\left(m^2?g^{-1}\right) = Q_H / \left(2.1\times[Pt]\right) \tag{1}$$

Where Q_H ($C.m^{-2}$) is the charge exchanged on the Pt surface, [Pt] ($g\ m^{-2}$) is the Pt loading on the electrode. The calculated A_{EL} of the catalysts are given in Table 1. The A_{EL} of the Cu@Pt/MWCNTs catalyst with a Cu: Pt mass ratio of 5wt% to 15 wt% was calculated to be 66.5 $m^2\ g^{-1}$, which is larger than the A_{EL} of the Pt/MWCNTs catalyst.

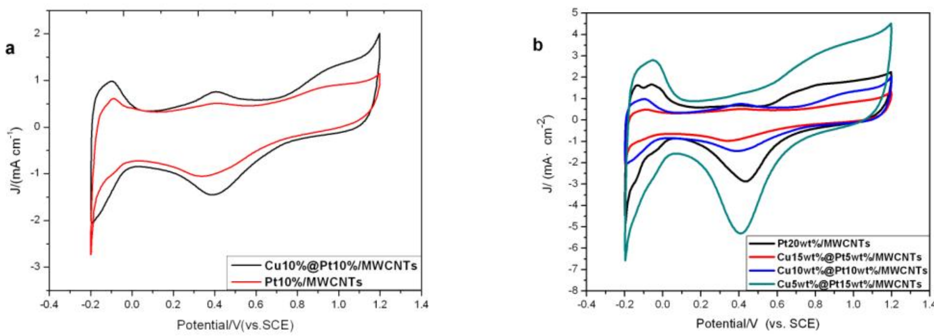

Fig.3(a) Cyclic voltammograms of the different Cu@Pt/MWCNTs and Pt/MWCNTs electrocatalysts.

TABLE 1 ELECTROCHEMICAL ACTIVE SURFACE AREAS (A_{EL}) FOR DIFFERENT ELECTROCATALYSTS.

Different mass ratios of electrocatalysts	20%Pt/MWCNTs	5%Cu@15% Pt/MWCNTs	10%Cu@10% Pt/MWCNTs	15%Cu@5% Pt/MWCNTs
$A_{EL}/m^2 \cdot g^{-1}$	31.2	66.5	26.4	19.8

The LSVs in Fig.4 imply that the reduction of hydroxyl species shifts 30 mV toward positive potential for the Cu@Pt/MWCNTs catalyst with respect to the corresponding pure Pt/MWCNTs catalyst. On one hand, this result shows that the oxygenate Pt species have a weaker adsorption energy on the surface of the Cu@Pt/MWCNTs catalyst than on the surface of the pure Pt catalyst, which means that desorption of oxygenated Pt species on Cu@Pt/MWCNTs is easier than on the Pt/MWCNT.

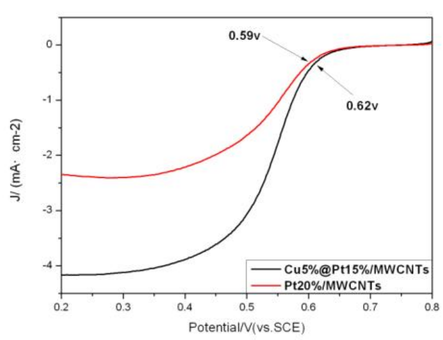

Fig. 4 Polarization curves of Cu@Pt/MWCNTs and Pt/MWCNTs catalysts in O_2 saturated 0.5 mol L^{-1} H_2SO_4 at a rotation rate of 1600 rpm. Sweep rate: 2mV s^{-1}.

4. CONCLUSION

A Cu@Pt/MWCNTs electrocatalyst was synthesized by an ethylene glycol reduction method. The average particle size of the Pt nanoparticles was about 8.3 nm, and the nanoparticles dispersed well on the MWCNTs surface. Because of the unique structure of MWCNTs and core-shell, the A_{EL} of the Cu@Pt/MWCNTs catalyst was 66.5 $m^2 g^{-1}$ at a Cu: Pt mass ratio of 5wt%: 15wt%. It was shown that the Cu@Pt/MWCNTs nanoparticles exhibited much higher catalytic activity than pure Pt catalyst which is very promising for PEMFC applications.

5. ACKNOWLEDGEMENTS

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Cover page

Title: *Experiment Study on Feeding Royal Chicken by Natural Mineral Feed*

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ABSTRACT

The Royal Chicken was free-range bred in the cherry orchard. Feeding Royal Chicken by common feed and special natural mineral feed respectively with the same other rearing conditions for comparison. The results show that the weight gain, survival rate and egg production of Royal Chicken fed by special natural mineral feed is much better than those fed by common feed.

INTRODUCTION

Royal Chicken, derived from the British royal manor, is ornamental, delicious, and nutritious. It is loved by people because of its rich nutritional value. Now we introduce special poultry Royal Chicken with high quality, through the contrast farming experiment, we explore a new model of the Royal Chicken's ecological farming, improve its breeding technology, provide a theoretical basis for the Royal Chicken ecological farming, and lay a good foundation for the promotion in the surrounding area.

MATERIALS

We select 200 eugenic Royal laying hens (50-day-old) from the Beidaihe ecological farming demonstration base (100 laying hens) and the China Life Sciences Group Qinhuangdao planting and breeding base (100 laying hens) for comparative test. Each occupies Cherry Orchard 9 acres, sheds 4 as a test site. In addition, we introduce the natural mineral feed and the life energy water system from Yanshan University National Science Park Functional Polymer Innovation Institute.

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TEST METHODS

200 Royal laying hens are divided into A and B groups (each for 100 hens). Group A use ordinary fodder and water feeding; Group B, raised with a unique fodder which was added natural mineral ingredients and natural mineral water purified by life energy water system (shown in Table 1). Chicken weight, hen egg production and other data of the two groups were recorded in detail. Farming contrast test was carried out from November 28, 2011 to November 28, 2012.

FARMING MANAGEMENT

Usually, we clean the hen houses carefully to ensure them dry and health, timely clean up the drinking fountains and chicken feeding trough and do well heat insulation work at night. In summer night, we hang incandescent lamps on the hillside to lure insects for chickens predation, ensure the Royal Chicken can take in rich, balanced nutrient. We do not use hormones and antibiotic substances during the test, vaccines and herbal medicine were used for disease prevention to prevent drug residues in the Royal Chicken.

THE GROWTH OF ROYAL CHICKEN

We provided a good growth environment for the Royal Chicken throughout the experiment, as showed in table1, epidemic prevention and disinfection work was timely and did not occur epidemics. Natural mineral feed(about 0.6% of the total feed), water purified by life energy water system.

TABLE 1: THE CONTRAST OF ROYAL CHICKEN FARMING SITUATION

Group	Farming method	Quantity	Area	Indoor lighting in winter	Indoor temperature in winter	Feeding amount	Fodder and water
A	Free ranging in the cherry orchard	100	Cherry Orchard 9 acres, sheds 4	12 hours a day	13℃	75g a day before laying eggs;100g a day after laying eggs	Ordinary fodder and water
B	Free ranging in the cherry orchard	100	Cherry Orchard 9 acres, sheds 4	12 hours a day	13℃	75g a day before laying eggs;100g a day after laying eggs	Natural mineral feed and purified water

EXPERIMENTAL INDEX STATISTICS

TABLE 2: THE TRIALS INDICATORS OF ROYAL CHICKEN

Group	Survival rate	Average annual egg production	Average weight(500g)
A	92%	153	3.32
B	98%	176	3.50
B than A	+6%	+15.03%	+5.42%

ANALYSIS OF THE RESULTS

Under the same farming conditions, compared to Royal Chicken feeded with ordinary fodder and water, the survival rate, average annual egg production and average weight of Royal Chicken feeded with fodder added natural mineral ingredients and natural mineral water purified by life energy water system increased by 6%, 15.03% and 5.42% respectively (as showed in Table 2).

CONCLUSIONS

The experiments show that the free-ranging method in the cherry orchard, use of the natural mineral feed and life energy water system play a more significant role in promoting the growth of the Royal Chicken, which can effectively improve economic efficiency. In addition, the breeding patterns fully enhance the space utilization of the Cherry Orchard, chicken manure as fertilizer improve the fruit quality, while Cherry Orchard provides a wealth of grass seeds, insects and other food for Royal Chicken, plays a virtuous cycle role in mutually reinforcing between planting and breeding, have a high popularization value.

Cover page

Title: *Extraction and Analysis of Polysaccharides from the Fruit of Capparis Spionosa L.*

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PAPER LENGTH: **10 Pages**

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ABSTRACT

In this study, we aim to isolate polysaccharide from the fruit of the common caper (*Capparis spinosa*), determine its content, and analyze its monosaccharide components. The phenol-sulfuric acid method was used to determine the content of polysaccharide, and HPCE was used to analyze its monosaccharide components. The results showed polysaccharide content of crude polysaccharide extracted from the common caper was 39.95%, while its purified form (after protein was removed using the Sevage method) was 66.42%. The monosaccharide composition of the polysaccharide was: Rha: Xyl: Glu: (Fru + Man): Ara: Gal = 3: 5: 48: 100: 21: 19. In conclusion, the phenol-sulfuric acid method is convenient and HPCE yields very good result when applied to the analysis of monosaccharide components of the polysaccharide.

INTRODUCTION

The common caper (*Capparis spinosa*), a plant belonging to the Capparidaceae family, is found in China mostly on the slopes in the Tianshan mountain range, where they are frequently seen in desert and rocky slopes at low altitudes. With a bitter taste and a warm nature, it is used in Chinese medicine mainly to dispel chills and remove dampness, stop blood stasis and swelling [1]. In Xinjiang Autonomous Region, it is used to treat a wide variety of conditions such as rheumatism, scapulohumeral periarthritis, dermatitis, malaria, and diuresis. In other countries, the common caper is found mainly in the Mediterranean region. As a traditional herbal medicine, it has been used in countries such as Turkey, India, Iran, Jordan, Saudi Arabia, and Egypt as a nourishing medicine, an anti-inflammatory and anti-hypertension agent [2], and a medicine to lower blood sugar, treat gout, reduce sputum, expel parasites, and treat diuresis and constipation [3,4]. There has not been any report in the literature on water extraction of polysaccharide from the fruit of this plant, so the authors conducted experiments to extract the polysaccharide and to determine its content and monosaccharide components.

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MATERIALS

Main Reagents

Capparis spinosa L. fruit was provided by engineering research center of natural anticancer drugs, Education Ministry of China. rhamnose, glucose, galactose, xylose, fructose, arabinose (analytical reagents).

Apparatuses

752 UV-visible light spectrophotometer; R-201 rotary evaporator; DZF-6030 vacuum drying oven; DHG-9140A blast drying closet; electrothermal constant temperature water bath; Beckman P/ACE^{TM} MDQ electrophoresis apparatus; capillaries (75 μm × 50 cm); pH meter; and centrifuge.

METHODS & RESULTS

Extraction and Purification of Polysaccharide from the Fruit of *C. spinosa*

100 g dry *C. spinosa* mature fruit was pulverized and extracted with 95% ethanol for 2 h at 80 ℃. The fluid was filtered and the residue was dried at 60 ℃. Then the residue was extracted with water three times for 1.5 h each time at 90 ℃, and the total water extract was condensed with decompression. 95% ethanol was added into the condensed water extract with the final concentration of ethanol to 80% and kept for 48 h. The crude polysaccharide sample was obtained with filtration and vacuum drying, and the yield was 5.0%.

Crude *C. spinosa* polysaccharide was dissolved in water, and Sevage solution (chloroform: n-butyl alcohol = 4:1) was added to the polysaccharide solution. Then solution was then shaken vigorously for 5 min and then centrifuged for 10 min at 4 000 r/min, after which the protein between the aqueous phase and the organic phase was removed. Subsequently, the pH of the polysaccharide solution was adjusted to 8-9, and 50 mL of 10% H_2O_2 was added to the polysaccharide solution and then set in a refrigerator at 4 ℃ for 24 h, after which the pigment was removed. The sample was obtained with dialysis and vacuum drying.

Determining the Content of the *C. spinosa* Polysaccharide

SELECTING THE MAXIMUM MEASURING WAVELENGTH

Each of glucose, crude polysaccharide and purified polysaccharide were dissolved in 100mL water. 0.6 mL each of glucose standard solution, solution of crude *C. spinosa* polysaccharide and refined *C. spinosa* polysaccharidewas taken, and distilled water was added to 2.0 mL. 1.0 mL of 5% phenol solution was mixed with each of the 3 samples. 5.0 mL of concentrated sulfuric acid was quickly pipetted. The samples were vigorously shaken for 2 min, and then set still for 20 min at 20 ℃. After the samples were cooled with running water, the absorbance for each was measured at 450-530 nm. According to the results, a measuring wavelength of 485 nm was selected.

SELECTING THE OPTIMAL COLORIMETRIC METHOD

Four factors, namely, amount of 5% phenol used (A), amount of concentrated sulfuric acid used (B), temperature for the reaction (C), and reaction time (D) were designed into the experiment. Three values were chosen for each factor (Table 1), and an $L_9(3^4)$ orthogonal array was used for the test. Test data and results calculated from them are shown in Tables Ⅰ and Ⅱ.

TABLE I FACTORS AND THEIR LEVELS

Level	A (mL)	B (mL)	C (℃)	D (min)
1	0.8	4.0	20 (room temperature)	20
2	1.0	5.0	60 (water bath)	30
3	1.2	6.0	100 (water bath)	40

TABLE II THE $L_9(3^4)$ ORTHOGONAL ARRAY

Number	A	B	C	D	OD(A)
1	1	1	1	1	0.524
2	1	2	2	2	0.702
3	1	3	3	3	0.539
4	2	1	2	3	0.438
5	2	2	3	1	0.692
6	2	3	1	2	0.598
7	3	1	3	2	0.378
8	3	2	1	3	0.496
9	3	3	2	1	0.471
K1	1.765	1.34	1.618	1.687	
K2	1.728	1.89	1.611	1.678	
K3	1.345	1.608	1.609	1.473	
k1	0.588	0.447	0.539	0.562	
k2		0.63	0.537	0.559	
k3	0.448	0.536	0.536	0.491	
R	0.14	0.183	0.003	0.071	

From the results in Table II, it can be seen that, with absorbance as indicator for the investigation, the effects of the several factors as shown by their ranges (*R*) follow the order B > A > D > C. It can be intuitively seen that the optimal combination of conditions for coloration should be $A_1B_2C_1D_1$, which combination would be used for subsequent experiments in this study.

PLOTTING THE STANDARD CURVE

The standard solution in the amounts of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL was accurately measured, and distilled water was added to each to bring its volume to 2.0 mL. Then 0.8 mL of phenol was added to each sample. After the samples were shaken, 5.0 mL of concentrated sulfuric acid was quickly added to each of the samples, which were then kept at 20 ℃ for 20 min. After the samples were cooled with running water, their absorbance (*A*) was measured at 485 nm. The same procedure was repeated on 2.0 mL of distilled water, which was used for the blank. The standard curve was created by plotting absorbance *A* against polysaccharide content (μg/mL), and the equation for the standard curve was calculated. The experiment showed that a good linear fit obtained for standard glucose in the 10-50 μg/mL range. After the data were processed, the regression equation was obtained as follows: $A = 0.0142\ C + 0.0114$, $r = 0.9992$.

MEASUREMENT OF THE SAMPLE

Measurement was made on 0.8 mL each of the crude polysaccharide and refined polysaccharide test solutions following the procedure. The measurement was performed 3 times, and the polysaccharide contents of the crude and refined samples were found to be 39.95% and 66.42%, respectively.

HPCE Analysis of *C. spinosa* Polysaccharide

10 mg of the polysaccharide was put in a 5 mL ampoule pipe. 1.0 mL of 2 mol/L H_2SO_4 was added into ampoule pipe for hydrolysis at 100 ℃. After 8 hours, the sample solution was neutralized with barium carbonate and set still over night. 200 μL sample solution was drawn and dramatized by α-naphthylamine and $NaBH_3CN$ for 2 h at 80 ℃. 1 mL methylene trichloride and 1 mL double-distilled water was added, and the upper-layer aqueous phase was filtered with 0.45 μm microporous membrane and measured by high-performance capillary electrophoresis (HPCE).The conditions for electrophoresis as follows: buffer solution: 75 mmol/L (pH = 10.5) borax solution; column temperature: 25 ℃; voltage: 15 kV; detecting wavelength: 240 nm; injection time (hydrodynamic injection 0.5 pi): 15 s.

The electrophorogram for the capillary zone for the standard α-naphthylamine derived monosaccharides thus obtained was compared to the electrophorogram for the polysaccharide. Then the monosaccharide components in the polysaccharide were determined according to the peaking times, and the relative proportions of the monosaccharide components were calculated according to the peak areas. The monosaccharides and their proportions were found to be Rha: Xyl: Glu: (Fru and Man): Ara: Gal = 3: 5: 48: 100: 21: 19.

The electrophorogram for the mixed monosaccharides and that for the *C. spinosa* polysaccharide are shown in Table III and Figure 1, and Table Ⅳ and Figure 2, respectively (the horizontal axis represents time in min, while the vertical axis is MAU).

TABLE III MIGRATION TIME FOR MIXED MONOSACCHARIDES

Peak No.	Component	Migration time (Min)
1	Derivative reagent	11.785
2	Rhamnose	22.725
3	Xylose	26.676
4	Glucose	32.516
5	Mannose or Fructose	33.193
6	Arabinose	34.513
7	Galactose	44.552

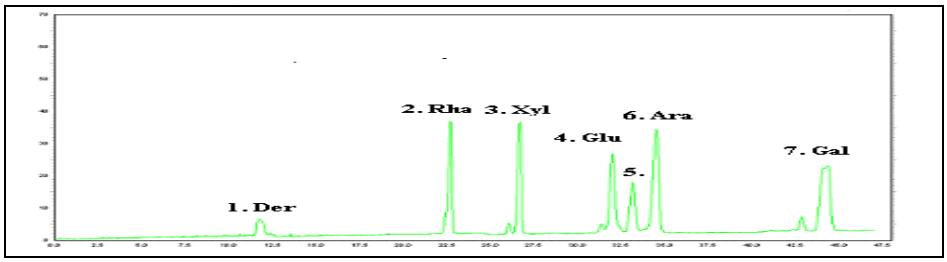

Figure 1. The electrophorogram of the mixed monosaccharides

TABLE IV THE MIGRATION TIME, PEAK AREA, AND PERCENTAGE OF EACH MONOSACCHARIDE IN *C. SPINOSA* POLYSACCHARIDE

Peak No.	Component	Migration time (Min)	Area Percent (%)
1	Derivative reagent	11.785	-
2	Rhamnose	22.994	1.78
3	Xylose	26.942	2.91
4	Glucose	32.929	10.73
5	Mannose and/or Fructose	33.785	60.78
6	Arabinose	35.168	12.51
7	Galactose	44.257	11.24

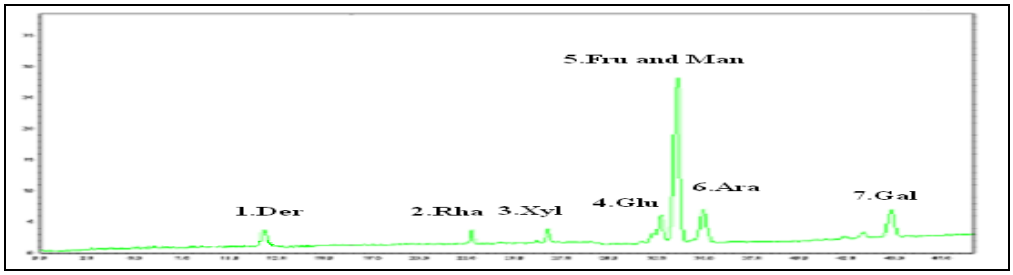

Figure 2. The electrophorogram for *C. spinosa* polysaccharide after derivation

DISCUSSION

There have been many reports in the literature on the determination of polysaccharide content using the phenol-sulfuric acid method, but the conditions for colorimetric development are affected by many factors, such as the temperature at which the measurements are made, time of coloration, amount of the colorant added, and the chemical properties of the polysaccharide in question. Thus, the best approach is to choose optimal conditions for colorimetric development based on experiment. . Hexoses usually have their maximum absorbance at 490 nm, and our experiment shows that *C. spinosa* polysaccharide probably contains all these compounds. In our experiment, it was found that the absorbance declined when the time for measurement exceeded 20 min, which was probably because furaldehyde derivatives were reduced with the prolongation of time. The carbohydrate content of purified polysaccharide (obtained by removing the protein using the Sevage method, decoloration using hydrogen peroxide, and dialysis) was raised to 66.42%, which differs significantly from the carbohydrate content of the crude polysaccharide. Thus, the method for purification is feasible.

Characterized by high efficiency, good speed in producing results, convenience, and small amount of sample required, HPCE is highly sensitive, capable of detecting test drug on the order of 10^{-15}-10^{-13} mol [5-7], when the test is made using UV. The application of HPCE to the analysis of monosaccharide components of polysaccharides is a new technology for polysaccharide analysis in recent years. Since carbohydrates usually do not absorb fluorescence or UV, it is a good practice to enhance the sensitivity of their UV or fluorescence test by having them go through pre-column derivation so that they would have appropriate functional groups emitting color or fluorescence. This method can also make carbohydrates carry inherent charges so that the electromigration performance of neutral carbohydrates can be improved. Since different

oligosaccharides with the same reducing end respond to UV or fluorescence at the same unique wavelength, this method can make the quantitative analysis of carbohydrates rather convenient. In our experiment, α-naphthylamine was used to derivatize the carbohydrates, whose reducing end reacted with α-naphthylamine to form Schiff's bases. $NaBH_3CN$ was then used to reduce such bases to secondary amines. This way, not only were detectable functional groups introduced, but the carbohydrates were changed to borate complexes with inherent charges. In the experiment, it was found that the main factor affecting the resolution of HPCE was the pH of the buffer. Increasing the pH of the buffer helped to increase the resolution for the carbohydrates, but the peaking time was delayed correspondingly. Increasing the concentration of the buffer had the same effect. Good effect was achieved by selecting 75 mmol/L for the concentration of the borax solution. It is worth noticing, though, that the concentration should not be made too high, either, for borax is difficult to dissolve, and would be re-crystalized if it is forcibly dissolved by applying ultrasonic waves. Since high-voltage electric field is used for driving force in HPCE, increasing voltage can help to increase resolution. From the experimental results, it can be seen that generally pentoses (such as rhamnose and xylose) are separated earlier than hexoses. However, some monosaccharides are difficult to separate, e.g., fructose and mannose in our experiment, possibly because the 3rd and 4th carbon atoms of both these monosaccharides carry -OH groups in cis-form. These problems conceivably can be solved when certain techniques, such as mass-spectrometry and nuclear magnetic resonance (NMR), are integrated with HPCE.

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Cover page

Title: *Sulfated Modification and Anti-Tumor Activity of Laminarin*

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ABSTRACT

In this study, we aim to study the sulfated modification of laminarin and investigate the change of structure and anti-tumor activity. Chlorosulfonic acid-pyridine method was applied for sulfated modification. The molecule weight and IR spectra of laminarin and laminarin sulphate (LAMS) were investigated, and the surface conformation was observed with scanning electron microscope, then the anti-tumor activities were evaluated by MTT method. LAMS were synthesized with the sulfate content of 37.8% and the molecule weight of 13000. IR spectra of laminarin and LAMS showed characteristic absorption peaks of polysaccharide, and LAMS had characteristic absorption peaks of sulfate, both laminarin and LAMS had β-glycoside bond. There were obvious differences in surface conformation between laminarin and LAMS, and laminarin showed like cloud or sponginess, while LAMS showed branchlike, flakiness and granular roundness. MTT results showed laminarin and LAMS both had inhibitory effects on LOVO growth, and the anti-tumor activity of LAMS was higher at the same concentration. It suggested that sulfated modification could change laminarin structure and enhance the anti-tumor activity markedly.

INTRODUCTION

Biological activities of polysaccharides can be developed and enhanced by means of chemical modifications, which can be more widely used in application or in clinical. Sulfated modification can help to get high activity, good functional polysaccharides and polysaccharide derivatives, and the introduction of sulfate groups may cause the change of polysaccharide physicochemical properties and three-dimensional conformational, particularly the change of activities [1,2]. Some studies suggest that some polysaccharides without anti-tumor activity showed anti-tumor activity after sulfated modification, while some sulfated polysaccharides with anti-tumor activity lose or decreased anti-tumor activity after removing sulfate groups.

The main sulfated modification methods of polysaccharides include sulfuric acid method, chlorosulfonic acid-pyridine method, chlorosulfonic acid-carboxamide method, sulfur trioxide-pyridine method and sulfur trioxide-dimethylformamide

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method, and chlorosulfonic acid -pyridine method is widely applied for the high production and substitution degree of derivatives [3,4].

Laminarin, also known as brown algae starch, is an active component in Kelp and shows many activities including anti-tumor, immunomodulatory and antibacterial, anti-virus, regulating blood lipids, anti-oxidation, anti-clotting, anti-radiation and hypoglycemic [5]. In this study, we choose laminarin as object and to modify by means of chlorosulfonic acid -pyridine method, then investigated the structure and the anti-tumor activity changes between laminarin and laminarin sulphate (LAMS), which may provide a scientific basis for the studies on polysaccharide modifications and the comprehensive developments and utilizations of polysaccharides.

MATERIALS

Main Reagents

Lamnarin (Sigma); chlorosulfonic acid, pyridine, TCA, potassium sulfate, gelatin, barium chloride, formamide; blue dextran and dextrans; LOVO; FBS; DMEM/F12 culture medium (HyClone); Trypsin (Invitrogen); MTT (Sigma); DMSO.

Apparatuses

Thermostatic water bath; low speed centrifuge; UV-VIS Spectrophotometer; HPLC; FTIR Spectrometer; scanning electron microscope; clean bench; cell incubator; microplate reader.

METHODS

Sulfated Modification of Laminarin

A certain amount of pyridine was added into bottle with condensing unit and agitating device, and cooled in ice-bath. A certain amount of chlorosulfonic acid was dropped slowly into pyridine solution. When a large number of light yellow solid appeared, the bottle was removed from ice-bath. 100mg Laminarin was suspended in 10mL dimethyl- formamide (DMF) and treated with the ultrasonic treatment for 20 min, then added to bottle. The bottle was put in hot water bath (75℃) immediately. After 1.5 h, the bottle was cooled and added some ice-water, then adjusted to pH7 with NaOH and added three times volume of ethanol. The sediment was obtained by centrifugation, dissolved in some water, dialyzed with distilled water for 3d, concentrated and freeze-dried.

Determination of Sulfate Content in LAMS

According to the method of barium sulfate turbidity, the sulfate content was determined by ultraviolet spectrophotometer at 360 nm.

Measurement of Molecular Weight

Molecular weight was measured by high performance gel permeation

chromatography and a refractive index detector. The column and the detector were maintained at 40 ℃, and the mobile phase was 0.2 mol/L ammonium acetate buffering at a flow rate of 0.8 mL/min.

Analysis of IR Spectra

5 mg sample and 400mg KBr were blended pulverized for 5-10min, pressed to a tablet and scanned with wavelength 4000-400cm^{-1} by infrared spectrometer.

Observation of Surface Conformation

The sample powder was fixed on the conductive adhesive. The three-dimensional images observed by QUANTA 250 FEG-scanning electron microscope at the voltage of 20kV.

MTT Assay

1×10^4 cells were cultured in a 96-well-plate for the first 24 h, and then were incubated with drug (400, 800, 1600 mg/L) for 72 h. After incubation, MTT was added to the culture at a final concentration of 0.5 mg/mL. After incubation at 37 ℃ for 4 h, all media were removed, and 150 μL DMSO was added to dissolve purple formazan crystals. The plate was measured by microplate reader at 570 nm. The inhibition rate was used to evaluate cytotoxicity.

Statistical Analysis

Statistical comparison within groups was carried out by one way ANOVA. A *p*-value of less than 0.05 was considered to be significant statistically.

RESULTS

The Sulfate Content in LAMS

The linear regression equation of sulfate standard curve was A= 2.2856C-0.0205 (R^2 = 0.999), and the content of SO_4^{2} showed good linear relationship in the range of 12~120μg. According to the forma: *f*= W/C, the conversion factor *f* was 0.969. The content of SO_4^{2} in LAMS was 37.8%, and according to the formula: $DS=(1.62\times S\%)/(32-1.02\times S\%)$, the substitution degree (*DS*) was 1.07.

The Molecular Weight

The linear regression equation of molecular weight standard curve was $lgM_W = -6.0269K_{av} + 8.4358$ (R^2 = 0.9991), and the molecular weight of laminarin and LAMS was 5000 and 13000.

IR Analysis

The IR spectrum of laminarin showed the characteristic absorption peak of –OH stretching vibration appeared at 3370cm^{-1} and the peak of C-H stretching vibration in

-CH_3 or -CH_2 groups appeared at 2924cm^{-1}, which showed the characteristic absorption peaks of sugar. And the characteristic absorption peaks of pyranose appeared. The characteristic absorption peak of C-H scissor vibration appeared at 889cm^{-1}, which indicated the glycosidic bond in laminarin was β type. So the laminarin has pyranose skeleton with β-glucoside bond. As shown in Figure 1. Compared the IR spectrum of LAMS with laminarin, it showed the characteristic absorption peaks of S=O and C-O-S stretching vibration appeared at 1246cm^{-1} and 802cm^{-1}, which indicated the sulfates was introduced into sugar molecules. And it also showed the LAMS has pyranose skeleton with β-glucoside bond. As shown in Figure 2.

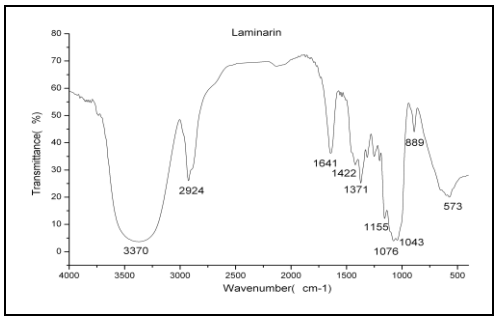

Figure 1. IR spectrum of Laminarin

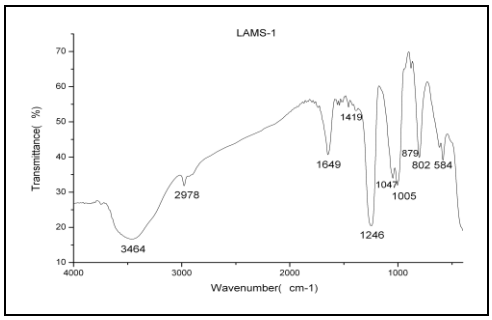

Figure 2. IR spectrum of LAMS

Conformation Observation

Under scanning electron microscope, there were obvious differences in surface structure between laminarin and LAMS. Laminarin showed like cloud or sponginess, and there were many dispersed sugar particles. While LAMS showed like branchlike, flakiness and granular roundness, and there were few dispersed sugar particles. As shown in Figure 3 and 4.

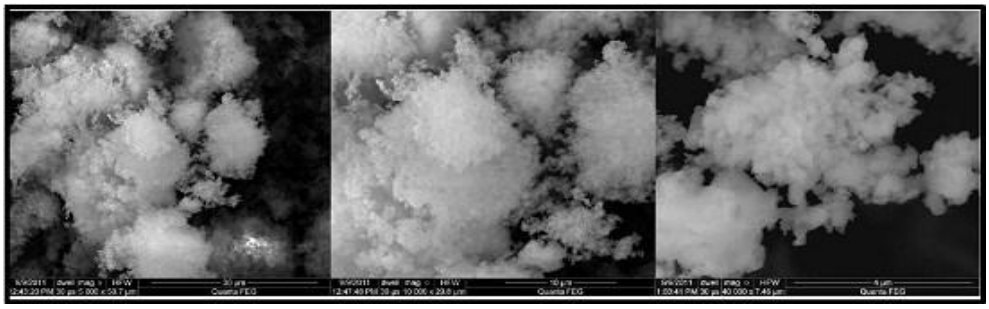

Figure 3. SEM pictures of Laminarin

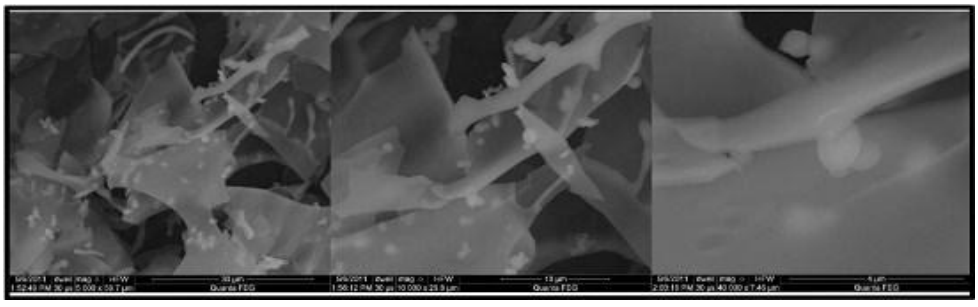

Figure 4. SEM pictures of LAMS

Anti-tumor Activity

After treated by various concentrations of lamnarin and LAMS for 72 h, it showed they both inhibited LOVO proliferation, which was significantly different compared with control group (P<0.05). After sulfated modification, the anti-tumor activity of laminarin was enhanced, and at the same concentration the inhibition rate of LAMS was significantly greater than laminarin, which was significantly different compared with laminarin group (P< 0.01). As shown in Figure 5.

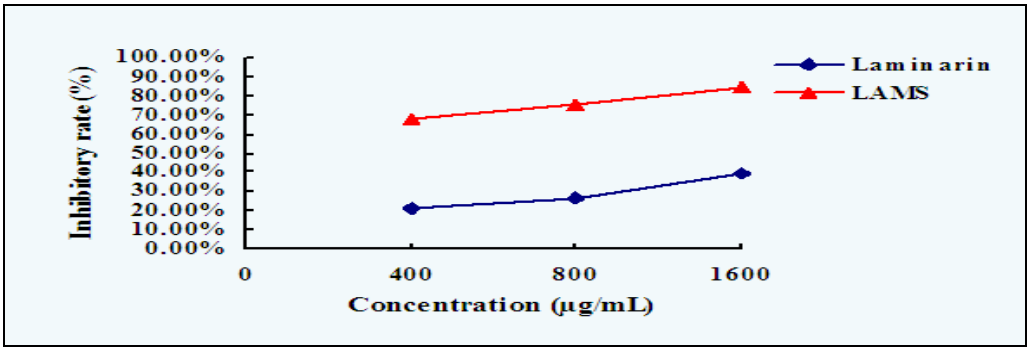

Figure 5. Anti-tumor effect of Laminanrin and LAMS

DISCUSSION

Sulfated polysaccharides or sulfated polysaccharide are polysaccharide derivatives with complex chemical structure and biological activity, and the hydroxies in the monosaccharide molecule of polysaccharide chains are substituted by sulfate groups. The biological activity of the sulfated polysaccharides is closely related to its structure and physicochemical properties, steric and electrostatic repulsion effect of sulfate groups can change the space structure of polysaccharide and the flexion of the sugar chain, increase water-soluble, which lead to the change in biological activity, so the structural transformation of polysaccharide is a research hotspot [6-8].

In this experiment, we synthesized laminarin sulfate (LAMS) with substitution degree of 1.07 by means of chlorosulfonic acid-pyridyl method. The IR spectrum of LAMS showed the characteristic absorption peak of -OH weakened (3400cm^{-1}), the characteristic symmetric stretching vibration peak of C-O-S (810cm^{-1}) and the characteristic asymmetric stretching vibration peak of S=O (1245cm^{-1}) appeared. And the laminarin and LAMS both have pyranose skeleton with β-glucoside bond. Under scanning electron microscope, there were obvious differences in surface structure between laminarin and LAMS, and laminarin showed like cloud or sponginess, while LAMS showed like branchlike, flakiness and granular roundness. The changes of shape indicated the chemical change in most cases, and it may be due to that after hydroxyl groups on the sugar units are substituted by sulfate groups, the conformation of the sugar chain changes, and the repulsion between sulfuric acid groups may result in conformation showing stretch and rigid morphology.

The anti-tumor experiment showed that after sulfated modification, the anti-tumor activity of laminarin was significantly enhanced, and the inhibition rate of LAMS was significantly greater than laminarin at the same concentration. This may be due to the sulfated modification cause changes in the polysaccharide molecular structure and

spatial conformation, which lead to biological activity changes. After the hydroxyl group of the laminarin sugar units being substituted by a sulfate group, the conformation of the sugar chains distort or change, and easy to form a non-covalent bond. Moreover these repulsion between the anionic groups elongate sugar chain, and part of the sulfuric acid group and the hydroxyl groups on the sugar chain may form hydrogen bonds, which may form a helical structure in the sugar chain and show active conformation, thus cause the increase in its activity.

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Cover page

Title: *Coupling Effects of Water and Fertilizer on Growth and Physiological Characteristics of Chinese White Poplar Seedlings*

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ABSTRACT

To quantify the coupling effects of soil water content and fertilizer application on the plant stem height (SH), leaf area (LA), net photosynthesis rate (PN) and biomass yield (BY) of *Populus tomentosa* Carr. seedlings, a test was conducted by using a current rotary combination design of quadratic regression of three factors with five levels, and the mathematical regression models and multiple target decision model were established. Our results showed that all the W, N and P factors had obvious positive effects on plant SH, LA, PN and BY with the size sequence of W > N > P. The border effects of three factors had a descending trend with W, N and P increasing. In order to achieve maximum growth potential of *P. tomentosa* seedlings, obtain high water and fertilizer use efficiencies and reduce the risk of nitrate pollution of groundwater in arid and semi-arid regions in China, it can be concluded that the combination of W, N and P recommend for growers was W = 78.1%FC, N = 4.48 g plant^{-1} and P = 1.67 g plant^{-1}.

1. INTRODUCTION

Water and fertilizer are two of the main factors limiting the realization of plant growth and productivity [1-3]. Both irrigation and fertilization are widely applied by growers to achieve high plant growth and high yield. But on the other hand, excessively high levels of water and fertilizer, much higher than required for optimum production often bring following results: the rise of plantation cost, over-consumption of fresh water and fertilizer resources, reduction of water and fertilizer use efficiency, the hazard of nitrate and phosphate loss by wind and water erosion and even leaching, contamination of groundwater and negative effect on plant yield and quality [4,5]. Thus, an appropriate application of soil water and fertilizer has the vital purpose of increasing water and fertilizer productivity and reducing environment pollution risk.

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Chinese white poplar (*Populus tomentosa* Carr.), as an indigenous tree species in China, is a kind of fast-growing and high-yield poplar and plays a key role in the projects of fast-growing and high-yield forests, and conversion of cropland to forestland [6]. *P. tomentosa* is mainly distributed in the middle and lower reaches of the Yellow River, covering more than 10 provinces and occupying an area of approximately one million km^2, approximately 1/9 of the total area of China.

The specific objectives of this study were to: (1) quantify the coupling effects of W, N and P treatments on the stem height (SH), leaf area (LA), net photosynthesis rate (PN) and biomass yield (BY) of *P. tomentosa* seedlings, and (2) determine recommendations of both water and fertilizer application for growers to obtain the optimal production.

2. MATERIALS AND METHODS

2.1 Experimental Design

The experiment was carried out for five months in the nursery, by using a current rotary combination design of quadratic regression of three factors [7]. Five levels of soil relative water content (W) as percentage of FC, five levels of nitrogen application (N) as granular urea fertilizer and five levels of phosphorus (P_2O_5) application (P) as superphosphate fertilizer were applied in this experiment (Table 1). Fertilizers including N, P_2O_5 and potassium (K_2O) were applied in small holes near the plant root distribution during the growing season: N applied three times (mid-June, mid-July and mid-August), P_2O_5 applied twice (mid-June and mid-August) and K_2O applied twice (mid-June and mid-July) with 0.75 g $plant^{-1}$ per time. The five W levels were achieved by irrigating and measured with a handheld time domain reflectometry meter ML2x every five days [8,9].

TABLE 1 CODE LEVEL SCHEME OF EXPERIMENTAL DESIGN

Code level	Soil relative water content (W, %FC)	Nitrogen application (N, g $plant^{-1}$)	Phosphorus (P_2O_5) application (P, g $plant^{-1}$)
-1.682	30	0	0
-1	40	1	0.5
0	55	2.5	1.25
1	70	4	2
1.682	80	5	2.5

During the experiment period, all plants were transferred in a glasshouse at evening or before rain in order to avoid the changes of soil water status caused by rainfall.Eighty one-year-old seedlings in a completely random arrangement were subjected to a total of 20 treatments (Table 2) with four replications.

TABLE 2 EXPERIMENTAL DESIGN MATRIX AND CHANGES OF THE GROWTH PARAMETERS OF *P. TOMENTOSA* SEEDLINGS UNDER ALL 20 TREATMENTS

Treatment	Experimental design matrix			Treatment	Experimental design matrix		
	W (x_1)	N (x_2)	P (x_3)		W (x_1)	N (x_2)	P (x_3)
1	1	1	1	11	0	1.682	0
2	1	1	-1	12	0	-1.682	0
3	1	-1	1	13	0	0	1.682
4	1	-1	-1	14	0	0	-1.682
5	-1	1	1	15	0	0	0
6	-1	1	-1	16	0	0	0
7	-1	-1	1	17	0	0	0
8	-1	-1	-1	18	0	0	0
9	1.682	0	0	19	0	0	0
10	-1.682	0	0	20	0	0	0

2.2 Growth and Physiology Parameters

Seedlings were harvested at the end of the experiment for estimation of biomass yield. Dry weights of leaves, stems and roots were recorded after oven-drying of samples for 72 h at 80 ℃. Leaf area (LA) of plants was measured with an automatic leaf area meter LI-3000 before harvest. Increment of stem height (SH) was determined as the final values minus the initial values. Measurement of net photosynthesis rate (PN) was carried out on the third fully expanded leaf (from the top) of each plant, with a portable photosynthesis system LI-6400. PN values were recorded per month (in mid-June, mid-July, mid-August and mid-September) and then averaged [10].

2.3 Statistical Analysis

Simulate regression models were established to quantify the coupling effects of water and fertilizer on the growth parameters. The regression coefficient was analyzed by F-test. First derivation and the quadratic equations with two unknowns was used to analyze the monofactor border effect on the growth between W × N, W × P and N × P. $P < 0.05$ was considered statistically significant. The optimal scheme of regression models was determined with LINGO software (version 8.0, LINDO SYSTEMS Inc., Chicago, IL, USA).

3. RESULTS

3.1 Regression Model

By using the results of the growth parameters of *P. tomentosa* seedlings subjected to a total of 20 treatments (Table 2), mathematical regression models were established after removing the non-significant ($P>0.05$) regression coefficient (Table 3). A carry through test of the significance of the models (1)-(4) showed that there were highly

significant (P<0.01) differences: $F_{values}>F_{0.01}(9,10)=4.94$, and that the actual circumstances are reflected primarily by the regression models, with a high liner correlation coefficient between theoretical and objective values.

TABLE 3 RELATIONSHIPS BETWEEN THE GROWTH (Y) AND THREE FACTORS: W(X_1), N(X_2) AND P(X_3)

Growth parameters	Regression equation	F	R^2
Stem height (SH)	$y_{SH} = 136.87 + 18.41x_1 + 15.42x_2 + 10.20x_3 + 7.64x_1x_2 - 16.60x_1^2 - 12.81x_2^2 - 11.03x_3^2$ (1)	19.86	0.95
Leaf area (LA)	$y_{LA} = 2805.75 + 572.48x_1 + 464.95x_2 + 148.64x_3 + 339.19x_1x_2 + 221.72x_1x_3 - 335.46x_1^2 - 359.92x_2^2 - 419.54x_3^2$ (2)	31.02	0.97
Net photosynthesis rate (P_N)	$y_{PN} = 17.06 + 1.32x_1 + 0.77x_2 + 0.70x_3 - 0.77x_1^2 - 0.81x_2^2 - 0.72x_3^2$ (3)	6.65	0.86
Biomass yield (BY)	$y_{BY} = 55.66 + 11.17x_1 + 6.55x_2 + 4.08x_3 + 5.67x_1x_2 - 5.58x_1^2 - 7.15x_2^2 - 10.66x_3^2$ (4)	13.87	0.93

3.2 Factorial Effect Analysis

3.2.1 MAIN FACTOR EFFECT

The importance of the factor degree was seen directly by the absolute value of the deviation regression coefficient of the models, while the symbol "+" or "-" shows only the function direction of the factor. In the models (1)-(4), the coefficients of x_1, x_2 and x_3 were positive indicating that all the W, N and P factors had obvious positive effects on SH, LA, PN and BY of *P. tomentosa* seedlings, with the size sequence of W > N > P (Table 3). The significant coefficient of the interaction terms (x_1x_2, x_1x_3 and x_2x_3) were different among the models (1)-(4) showing that the coupling effects of three factors on the growth parameters of plants were complex. All x_1x_2 values in the models (1), (2) and (4) were positive meaning that the coupling of W × N had a cooperative effect on the all the growth parameters except PN. The x_1x_3 values in the models (2) were positive meaning that the coupling of W × P had a positive effect on the LA (Table 3). However, there were no coupling effects of three factors on the PN values since there was no significant coefficient of the interaction terms in model (3). The quadratic term coefficient of x_{12}, x_{22} and x_{32} was negative and demonstrated that over-W, over-N and over-P applications not only waste resources, but also reduce the growth of *P. tomentosa* seedlings.

3.2.2 MONOFACTOR EFFECTS

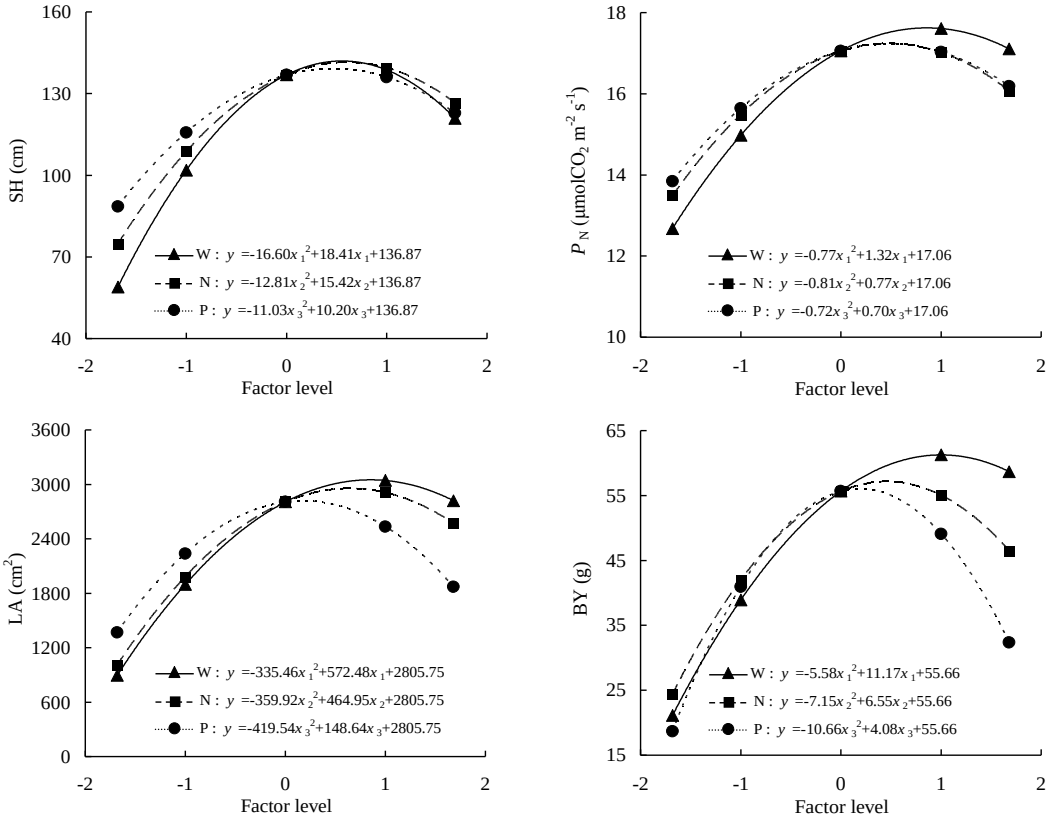

Figure 1. Monofactor effects of three factors (W: x_1, N: x_2, P: x_3) on stem height (SH), leaf area (LA), net photosynthesis rate (PN) and biomass yield (BY) of *P. tomentosa* seedlings.

A set of simple quadratic regression sub-models of y_{SH}, y_{LA}, y_{PN} and y_{BY} can be derived by fixing two factors as 0 level in the models (1)-(4) and other factor as an explanatory variable (Fig. 1). Our results indicated that all the four growth parameters (SH, LA, PN and BY) initially improved with an increase of W, N and P, but actually decreased when beyond a certain W, N and P level (Fig. 1). As shown a parabola trend, there were some zeniths of growth parameters in the factor values of x_1=0.55, x_2=0.60 and x_3=0.46 for SH, x_1=0.85, x_2=0.65 and x_3=0.18 for LA, x_1=0.86, x_2=0.48 and x_3=0.48 for PN, and x_1=1.00, x_2=0.46 and x_3=0.19 for BY. Moreover, when compared to N and P factors, W caused the most serious reduction in SH, LA and PN under the lowest level (-1.628) and could maintain relative high values of LA, PN and BY after the apex under the highest level (1.628).

3.3 Multiple Target Decision Model Analysis

The regression models (1)-(4) were subjected to analyze the single optimum value of the four growth parameters in each model (SH = 152.43 cm, LA = 3726.45 cm^2, PN = 17.97 $\mu molCO_2\ m^{-2}\ s^{-1}$ and BY = 68.20 g), which was performed by using LINGO software. After determining the weight values of each parameter (w_{SH} = 0.24, w_{LA} = 0.26, w_{PN} = 0.25, w_{BY} = 0.25), the following multiple target decision model was obtained based on partial least-square method: $y=0.24(y_{SH}-152.43)^2$ +

$0.26(y_{LA}-3726.45)^2 + 0.25(y_{PN}-17.97)^2 + 0.25(y_{BY}-68.20)^2$. The four growth parameters reached the optimum values when W = 1.55, N = 1.33 and P = 0.56. Therefore, when W = 78.1%FC, N = 4.48 g plant^{-1} and P = 1.67 g plant^{-1}, then the growth of *P. tomentosa* seedlings were optimized: SH = 141.25 cm, LA = 3712.78 cm^2, PN = 17.01 μmolCO_2 m_{-2} s_{-1} and BY = 66.23 g.

4. CONCLUSION

In conclusion, *P. tomentosa* has a great demand for both soil water and soil nutrient due to fast growth, and is highly dependent on inputs of irrigation water and fertilizer to achieve optimum production. All the W, N and P factors had obvious positive effects on plant SH, LA, PN and BY with the order of W > N > P. There were synergistic effects of W × N on SH, LA and BY while the coupling of W × P only on LA. Based on the multiple target decision model, the combination of W, N and P recommend for growers was W = 78.1%FC, N = 4.48 g plant^{-1} and P = 1.67 g plant^{-1}, in order to achieve maximum growth potential of *P. tomentosa* seedlings, obtain high water and fertilizer use efficiencies and reduce the risk of nitrate pollution of groundwater in arid and semi-arid regions in China.

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Cover page

Title: *The Development Vein and Prospect of Ecological Civilization Research*.

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ABSTRACT

Research on ecological civilization in China mainly focuses on two kinds of paths: to concern about ecological civilization itself and to do correlation research by utilizing the conception of ecological civilization. As for ecological civilization itself, the academic circles explored from multi-dimension, such as the connotation, the constitution and the construction paths of ecological civilization. The past thirty years' study on ecological civilization have achieved abundant progress. Still, needing to be further deepened in many aspects, such as to heighten the pertinence, to enhance the accuracy and to strengthen the meta-study of Ecological civilization.

INTRODUCTION

The appearance of the ecological crisis and its solutions, have caused people to do research on ecological civilization widely and deeply. Ecological civilization research in China started from scratch, developing from steadily growth to considerable progress. Since the 17th CPC National Congress adopted the concept of ecological civilization for the first time in its report, the research on the ecological civilization has jumped and peaked in 2008. Since 2009, although somewhat fell back, it was still seized a high attention, and rebounded in 2012. Based on the interaction of theory and practice, Zhao Donghai indicated the year 2000 as the time point of the research, after that, the research began to base on the social reality in which global issues were becoming increasingly harsh.[1] Also the research has covered 40 subjects across the political, economic, legal, environment and other fields of study, the most concentrated were resources and environmental sciences, macroeconomic management and sustainable development, agricultural economy, etc.

Research on ecological civilization has departed in two ways, one is from the perspective of ecological civilization itself, to analysis its connotation, characteristics, index system, function, significance, construction paths and so on. Some scholars even

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proposed, study on ecological civilization inevitably lead to the subject of ecological civilization[2]; The second is the use of the conception of ecological civilization in correlative areas. Such as observing the system reform from the perspective of ecological civilization, explaining how to make the system more adapted to the requirements of ecological civilization, etc. This paper chooses three basic aspects from the ecological civilization itself: the connotation, composition and construction, to carry out the domestic research status up till now.

THE CONOTATION OF ECOLOLIGICAL CIVILIZATION

Cultivation Ways of Personal Ecological Civilization in the Mature Socialist Conditions, published on the second issue of Journal of Moscow State University' scientific socialism in 1984 was briefly introduced by the foreign academic trends column of Guangming Daily on February 18, 1985. This was the concept's first time appearance in China. The ecologist Ye Qianji is the pioneer in defining "ecoogical civilization" in China. He pointed out in the agro-ecology seminar in 1987 "The ecological civilization is to gain from the nature, to benefit the nature, which keeps the balance and harmony between human and nature, changing and protecting the nature at the same time". [2]Since then, the scholars in China have begun to focus on the study of ecological civilization, discussed extensively and put forward different views on the connotation of ecological civilization.

One is on the direct regulating object of the ecological civilization--between the man and nature. The proposition that there are two kinds of dispute theory. One is that the ecological civilization is the theme of the relationship between man and nature. As Shen Xiaohui thought, ecological civilization meant a good relationship between nature and human civilization.[3] Gao Changjiang pointed out that the ecological civilization from the development of philosophical sense, referring to the prosperity of both the individual and the world, and the harmonious civilization between man and nature's development.[4] Xie Guangqian, Wang Xingling divided the nature into biological systems and non biological systems, and on this basis the narrow and broad generalizations of ecological civilization.[5] The other one is that the ecological civilization not only includes the adjustment of the relationship between man and nature, but also the relationship between both people and people, people and society. Ji Zhenhai divided ecological civilization into primary and advanced form. The primary form was more used in the basic achievements of industrial civilization: cultural attitudes towards nature, not the brutal exploitation and maltreatment of nature, trying to improve the relationship between human and nature, the protection and construction of a good ecological environment. The advanced form referred to people in the transformation of the material world, actively improve and optimize the relationship between man and nature, man and man, the construction of ecological operation mechanism and orderly and good ecological environment, which was social construction of ecological civilization sense.[6] The differences in fact contains on the human and the nature, the relationship between social and ecological system of value judgment, thus we could see that the construction of ecological civilization not only faces the ecological crisis, dealing with the relationship between human and nature, the construction of ecological system civilization; it is needed to rise to the social construction of meaning, calling for the now mode of production, lifestyle, cultural

value, institutional arrangements and other aspects of the experience and lesson reflection.

The two is based on the interpretation of the different connotation of civilization. Li Shaodong believed to be the side in the view of civilization and the civilized behavior. Ecological civilization, to bring the rational understanding of the ecological environment and actively into the construction of spiritual civilization, became an important part of its construction.[7] Wang Zhihe thought, ecological civilization was a new form of human civilization, a modern industrial civilization backwash and beyond.[8] Chu Dajian further distinguished the ecological civilization in the developed and developing countries. In developed countries: ecological civilization, to the elevation of industrial civilization, correspondingly, its construction was to make ecological improvement of the existing achievements in the country's modernization; The developing countries, like China, was still in the period of modernization, the ecological civilization is the ecological industrial civilization.[9] Close the different interpretations to the civilization connotation embodied the ideal vision of ecological civilization was not only on the development of human society, but also the society at the present stage the realistic goal of socialist construction.

To make a long story short, in academic research on the connotation of ecological civilization there were different understandings, but most people agreed with and often quoted Pan Yue's formulation: ecological civilization referred to the humanity follow nature and society, the harmonious development of the objective laws and made the sum of matter and spirit result; referred to the people and nature, people and people, people and society's harmonious coexistence, benign cycle, all-round development, the basic purpose of continued prosperity and cultural ethics.[10] This formulation pointed out to deal with the relationship between man and nature and man and man's main problem, and especially the ecological civilization as the cultural and ethical form of existence.

CONSTITUTION OF ECOLOLIGICAL CIVILIZATION

Constitution of ecological civilization is an important field of the research of ecological civilization. Scholars divided the constitution from different perspectives. Ji Zhenhai held there are four levels in the constitution of ecological civilization. The first level was consciousness civilization, i.e. building the ecological civilization view. The second level was behavior civilization, which is to advocate thrift and oppose waste, and to settle the varied problems during the unsustainable development with civilized behaviors. The third was system civilization such as setting up relevant institutions, legislating laws and regulations, taking educational steps to build ecological consciousness. The fourth was industrial civilization, including the civilization of ecological agriculture, ecological industry, ecological service industry, environmental protection industry and so on.[6]

Shen Shuguang divided it from the perspective of philosophical foundation, ecological and scientific foundation, and energy foundation of ecological civilization. Also founded on the civilization of lasting and constant evolvement of ecological system, the ecological standardization of science and technology meant studying, developing, managing and applying science and technology according to the demand of ecological principles. Every civilization form had its specialized main energy and

ecological energy would be the basis during the development of ecological civilization.[11]

Li Shaodong analyzed the ecological civilization from the perspective of pure ecological moral value, high ecological ideal, scientific ecological culture, good ecological behaviors and so on. Ecological moral value included the western concept of ecological civilization, the Marxism's concept of reconciliation with nature, etc. Ecological ideal was seen as one of the specific contents of the common ideal of socialism. Ecological culture emphasized more on the publicity and education about the contents of ecological culture. For ecological behavior, it was the behavior of adhering to the moral rules of socialism and nature rules.[7]

Zhang Shouxian explained it from the perspective of deep and surface structure. Deep structure included ecological consciousness, ecological ethics and ecological culture, which was the qualitative regulation. Surface structure included ecological material civilization, ecological behavior civilization and ecological system civilization, which was the quantitative regulation. The two coexist and influence each other.[12]

WAYS OF CONSTRUCTING ECOLOLIGICAL CIVILIZATION

Nowadays with the increasingly serious pollution of the environment, how to construct and enhance the ecological civilization and how to promote the sustainable development of the economy and the society have been hot topics, which have been paid a lot attention. People have done many extensive and deep researches on these, gained many valuable achievements and reached some consensus.

Firstly, deepen the construction of system and mechanism to provide a system support for ecological civilization construction. Zhou Shengxian held, on one hand, the government should perfect and implement the mechanisms including the compensation system of using resource, the compensation mechanism for ecological environment and so on. Then adjusting to fundamental realities of the country should be set up and improved. On the other hand, for citizens, the decision-making mechanism and management mechanism should be improved, the participation channel should be enlarged and non-governmental organizations should be encouraged to develop in a healthy way.[13]

Secondly, further promote democracy and law construction, and perfect the laws and regulations about the construction of ecological civilization. Gao Weixing held the construction of ecological civilization was a systematic project that needed to establish and improve a complete set of scientific and effective laws, policy and technology on environment. At the same time, strengthening law enforcement, firmly combating various crimes of damaging ecological environment and bringing ecological civilization construction into the legal track.[14] Cui Hong thought that specialized laws of environment protection should be made and the whole law system from different perspectives should be used to protect environment in order to realize the transformation of ecological laws.[15]

Thirdly, greatly strengthen ecological education and increase power source of constructing ecological civilization from the masses. Yin Chengyong emphasized the moral concern in the relationship between nature and human, which meant constantly enhancing the human's moral responsibilities for nature and constantly improving

ecological moral quality of the whole nation.[16] Zhu Dafeng demonstrated the social responsibilities and ways of ecological civilization construction for the ideological and political education in colleges and universities.[17] Chen Yuzhou thought cultivating ecological human was the aim of ideological and political education, which could make people realize ecological civilization was an important part of social structure, rethought the relationship between human and nature and transformed the ethical values.[18]

Fourthly, strengthen the construction of ecological city, and build the regional supporter ecological civilization. Zou Aibing pointed out that, in the early studies, scholars have started to pay attention to the mode change of city development, claiming the city construction in accordance with the principles of ecology.[19] Ju Meiting etc, systematically discussed the construction of ecological city, from the urbanization and the development history of ecological city, supporting system, main base, index system and evaluating and so on, using these aspects to inform that the ecological city was the important supporter in constructing ecological civilization.[20] In 2009, Qiu Baoxing first proposed the concept of low-carbon ecological city in the 2009 International Forum on city planning and development, starting the research and practice of low-carbon ecological city. But the construction was still under exploration stage.[21] Reviewing the studies on the index system of ecological cities at home and abroad, Xie Pengfei,etc, introduced the construction method and procedure of ecological city , and selected 13 typical demonstration cases to summarize and evaluate, putting forward policy suggestions[22].

THE CONCLUSION AND PROSPECT

In summing up the research progress on the connotation, constitution and construction of ecological civilization, we can see that, the existing research has achieved fruitful results, providing a strong theoretical support for the construction of ecological civilization in China. With the continuous advancement of the practices of the ecological civilization construction, the research on ecological civilization will also continue to be strengthen and deepen. In the expectable future, the study on ecological civilization can be put forward from the following three aspects. One is to strengthen the pertinence. To accurately grasp the present situation of ecological civilization construction around, in view of the existing difficulties and problems, to make the ecological civilization theory research to better serve the practices of ecological civilization construction. Two is to enhance the accuracy. At the same time of making theoretical explanations and regulation cognition, we shall perfect the construction of evaluation and index system to provide scientific theoretical basis for the evaluation of ecological civilization achievements. Three is to strengthen the meta-study of ecological civilization. The accumulation of related papers, books and other research outcomes laid a solid and reliable theoretical foundation of creating the subject of ecological civilization. To strengthen the following and to summarize the academic trends, providing the necessary theoretical support for constructing and improving the theoretical system of the ecological civilization subject.

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ABSTRACT

In recent years, astaxanthin is claimed to have an antioxidant activity as high as 10 times more than other carotenoids like lutein, zeaxanthin, canthaxanthin, and β-carotene; 500-fold stronger free antioxidant activity than vitamin E; 38-fold stronger than β-carotene and 100 times greater than α-tocopherol. *Penaeus monodon* (Giant Tiger shrimp) is the largest commercially available shrimp and the waste is an important source of carotenoids such as astaxanthin and its esters. In order to establish an efficient and simple recovery process, the astaxanthin was extracted from the freeze-dried Giant Tiger shrimp waste (including head, tail and shell) using mixture of different organic solvents. The techniques of identification and separation of carotenoids used were UV spectrophotometric test and the high performance liquid chromatography (HPLC) analysis. The qualitative and quantitative of the astaxanthin extract then were evaluated. The total carotenoid content in *P. monodon* waste was 272.51 ± 0.16 μg/g dry weight and 6 astaxanthin derivatives were identified. The first 5 peaks (peaks 1-5) were identified as *trans*-astaxanthin monoester and peak 6 was found as 13-*cis*-astaxanthin monoester.

INTRODUCTION

Over the last decade, the global production of shrimp has been increasing steadily and this trend is expected to continue [1]. Shrimp processing is one of the most vital marine industry that generates a large bulk of waste products. Normally, the heads, shell and tail portions of shrimp are removed during processing. The body parts processed for human utilization comprises only 70% of the overall shrimp landing. So, there is a remarkable tonnage of shrimp waste generated in which one of the major carotenoids, astaxanthin can be obtained [2].

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Astaxanthin is a red pigment that belongs to the family of the xanthophylls, the oxygenated derivatives of carotenoids [2]. Most crustaceans like shrimps, crabs, and lobsters are tinted in red by accumulation of this pigment. It is also available in birds like flamingo, in microorganisms, insects and micro-green alga (*Haematococcus pluvialis)* [3]. Astaxanthin is widely used as a pigmentation source in aquaculture and poultry industries and gained applications in nutraceutical and pharmaceutical industries due to its outstanding antioxidant activity [3]. This pigment was proven to have 500-fold stronger free radical antioxidant activity than vitamin E and 38-fold stronger than β-carotene [4]. Astaxanthin, has been identified as the predominant pigment isolated from *Penaeus* shrimp [5, 6]. Apart from that, Malaysia is also one of the main producer countries of this species [7]. To the best of our knowledge, studies of the Carotenoid content and composition from Malaysian waters are still lacking. So, the results from this study would provide new information regarding the carotenoid that can be found in the shrimp waste from Malaysia. Therefore, this study aimed to characterize astaxanthin from Giant Tiger shrimp waste due to the availability and abundance of this species in our country. Furthermore wild Malaysian Tiger shrimp wastes are rich in astaxanthin and it would be a great opportunity in health food industry if astaxanthin could be extracted as a functional ingredient.

MATERIALS AND METHODS

Sample Preparation

Sample of fresh Giant Tiger shrimps (*P. monodon)* (20-30 counts kg^{-1}) were harvested from Tanjung Karang, Selangor. The shrimps were immediately transported in an expanded polystyrene box to the laboratory under iced condition (-4 ℃). The samples (carapace, shell and tail) were freeze-dried for 72 hours, after which the sample was grounded into fine powder and kept at -80 ℃ until further analysis. Most of the solvents and chemicals were of analytical grade (acetone, methanol, hexane, chloroform, petroleum ether). Ethyl acetate and acetonitrile were of HPLC grade purchased from Fisher Chemicals Company, USA.

Extraction

The extraction procedure follows the established methods described by [8]. 1.0 g of each powdered freeze-dried sample was rehydrated by adding distilled water and extracted with a mixture of acetone and methanol mixture (7:3) at room temperature until colorless. The crude extract was centrifuged for 5 min at 10 000 g and stored at 4 ℃ in the dark prior to analysis. To extract carotenoids, an equal volume of hexane and distilled water were added to the combined supernatants. The solution was then allowed to separate and the upper layer containing astaxanthin was collected. The combined upper phase was then dried to completion under a gentle stream of oxygen-free nitrogen.

Determination of Total Content of Carotenoid

Total carotenoid concentration was determined by spectrophotometric method as described by [8]. The dried carotenoid was resuspended in 300 µL of ethyl acetate.

Then, 50 µL of the redissolved sample was diluted with 950 µL chloroform for spectrophotometric analysis. The carotenoid-containing solution was measured at three different wavelengths: 480 nm, 648 nm and 666 nm using Varian Cary 50 UV-Vis spectrophotometer. The Wellburn Equation [9], in chloroform was applied to obtain the total carotenoid content.

HPLC Analysis

The HPLC analysis was performed on an Agilent model 1200 series comprised of a binary pump with autosampler injector, micro vacuum degassers, thermostatted column compartment and a diode array detector in accordance to [8]. The column used was a ZORBAX Eclipse XDB-C_{18}end capped 5 µm, 4.6x 150 mm reverse phase column (Agilent Technologies, USA). Astaxanthin standard was obtained commercially from Chromadex, Inc. Sigma-Aldrich. The solvents used were (A) acetonitrile:water (9:1 v/v) and (B) ethyl acetate. The solvent gradient used was developed as follows: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min), 60-100% solvent B (25-25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B(35-35.1 min) at a flow rate of 1.0 mL min^{-1}. The temperature of the column was maintained at 20 ℃. The identity of astaxanthin esters were confirmed by their spectral characteristics, absorption of the carotenoids in the mobile phase. Compounds were identified by co-chromatography with standards and by elucidation of their spectral characteristics using a photo-diode array detector. Detection for their peaks was in the range of 350 to 550 nm.

RESULTS AND DISCUSSION

A total of 6 carotenoid derivatives were detected from the shrimp waste as shown in Figure 1.0. All of the peaks were identified based on the spectral characteristics, retention time and the absorption spectrum as described in detail by [10] [11] and [12]. Most of the peaks were identified as astaxanthin esters (Table 1). The first 5 peaks were identified as *trans*-astaxanthin monoester (λmax = 478), (λmax = 478), (λmax = 476), (λmax = 478) and (λmax = 478). While peak 6 was tentatively identified as 13-*cis*-astaxanthin monoester (λmax = 468). It was reported that *P. monodon* preferentially accumulates astaxanthin monoester in its exoskeleton when the total carotenoid content exceeds 80 µg/g [13].

In this study, the total carotenoid content for *P. monodon* waste was 272.51 ± 0.16 µg/g dried weight (DW) which is 87.9% higher than those harvested from Indian waters [14]. Paradoxically carotenoid content in *P. monodon* from waters of the Indo-Pacific region varied from 23 to 331 µg/g in the exoskeleton. The lowest level in these prawns were those having a pale blue body colour and the highest level were those having a dark grey body colour [15]. Interestingly, the body colour of different shrimp species varied with carotenoid content. As a good example, *P. stylifera,* having a higher carotenoid content, showed a dark orange/red exoskeleton while *P. indicus,* having a low carotenoid content, showed a whitish exoskeleton [14].

Astaxanthin and its esters also can be found from the extracts of algae (*Haematococcus pluvialis)* and most of astaxanthin esters were determined at the retention time around 15 to 30 min [12] and [16]. The carotenoid composition in

H. pluvialis was found similar to that in some other varieties of shrimps such as Antartic krill [17]. In nature, *H. pluvialis* has become one of the most important diets for algae consumers and their predators higher up in the food chain like shrimps and salmon which later accumulate astaxanthin in which contribute to the accumulation of the astaxanthin carotenoids in their bodies [18]. However, in this study there is no free astaxanthin identified as most of the astaxanthin were esterified and not properly hydrolyzed.

TABLE 1. HPLC ANALYSIS OF CAROTENOID COMPOSITION IN *P. monodon* WASTE

Peak No.	Type of carotenoid	Retention time (min)	λ (nm)
1	*trans*-astaxanthin monoester	23.891	478
2	*trans*-astaxanthin monoester	24.322	478
3	*trans*-astaxanthin monoester	25.188	476
4	*trans*-astaxanthin monoester	25.495	478
5	*trans*-astaxanthin monoester	27.200	478
6	13-*cis*-astaxanthin monoester	27.833	468

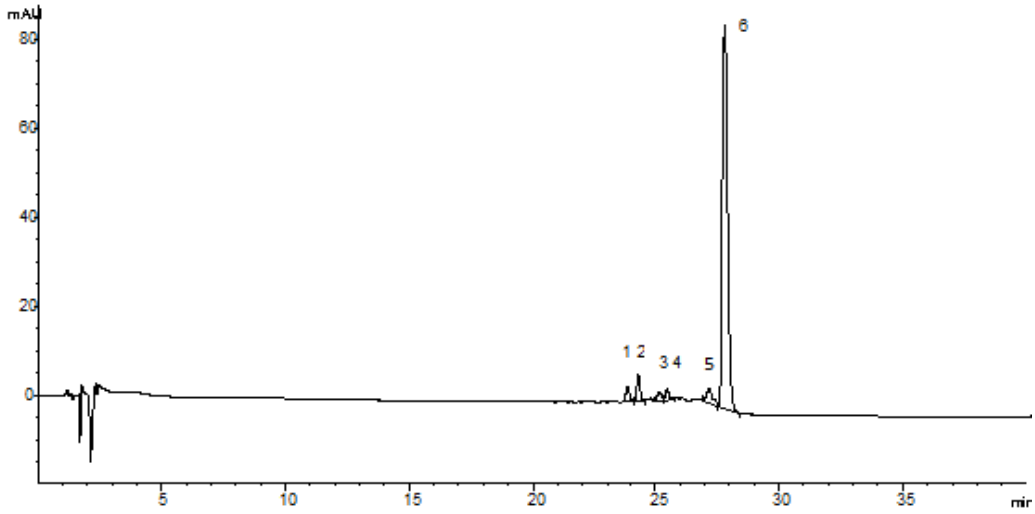

Figure 1. HPLC chromatogram of carotenoids extracted from *P. monodon* waste harvested from Tanjung Karang, Selangor, Malaysia. Chromatographic conditions are described in Table 1.

CONCLUSION

These findings highlighted the potential to manipulate the total carotenoid content and the type of carotenoids that are produced in Giant Tiger shrimp waste based on the multiplicity of pigment, feed, environment where the species were found as well as extraction method. The total carotenoid content of Tiger shrimp waste reported by [14] was 145 µg/g and according to [18] the most prevalent carotenoid found in the commercially important penaeids is astaxanthin, which representing about 65-98% of total carotenoids present and the most predominantly is in ester form (diesters and monoesters). The questions remain for further analysis and research is which factor or mechanism will stimulate carotenogenesis of free astaxanthin.

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Cover page

Title: *Research on the Relationship between Cyanobactria-bloom and Lake Surface Temperature in Lake Taihu Using Time-series MODIS Data*

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ABSTRACT

Lake surface temperature (LST) is one of the important parameters of Lake Ecosystem and significantly affects water quality. 100 images from Moderate Resolution Imaging Spectroradiometers (MODIS) from 2009 to 2010 were used to retrieve lake surface temperature in Lake Taihu by split-window algorithm, where long-term and widespread field measurements are rarely available. The results have very good accordance to the results of field measurement, with the correlation coefficient of 0.975. In addition, the seasonal and spatial variation in temperature was detected based on the retrieved values. Finally, the relationship between cyanobacteria bloom and lake surface temperature was analyzed. Appropriate lake surface temperature (25~30℃) was necessary condition of cyanobacteria bloom. Cyanobacteria bloom broke out seriously from May to October in 2009 and 2010 when the temperature increased to 25℃, especially in October in 2010 with the covering area exceeded 400km^2.Through the study, we hope to figure out the inner mechanism of algal-bloom and offer theoretical support to lake eutrophication management and the forcasting and monitoring of cyanobacteria bloom.

INTRODUCTION

Eutrophication in lakes and reservoirs is one of the most serious water pollution problems at present and will still be in the near future all over the world. According to the report of U.S. EPA, eutrophication accounts for almost one half of the impaired lake areas and 60% of impaired river reaches within the United States alone [1], and eutrophication-related water quality impairment can have very substantial negative economic effects. Frequent algal-bloom causes predictable increases in the biomass of noxious and toxic algae, which leads to water quality deterioration and drinking water crisis [2]. It has great influence on industrial water supply, tourism, fisheries and the people living around the basin. As a result, to expound the dominant physical, chemical and biological factors that might induce the formation of algal-bloom, get comprehensive knowledge about the cyanobacteria bloom-forming mechanism and

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their relationship with algal growing and propagation in eutrophic lakes and to establish the prewarning and monitoring model of algal-bloom is one of the most important approaches to control algal-bloom.

Lake surface temperature is one of the most important parameters determining ecological conditions in lake water [3]. It influences water chemistry as well as biological processes inside of a lake. The structure of physical properties of fresh-water lakes can be characterized by the temperature changing with depth. Furthermore, temperature differences between air and water are controlling the exchange of heat and moisture and are, therefore, crucial for understanding the hydrological cycle. Numerous modelings and empirical studies show that lake surface temperatures are closely related to algal propagation and algal-bloom. Kong & Gao [4] raised the four-phase development hypothesis on the process of the cyanobacteria bloom-forming: dormancy in winter, recruitment in spring, growth and float to the water surface in summer and sink to the sediment in autumn. Bloom formation and the accumulation of harmful scum on the water surface, is a result of the rising of temperature. Based on the monitoring data from 1992-1999 in Meiliang Bay, Lake Taihu, the result showed that water temperature is the most significant related factor to total algal biomass and Microcystis biomass[5]. Furthermore, Liu et al. [6] found out that Microcystis biomass was significantly correlated with water temperature, the correlation coefficient of which is 0.9. As for Lake Taihu, appropriate water temperature(ranged from 24 ℃ to 30 ℃) was necessary condition of blue algal bloom, while high temperature(>30 ℃) significantly inhibited the growth of blue algae [7]. When the annual mean temperature increased by 1.0 ℃, the annual mean algal biomass increased by 0.145 times, and the more eutrophic the lake, the greater the effect temperature had on algal growth [8]. To conclusion, when water temperature is below 20 ℃, cyanobacteria are under dormancy and recruitment. The most appropriate temperature for cyanobacteria is between 24 ℃ and 30 ℃. Higher water temperature (>30 ℃) would restrict the growth of cyanobacteria. To inhibit algal growth in the phase of dormancy or recruitment with directed measurements is one of the most effective ways to control algal-bloom.

The regular water quality monitoring of Lake Taihu by traditional means of sampling and analysis is intensely funded. However, because of the large surface area of the lake, it is difficult to monitor then by conventional methods. Simple point measurements of a certain variable can lead to inaccurate estimates of lake conditions in general. Considering the present sampling frequency of nine to ten times a year, there is also a significant risk that temporal variations may be missed. These limitations in spatial and temporal resolution can make it hard to detect long-term changes in parameters with a high degree of variation such as water temperature. The data generated by remote sensing satellites such as Landsat TM, MODIS with mono-window or split-window algorithm to retrieve LST can complement continuous water temperature monitoring programs. It may even develop the value of the present programs as the resulting spatial and temporal scales at which the quality parameters are monitored could reach levels that have previously not been possible.

In this paper, our objective is to test whether the MODIS L1B 1km spatial resolution product can be applied for lakes whose surface areas are large enough to be observed at the MODIS spatial resolution. We extracted lake surface temperatures for Lake Taihu from the MODIS images using split-window algorithm and compared the satellite-derived lake surface temperatures with field temperature measurements. We

presented temperature development over the time-series of 2009 to 2010 in Lake Taihu and detected the temporal and spatial variation in temperature, which cannot be shown by any other method. In addition, the relationship between cyanobacteria bloom and lake surface temperature were analyzed, in order to understand the mechanism of algal-bloom and offer scientific support to eutrophication control and cyanobacteria bloom prewarning and monitoring.

REGION UNDER STUDY

Lake Taihu is the third-largest lake in China. It is located between 30°55′40″N and 31°32′58″N, and 119°52′32″E and 120°36′10″E in Yangtze Delta, eastern China. It has a total water area of about 2,338.11 km^2, and also a shallow lake with the mean depth of 1.9 m and maximum depth of 2.6 m corresponding to an elevation of 3.0 m above sea level. The Lake Taihu drainage basin (Fig.1) is the most industrialized area in China with high population density, urbanization, and economic development. It is important for water supply, flood control, tourism and recreation, shipping and aquaculture [9]. With the increasing pollution from both urban and rural areas surrounding Lake Taihu Basin over the past two decades, eutrophication and algal blooms (Microcystis sp.) have occurred frequently which severely affects the function of the lake as a drinking water supply. In 2007, the worst blue-green algal bloom ever recorded in Lake Taihu occurred, depriving nearby residents of clean tap water and causing local people panic. Therefore, more efficient water quality monitoring of the lake is urgently needed [10].

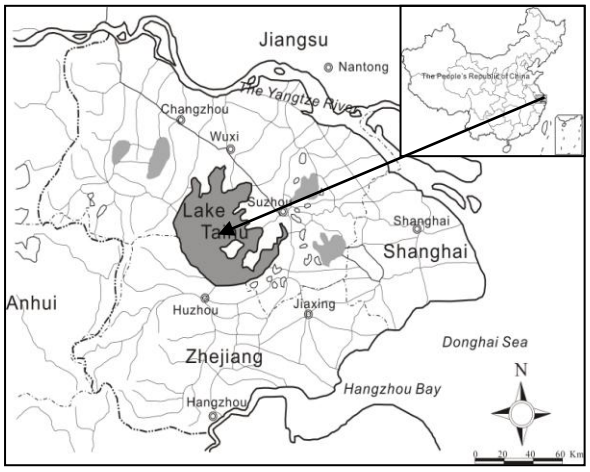

Figure 1. Location of Lake Taihu Basin in China

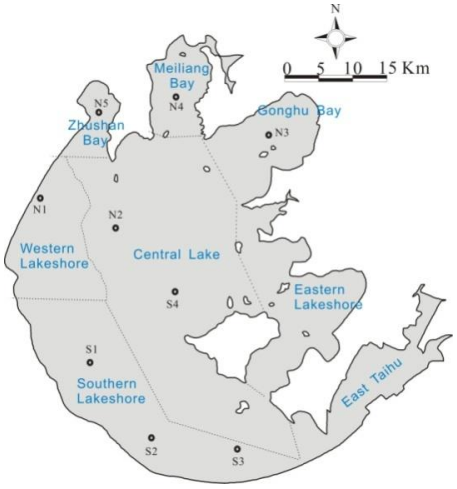

Figure 2. Distribution of the 9 sampling sites in Lake Taihu

METHOD

Field Observations

The monitoring has been conducted 9 to 10 times per year, at nine points in Lake Taihu(fig. 2). Lake Taihu was separated into eight districts in our study, that is,

Zhushan Bay, Meiliang Bay, Gonghu Bay, Western Lakeshore, Southern Lakeshore, Eastern Lakeshore, Central Lake and East Taihu. The nine sampling points were located in these districts, one in each bay, which has been severely influenced by cyanobacteria bloom. East Taihu was neglected as it is abundant in aquatic macrophytes such as phragmites communis and potamogeton malaianus, which can effectively inhibit cyanobacteria bloom [11]. Water temperature measurements are conducted using AAQ1183 Aqua quality sensor. It is a modernized compact and light-weight multi-parameter water quality meter, developed following the completion of COMPACT series lineup. Temperature is measured by the thermistor with 0.001℃ resolution and ±0.02 ℃ accuracy. Here we have used temperature data measured in the depth of 0.3m in 2009–2010.

MODIS Imagery

The Moderate Resolution Imaging Spectroradiometers (MODIS) carried by NASA's Terra and Aqua satellites acquire data in 36 spectral channels from 0.415 to 14.235 μm with a spatial resolution between 250 m and 1 km [12]. Two bands are imaged at a nominal resolution of 250 m at nadir, with five bands at 500 m and at the remaining 29 bands at 1000 m, which is perfectly suitable for large lakes like Lake Taihu. Its large spectral range makes this sensor capable of collecting information about atmosphere, land, ice and/or water properties simultaneously. The positions of the MODIS spectral bands, the instrument's high radiometric sensitivity compared to common ocean color satellites, and the relatively easy access to acquired data makes it attractive also for applications in limnology. In this study we used two sets of MODIS data. One is the MODIS TERRA and AQUA radiance 5-Min L1B swath 1km data of the years 2009 and 2010 to retrieve lake surface temperature in Lake Taihu with split-window algorithm. The other is MODIS/Terra land surface reflectance daily L2G global 250m data to retrieve the covering area of cyanobacteria blooms. The data were provided by the NASA/WIST-ECHO at https://wist.echo.nasa.gov/api/. Images potentially affected by significant cloud contamination were identified objectively using climatological and spatial threshold tests and then visual inspection was used to remove cloud-contaminated images. All available clear-sky MODIS Terra and Aqua imagery between 2009 and 2010 were used, resulting in a total of 100 Terra (Aqua) clear-sky images after preprocessing.

Retrieval of LST from MODIS Data

DATA PREPROCESSING

All data get from NASA are preprocessed through geometric correction and atmospheric correction. The MOD09GQ surface reflectance files were subsetted and reprojected with the MODIS Reprojection Tool (https://lpdaac.usgs.gov/lpdaac/tools/modis_reprojection_tool). For MOD02 1km L1B calibrated radiances files, each image was geo-referenced based on the WGS 84 UTM zone 50 North coordinate system in ENVI software.

On the other hand, due to the interaction between solar radiation and atmosphere, changes in satellite sensor calibration, differences in illumination and observation angles, MODIS sensor receives distorting signals. Atmospheric correction is necessary

to acquire true surface reflectance and improve the accuracy of retrieving lake surface temperature. ENVI's Fast Line-of-sight Atmospheric Analysis of Spectral Hypercubes (FLAASH) module is a first-principles atmospheric correction modeling tool for retrieving spectral reflectance from hyperspectral radiance images [13]. MOD02 1km L1B calibrated radiances files are processed by FLAASH module, and the input parameters are as following: zenith angle, azimuth angle, sensor type, sensor altitude, ground elevation, flight date, flight time GMT, atmospheric model, aerosol model, initial visibility and scene center location and so on.

SPLIT-WINDOW ALGORITHM

Up to now, many algorithms for estimating sea surface temperature, land surface temperature from satellite observation have been proposed. Mono-window algorithm is a simple inversion of the radiation transfer equation providing that the surface emissivity and the atmospheric profiles are known in advance [14,15]. Multi-window algorithm combines more than two thermal channels together, which can simultaneously retrieve surface temperature and emissivity [16]. The split-window algorithm was firstly utilized to derive sea surface temperature, which was expressed as the linear combination of the brightness temperature of two adjacent windows [17]. It uses the differential absorption in adjacent thermal band to correct the atmospheric effects [14,18] .From then on, a variety of split-window methods have been developed and modified to retrieve sea surface temperature and land surface temperature. Based on the studies of previous researchers, Qin et al. [14] make some reasonable simplification for the radiation transfer equation and propose a split-window algorithm based on NOAA/AVHRR, which needs only two parameters (emissivity and transmittance) and the accuracy of retrieval land surface temperature is under 2 ℃. The general algorithm of split-window can be depicted as follows [14]:

$$T_S = T_4 + A(T_4 - T_5) + B \quad (1)$$

T_S represents land surface temperature. A and B are parameters. T_4 and T_5 are brightness temperatures of AVHRR 4/5, respectively. The unit of T_S, T_4, T_5 is K.

Mao et al. [16] improved the algorithm by Qin [14] to be suitable of land surface temperature retrieval from MODIS data. For comparational convenience, he simplified the equation as follows:

$$T_S = A_0 + A_1 T_{31} - A_2 T_{32} \quad (2)$$

T_S represents land surface temperature. $T_{31}$and $T_{32}$are brightness temperatures of MODIS 31/32, calculated by the DN value of the two bands. A_0 ,A_1 , A_2 are parameters, determined by land surface emissivity and atmospheric transmittance. Firstly, the emissivity can be estimated through the components of one pixel at the scale of MODIS. Vegetation, soil, and water are the basic ingredients for MODIS TIR band image [19]. We use NDVI to get the ratio of three ingredients to estimate the surface emissivity of Lake Taihu [16,20]. Secondly, atmospheric transmittance of thermal band is retrieved by analyzing the relationship between atmospheric water vapor content and transmittance in NIR bands through atmospheric simulation [21,14]. Kaufman et al. [22] did many experiments and concluded that the retrieval of water content by ratio is available. Then water vapor content is retrieved from the NIR band (band 2 and 19) of MODIS image [16]. The details of the above-mentioned parameters

can refer to the Mao et al. [16] and Qin et al. [14]. The routine to retrieve lake surface temperature can be described as Fig. 3.

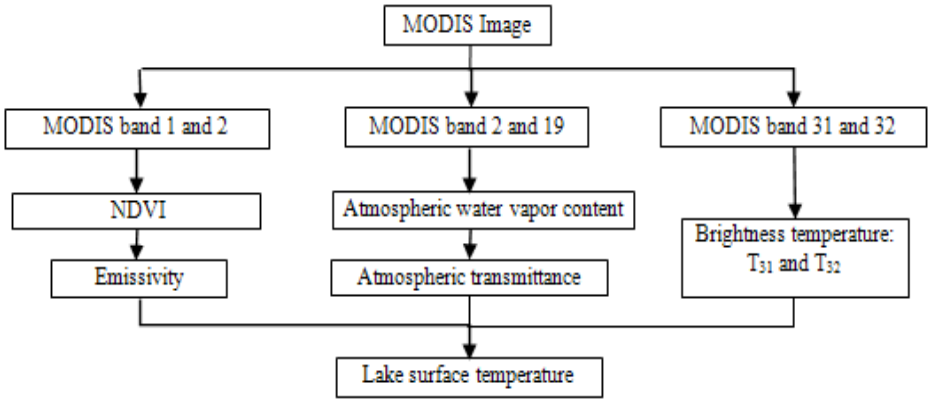

Figure 3. Map of retrieving lake surface temperature by MODIS through Split-window algorithm

Retrieval of Cyanobacteria Bloom Covering Area from MODIS Data

Chlorophyll-a (chl-a) is an important parameter to determine the lake trophic state. It is one major factor affecting water environment, and produces visible changes in the surface of waters. With the breakout of cyanobacteria bloom, the concentration of chl-a increased, which has a positive correlation with the absorption coefficient in 440nm and 680nm, as well as with the reflectance peak in 700nm [23,24]. According to the study of Duan et al. [25], the spectral character of typical objections in MODIS can be concluded as following. In the visible light bands (band 3, 4, 5), trophic water has similar spectral character with turbid water, with reflectance peak in green light (band 4), the value of which is similar and obviously higher than clean water and vegetation. In near infrared bands (band 2, 5, 6, 7), the difference between cyanobacteria and water is larger and is more similar to the spectral of vegetation.

Particularly, there is 'slope effect' in band 2 (841-876nm). As a result, the key of deriving cyanobacteria distribution by MODIS is to distinguish the spectral character of cyanobacteria and water, especially turbid water. In our study, band 2 has been used to extract the information of cyanobacteria as it is the best band to distinguish whether the water is trophic, turbid or clean. The area with the value of band2>0.1 is extracted as the cyanobacteria bloom covering area. Fig.4 shows the distribution map of cyanobacteria bloom On August 19th, 2009.

VALIDATION

Sources of Error

Firstly, validation of MODIS-derived lake surface temperature has been limited [26]. Errors in satellite-derived LST may arise from instrument noise and drift, sun glint [27], cloud contamination (e.g., thin cirrus), misspecification of atmospheric attenuation [28] and surface emissivity effects. Meanwhile, split-window algorithm was proposed by Qin [14] and Mao [16] based on land surface temperature and sea surface temperature retrieval. When being applied to retrieve lake surface temperature,

the parameters will change together with the difference in study area and the mineral substance within the water, which made it less precise to retrieve lake surface temperature. More mature studies should be made to improve the accuracy of the algorithm.

Secondly, according to the field measurement in sampling sites N1 and N2 in Lake Taihu from July 9 to 13, 2009, the difference between skin temperatures(0.3m below the water surface) and bottom water temperature(0.3m above the bottom of the lake) is less than 1.5℃ (Fig. 5). At 10:00, the average top-bottom temperature difference is 0.4℃, at which time satellite Terra overpasses Lake Taihu Basin, while the value at 14:00 is approximately 0.4 ℃, when Aqua overpasses this region. For large shallow lakes like Lake Taihu without temperature phase separation, the surface water temperature could be representative for the temperature variation and distribution of the whole lake.

Thirdly, the relationship between skin and bulk temperature is modified by near-surface temperature gradients that have two different factors: the heat exchange with the atmosphere and thermal stratification of water column [26].

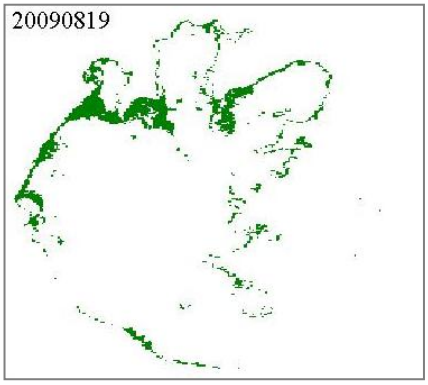

Figure 4. Distribution map of cyanobacteria bloom

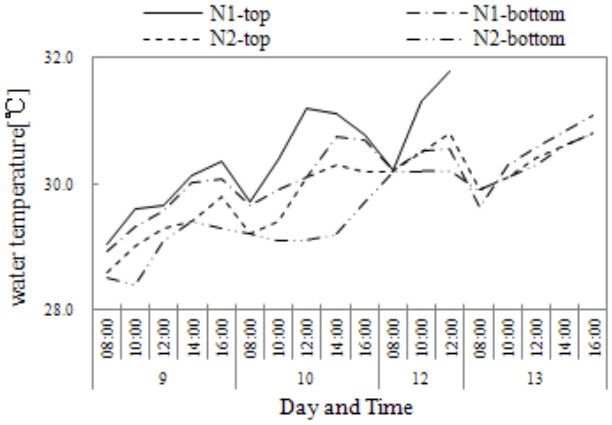

Figure 5. The difference of temperature between the top and the bottom

Comparison of MODIS Retrieved LST with Field Temperatures

According to the nine sampling points in Lake Taihu, the 5*5 square was chosen to calculate the average MODIS derived temperature. Only field measurements taken within 1d of the satellite retrieval were used for the comparison, resulting in 124 total match-up. Despite the relatively small number of remaining match-ups, the retrieved lake surface temperature estimates from MODIS over Lake Taihu were compared to the skin temperature measurements (0.3m below the water surface) conducted at the respective sampling sites (Fig. 6).

From the figure it can be seen that, for the selected sampling sites, there is a sound correlation between MODIS retrieval lake surface temperature and the field skin temperature measurements. Especially the sites N1 (Western Lakeshore), N3 (Zhushan Bay), N4 (Meiliang Bay) and N5 (Gonghu Bay) show good agreement with satellite data. For sampling points S1 and S2 located in Southern Lakeshore, there is somewhat higher mean difference between the satellite and field measurements. When investigating the single differences between the field temperatures and the MODIS

retrieved LST estimates, it was found that for most of the match-ups, the satellite retrieved temperatures were a bit higher than the field temperatures. This may attribute to that the sampling was mostly taken in the early morning, while the satellite crossing time was at noon, when the heat accumulation led to higher water temperature.

The complete data set of all 124 match-ups shows a mean absolute difference of 3.00 ℃ between the satellite and field temperatures; the standard deviation amounts to 1.35 ℃. It is noticeable that the average temperature difference is larger and the correlation coefficients is 0.975, the reason for which may be that the split-window algorithm was optimized not for lake surface temperature, but for land surface temperature. The estimation of lake surface emissivity from NDVI was lower, which led to high surface temperature [29].

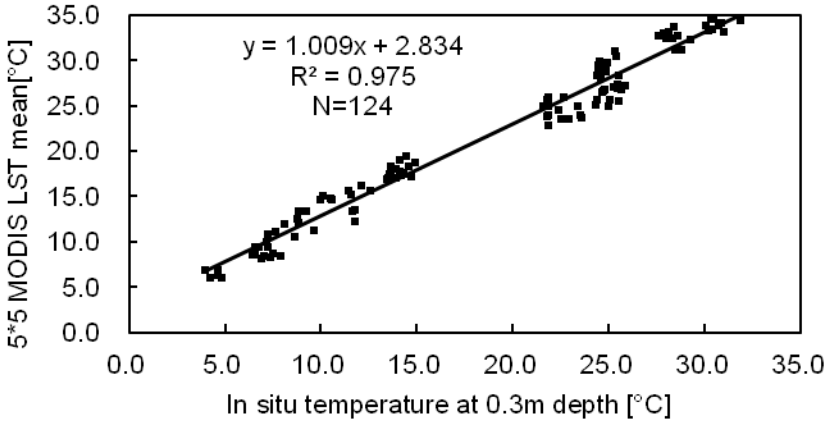

Figure 6. comparison of in situ surface temperature with MODIS retrieval LST value for the available sampling sites shown in Fig.2.

RESULT AND DISCUSSION

Lake Surface Temperature

Fig. 7 shows the maximum, mean and minimum lake surface temperature in from 2009 to 2010 in Lake Taihu. Generally, temperatures decrease from shoreline to the center of the lake, as a result of heat exchange between the surrounding land and the lake. The water temperature series show significant seasonal variation of lake surface temperature, summer with higher temperature and winter with lower temperature. January is the month with the lowest maximum temperature (9.6 ℃), which starts to rise in February (15.7 ℃). From March to May, the maximum temperature increases to around 31 ℃. From May to August, the temperature increases form approximately 31 ℃ to 35 ℃. In October, the temperatures start to drop. The mean temperatures present the same observed patterns as the maximum temperatures.

The time series shows that the maximum temperature is the lowest in January, rising dramatically in February (Fig.8). From February to May, the temperature increases (~15 ℃) in relation to May. Between May to September, the maximum temperature shows little variability. The months of October, November and December present a downward turn in maximum temperature. The mean temperature presents the same patterns observed in the maximum temperatures. The minimum temperatures present steady increase during January and April, while after April, the minimum

temperature starts rising substantially. After September, the minimum temperature starts decreasing.

Describing the seasonal variability of average monthly MODIS-retrieved lake surface temperatures is complicated by the pronounced cloud cover and lack of clear-sky images during the rainy season (June to August). While we present data from the entire year, we are less confident of the accuracy of rainy season seasonal variability statistics due to this limitation. However, the pattern of mean temperature trends is well-defined, with one peak in the time series, which occurs in July. The lowest temperature occurs in January.

Seasonal Variation of Lake Surface Temperature based on Cyanobacterai-Bloom

With the degradation of eutrophication in Lake Taihu in recent years, algal-bloom (especially the Microcystis aeruginosa bloom) occurs frequently in summer, showing significant seasonal characteristics [4,30]. Therefore, it is sensible to analyze the season variation of lake surface temperature for understanding the cyanobacteria bloom in Lake Taihu. According to the climatology in China and the condition in this region, the seasonal maps were computed using the average monthly mean from 2009 to 2010 of the following months (Fig.9): winter (from December to February), spring (from March to May), summer (from June to August), autumn (from September to November). The analysis of the seasonal variation of lake surface temperature shows the following characteristics: In winter, the average temperature is lower than 10 ℃, the metabolism of cyanobacteria have stopped, affected by the low temperature and the dark circumstance. The change of temperature determines whether the cyanobacteria could get through the cold winter and the total amount of the algal source in the coming year. In spring, the temperature rises steadily, which is suitable for algal to recruit abundantly. The physiological and biochemical activity of algae resuscitate slowly and the population starts to form. During this period, the rising of temperature will provide temperature benefit for algal growth and the outbreak of algal-bloom. Summer is the season with the most severe algal-bloom and eutrophication in Lake Taihu, when temperature reaches its maximum value in July. With temperature rising from 25 ℃ to 35 ℃, algae grow and float to the water surface with other appropriate meteorological and hydrological conditions [31]. In autumn, surface temperature during September was still relatively high, which is suitable for the continuous outbreak of cyanobacteria bloom. Cooling happened continuously until November, when temperature fell below 15 ℃. From Fig.8, we can see that quadratic curves were fitted to the MODIS retrieved lake surface temperature season variation.

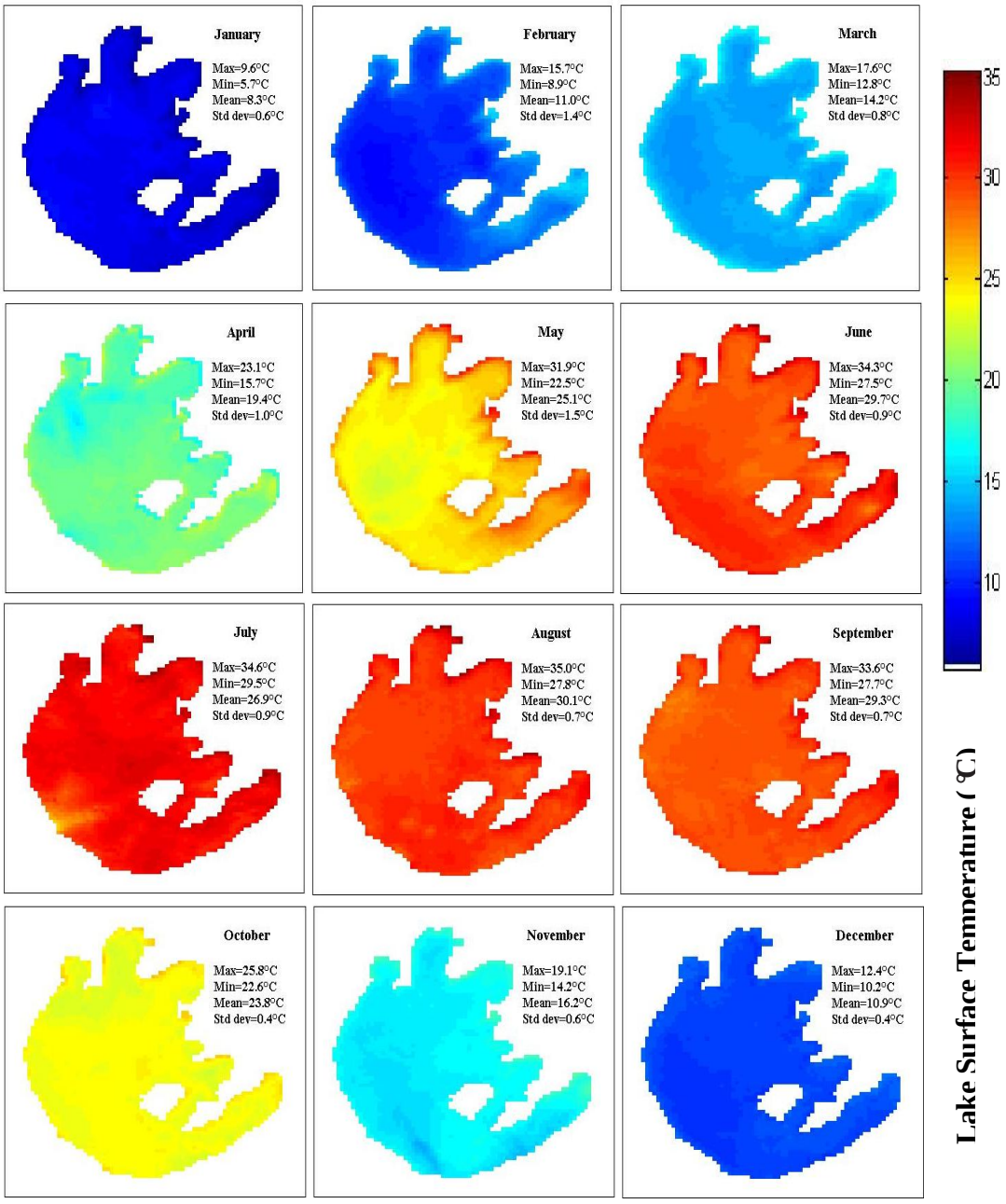

Figure 7. Monthly lake surface temperature (LST) from 2009 to 2010. The maximum, minimum, mean and standard deviation in median monthly LST are shown in upper right corner

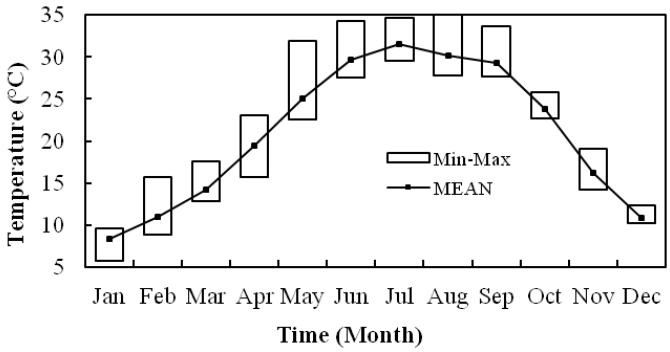

Figure 8. Monthly mean LST statistics from 2009 to 2010 showing the mean, minimun, maximum values .

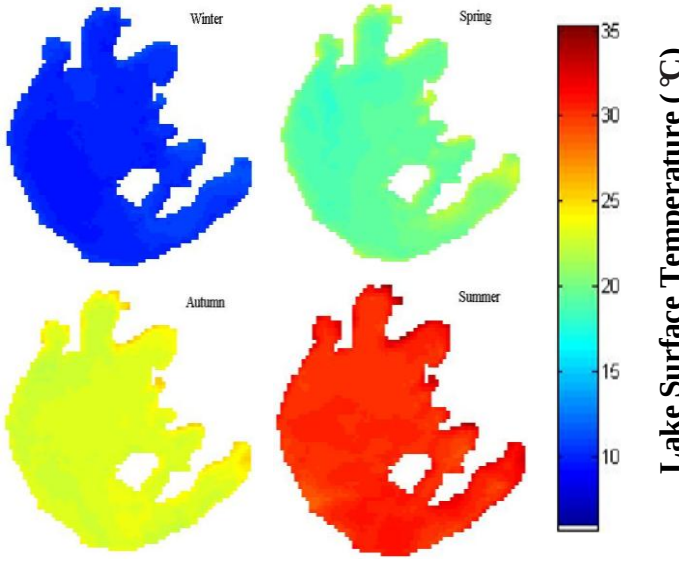

Figure 9. Monthly mean lake surface temperature

Spatial Variation of Lake Surface Temperature

Although Lake Taihu has a large area, there is inconspicuous spatial variation in temperature over the entire surface. As the water temperature nearby the land is higher than that of the central lake because of heat exchange, the lake surface temperature retrieved through split-window algorithm can reflect the phenomenon. It can also be seen from Fig.10. According to the analysis of lake surface temperature in Western lakeshore, Central lake, Southern lakeshore and the three bays, the maximum temperature occurs in the three bays, with the annual average temperature of 21.6 ℃, maximum temperature of 32.85 ℃ and minimum temperature of 7.15 ℃. The minimum temperature occurs in the Central Lake, with the annual mean temperature of 20.5 ℃, maximum temperature of 32.29 ℃ and the minimum temperature of 8.61 ℃. This is caused by the fact that the Central region lays far away from the land and has a slower change of temperature rising and decreasing. During the period of MODIS crossing the study area, the water temperature of Taihu is rising with the solar radiation. The retrieved surface temperature in the central lake is lower than other region lack of the heat exchange with land.

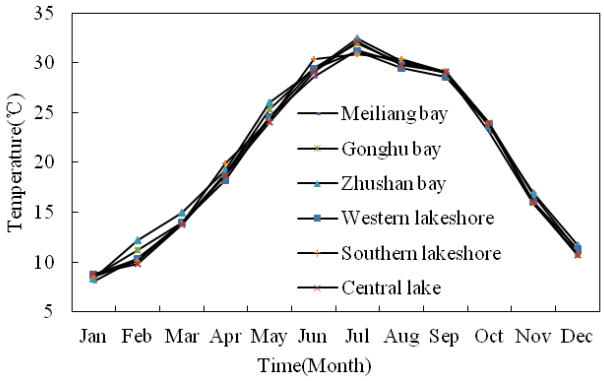

Figure 10. Spatial variations of LST in each region of Lake Taihu

Relationship Between Lake Surface Temperature and Cyanobacteria Bloom

Based on split-window algorithm and the method to calculate cyanobacteria bloom covering area, the monthly average lake surface temperature and cyanobacteria bloom covering area were shown in Fig.11, additionally with MODIS time-series cyanobacteria bloom covering area in 2009 and 2010 (Fig. 12&13). It is obvious that there was a favorable synchronization between LST and cyanobacteria bloom. The annual cycle of LST showed one regular summer peak in accordance with fluctuations in cyanobacteria bloom covering area, with high LST, almost up to 30 ℃ during summer declining to 7 ℃ by January. The highest cyanobacteria bloom covering area retrieved was in August.

It has generally been accepted that cyanobacteria have higher temperature optima for growth than any other phytoplankton groups. Harmful cyanobacteria such as Microcystis populations have been shown to dominate above 25 ℃ [32]. According to this idea, the cyanobacteria bloom in Lake Taihu broke out seriously from May to October when the temperature rose above 25 ℃. On August 21th, 2009, the cyanobacteira bloom covering area reached 998km^2, while the LST was 33.1 ℃ (Fig.12). The overall situation in 2010 was improved and quite different from 2009. As is shown in Fig.13, algal-bloom outbreak concentrated in October. The first one turned up in March, which was advanced for almost a month, and the last one postponed to early November.

The general condition of cyanobacteria bloom from 2009 to 2010 could be described as following: In early April, the algae started to recruit with the rising of temperature. The first peak of cyanobacteria bloom outbreak occurred in May and continued to early June. Along with the arrival of the rainy season, the situation was better. The second continuous outbreak turned up in August due to the high air temperature and scarce precipitation. When it comes to November and December, the results may be unconvincing as influenced by high concentration of suspended substance. Meanwhile, the outbreak stopped ultimately as the decreasing of water temperature and severely cold weather condition. From Fig.11, we can see that there has been a good relationship between cyanobacteria bloom covering area and the lake surface temperature. According to the different response relationships, the whole year is separated into four phases.

(1) From March to late May, the mean surface temperature of Lake Taihu increased gradually from 14 ℃ to 25 ℃, while the cyanobacteria bloom covering

area presented a significantly positive correlation with the rising and fall of the temperature.

(2) After the rainy weather in June, the mean surface temperature rose rapidly above 30℃ in early July. Since then to mid-September, the lake surface temperature was the highest all the year around (>30℃). The relationship between LST and cyanobacteria bloom was not well due to the algal migration effected by wind, waves and lake current [5].

(3) From mid-August to late-October, lake surface temperature shows a remarkable fluctuation, so was the stats of cyanobacteria bloom, presenting a relatively weak correlation. According the algal-bloom report in 2010, on August 15th, large-scale outbreak of cyanobacteria bloom occurred, when the average lake surface temperature reached above 32.5℃. However, on October 31th in 2010, even though the lake surface temperature was only 24.5℃, the algal-bloom covering area exceeded 400km2, which might lead to the weak correlation.

(4) Since late-October, cold weather frequently struck Lake Taihu basin, the surface temperature gradually dropped below 20℃. There existed a relatively good synchronization between average surface temperature and cyanobacteria bloom covering area (R^2=0.5979). When the temperature dropped below 5℃, dormancy of the cyanobacteria started.

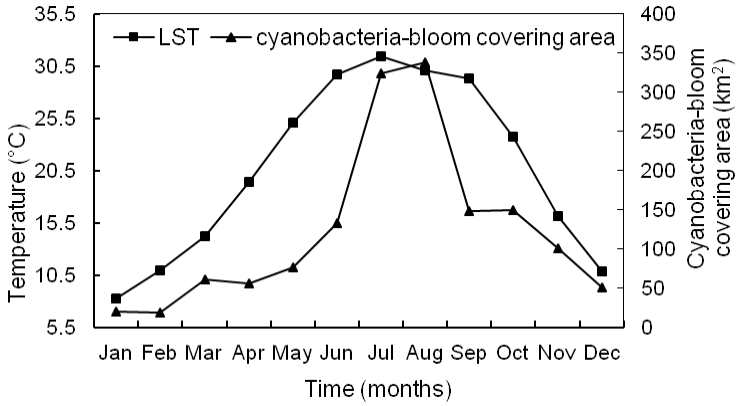

Figure 11. Montly average lake surface temperature and cyanobacteria-bloom covering area from 2009 to 2010 in LakeTaihu

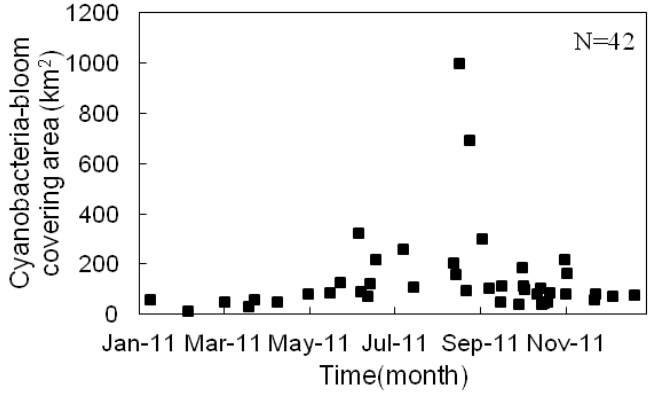

Figure 12. MODIS time-series cyanobacteria-bloom covering area in 2009

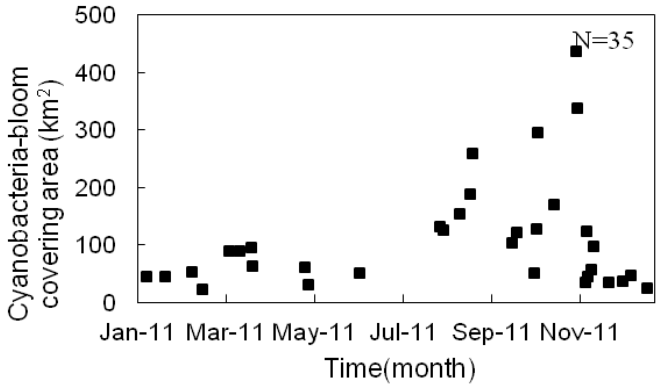

Figure 13. MODIS time-series cyanobacteria-bloom covering area in 2010

CONCLUSIONS

The objective of this study was to map the LST and improve understanding of spatial and temporal variations in Lake Taihu using MODIS time series data. Simultaneously, the relationship between cyanobacteria bloom and LST was analyzed. LST were retrieved from MODIS L1B products through split-window algorithm. Band 2 of MODIS land surface reflectance products has been used to extract the information of cyanobacteria. The main conclusions are: Firstly, it can been seen that the results of split-window algorithm to estimate lake surface temperature has very good accordance to the results of field measurement, with the correlation coefficient of 0.975. Secondly, for spatial variation, temperatures decrease from shoreline to the center of the lake. Maximum temperature occurs in the three bays and minimum temperature occurs in the Central Lake. However, there is inconspicuous spatial variation in temperature over the entire surface. The seasonal analysis shows that quadratic curves were fitted to the MODIS retrieved lake surface temperature seasonal variation. Finally, from the MODIS time series data analysis, there is a relatively good relationship between cyanobacteria bloom and lake surface temperature. Appropriate lake surface temperature (25~30 ℃) was necessary condition of cyanobacteria bloom. However, variation of cyanobacteria bloom covering has also been irregular. Algal growth is inherently complex, predominantly showing nonlinear responses to various environmental parameters such as temperature, light and several nutrients [8]. Furthermore, the condition of Lake Taihu is so complicated that it is impossible for us to find out the exact correlation between LST and cyanobacteria bloom merely through MODIS time-series data. Higher resolution remote sensing images are required for the study, and the influence of wind, nutrient and solar radiation should also been considered.

ACKNOWLEDGEMENTS

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Cover page

Title: *Sorption of Cu^{2+} Ion from Aqueous Solutions by Phosphoric Acid Activated Poultry Manure Biochar*

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ABSTRACT

Biochar (BC) converted from poultry manure can be used as an alternative adsorbent for removal of heavy metals in aqueous solution. This study investigated the effect of phosphoric acid treatment of BC obtained from slow pyrolysis of chicken manure wastes. The acid modification substantially increased surface areas of BC. Surface areas of the BC treated with phosphoric acid were much higher (17.7 ±1.5 times) than those of the BC without the acid treatment. The acid-activated BC with a high surface area of 320.1 m^2/g can be utilized as a cheap alternative adsorbent of commercial activated carbon. For copper removal from aqueous solutions, the acid treated BC showed maximum adsorption in the pH of 5.5 and good adsorption capacity of 137.6 mg/g, which can be comparable to that of commercial activated carbon. These findings suggest that acid-activated BC may be an effective, less costly, and environmentally sustainable adsorbent for copper removal from aqueous solution.

1. INTRODUCTION

Some toxic heavy metals can be accumulated in the human bodies and other organisms. The concerns over the presence of toxic heavy metal ions in wastewater from industrial processes such as metal-finishing, electroplating and chemical manufacturing industries have been increased [1]. Heavy metals which have high water solubility can easily enter the food chain through many ways. Many of non-biodegradable or accumulated species in the human bodies or in the environment bodies have been proven to hazardous materials and lead to cause a number of health problems, diseases and disorders [2]. The heavy metals concentrations must be removed to acceptable levels before being discharged into the environment since they are non-degradable in nontoxic end products. Heavy metal intake the human may occur primarily from contaminated food, drinking water, skin and lung adsorption. According to world health organization (WHO) [3] and International Programme on Chemical Safety (IPCS) [4], the most toxic heavy metals are chromium, cobalt, nickel, copper, zinc, cadmium, mercury and lead. The primary sources of copper released from industries such as printed circuit board production, metal finishing processes, tannery operations, chemical

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manufacturing, and mining drainage. Copper may cause stomach and intestinal distress, liver and kidney damage, and anemia. As copper is a highly toxic heavy metal, removing copper ions from wastewater is an important procedure.

A number of treatment methods for removing heavy metals from wastewater including chemical co-precipitation, reverse osmosis, ion exchange, evaporation, membrane filtration and adsorption. Adsorption is one of the most common and effective method for removing dissolved heavy metal ions from wasteswater [5]. Adsorption is often used at the end of a treatment process for pollution control because of the high degree of purification that can be achieved. Biochar (BC), an emerging carbon material produced mainly from low-cost biomass residuals such as agricultural waste and poultry manure, has received much attention recently in the science community because of their potentials in many environmental applications, including water treatment, carbon sequestration, soil improvement, and environmental remediation [6]. BC has structured carbon matrix with a medium-to-high surface area that may act as a surface sorbent. It is known that some BCs are able to absorb heavy metal ions. Many researchers have modified BC characteristics to increase their acidic surface functional groups to adsorb effectively heavy metals by forming chelate. Xue et al., 2012 [7] tried to enhance the fraction of the oxygen-containing functional groups in biochar using H_2O_2. Kastner et al., 2012 [8] was successfully added acidic functional groups into BC using H_2SO_4.

In this work, biochar was thermally-produced from slow pyrolysis of chicken manure and then was treated with phosphoric acid (H_3PO_4). We hypothesized that H_3PO_4 treatment of the biochar could increase oxygen-containing functional groups on its surface and thus enhance its ability to remove copper from water. The purpose of this study was to develop a new technology to modify biochar derived from chicken manure and to develop value-added, biochar-based sorbents for the removal of copper.

2. MATERIALS AND METHODS

2.1. Biochar Production

Chicken manure was collected from a typical chicken farm in Ulsan, Korea as feedstock for biochar production. Naturally dried chicken manure was pyrolyzed in the furnace reactor maintained at 450^0C for 1h. The reactor was then cooled to room temperature and the biochar was collected. For the acid activation, 10 g of the obtained BC was mixed with a 200 ml of 75% phosphoric acid solution (H_3PO_4) in a glass and then heated to form a paste. The paste solution was placed in the furnace and heated from room temperature up to $100\ ^0$C, and then maintained for 30 min for further oxidation process at $100\ ^0$C. After the treatment with phosphoric acid, the samples were washed with a mixture of 0.1 M of NaOH and distilled water (H_2O) until the pH of the washing solution reached 6 to remove impurities. After being washed with NaOH and distilled water, the fine powdered activated BC was dried in the oven maintained at 100 ℃ to get constant weight. The activated BC sample was stored for later experiments.

2.2. Characterization of Biochar

Table 1 shows physical and chemical properties of the BC samples. Carbon,

hydrogen, and nitrogen contents of the samples were determined using an Elementar Analyzer (LECO-CHNS 932, USA). To measure the pH, 5 g of the sample was added into distilled water to obtain a final volume of 100 ml. The mixture was then shaken and allowed to stand for 5 min before measurement with a pH meter. Specific surface areas of the BC samples were measured with a surface area analyzer (ASAP 2020, Micormeritics Instrument Co., Norcross, GA, USA) using N_2 (BET) adsorption methods to determine macro- and micropore surface area.

2.3. Batch Sorption of Lead and Cadmium

Laboratory batch sorption experiments were conducted to find the isotherms of copper sorption onto the BC. About 0.2 g of the adsorbent was mixed with 100 ml Cu (II) and aqueous solution ($Cu(NO_3)_2$) for each experiment. The mixture was then shaken in a mechanical shaker (120 rpm) at room temperature (20 – 22^0C). To obtain the sorption isotherms, solutions of various lead concentrations were shaken with the adsorbent for 2 h. At the end of each adsorption experiment, the mixtures were immediately filtered and copper concentrations in the filtrate were determined using Atomic Absorption Spectrometry (AAS).

3. RESULTS AND DISCUSSION

3.1. Characteristics of BCs

Table 1 shows the characteristic of BCs sample before and after the phosphoric acid treatment. The oxygen and carbon contents identified from the raw chicken manure sample were 29.3 and 20.7%, respectively. By the using activation process, the carbon content of BC sample before the acid treatment significantly increased from 51.6 to 80.0%, while the oxygen content decreased from 17.1 to 10.9% as compared that after acid treatment. The surface area (BET) of BC sample after the acid treatment of 320.1 m^2/g was much higher than that (18.1 m^2/g) before the acid treatment. This indicates that the acid treatment (a kind of an activation process) make the organic compounds to be oxidized and inorganic substances to be dissolved in phosphoric acid solution, resulting in increasing carbon content, surface area and decreasing nitrogen and oxygen contents.

TABLE 1. THE CHARACTERISTICS OF BC SAMPLES

	C (%)	O (%)	H (%)	BET (m^2/g)	pH
Raw materials	20.74	29.27	2.10	-	-
BC before activated	57.56	17.35	2.66	18.1	-
BC after activated	80.02	10.85	2.63	320.1	6.0

3.2. Effect of Initial Solution pH

Solution pH is the important parameters in the sorption process optimization. It affects not only the surface charge of the adsorbent, but also the degree of

ionization and speciation of metal ions in solution. The solution pH effect of Cu(II) sorption removal by the adsorbent was identified by varying initial solution pH with keeping other sorption parameter constants. Effect of initial solution pH on adsorption was determined by mixing 0.2 g of adsorbent with 100 ml of solution containing Cu^{2+}concentration of 100 ppm at various pH values ranging from 2.0 to 6.5 in 120 minutes.

The effect of pH on the adsorption process is presented in Fig. 1. Since precipitation of Cu(II) occurred due to the formation of hydroxide complexes, carbonates or phosphates, the pH higher than 7.0 was not investigated. Removal of Cu(II) ions was relatively low at strong acid pH around 2.0 compared to that at higher pH values. This can be attributed to the fact that a high concentration of H^+ ions competes with Cu(II) for active sites at the low pH, with an apparent preponderance of H^+ ions, resulting in the suppression of Cu(II) adsorption on the surface of BC. Therefore, increasing the initial concentration of proton in aqueous solutions resulted in the decrease of Cu(II) removal at strong acidic conditions. The pH increase makes competition of Cu(II) ions with protons for binding sites and released more binding sites for Cu(II) ion adsorption, which results in the increase of adsorption effectiveness. The highest of adsoption of Cu(II) onto the biochar was observed at pH 5.5. This can be ascribed that high pH conditions reduce the mobility of Cu(II) due to the decrease in the exchangeable form, resulting in a decrease in the contact probability between adsorbent and adsorbate [9].

3.3. Effect of Dose BC Samples

The influence of adsorbent dose, ranging from 0.01 to 0.3g, for removal of Cu(II) ions

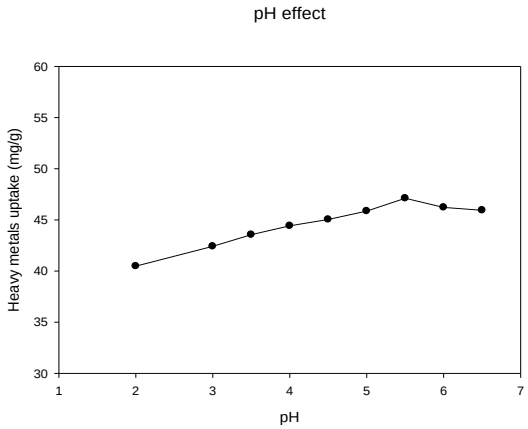

Figure 1. Effect of pH

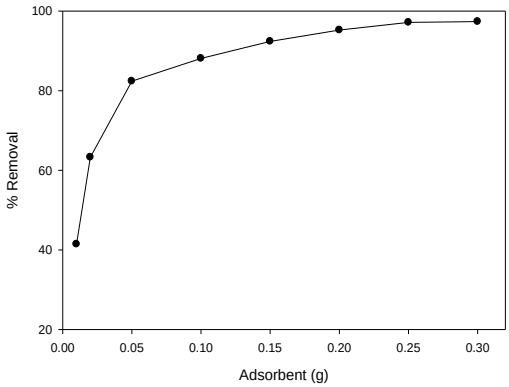

Figure 2. Effect of adsorbent dose

was investigated at the optimum pH (5.5) in 120 min. Fig. 2 shows the effect of the adsorbent dose. The increase in adsorbent dose increased adsorbed amount of metals onto the activated BC. As the adsorbent dose increases from 0.05 to 0.30 g, the removal of copper (II) ions increased from 41.42% to 97.35%, respectively. The adsorption percentage increases rapidly with the increasing adsorbent dose from 0.01 to 0.25 g. However, the removal efficiency showed only small increases above 0.25 g of the dose. This slight increase is due to the decrease in availability of active surface sites and increase in conglomeration of the adsorbent at such higher dose above

0.25g. The optimum dosage of BC for adsorption of copper (II) ions was considered as 2.5 g/l. All active sites on the adsorbent surface are then occupied and increase in adsorbent dosage do not provide higher uptake of copper (II) ions.

3.4 Effect of Initial Concentration of Metal Ions

Fig. 3 shows the effect of initial concentration of metal ion solution for removal of Cu(II) ions at a pH of 5.5 with a constant agitation speed of 150 rpm for 120 min. The amount of Cu^{2+} adsorbed increased with increase in the initial concentration upto 400 ppm of copper (II). It means that the removals of copper ions are highly concentration-dependant. At lower concentrations of copper ion, the amount of metals ions available for the adsorption by BC samples is less as compared to the available sites on the adsorbent. However, at higher concentration, the available sites for adsorption become fewer. These results indicate the adsorption removal of the heavy metal ions depends on the initial concentration of interest metals. It is obvious that the amount of Cu (II) ions adsorbed is not increased considerably when the initial of Cu (II) concentration are higher than 400 mg/l, which have 128.73 mg/g for adsorbent Cu, respectively. It was found that the adsorption rate is rather fast at the initial adsorption stage and then it slows down gradually after more sites were occupied by the adsorption metals. Similar results were obtained in the systems described in the literature, e.g. for the Cu(II) and Zn(II) adsorption on BC obtained from pyrolysis of hardwood and corn straw [10].

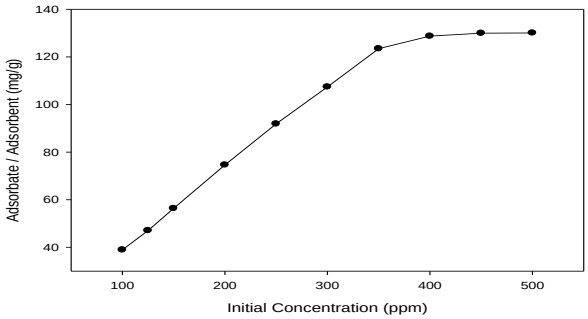

Fig 3. Effect of initial concentration

4. CONCLUSION

The phosphoric acid-activated chicken manure biochar showed maximum adsorption for Cu(II) at pH 5.5. The adsorbent amount, pH and concentration of solutions affected the adsorption process. Adsorption data were strongly correlated with the Langmuir adsorption isotherm indicating surface homogeneity. The equilibrium sorption capacity of 137.6 mg g^{-1} as determined from the Langmuir isotherm for Cu(II) are very high compared to the those of previous reported adsorbents. The BC samples can be used for removal of Cu (II) ions as an alternative to activated carbon.

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Cover page

Title: *Effect of Lipid Levels on Growth, Selected Liver Biochemical Parameters and Histology of Blood Parrot*

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ABSTRACT

Use the FuLinMen Grade A soybean oil as the fat source, grass goldfish expanded diet as the base material to compound 5 different feed of which dietary lipid level at 6.28%, 9.43%, 12.11%, 14.79% and 18.40% respectively. Use them to feed blood parrot 60 d, study the effects of dietary lipid level on the growth, selected biochemical parameters of liver and tissue structure of blood parrot. The result shows that the dietary lipid level between 6.28% and 9.43%, BWG and CF increase significantly along with the increasing of dietary lipid level. When the level is over the 12.11%, HSI also increases significantly. The mortality of test fish increases significantly and up to 45%, when the dietary lipid level over 14.79%. The content of total lipid, CHOL and TG increases along with the increasing of dietary lipid level, among which, TG and total lipid increase significantly of the level at 12.11%, CHOL increases significantly of the level at 14.79%. When the dietary lipid level is 14.79%, liver tissue section shows vacuolated cells, cells without core and some degree of fat degeneration, and when the dietary lipid level is 18.40%, the fat degeneration goes serious. From the study above, it shows that suitable dietary lipid level of feed for blood parrot is 9.43%.

1. INTRODUCTION

Blood Parrot is a new kind of fish comes from the hybrid of male *Amphilophus labiatus* and female *Cichlasoma synspilum Hubbs*. Because its body color is bright and red and its body shape is cute, it is very popular in the market [1-3].

Fat is one of the important nutrition for fish. Fat in the feed provides the fish not only the needed power for growth and vital activity, but also the essential fatty acid which is required to maintain normal physiological function. The insufficient or overabundance of the fat level in feed can cause different effects on the growth, development and reproduction of aquatic animals, and then leads to some metabolic diseases [4-5]. At present, different researches focus on the effect of different dietary lipid level on fish growth are carrying out in domestic and abroad [6-9], but there are only few reports focus on the research of blood parrot fat nutrition. So in this experiment, the blood parrot is the research object. From the study of the effect of

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dietary lipid level on growth of the body, physiological indexes and tissue structure of the liver, to achieve the proper dietary lipid level that suitable for the growth of blood parrot, provide some theoretical foundation to the development of blood parrot feed.

2. MATERIAL AND METHODS

2.1 Feed Used in the Experiment

Use the grass goldfish expanded diet produced by Tianjin Tianxiang Aquatic Products co., Ltd as the base material, FuLinMen grade A soybean oil as the fat source, to compound 5 kinds of feeds with different fat levels. The percentage of soybean oil in these 5 different feeds is 0%, 3%, 6%, 9% and 12% respectively, and the total lipid level in the feeds is 6.28%, 9.43%, 12.11%, 14.79% and 18.40% respectively. Detailed nutrition ingredient of the feeds is shown in Table Ⅰ below.

TABLE Ⅰ DIET NUTRITION INGREDIENT PERCENTAGE (%)

Formula	6.28%	9.43%	12.11%	14.79%	18.40%
Soybean oil	0.00	3.19	6.38	9.57	12.76
Expanded diet					
Crude Protein	33.00	33.00	33.00	33.00	33.00
Crude fiber	10.00	10.00	10.00	10.00	10.00
Crude ash content	10.00	10.00	10.00	10.00	10.00
Ca	1.13	1.13	1.13	1.13	1.13
P	1.23	1.23	1.23	1.23	1.23
Salt	0.90	0.90	0.90	0.90	0.90
Water	11.00	11.00	11.00	11.00	11.00
Lys	1.60	1.60	1.60	1.60	1.60

2.2 Feeding and Management

Blood parrots in this experiment are purchased from XunYang aquatic products co., Ltd located at JinNan district, Tianjin. After arrive the lab, blood parrots are domesticated in the plastic cases in the first two weeks, it sets five experimental treatments, each experimental treatment has three parallel samples, a total of 15 white plastic cases (50×36×45 cm). Each case has 30 fish [initial weight (3.64±1.07)g]. Feed the fish 5 times a day (09:00, 12:00, 15:00, 18:00, 21:00). Change 1/3 of the water in cases once a day and remove the residual bait and feces. Water in the experiment is 24h aeration tap water , temperature at 28±1℃, Feeding trial lasts 60d.

2.3 Sample Collection

After the 60d feeding trial, stop the feeding 24h, randomly pick 10 fish from each plastic case, anesthesia by MS-222, and then dissected and get the samples of liver, wash them using 4℃ precooling deionized water, store them in the -80℃ refrigerator. Then randomly pick 3 fish from each plastic case, dissection and take the terminus of the liver in the left side as the samples and put them into the Bonn's liquid to fix for 24h, wash them using 50% ethanol and finally store them in the 70% ethanol.

2.4 Determining of Indicators and Making of Tissue Slice

2.4.1 GROWTH INDICATIOR CALCULATION

Body Weight Gain (BWG, %)=[(W_t-W_0))/W_0]×100 ;
Condition Factor(CF,%)= (W/L3)×100;
Survival (survival,%)= number of survival/total number×100
Hepatosomatic index (HSI,%)= (Weight of liver/Weight of fish)×100;

Attention, In the formulas above, 'W_0' is the initial weight of a fish, 'W_t' is the final weight of a fish, 't' is number of days in trial, 'W' means the weight of fish, 'L' represents the length of a fish.

2.4.2 DETERMINING OF LIVER PHYSIOGICAL INDEXES

Defrost samples at 4℃ and cut them into pieces, and then add precooled pure water according to the ratio of quality to volume at 1:9. Homogenate the compound using glass homogenizer, this process lasts 10min under 3500r/min (LD4-2A, Low Speed Centrifuge), take the supernatant. Triglyceride(TG) and cholesterol(CHOL) is determined using reagent kit produced by Nanking Jiancheng Biological Engineering Research Institute, measuring instrument is automatic biochemical analyzer. Total lipid is determined using vanillin assay [10].

2.4.3 MAKING OF TISSUE SECTION

Fixed fish liver is sectioned using regular paraffin method, thickness of each section is 7 ~ 9μm, HE staining. Observe under the 'Nikon eslipse 50i' microscope, take pictures using 'Nikon coolpix 5400' digital camera.

2.5 Statistical Analysis

Experimental data shows as 'average±standard error ($\bar{x}$ ±SE), the analysis software is SPSS 17.0. Perform the significance test on the test outcome, using the ANOVA one-way analysis of variance and Duncan multiple comparisons method.

3. RESULT AND ANALYSIS

3.1 Effects of Dietary Lipid Levels on Growth of Blood Parrot

Effects on blood parrot's growth of dietary lipid level are shown in TableⅡ below. From the TableⅡ, it's easy to find that with the increasing of dietary lipid level, BWG and CF level increase at first and then decrease. Treatment of dietary lipid at 14.79% achieves the highest BWG but without conspicuous difference compared with the treatments of 9.43% and 12.11%(P>0.05). Highest CF level is achieved by treatment of 9.43% and it's conspicuously higher than following two treatments of which dietary lipid at 6.28% and 18.40%(P>0.05). Treatments of 14.79% and 18.40% achieve conspicuous higher HSI level than other treatments(P>0.05). And also, the dead rate of blood parrots increases with the increasing of dietary lipid level, obviously, treatment of 18.40% has a dead rate which is significantly higher than other treatments

have(P>0.05).During the feeding trial, fish in treatments of 6.28%, 9.43% & 12.11% is always good feeding, no abnormal symptoms on body shape. Most fish displays the symptoms of anorexia and powerless to swim normally after 6th week in treatment 14.79%. From the 5th week, fish in treatment of 18.40% starts to die.

3.2 Effects of Dietary Lipid Level on Biochemical Parameters of Blood Parrot'S Liver

Effects of dietary lipid levels on the biochemical indexes of blood parrot's liver are shown in Table Ⅲ.From the Table Ⅲ, it shows that the level of CHOL and TG increase with the increasing of the dietary lipid level. The CHOL and TG of 14.79% and 18.40% treatments are higher than others significantly (P<0.05). The trend of the relationship between total lipid level and dietary lipid level is displayed as positive correlation. Treatments of 18.4% achieves the highest level, which is significantly higher than the three treatments with relatively lower dietary lipid level(P<0.05).

TABLE Ⅱ EFFECTS OF DIETARY LIPID LEVELS ON GROWTH PERFORMANCE OF BLOOD PARROT

Treatments	6.28%	9.43%	12.11%	14.79%	18.40%
BWG(%)	53.27 ± 2.85^{a}	80.05 ± 1.67^{b}	92.35 ± 14.64^{b}	128.2 ± 19.50^{b}	57.09 ± 10.2^{a}
CF(%)	2.47 ± 0.18^{a}	3.23 ± 0.11^{b}	3.11 ± 0.55^{ab}	2.73 ± 0.09^{ab}	2.38 ± 0.04^{a}
HSI(%)	0.83 ± 0.10^{a}	1.3 ± 0.16^{a}	1.74 ± 0.11^{ab}	2.35 ± 0.17^{c}	2.75 ± 0.20^{c}
Mortality (%)	15.0 ± 5.0^{a}	13.5 ± 3.5^{a}	18.0 ± 7.1^{a}	36.5 ± 4.9^{ab}	45.0 ± 16.9^{b}

Note: Numbers in the same line with different corner marks mean the difference between samples is remarkable (P<0.05)

3.3 Liver Tissue Slice

After the 60d's feeding trial, the effects of different dietary lipid level on blood parrot's liver display in the plate I, From the Plate I, it shows that with the increasing

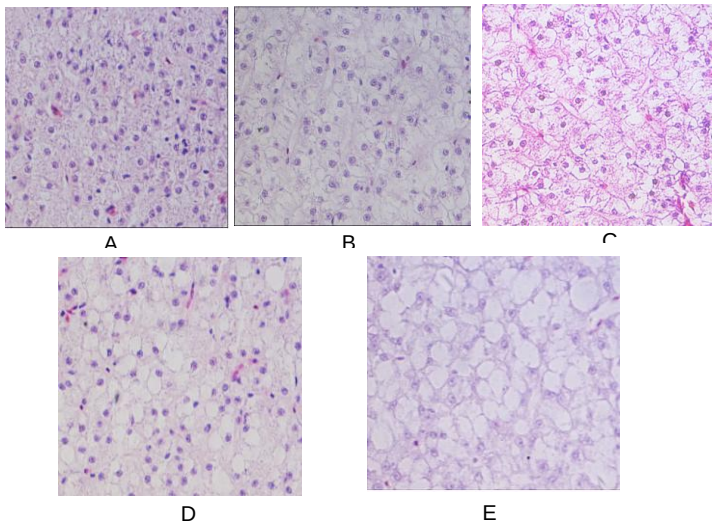

Plate Ⅰ Effects of dietary lipid levels on the liver structures of blood parrot

A. After 60d feeding trial, treatment of dietary lipid level at 6.28%, slice of the liver tissue.
B. After 60d feeding trial, treatment of dietary lipid level at 9.43%, slice n of the liver tissue.
C. After 60d feeding trial, treatment of dietary lipid level at 12.11%, slice of the liver tissue.
D. After 60d feeding trial, treatment of dietary lipid level at 14.79%, slice of the liver tissue.
E. After 60d feeding trial, treatment of dietary lipid level at 18.40%%, slice of the liver tissue.

of dietary lipid level, the liver's cells display conspicuous denaturation. In picture A, the liver cells have normal shapes, uniform and regular array, the cell nucleus is obvious and located at the central of the cell, and no nucleus offset and vacuoles (fat droplets) displays. However, in picture D, it shows vacuolated cells with no cell core and some degree of fatty degeneration, in picture E which represent the treatment of 18.40% dietary lipid level, it shows most of the cells lose their cell core and happen serious fatty degeneration.

TABLE Ⅲ EFFECTS OF DIETARY LIPID LEVELS ON THE BIOCHEMICAL PARAMETERS OF LIVER IN BLOOD PARROT (Unit : *mmol / l*)

Treatments	6.28%	9.43%	12.11%	14.79%	18.40%
TG	0.38 ± 0.02^{a}	0.61 ± 0.03^{ab}	1.05 ± 0.01^{b}	3.03 ± 0.21^{c}	3.06 ± 0.23^{c}
CHOL	0.50 ± 0.20^{ab}	0.61 ± 0.03^{b}	1.06 ± 0.01^{b}	2.68 ± 0.14^{c}	3.27 ± 0.03^{d}
Total lipid(ug/ml)	55.01 ± 0.41^{a}	56.56 ± 0.47^{a}	59.82 ± 1.06^{b}	61.55 ± 0.78^{bc}	64.17 ± 1.14^{c}

Note: Numbers in the same line with different corner marks mean the difference between samples is remarkable ($P<0.05$)

4. DISCUSSION

4.1 Effects of Dietary Lipid Levels on the Growth of Blood Parrot

Researches shows that fish with fatty liver will behave abnormally. Such like *Trachinotus ovatus* [11], *Sciaenopsocellatus* [12], *Hephaestus fuliginosus* [13], when they have fatty liver, it shows some abnormal symptoms. In this experiment, fish in treatments of 6.28% to 12.11% shows good body shape without any abnormal behavior and morphological characteristics, but fish in treatments of 14.79% and 18.4% show some bad result, most of them show the symptoms like anorexia and powerless to swim normally. Such symptoms observed in this experiment are basically similar with other fish suffered from fatty liver, just because of the fish species, growth stages, quality of feed and breeding environment are different, such abnormal symptoms shows different in degree [14].

Wang finds the proper dietary lipid level for *Myxocyprinus asiaticus* is between 6.62% and 7.02% [15]. Gan finds the *Oreochromis niloticus* achieves best growth performance with the dietary lipid level at 4% [16]. Furthermore, use the feed with dietary lipid level between 4.7% &10.9% can boost the growth of *Pelteobagrus vachelli* early juveniles [17]. In this experiment, When the dietary lipid level is at 12.11% and 9.43%, BWG and CF achieve the peak value. HSI is the key indicator to evaluate the healthy level of fish. Relatively low HSI indicates that the load of liver is in the manageable range [18], and this range depends on its own liver's function [19]. Constantly increasing the dietary lipid level can cause the elevation of HSI, it is also found in this experiment that the HSI of blood parrot in each group increases with the increasing of dietary lipid levels, especially when the dietary lipid level is 14.79% and 18.40%, the HSI indicate a significant increasing, and some fish show the symptoms of fatty liver.

4.2 Effects of Dietary Lipid Level on Biochemical Parameters of Blood Parrot'S Liver

The plasma lipoprotein of fish plays an important role in transporting the fat in

liver(mainly refers to TG). Lipoprotein is synthetized on the rough endoplasmic reticulum of liver cells, after combining with liver fat, it secrete into the cytoplasm through the Golgi apparatus, finally transported outside the liver with the blood, thereby maintains the equilibrium between the fat in the liver and fat in the blood. When intake too much fat, fat in liver cells cannot be transported outside the liver in time, it will cause the accumulation of the fat in the liver, leads to the increasing of fat content in the liver [20]. The outcome data of this experiment indicates that the total fat, TG and CHOL will significantly increase with the increasing of dietary lipid level. It means too high dietary lipid level will make negative influence on the liver function of blood parrot, and reduces its ability of transporting and clearing exogenous fat, and then blocks the outward transporting of TG, leads to the increasing of TG content in liver. Moreover, because the liver is the esterification organ of TG, when liver cell is destroyed or the liver function is damaged, Endogenous cholesterol as a transit LDLC and lecithin cholesterol acyl transferase (LCAT) activity decreased, LDLC number decreased, affecting the transport of cholesterol, leading to cholesterol in the liver weight retention.

3.3 Effects of Different Dietary Lipid Level on Tissue Structure of Blood Parrot'S Liver

For cultured fishes, intake of too much sugar and fat will cause the accumulation of much fat in liver cells, which can cause the degeneration and necrosis of liver cells, then makes the liver function reduced even exhaustion [21]. Research on the structure of the liver in Channel catfish shows that feed by high dietary lipid level fodder have honeycomb structure in their liver, the contour of liver tissue is not clear using SEM [22]. Xu [23] found the similar result on songpu mirror carp juveniles, with the dietary lipid level increasing, symptoms of nutritional fatty liver such like irregular arrangement, vacuolation and cell core offset take place on mirror carp liver cells. In this experiment, in the treatment of 14.79%, some liver cell appears as vacuolated, no cell core and fat degeneration, in the treatment of 18.40%, most liver cell appears as no cell core and serious fat degeneration. Results above indicate that feeding blood parrot using the fodder has dietary lipid level over 12.11% will cause serious liver damage, and HSI will increase significantly at the same time, which also shows too much fat is accumulated in the liver.

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Title: *Slope Risk Level Assessment Based on Erosion Induced Landslide: A Case Study in Malaysia*

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ABSRACT

Erosion induced landslide in one of the factors that governing landslides. Landslides in Malaysia cause thousands of deaths, injuries each year and ringgit in property damage. In attention of the erosion induced landslide as one of the factors governing landslides need to be well thought-out thoroughly. In this study, the development of landslide risk level based on erosion induced landslide which are related to six (6) factors of Universal Soil Loss Equation (USLE) are performed by data collection and soil sampling from various of slope and landslide located in Malaysia. Each factor in an erosion induced landslide is provided with own scale and all of factors are divided into its own weight depending on its impact which inducing landslide mainly on the soil erodibility and rainfall erosivity index . All of percentages for each factor were summed for the development of landslide risk level based on erosion induced landslide. Therefore, the finding of this research is a new technique of predicting with greater accuracy future erosion induced landslide and it can be made easier to identify in the country.

Keywords: Erosion induced landslides, Erosion factor, Universal Soil Loss Equation, Slope Risk Level .

1. INTRODUCTION

Landslide and Slope failure in Malaysia are a serious geologic hazard common to many parts of the world. in causing losses to various financial terms of which cost millions of dollars, and most importantly, can result in loss of life of residents. Most cases in Malaysia were involved hillside areas such as at Bukit Antarabangsa Tragedy in 2008, Cameron Highland in 2011 which caused a woman was killed, two people are in critical conditions with eight to ten people missing in a landslide involving five aborigine homes at a Sungai Ruil village and the latest at Puchong, Selangor 2013.

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In addition, slope failures due to deforestation for hillside development are partly the major causes. There were some instances where the development projects at hill sites were abandoned for a considerable period, affecting the maintenance of the slopes could cause landslides. Roslan [1] in his book of 'Forecasting Erosion Induced Landslide', the researcher stated that "erosion induced landslide poses enormous threats and over the past years as well as the present scenario has caused severe damages". Furthermore, based on engineering scope, the researcher highlighted that the soil erosion is a process of detachment of soil particles from the soil mass can be a function of rainfall erosivity.

In another study conducted [2], discussed that soil erosion by water agent is one of problem in tropical countries such as Malaysia, mostly on steep land and in areas devoid of vegetative cover. The researcher also mentioned that vegetation, soil properties, mechanical properties, rainfall and slope are interaction factors for soil erosion. Tew and Goh [3], defined that the soil erosion can be as the transport and detachment of soil particles from the environment and will occur when the intensity of rainfall exceeds the infiltration rate of the disturbed soil. There are two main agents of soil erosion which are wind and water**.** In addition, a study conducted by [4], emphasized that the process of soil erosion is a long-term and degradation land surface almost invisible which can be caused by wind or water and bad of human activities.

Therefore, this research was carried out to establish landslide risk level based on erosion induced landslide which is regarded to all the factors governing erosion process. The outcome of this study which is the method can be proposed and used to solve all problems related to erosion induced landslide issues and can improve understanding to a landslide risk level to give pre-warning to residents.

2. MATERIALS AND METHODS

2.1 Methodology

The methodology of this study mainly based on laboratory test and data analysis from selected location and obtained the information which focus on Universal Soil Loss Equation. From Universal Soil Loss Equation (USLE), the data that's collected and measured from selected sites and landslide tragedies locations. The locations of slope are selected based on the potential of high risk threat to resident near the sloping areas. Rainfall data are taken from the automatic rainfall station located within the 20km radius of the study area. In this study, the sampling areas are located in area Selangor and Kuala Lumpur. Furthermore, the slope parameter which used to measure slope length and steepness by using the Range Finder or as known as Trupulse™ 200 / 200 b. Figure 1 shows Mara University of Technology, Puncak Alam, Selangor and model of Range Finder, shown in Figure2.

Figure 1 : Mara University Of Technology, Puncak Alam, Selangor , Malaysia

Figure 2 : Range Finder model Trupulse™ 200 / 200 b

The laboratory such as particle size distribution, moisture content, and permeability are conducted to determine the soil characteristic that will relate with (USLE) by laboratory test according to BS 1377 Part 1 and Part 2. To solidify this study, historical site data will be collected from few related government or institutions namely:

1) Minerals and Geoscience Department Malaysia (JMG)
2) Public Work Department (Slope Engineering Department, JKR)
3) Department of Irrigation and Drainage Malaysia (JPS).
4) The International Research Centre for Disaster Prevention (IRCDIP)

The data that will be collected is based on Universal Soil Loss Equation (USLE) parameters as shown below:

1) Rainfall erosion index (R)
2) Soil erodibility factor (K)
3) Slope length (L)
4) Steepness factor (S)
5) Vegetative cover factor (C)
6) Erosion control practice factor (P)

Rainfall erosivity can be measured by using ROSE indexes that created by [5] and soil erodibility can be measured by using a ROM scale [1] and Equation 1 shown degree of soil erodibility index can be determined by using a Rom scale

formulation below and Figure 6 shows the measurement for length and steepness slope.

$$\text{Degree of erodibility index} = \frac{\%\text{Sand} + \%\text{Silt}}{2(\%\text{Clay})} \quad , \tag{1}$$

Every factor stated in difference scale and (6) six factors are divided into weight that the high percentage given to a major factor. The weightage of soil erodibility and rainfall erosivity are 40% for each because both are given a big impact to erosion induced landslide and both are equally important and the rest of factor will be 5% for each.

Risk level monograph considers x-axis and y –axis. Nomograph on y- axis defined the value in percentage factor that consider 5% for which length (*L*) and steepness (*S*), 10% for combination erosion control practice factor (*P*) with vegetative cover factor (*C*) . Rainfall erosivity index (*R*) and Soil erodibility factor (*K*) consider 40% weighting for each factor . On the X- axis only define the factor the governing erosion induced landslides. The difference of weight because the main factor governing erosion are rainfall erosivity (*R*) and soil erodibility (*K*) and other factors such as slope length (*L*) ,slope steepness (*S*), practice factor (*P*) and vegetative cover factor (*C*) only contribute to amount soil loss. The value of Risk Level can be created based on the summation of the percentage six (6) factors and the maximum of summation is 100%. Table 1 shows the total risk level which will be classified.

TABLE 1 : CLASSIFICATION OF SLOPE RISK LEVEL

Percentage (%)	Slope Risk Level
<20	Low
20.1 - 39.9	Moderate
40-59.9	High
60-79.9	Very high
80-100	Critical

3. RESULTS AND DISCUSSION

Based on the results, Table 2 shows the result from historical landslides and selected slopes in Puncak Alam area. The selected slopes at MARA University of Technology in Puncak Alam were divided into five (5) slopes and others are historical landslides. All of slopes are given the different value and risk levels are shown in Table 2.

TABLE 2: LANDSLIDES RISK LEVEL FOR SLOPES

Location	Percentages	LRL
Kampung Pasir , Ulu klang	**45.07**	High
Kampug Sungai Bkt Putih,Ampang	**56.46**	High
Seksyen 10,Wangsa Maju	**82.89**	Critical
Taman Hillview , Hulu Kelang	**55.67**	High
Taman Bukit Mewah , Bukit Antarabangsa	**76.42**	Very High
SLOPE 1 , Puncak Alam	**47.75**	High
SLOPE 2 , Puncak Alam	**44.66**	High
SLOPE 3 , Puncak Alam	**44.82**	High
Fakulti Sains Kesihatan 6A Taman Herba , Puncak Alam	**17.36**	Low
Fakulti Farmasi , Puncak Alam	**30.45**	Moderate

Referred to Table 2, the results show that historical data which is Seksyen 10,Wangsa Maju is shown the Critical level and SLOPE 1 ,SLOPE 2 and SLOPE 3 shown High level. Meanwhile, Slope at Fakulti Farmasi and Fakulti Sains Kesihatan 6A Taman Herba respectively Low and Moderate level. Therefore, the level of risk for every slope can be known by contributing all measured parameters that govern erosion induced landslide. The historical slope and selected slope shown the difference risk level which is the factor rainfall erosivity (R) and soil erodibility (K) contributing most to the value of the percentage of Risk level. Meanwhile, other factors also need to care even a small percentage but it does contribute to the erosion induced landslide.

4. CONCLUSION

From the results, the slope risk levels were identified based on erosion induced landslide. The combination of landslides historical data collection and these adopted parameters were developed the risk level in that area. By applying both Nomograph and the newly developed landslide risk level at the research location it was justified the use of the newly risk level assessment in predicting future erosion induced landslide at other location and give pre-warning to adjacent residents. Furthermore, this research can be used as a tool to develop an erosion risk level guideline for slopes throughout the nation and can reduce the unwanted impact to human life

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Cover page

Title: *Mitigation of Disinfection By-Product Formation Whilst Incorporating the Bromide Ion*

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ABSTRACT

The contribution of bromide to the subspecies of total trihalomethanes (TTHM) demonstrates that bromide compounds approach fifty percent of TTHM as bromide reaches 10 µg/L. This is problematic since the cancer slope factors for bromide compounds are ten-fold higher than chloroform, meaning the potential cancer rate when bromide is present is greatly increased. A multivariate regression model for TTHM (R^2 = 0.91) which includes bromide, is described and demonstrates that water temperature, dissolved organic carbon, pH, pre-chlorination levels and raw water bromide levels are statistically significant variables for prediction of TTHM.

Keywords: Multivariate regression, Trihalomethanes (THMs), Bromide, Chlorine

INTRODUCTION

An Overview of Disinfection By-Products in Drinking Water

Trihalomethanes are by-products created when chlorine used in the water disinfection process reacts with naturally occurring organics (e.g. organics formed by the decay of vegetation) in raw water. The most common THMs formed are chloroform, bromodichloromethane (BDCM), chlorodibromomethane (CDBM) and bromoform and hence the bromide ion is obviously a contributing factor to TTHM. The THMs formed as noted above, are carcinogens, with the exception of CDBM, for which there is suggestive, yet inadequate, evidence for carcinogenicity [1]. To decrease the health risks to consumers, the Ontario government (and others) is considering lowering its TTHM guideline from a maximum acceptable concentration of 100 µg/L to a maximum of 80 µg/L. Of interest herein is the degree to which the bromide ion affects the speciation of the THM.

Related Literature on THM Formation

The use of regression equations to model TTHM closely follows the approach used by Amy et al., Sadiq et al., McBean et al. and Harper have developed regression equations to model TTHM in drinking water [2][3][4][5][6]. However, the last two papers did not include bromide in the regression equation because

bromide data over a four year period (2005-2008) have only recently been released by the Ontario-based Drinking Water Surveillance Program (DWSP) [7]. The DWSP is a scientifically-based water monitoring program which examines drinking water quality with a focus on non-regulated drinking water quality parameters, and possible new contaminants.

LITERATURE REVIEW AND BACKGROUND

Known Health Effects of Disinfection Byproducts (DBPs)

Table 1 outlines known health effects associated with different classes of DBPs. The current standard for TTHM set by Health Canada is 100 µg/L [8]. By comparison, the US Environmental Protection Agency (USEPA) has set their standards at 80 µg/L for TTHM [1].

Bromide Ion

Health Canada has listed the bromide ion as one of the contributing factors in the formation of THM [9]. Until recently, however, only very limited bromide data have been available; of interest is the structure of regression models in response to the addition of the bromide ion.

The TTHM regression model reported in Harper without bromide data, for water as released to the distribution system is shown in Equation 1 below [6]. When chlorine is applied to raw water immediately prior to entering the treatment facility), it is called pre-chlorination ($PreCl_2$); when chlorine is applied to effluent (water leaving the treatment facility), it is post-chlorination ($PostCl_2$).

$$TTHM = 10^{0.825} * (PreCl_2)^{0.238} * (PostCl_2)^{-0.099} * (Temp)^{0.225} * (DOCR)^{0.362} * (DOCT)^{0.585} \quad (1)$$

Where n=206 $R^2 = 0.75$

Where,
$PreCl_2$ = Pre-Chlorine Dosage in mg/L (raw, incoming water),
$PostCl_2$ = Post-Chlorine Dosage in mg/L,
Temp = Temperature of Treated Water, (C °)
DOCR = Dissolved Organic Carbon in Raw Water in mg/L,
DOCT = Dissolved Organic Carbon in Treated Water in mg/L.

The negative power value for $PostCl_2$ suggests that increasing post–chlorination will decrease TTHM but since total chlorination must be maintained, this only occurs if pre-chlorination is increased (which would cause increased TTHM). Hence, there is potential to lower DBP formation by lowering pre-chlorine dosage and raising the post-chlorine dosage.

Figure 1 illustrates how alternative disinfection by-product subspecies vary in percent speciation given different bromide concentrations. It is important to note that bromide concentration is not the only factor that affects THM speciation, as the Br:organic carbon ratio and Br:chlorine ratio also influence THM speciation. The data used to analyze THM speciation by Health Canada was taken from three drinking water treatment facilities in Canada in 1993 [9]. Looking specifically at

TABLE I. THE HEALTH EFFECTS RELATED TO THE MAJOR DBPS
(MODIFIED FROM USEPA [1])

Class of DBPs	Compound	Potential Health Effects
Trihalomethanes	Chloroform	Cancer, liver, kidney and reproductive effects
	Chlorodibromomethane	Nervous system, liver, kidney and reproductive effects
	Bromodichloromethane	Cancer, liver, kidney and reproductive effects
	Bromoform	Cancer, nervous system, liver and kidney effects

TTHM, when the bromide ion is < 10 μg/L, the chloroform concentration is nearly 100% of the TTHM formed (see Figure 1). Chloroform has the lowest cancer slope ($0.0061(mg/kg/day)^{-1}$) of the THM subspecies [1]. When the bromide ion reaches 500 μg/L, the subspecies are formed in the following percentages: chloroform (CF) 20%, bromodichloromethane (BDCM) 20%, chlorodibromomethane (CDBM) 25%, and bromoform (BF) 35%. Considering how the three bromo-subspecies have cancer slopes that are at least 10-fold greater than chloroform (see Table 2), the implication is a significant increase in the overall cancer risk when bromide concentrations are high (500 μg/L). Table 2 lists the cancer slope factor of each THM subspecies.

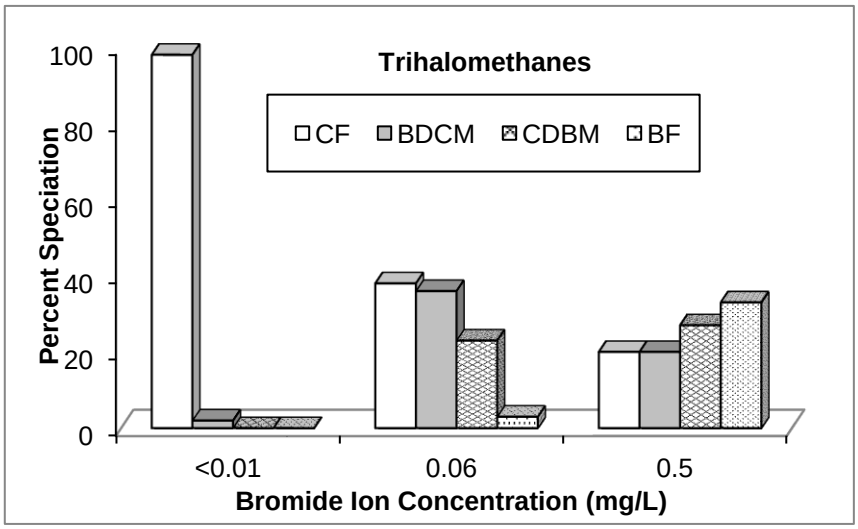

Figure 1: Subspecies formation based on bromide ion concentration
(Modified from Health Canada [9])

TABLE II. CANCER SLOPE FACTOR FOR EACH THM SUPSPECIES [1]

Compound	Slope Factor $(mg/kg/day)^{-1}$
Chloroform	0.006
Chlorodibromomethane	0.084
Bromoform	0.079
Bromodichloromethane	0.062

Data from all Ontario WTPs (which recorded bromide data from 2004 – 2007), given bromide concentrations in raw drinking water, were used in this study to

illustrate how THM speciation favours chloroform as bromide levels decrease, and are shown in Figure 2 [7]. Concentration data were used for Ontario WTPs (21 surface, 4 ground, 2 mixed) for the years of 2004 – 2007 for bromide, as well as all THM subspecies. The THM subspecies concentrations were summed, and the percent speciation of each THM subspecies was calculated by dividing each individual subspecies by the sum. The percent speciation was then graphed against the corresponding bromide concentration. As apparent from Figure 2, when bromide levels are at <5 µg/L, chloroform concentrations exceed 80% of TTHM formed, whereas when bromide levels increase to >10 µg/L, the percentage of chloroform drops to 55%. Conversely, brominated-THM subspecies (CDBM, BDCM and BF) all increase in percent speciation (to 13%, 23%, and 9%, respectively). These findings differ from those of Health Canada because in the Health Canada study the THM subspecies concentrations were taken from the distribution system, as opposed to the treated water [9].

Of interest is to show how cancer risk is affected when raw water bromide concentrations increase. To demonstrate this, the cancer risk for TTHM and each THM subspecies was calculated by multiplying the THM percent speciation by an assumed TTHM of 20 µg/L to obtain an estimated concentration for each THM subspecies. From these concentrations, the exposure to each of these compounds, given by the Lifetime Average Daily Dose (LADD), can be calculated as:

$LADD = (C_w \times IR \times EF \times ED)/(BW \times AT)$

Where,

C_w = Contaminant Concentration,

IR = Intake Rate,

EF = Exposure Frequency,

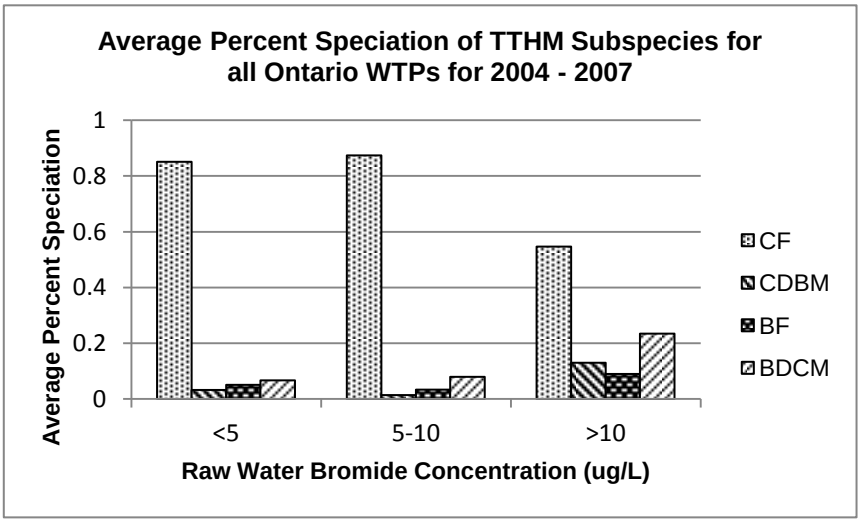

Figure 2: Average percent speciation of TTHM subspecies given bromide concentrations in raw drinking water

ED = Exposure Duration,

BW = Body Weight and,

AT = Averaging Time.

Assuming a body weight of 70kg, an intake rate of 2L of water a day for 365 days per year, an exposure duration of 70 years (ED), and an averaging time (standard lifetime) of 25,550 days; the LADD is: C_w x consumption of water per body weight per day. The consumption of water per mass per day is: 0.029L/(kg*day) [10].

The corresponding incremental excess lifetime cancer risk, IELCR, for the THM subspecies concentrations is calculated by multiplying the LADD by the corresponding Cancer Slope Factor (CSF) of each THM subspecies given in Table 2. The resulting cancer risks for all THM subspecies, as well as TTHM, are shown in Figure 3. As Figure 3 demonstrates, cancer risk increases by 129% when raw water bromide concentrations increase from less than 10 µg/L to greater than 10 µg/L.

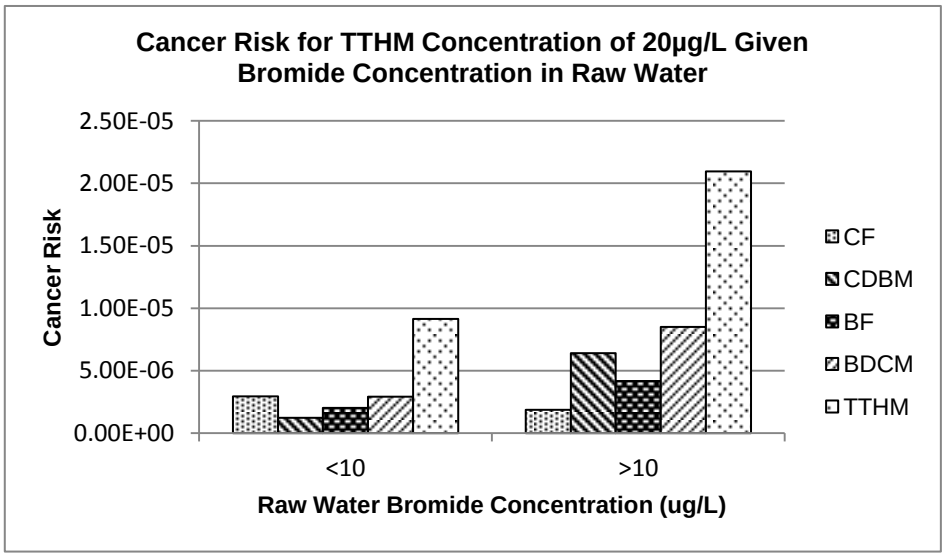

Figure 3: Cancer Risk for TTHM Concentration of 20 µg/L, for different Bromide Concentrations in Raw Water

When bromide is low, chloroform concentrations predominate TTHM, but when high, there is both higher brominated THMs and greater TTHM, as demonstrated in Figure 4. This indicates that bromide concentrations are proportional to brominated THMs and TTHM. In addition, the brominated subspecies have been shown to have significantly higher CSFs compared with non-brominated subspecies. A number of researchers (Morrow, Chang, Kampioti, Uyak, Wang, and Sun) have reported that speciation shifted to the bromine-substituted THMs when all other parameters were held constant[11][12][13][14][15][16]. Under conditions of high natural organic matter (NOM) and low bromide concentrations, chloroform predominated, especially during longer reaction times. In the presence of chlorine and organic material, as much as 50% of the bromide ion may become incorporated into the brominated trihalomethane subspecies; this efficiency of bromide incorporation implies that 100 µg/L of bromide may result in up to 50 µg/L of THM-bound bromine (THM-Br) and consequently, lower bromide concentrations will have a significant impact on the concentration and speciation of formed THMs [12].

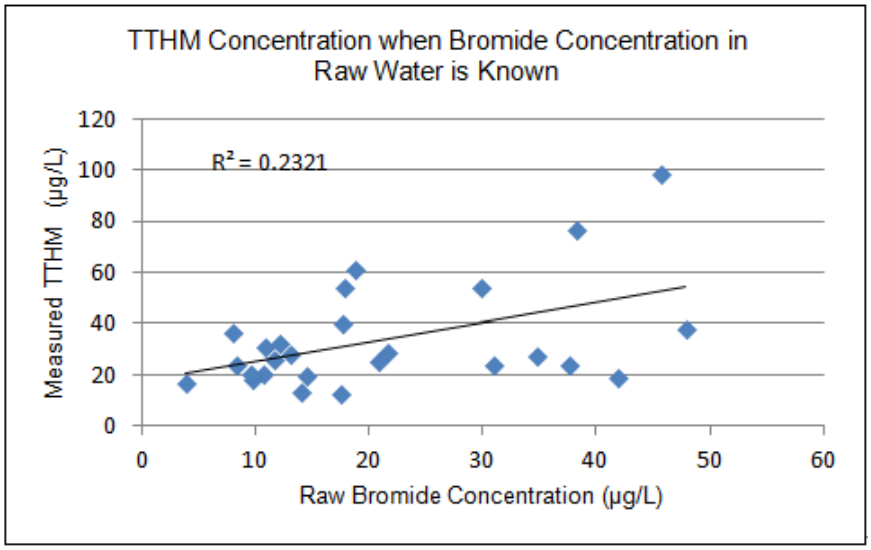

Figure 4: TTHM when Bromide Concentration in Raw Water is Known

STUDY AREA AND APPROACH

Multiple Regression Analysis

A multiple regression model (Equation 2) was developed to estimate the various disinfection by-product concentrations in chlorinated drinking waters, using data from six WTPs (five from surface water sources, one from mixed surface water and groundwater sources) in Ontario during 2005 to 2008. Only for six treatment plants were all chlorination dosage data and bromide data available and hence used in the regression analysis.

Derivation of the Predictive Equation and Validation thereof

Using Microsoft Excel's regression tool, 2005 – 2008 data were used to derive predictive equations for TTHM, chloroform and total brominated-THMs (the sum of BDCM, CDBM and BF) released to the distribution system. The backward elimination multivariate regression method (for log-transformed variables) was employed as follows:

1. Run Microsoft Excel's regression analysis, testing all potential variables in the equation.
2. Check to see if all variable p-values are below 0.05, and if not, remove the variable with the highest p-value.
3. Re-run the regression analysis with all remaining variables.
4. Continue this procedure, removing the variable with the highest p-value and re-running the regression model, until all remaining variables have a p-value less than 0.05, at which point, all remaining variables are statistically significant.

Through trial and error, a number of statistically significant independent variables were identified. These variables were: dissolved organic carbon in raw water in mg/L (DOCR), pre-chlorine dosage in mg/L ($PreCl_2$), post-chlorine dosage

in mg/L ($PostCl_2$), bromide in raw water in µg/L (RawBr), bromide in treated water (TreatedBr), temperature of raw water in ℃ (Raw Temp), and pH of treated water being released to the distribution system (Treated pH). Statistically significant non-linear models relating these variables were obtained as per Equations 2, 3.

$$TTHM = 10^{1.62} \times (DOCR)^{0.663} \times (PreCl_2)^{0.653} \times (RawBr)^{0.282} \times (Raw\ Temp)^{0.173} \times (Treated\ pH)^{-1.40} \text{ Where } n = 26, p=0.88. \quad (2)$$

$$\text{Total Brominated-THMs} = 10^{-3.659} \times (PreCl_2)^{1.17} \times (TreatedBr)^{0.91} \times (Raw\ Temp)^{0.23} \times (pH)^{3.54} \text{ Where } n = 26, p=0.67. \quad (3)$$

Table 3 lists the 'p values' for Equation 2 that indicate the statistical significance of the corresponding variables. This indicates that all the constituents, intercept, dissolved organic carbon in raw water (DOCR), pre-chlorine dosage ($PreCl_2$), bromide in raw water (RawBr), temperature of raw water (Raw Temp), and pH of treated water prior to being released to the distribution system (Treated pH) are statistically significant in this application. Post-chlorination was tested for statistical significance in place of pre-chlorination and was determined not to be statistically significant (p=0.88). This implies that if pre-chlorination were lowered to decrease TTHM, and post-chlorination would thus have to be increased, the

TABLE III. THE SIGNIFICANCE OF MULTIVARIATE REGRESSION FOR EQUATION 2

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower95%*	*Upper95%*
Intercept	1.62	0.635	2.55	0.0191	0.295	2.94
DOC Raw	0.663	0.0788	8.41	5.33E-08	0.498	0.827
Pre Chlorine	0.653	0.224	2.91	0.00863	0.185	1.12
Raw Bromide	0.282	0.104	2.71	0.0135	0.0648	0.500
Raw Temp	0.173	0.0414	4.18	0.000460	0.0867	0.259
Treated pH	-1.40	0.580	-2.42	0.0254	-2.61	-0.191

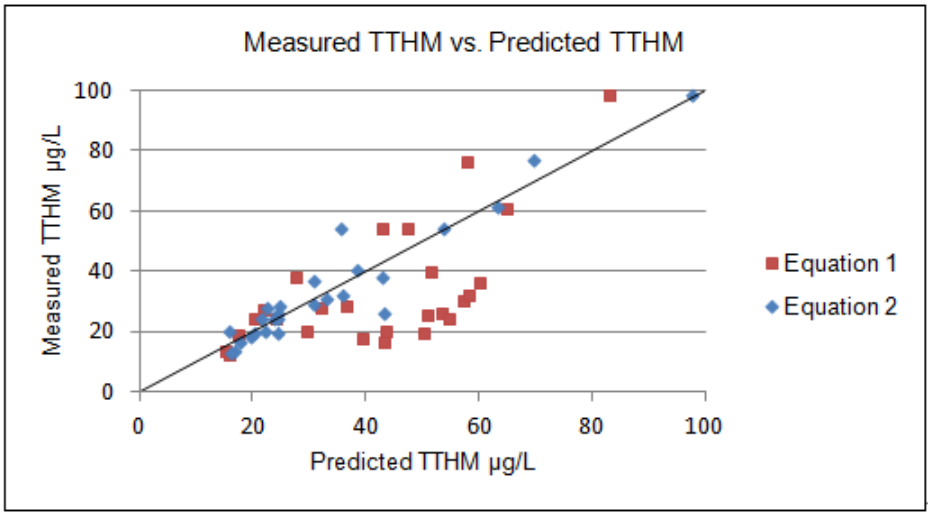

Figure 5: Measured TTHM vs. Predicted TTHM for Equations 1 and 2

increase in post-chlorination should not affect TTHM. However, since THM formation is initiated by pre-chlorination and fewer reactive sites are likely available for post-chlorination. This may result in post-chlorination coming into significance if pre-chlorination were lowered. It should also be noted that the negative coefficient for the pH factor is at variance with Amy et al [2].

With a validated model, the significance of alternate operational and water quality parameters which control or reduce DBP formation can be identified. For example, treatment plant operators can lower DBP formation by reducing pre-chlorination dosages, while maintaining sufficient residual for adequate disinfection.

In Figure 5, the predicted TTHM using Equations 1 and 2 were graphed against measured TTHM and demonstrate how the accuracy of Equation 1 and Equation 2 compare to each other. As apparent from Figure 5, Equation 2 demonstrates improved accuracy over Equation 1. To demonstrate how altering water quality parameters may affect cancer risk, Figure 6 shows when bromide concentration is approximately constant and low, how altering chlorination levels can reduce cancer risk. A reduction of pre-chlorination by 10% can lower cancer risk by roughly 6.5%, a reduction of 20% lowers cancer risk by 13.5%, and a reduction up to 30% can lower cancer risk by 20.8%. It is important to note that several individual WTPs have noticeably higher bromide concentrations than others, and thus have higher TTHM. The bromide ion affects the speciation of the THM and it shift from $CHCl_3$ to brominated THMs.

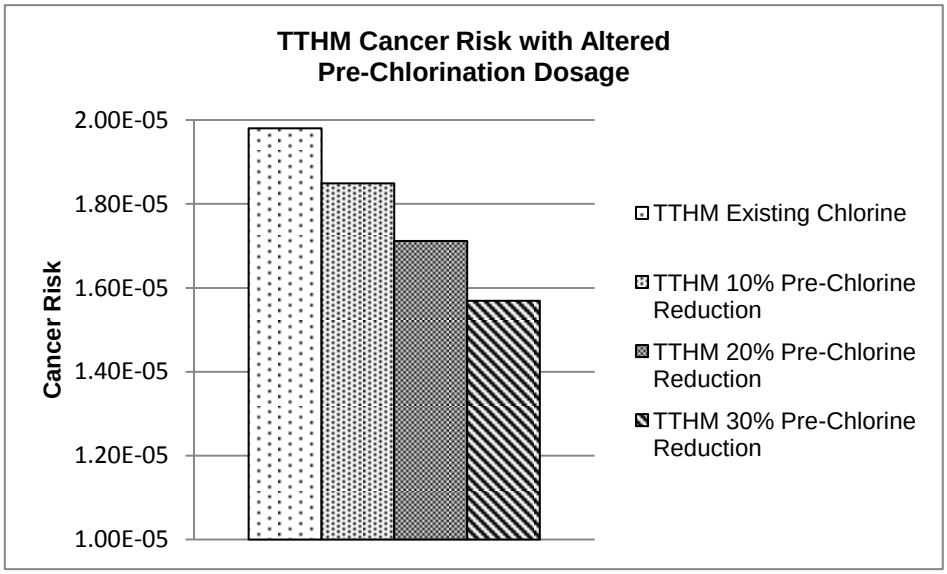

Figure 6: TTHM Cancer Risk with Altered Pre-Chlorination Dosage

CONCLUSIONS

1) When bromide is at low concentrations, chloroform concentrations are nearly 100% of TTHM, but when bromide is at higher concentrations (approximately 10 µg/L), brominated-THMs approach 50% of TTHM.
2) A regression model ($R^2 = 0.91$) was determined to characterize the formation of TTHM with statistically significant variables of water temperature, dissolved

organic carbon, pH, pre-chlorination and raw water bromide concentrations; and hence can be used to predict TTHM. Regression models were also determined to characterize the formation of chloroform and total brominated-THMs.

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Cover page

Title: *Interleukin-1 Receptor-Associated Kinase-2 Genetic Variant Increases NF-κB Activity induced by poly (I:C) and Influenza virus*

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ABSTRACT

The Interleukin-1 Receptor-Associated Kinases (IRAKs) are key regulators in modulation of Toll-like receptor (TLR) signaling, however little is known about the particular role of IRAK2. Here, we identified that two non-synonymous exon SNPs in human *Irak2* gene, rs3844283 (coding L392V) and rs708035 (coding D431E), are associated increased TLR3-mediated NF-κB activation. We found increased NF-κB transcriptional activity and expression of NF-κB-dependent pro-inflammatory cytokines in poly (I:C)- stimulated IRAK2 knockdown cells transfected with D431E IRAK2 than with WT IRAK2. However, the change of amino acid at position 392 of D431E IRAK2 from leucine (L) to valine (V) restrains the ability. Importantly, upon Influenza virus infection, expression of D431E IRAK2 into cells with knockdown of endogenous IRAK2 leads to more induction of IL-6. Further study demonstrated that the elevated NF-κB activation is due to increased TRAF6 ubiquitination. Our study provides an important insight of IRAK2 SNPs in the regulation of NF-κB activation and suggests the IRAK2 variant (rs708035) as a potential novel and broadly applicable biomarker for disease or as a therapeutic intervention point.

INTRODUCTION

Toll-like receptor (TLR) is an important group of pattern recognition receptors (PRRs). Upon engagement of distinct TLRs by specific ligands, intracellular signaling is activated, resulting in the activation of transcription factors such as NF-κB. IRAKs have been implicated in regulation of TLR signaling (1-3). Until now, four different IRAKs (IRAK1, IRAK2, IRAKM, and IRAK4) have been identified in mammals (4). The exact roles of IRAK2 have remained elusive for many years since its discovery. Knockdown of IRAK2 by siRNA in human cell lines showed that IRAK2 participates in NF-κB responses to IL-1R and multiple TLRs, including TLR3 (5-7). Furthermore, Keating *et al.* reported that IRAK2 plays a more central role in TRAF6 ubiquitination, a key event for NF-κB activation (5).

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Single nucleotide polymorphisms (SNPs) within the *Irak* genes have been discovered recently. However, there is no report about the function of known IRAK2 genetic variants so far. In this study, we identified two non-synonymous exon SNPs in human *Irak2* gene, rs3844283 (coding L392V) and rs708035 (coding D431E), and demonstrated D431E IRAK2 leads to higher NF-κB transcriptional activity and more expression of NF-κB-dependent pro-inflammatory cytokines induced by poly (I:C) and Influenza virus compared to wild-type IRAK2. However, NF-κB transcriptional activity and cytokine expression are comparable in both L392V/D431E IRAK2- and WT IRAK2-expressing cells. Additionally, we showed the elevated NF-κB activation mediated by D431E IRAK2 is caused by increased TRAF6 ubiquitination. These studies indicate the IRAK2 variant (rs708035) might be associated with deregulation of inflammation and immune responses in human caused by hyper-activation of NF-κB.

MATERIALS AND METHODS

- **Cell and Plasmids**

TLR3-HEK-293 cells stably expressing TLR3 were provided by National Centre of Biomedical Analysis (NCBA). pcDNA3-WT-IRAK2 encodes WT IRAK2 with a Myc tag. The constructs that respectively encodes L392V IRAK2, D431E IRAK2 and L392V/D431E IRAK2 were generated by site-directed mutagenesis. The TRAF6 expression plasmids Flag-TRAF6 was from NCBA.

- **Immunoprecipitation and Immunoblotting**

For immunoprecipitation of ubiquitinated proteins, cells were lysed in RIPA buffer and then subject to rotation at 4 ℃ for 6h with M2 beads (Sigma-Aldrich). The beads were washed three times with RIPA buffer and immunoprecipitates were resuspended in 40 μl of 1 ×loading buffer and resolved by SDS-PAGE and immunoblotting.

- **Reporter Gene Assays**

NF-κB activation, IL-6 and IL-8 promoter induction were measured by reporter gene assay. The reporter gene assay was performed according to the manufacturer's instructions (Promega).

- **RNA Quantification**

Total RNA was purified with TRIzol reagent (Invitrogen) following the manufacturer's instructions. Real-time quantitative PCR analysis was performed using the LightCycler (Roche) and SYBR RT-PCR kits (Takara).

- **RNA Interference**

siRNA duplexes targeting the human *Irak2* gene targeted the following sites: IRAK2-siRNA #1, 5'-CCA GGA TCA ATC GAA AGA TTT-3'; IRAK2-siRNA #2, 5'-GCA ACG TCA AGA GCT CTA ATT-3'; IRAK2-siRNA #3, 5'-GTG GCA AAT TGA GAT CAA TTT-3'. Non-silencing siRNA was used as a control (GenePharma).

RESULTS

- **Construction and Expression of the IRAK2 Wild Type and Variants**

We found two exon SNPs (rs3844283 and rs708035) in human *Irak2* gene which are non-synonymous with high reported frequency in Chinese Han population, resulting in the change of amino acid from leucine (L) to valine (V) at position 392 and from aspartic acid (D) to glutamic acid (E) at position 431. Western blotting showed WT, L392V and L392V/D431E IRAK2 only had one protein band whereas D431E IRAK2 had a smaller one besides the band which was of equal molecular weight to others (Fig. 1A). Then, we determined the DNA bases at these two SNP sites in six human tissue samples and detected endogenous IRAK2 expression (Fig. 1B). In term of rs708035, all of the tissue samples were homozygous for the A allele (431E). For rs3844283, there were four homozygous tissue samples of the C allele (392L), one homozygous tissue sample of the G allele and one heterozygous tissue sample. In accord with the sequencing results, endogenous IRAK2 from the sample of L392V/D431E IRAK2 had only one protein band while that from D431E IRAK2 performed two bands. We immunoprecipitated D431E IRAK2 and then carried out mass spectrometry analysis on the smaller bands of D431E IRAK2. MS/MS analysis identified this band was indeed IRAK2 (Fig. 1, C and D).

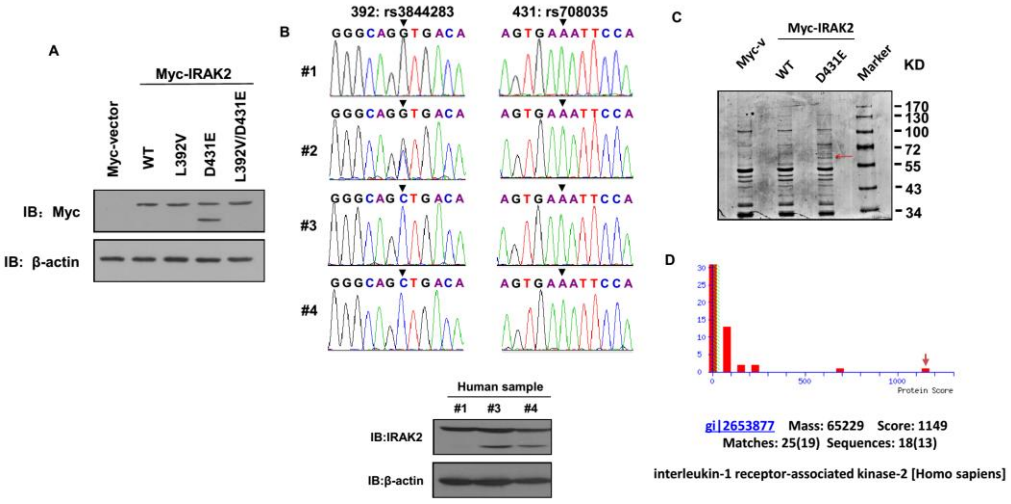

Figure 1. Detection of IRAK2 variants in tissue samples.

- **D431E IRAK2 Increases TLR3-mediated NF-κB Activation**

We focused on the ability of WT IRAK2 and its variants to affect NF-κB activation induced by TLR3 ligand, poly(I:C). The transcription of the NF-κB-controlled reporter gene and IL-8 promoter were examined when WT IRAK2 and its variants were overexpressed at equivalent levels in TLR3-HEK-293 cells with or without poly(I:C) stimulation. Overexpression of all forms of IRAK2 could lead to transcription of NF-κB-dependent reporter gene without poly(I:C) stimulation, and D431E IRAK2 seemed to have a stronger effect. When the transfected cells were treated with poly(I:C), the transcription of reporter gene was more increased. There was more expression of reporter gene in cells overexpressed with D431E IRAK2 (Fig. 2, A and B).

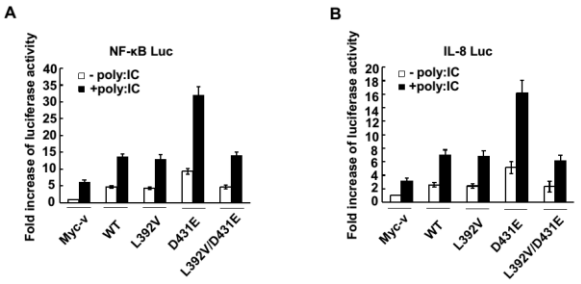

Figure 2. D431E IRAK2 increases TLR3-mediated NF-κB activation.

- **Expression of D431E IRAK2 at Endogenous Level Induces Stronger NF-κB Activation upon poly(I:C) Stimulation**

IRAK2 siRNAs were used to knock down endogenous IRAK2 expression. The knockdown efficiency of three siRNAs in TLR3-HEK-293 cells was detected. As shown in Fig. 3A, mRNA and protein expression of endogenous IRAK2 were completely ablated in the presence of IRAK2 siRNA compared to cells treated with the negative control siRNA. Importantly, knockdown of IRAK2 obviously decreased the expression of the NF-κB reporter gene induced by poly (I:C) (Fig. 3B). Then, we generated siRNA-resistant plasmids respectively encoding WT and D431E IRAK2 and transfected them into cells with knockdown of IRAK2. As shown in Fig. 3C, IRAK2 siRNA, which was effective in knockdown of endogenous IRAK2, had no effect on the expression of Res-WT and Res-D431E IRAK2. Reporter gene assay showed that TLR3-HEK-293 cells pre-treated with IRAK2 siRNA barely responded to poly (I:C) stimulation. However, reintroduction of as much Res-WT or Res-D431E IRAK2 as endogenous IRAK2 in cells pre-treated with IRAK2 siRNA restored cell responses to poly(I:C) stimulation. Res-D431E was significantly more potent than Res-WT IRAK2 in mediating expression of reporter gene with poly (I:C) stimulation (Fig. 3D). Then, we analyzed the time courses of poly (I:C)-induced IL-6 and IL-8 transcript in cells with Res-IRAK2 reintroduced after treated by IRAK2 siRNA. The results showed that more IL-6 and IL-8 mRNA was induced at the responsive time points after poly (I:C) stimulation with Res-D431E IRAK2 expression (Fig. 3, E and F).

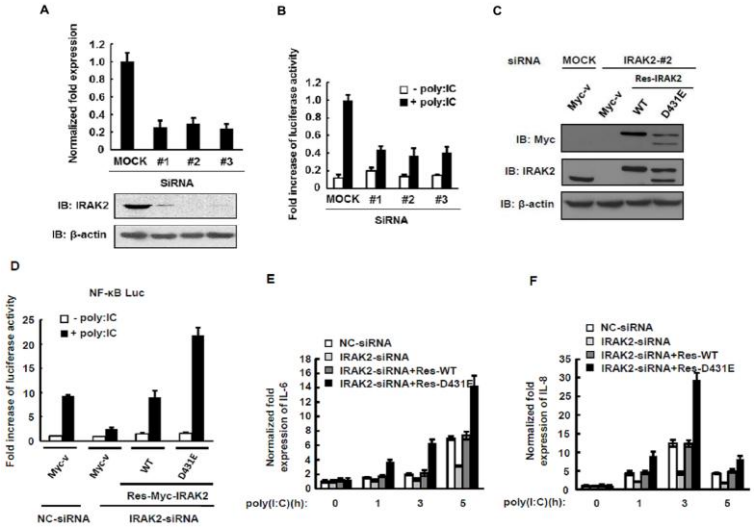

Figure 3. Expression of D431E IRAK2 at endogenous level induces stronger NF-κB activation upon poly (I:C) stimulation.

- **D431E IRAK2 Causes More IL-6 Secretion in Cells Infected with Influenza Virus**

TLR3 recognizes double-stranded RNA (dsRNA) produced by RNA virus that infect the host cells. Here, we wondered whether the same conclusions in the case of viral infection could also be drawn. We infected A549 cells with Influenza A virus (IAV) and investigated NF-κB activation. As shown in Fig. 4A-B, knockdown of IRAK2 by siRNA in A549 cells led to substantial decrease on IAV-triggered IL-6 and IL-8 mRNA expression. Moreover, when Res-WT and Res-D431E were re-introduced into the cells, the transcription was restored. As expected, Res-D431E seems to be more efficient in stimulating the transcription of NF-κB target genes than Res-WT. Similar results were also observed on the secretion of IL-6 by ELISA assay (Fig. 4C).

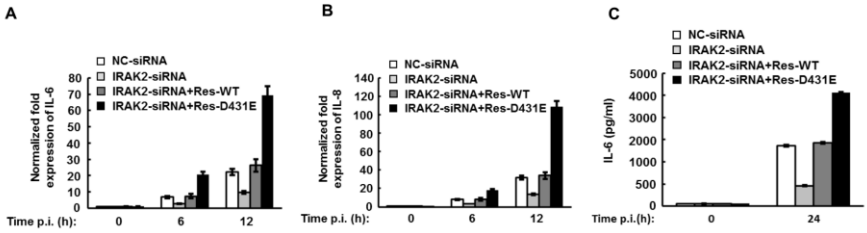

Figure 4. D431E RAK2 causes more IL-6 secretion in cells infected with Influenza Virus.

- **D431E IRAK2 Promotes TRAF6 Ubiquitination**

Interaction of IRAK2 and the E3 ubiquitin ligase TRAF6 stimulated the formation of polyubiquitin chains associated with TRAF6, which is a key step for NF-κB activation induced by IL-1/TLR agonists. We next performed immunoprecipitation assay to detect whether D431E IRAK2 interacted with TRAF6 stronger than WT IRAK2. However, the results showed that there was no difference between interaction of TRAF6 with these two forms of IRAK2 (Fig. 5A). In order to further determine the molecular mechanism for the effect of D431E IRAK2 on NF-κB activation, we detected the levels of TRAF6 ubiquitination induced respectively by WT and D431E IRAK2. As shown in Fig. 5B, conjugation of Myc-ubiquitin to Flag-TRAF6 was clearly induced in the presence of IRAK2, but knockdown of IRAK2 obviously impaired TRAF6 polyubiquitination. Re-introduction of Res-IRAK2 initiated TRAF6 polyubiquitination. As expected, expression of Res-D431E IRAK2 induced more obvious TRAF6 polyubiquitination than that of Res-WT IRAK2.

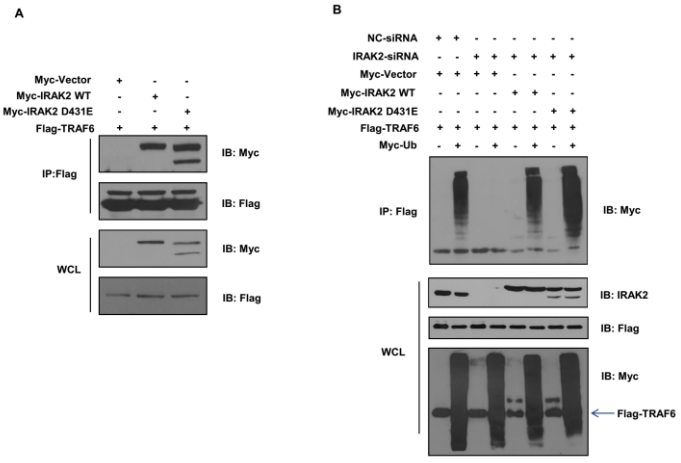

Figure 5. D431E IRAK2 promotes TRAF6 ubiquitination.

CONCLUSIONS

SNP is the most common type of genetic variations among people. Some of SNPs have proven to be very important and may be associated with human diseases. As reported, SNPs within the *Irak* genes occur frequently in the human population, whereas little is known about whether these genetic variants could lead to deregulation of IRAK activity, especially for IRAK2. In the present study, we focused on two known SNPs of human *Irak2* gene resulting in the change of amino acid from leucine (L) to valine (V) at position 392 and from aspartic acid (D) to glutamic acid (E) at position 431. Functional analysis showed that D431E IRAK2 was associated with greater NF-κB activation mediated by TLR3 and increased secretion of pro-inflammatory cytokines upon Influenza virus infection. The change of amino acid at position 392 of D431E IRAK2 from leucine (L) to valine (V) restrains its ability in mediating increased NF-κB activation. Moreover, we demonstrated that increased NF-κB activation mediated by the IRAK2 variant was resulted from increased TRAF6 ubiquitination. Our results indicate that D431E IRAK2 might be critical for development of human diseases caused by deregulation of inflammation and immune responses that were associated with hyper-activation of NF-κB. Notably, the higher frequency of the IRAK2 rs708035 susceptibility allele in Chinese than in people of European might also be one of factors contributing to more serious illness upon Influenza virus infection in Chinese people.

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Cover page

Title: *Toward Rural Sewage Treatment in China*

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ABSTRACT

Untreated rural sewage is a major contributor to China's water pollution problem, which has become the focus of many Chinese academic and government researchers. During the 11th five-year plan, various government ministries have made substantial investments in domestic sewage treatment, resulting in many advancements in rural areas. But more improvements are needed. To address these problems, several suggestions were proposed.

1. THE SIGNIFICANCE OF DOMESTIC SEWAGE TREATMENT

There are more than 2.7 million rural villages in China that produce over 8.0 billion tons of sewage annually, accounting for 22.9% of the total national sewage discharge rate. However, only 6–15% of rural sewage is treated, far less than the 78% average treatment rate for urban sewage. More effective sewage treatment in rural areas is needed for building a well-off society, particularly as envisioned by the new countryside "clean and tidy village" proposal in the 11th five-year plan [1]. Better rural sewage treatment can help prevent and control non-point source pollution. Therefore, rural sewage treatment has become the focus of many Chinese academic and government researchers.

2. THE RECENT ADVANCE IN DOMESTIC SEWAGE TREATMENT

During the 11th five-year plan, various government ministries have made substantial investments in domestic sewage treatment, resulting in many advancements in rural areas. For example [2], a campaign to improve the environment of villages and small towns, undertaken by the Ministry of Housing and Urban-Rural Development, led to the investment of tens of billions of RMB and subsequent environmental improvements in 150,000 administrative villages. A campaign for the comprehensive improvement of villages, sponsored by the

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Ministry of Environmental Protection, invested nearly 6 billion RMB, and environmental conditions in 40,000 villages were improved. The Rural Clean Engineering campaign by the Ministry of Agriculture invested 20 million RMB every year during the 11th five-year plan, and environmental improvements occurred in 1,100 model villages. A Ministry of Health campaign called the Rural Lavatory Improvement Project invested nearly 100 million RMB every year.

Lavatory Improvement Project invested nearly 100 million RMB every year during the 11th five-year plan, and 65% of rural toilets were modified. By the end of 2010, 1,625 sewage treatment plants and 115,000 km of sewage pipe networks had been established in rural villages, processing 9,540,000 tons of sewage daily, and another 33,800 administrative villages are building sewage treatment facilities with a total of 41,000 km of pipe network.

Many scholars have studied the technologies for treating rural domestic sewage, and they have concluded that these technologies are effective in providing good sewage treatment while having relatively low capital and operational costs and easy maintenance. Rural domestic sewage treatment systems can be either centralized or decentralized [3]. For villages where homes are scattered and population density is low, decentralized treatment should be adopted. Technologies currently used for village sewage treatment are septic tanks, anaerobic firedamp pools, stabilization ponds, constructed wetlands, anaerobic biological filters, and subsurface wastewater infiltration systems [4].

3. THE EXISTING PROBLEMS

Although rural sewage treatment has been improved during the 11th five-year plan, more improvements are needed. There is still no national plan for rural sewage treatment, the work performed by government departments has been fragmentary. Another problem is that a clear legal framework for preventing and controlling rural sewage pollution is absent, and there are no national standards for sewage effluent discharges in rural areas. The current standards used for rural sewage treatment are the same as the national municipal discharge standards, which specify stringent treatment requirements not suitable for rural villages having limited economic resources. Financial capital subsidies for rural sewage treatment also need to be increased. In 2010, 175 RMB per person was provided for municipal sewage treatment, while only 13.7 RMB per person were allocated to rural sewage treatment. A final issue is that sewage treatment technology is too expensive, and more than half of rural sewage treatment plants are not operating optimally. For example, some technologies such as cleaning tanks, which were introduced from developed countries, do not work well in rural settings, and their investment and operating costs are high. Because only about 10 villages have a specialized sewage treatment manager or technician, rural treatment systems must be simple to operate.

4. SUGGESTIONS

We have several suggestions to address these problems. First, the responsibilities of the central government and local governments need to be clarified, and a joint task force should be formed to coordinate among government

departments. Second, to finally solve the funding problem, subsidies from the central government and local governments need to be firmly established, and the responsibilities of village collectives and residents should be defined. Third, through an effective, integrated planning process across all levels of government, sewage treatment technologies having low capital and operational costs and easy maintenance need to be identified, developed, and deployed. Furthermore, more technical guidance and manager training should be provided, and outreach and educational programs should be developed to improve public awareness about rural sewage treatment.

The 12th five-year plan clearly puts forward the goal of continuously and comprehensively improving the rural environment, including strengthening the construction of rural facilities and public services and improving water pollution control in rural areas.5 At present, many rural sewage treatment research projects have been approved and construction has begun. These developments should be built upon and expanded as China moves forward with its rural sewage treatment programs.

ACKNOWLEDGMENTS

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Cover page

Title: *A Case Study of the Energy-saving Mode of Happy Farmhouse Tourism in China ——A Perspective of Sustainable Development*

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ABSTRACT

According to the development of Happy Farmhouse (HF) tourism, we try to explore the new mode of "health tourism" of rural recreation so that the health level of tourists can be improved. Meanwhile, we construct the Chinese type energy-saving mode of rural tourism to protect the natural environment and practice the ecological energy cycle for sustainable development. Based on the "1231" tourism strategic planning mode of Beijing University, we attempt to design health energy-saving tourism mode for Chinese tourists in the rural places, especially in the natural landscape areas. On the basis of the case study of the Sea of Cloud Bamboo HF in Jinyun Mountain in Chongqing, we have found out that there are two aspects which can achieve energy-saving goals of HF tourism. One aspect is energy-saving in the context of health ecological tourism from the perspective of tourists. The other aspect is the related tourism facilities and attractions. This is the sustainable utilization of main local natural resources to design energy-saving mode of ecological energy cycles. By means of the two mode designs described above, we can fully realize how the energy-saving of the ecological tourism on HF can improve the income of local residents and achieve the objective of sustainable development.

INTRODUCTION

Health tourism is "the sum of all the relationships and phenomena resulting from a change of location and residence by people in order to promote, stabilize and, as appropriate, restore physical, mental and social well-being while using health services and for whom the place where they are staying is neither their principle nor permanent place of residence or work"(Kaspar, 1996). Happy Farmhouse (HF) can be one kind of health tourism to save energy. HF is the result of rural economic development. It is the phenomenon that urban people who have leisure time to relax and enjoy life by staying in nature nearby the city. The local farmers provide them with room and board and some local activities at a certain cost. With the development of rural tourism

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industry, the HF tour is changing its content and structure remarkably. From the simplest farm life style to the star standard assessment, the development of HF can satisfy different physical and psychological demands of tourists, who can enjoy fresh fruits and vegetables, drink natural water from the spring, appreciate the special culture and customs, improve health level and physical quality, and to live with friendly and kind peasants is the best choice for city dwellers on their weekends and holidays. Not only for personal reasons, as a country tour, the HF is one of the ecological tourism spots to save energy and protect the environment. Because of the efforts made to reduce carbon emissions, people are paying more attention to energy savings and sustainable development practices. This special opportunity in leisure health tourism has become very popular recent years in China. This paper taking a case study of Jinyun Mountain near Beibei District, Chongqing city, analyzes how to release the stress of tourists and relax themselves while enjoying beautiful natural scenery and comfortable environment in HF, and it also helps people to save their energy with the elimination of the use of household appliances at home, whose purpose is to enjoy nature and improve health level while "saving energy with innovative solutions for technical and economic integration."[1]

METHODOLOGY

We used a functional approach to gather information through communication and interaction with HF stakeholders, local governmental officers, the scenic spot administrator, tourists and farmers by receiving useful suggestions about how to save energy while traveling in the Jinyun Mountain and indications regarding what the tourists would like in health tourism. Then we began to work with the literature material method to find out the related academic achievements of this topic. Luckily, there were only few scholars who had discussed this topic from the academic level. To make certain relationships of health tourism and energy saving, we used the "1231" tourism strategic planning mode of Beijing University to design energy saving ways of health tourism in HFs in Jinyun Mountain. In order to collect the useful support materials, our group returned to investigate the tourism route in the mountain and assessed the conditions and activities in HFs, and observed the tourists who seemed to enjoy mountain climbing and wanted to stay in HF overnight. We asked for their advice on energy saving. Furthermore, on completion of the assessment of the related resources in the mountain and HFs, we developed programmes to make the strategic design on energy saving while traveling in Jinyun Mountain from two perspectives: tourists and HF stakeholders. All the data and materials used in the paper were respectively from field investigation, related academic papers and governmental documents.

A CASE STUDY

The energy saving mode of Sea of Cloud Bamboo HF tourism is a tough question when putting the theoretical discussion into practices in the perspective of tourism's strategic design. "However, a correct strategy plan can be not only in favor of the social and economic development for tourism region,"[2] but also play a prominent role in energy saving. The purpose of this study is to construct the overall plan of the

ecological tourism strategy about HF due to the concept of health tourism, not the operation manual. The methodology of this case study is based on the "1231" tourism planning mode, designed by the Tourism Research and Planning Center in the Institute of City and Environment in Beijing University. This "1231" mode mainly includes four parts: one developmental goal, two basic analyses, three plate designs and one support system (Bihu WU, 1999, 2010). The overall design idea figure of HF energy saving tourism can be seen in figure 1.

The Overall Goal: Saving Energy and Enjoying HF Tourism

Sea of Cloud Bamboo HF is located in the national scenic area Jinyun Mountain, which is famous for its natural landscape, cultural history and rich plant resources, covering an area of 76 square kilometers and its elevation is from 350 to 951 meters, with more than 1300 hectares of forestland and 1700 kinds of subtropical plants. In addition, it has a long history of Buddhism that has lasted 1500 years. Tens of thousands of tourists visit here every year. Due to this booming tourism, the local residents saw an opportunity and started the HF tourism business fifteen years ago. Now, most of the farmers in Jinyun Mountain have their own HF with private houses, lands and products. Many people can enjoy the local food and natural scenery in HF to have a good holiday.

However, sea of cloud bamboo HF has the same problems as most of the HFs face: unreasonable planning by farmers themselves, low cultural content and poor knowledge in operation and management. How can these HFs develop with professional operation, less pollution and health tourism to save more energy? The key of this problem is the scientific strategy plan. To reach the overall goal of saving energy and enjoy HF tourism, the following energy conservation projects in sea of cloud bamboo HF can be enumerated under the health tourism.

(1) More sports activities tourism attractions like climbing instead of driving (physical exercising and saving gasoline); (2) Advocating eating more green plant food and drinking mountain spring, less meat and tap water (reducing human production consumption); (3) Choosing the local energy saving materials for HF housing construction (energy saving building); (4) Using energy efficient or saving appliances like CFL, fans, LCD TV, etc (reducing power consumption); (5) Constructing the biogas providing system in HF (decreasing coal gas pollution); (6) Building sewage treatment systems and living garbage processing mechanisms (improving utilization of waste); (7) Advocating on ecological tourism to save energy indirectly (more ecological enjoying, less power consumption); (8) Management and energy saving knowledge training for farmers (strengthening implementation of energy conservation policy); (9) Government leading and supporting (guaranteeing the realization of energy saving tourism projects).[3]

Tourist Market and Sales Promotion Planning: Education and Attraction

EDUCATION

Education is very important for the implementation of the HF tourism strategic plan. The key of this education emphasizes two aspects: farmers and tourists. On one hand, the local government plays an irreplaceable role in taking the responsibility to

hold the training classes for the farmers, whose purpose to improve the management and sales skills of HF as well as of consciousness of energy saving and environmental protection. On the other hand, the local government should support the HF to propagandize the publicity with the information of the local tourism of health tourism, telling more people that the Sea of Cloud Bamboo HF tourism not only can be enjoyed as a part of HF, but also can improve their health level by climbing and walking. This kind of "communicative activity or advertisement can educate consumers on how to enjoy travel, while being healthier and protecting the environment."[4]

ATTRACTION

The tourism attraction is one of the most important reasons why people around the world come here. Under the health tourism concept, the significant factors of Sea of Cloud Bamboo HF tourism are "exercise tourism" and saving energy. First, we can construct the attractions on the natural and cultural resources along the tourism route with services, social system, residence and other tangible lifestyle of the tourism, by encouraging tourists to climb up to the top to enjoy the landscape in the Park of Jinyun Mountain while doing some exercise rather than driving a car . Second, we can provide some local characteristics such as entertainment and leisure places for tourists who have already exercised for hours to climb to the destinations by offering some educational interest type of activities, especially some team training, chess and outward bound funny that let people participate in activities like team training, chess competition, outward bound and so on. Third, the local government can hold some more significant cultural art festival or design some historic tour routes in order to attract more people. In all, the principle of strategic design of HF tourism attraction to energy-saving is simple: interest, health benefits and its impressive environment and allure.

Tourism Resource Evaluation and Functional Zone Planning: Rediscover and Construction

According to the resources evaluation, there are eleven functional zones of Jinyun Mountain tourism, which include the following zones: the introduction and domestication, subtropical evergreen broad-leaved forest, gymnosperms exhibit, Chongqing plants exhibit, rare and endangered plants exhibit, bamboo plants exhibit, camellia exhibit, sweet-scented osmanthus exhibit, the plum flower zone and HF tourism. From the table, the main functions of the Jinyun Mountain tourism zones are very clear: research and teaching, recreation and appreciation, cultivating seeds, garden business, and protection. In order to raise the strategic planning level, we should further rediscover and reconstruct the tourism resources of Jinyun Mountain by the scenic system evaluation and plan, including HF tourism.

REDISCOVER

To rediscover the advantages of HF in Jinyun Mountain, we should assess its tourism resource value by finding (1) the location advantage, within two hours' walk and two-lane road passing through the forest, the convenient shipping in Jialing River, super highways, light rail, railways etc, (2) exploiting advantages which include the beautiful natural resources and historical cultural sites, the kinds of attractive artificial

landscapes, and (3) policy advantage with many preferential policy supports by governments, which is more competitive on a large scale with a unified standard and professional management.

RECONSTRUCTION

To reconstruct the tourism plan at the strategic level under the health tourism concept, in order to save more energy while traveling around and enjoying Jinyun Mountain, the following steps can be carried out by the unified plan, wich is to (1) construct the modern comprehensive tourism industry system, including all aspects such as sightseeing, leisure, entertainment, fitness, learning, food, accommodation, sports etc., to employ the professional and "experienced expert to evaluate resources and build brand coming from the comprehensive arrangement"[5] and rational development to realize the sustainable development of tourism industry in Jinyun Mountain. (2) Step-by-step implement, which is, to further consummate the "fitness ladder" project , extend the walking route and add the scenic spots along the footpath, to vigorously develop the bamboo sea resources with HF and leisure activities, to take emphasis on the "exploiting the historical and cultural products and handiworks"[6] to improve the content of tourism. (3) Building characteristics, which is, to create the local characteristics of the scenic spot and HF entertainment and leisure sport area, to make such characteristics emphasized on "landscape appreciation, entertainment and leisure, history and culture, and sports activities, "[7] especially, to energetically develop the tourism projects which are related to "saving energy under the low carbon era." [8]

Tourism Product and Development Projects: Health Tourism

The theme of health tourism at a strategic level is designed to create new attractions to promote the economic increase and save energy. Based on the rich tourism resource, the health tourism at the macroscopic strategic guidance should be carried out in Jinyun Mountain, which is expressed in figure 3 clearly in the perspective of customers who can save energy relatively and directly.

ADVERTISING

According to extensive publicity, people should know about health tourism all over the country. Like any other functions of advertising, our purpose is firstly to occupy the "psychological resources" of potential tourists. When people get familiar with the content and advantages of such health tourism, for example, good for both physical and mental health, interesting climbing, playful activities, saving energy and protecting environment, the chance of traveling in Jinyun Mountain would increase.

EDUCATION

The customers need the education to enhance their experience in Jinyun Mountain. This education is not the traditional concept which has courses in the classroom. The customers' education emphasizes the knowledge of health tourism such as the benefits of walking, playing, climbing, hiking, going outside etc. The first step is to let the customers know the benefits, and the second is to provide guidance for them to

participate in those beneficial games or playful activities. Ultimately, they will be able to really feel the true interest that comes from such health tourism activities accompanied by saving energy.

ATTRACTIONS

The attraction is the most important element in every tourism area. The attractions can be built in three categories: sports, games and recreations. Sports may include climbing, walking, running, cultivating, picking, boating, shooting, riding etc., games with such sports such as swimming, ping-pong, basketball, volleyball, skating, trampoline, relay race, dancing and other competition activities and the recreation would include chess playing, KTV, fishing, bath spa etc. These attractions have the ability to save more energy. If customers spend more time on such attractions which are energy efficient, relatively, they would shorten the time to use electrical power at home on computers, TV sets, lights and other appliances, or use less water and producing less garbage.

REWARDS

The rewards of tourism in Jinyun Mountain can be designed as discount, service and free. In order to attract more people, if customers have participated in the competitions actively or won some prizes, we could provide discount price or additional service for them to enjoy in HF, even for free. The discount price reward can be carried out by means of food, accommodations or other service items. For some lucky tourists, we can provide them with their entire trip free of charge. These measures impel customers to participate in attractions projects and make this tourism cycle develop sustainably.

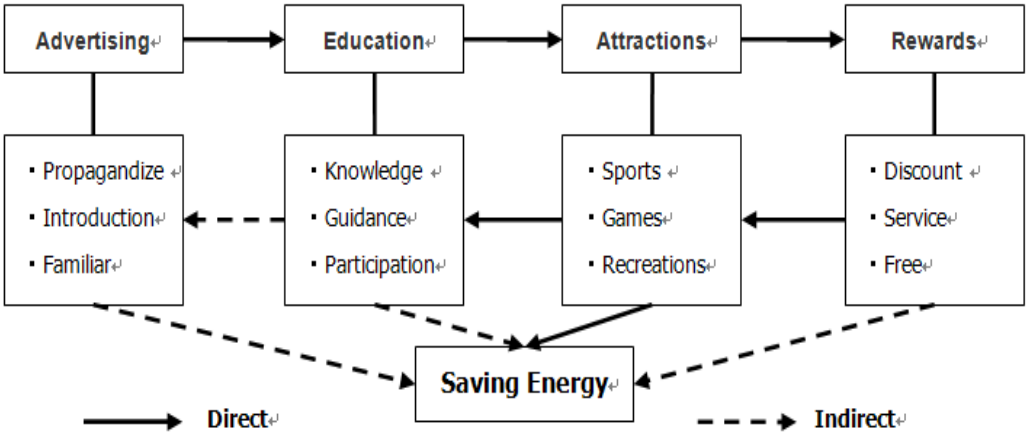

Fig 1. The macroscopic strategic map of designing health tourism in Jinyun Mountain

Tourism Facilities and Service Development Planning: Energy Saving Mode

As discussed above, health tourism is a relative method of saving energy that allows tourists to spend more time on outdoor activities instead of staying at home. This way may reduce the possibility of using electronic products and producing the sewage and garbage in the house to reach the goal of saving power energy directly.

Thus, this health tourism is to precipitate people to go out to have a wonderful time in HF tourism activities in Jinyun Mountain while saving energy. However, the energy saving mode of tourism facilities and service development is at a standpoint in the HF industry. In this saving mode, the emphasis of design is on four aspects: the rural ecological energy-saving residential buildings; the using of natural energy;[9] the life of rubbish and sewage treatment and the use of agricultural products or wastes, except for "using energy efficient or saving appliances" [10] like CFL, fans, LCD TV and so on. From these aspects, the energy on the materials used can be saved directly.

RURAL ECOLOGICAL ENERGY-SAVING RESIDENTIAL BUILDINGS

There are mainly two kinds of walls of HF residential buildings: traditional clay and cement brick (see fig 2). Now the construction of most rural houses is changing from the traditional clay structure into cement brick structure. The advantages of cement brick are obvious: it is economically feasible and materially durable. The farmers can build many floors and layers to enlarge their indoor residential area. So we advocate building such residential buildings by using local materials and the "improved structure of traditional constructions by using some agricultural production materials"[11] and other natural resources such as straws, clays, bamboos, glutinous rice pulp and limes. All these materials are available in local and are even cheaper and more convenient. The outside structures of these buildings are using "plant fiber cement composite panels" and "plant fiber board" sandwich structure. Compared with normal houses, the thermal insulation performance of this structure would increase by 50 percent.

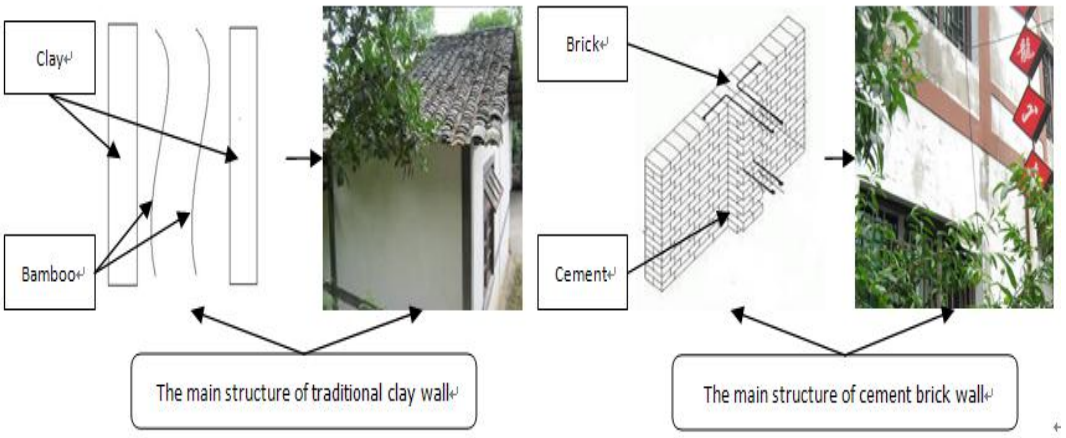

Fig 2. The main structure of two kinds of walls of HF Houses

USE RURAL NATURAL ENERGY

In rural areas, natural energy with the rich resources is provided by the environment. Considering the real situation of farmers and the economic cost, "it's advocated to use methane gas and solar power instead of natural gas and electricity power produced by the station."[12] On one hand, as the supplement energy, the methane gas is cheap and available. What's more, it is environmentally friendly and energy saving. It is easy for farmers to collect the materials that make methane gas. In

addition, the cost to build a biogas digester is acceptable for most farmers. On the other hand, there is enough sunshine that are 1000 to 1200 hours long in total in Chongqing city which is famous for its hot weather in China. This enough sunshine may encourage farmers to use polar power if the economy condition allowed. However, to build the solar power system would be a heavy financial burden to many farmers. Many farmers do not want to build the solar system compared with cost-benefit so that only few of them would consider this saving energy facility at the current economic level.

LIFE RUBBISH AND SEWAGE TREATMENT

There are three methods of dealing with life rubbish of HF: the landfill treatment which is to cover life garbage with dirt, the burning the garbage which is to make power to generate electricity and supply an adequate quantity of heat and the compost treatment which can not only solve the problem of dealing with garbage, but also realize the reusing of waste resources.

Considering the real situation of HF tourism, we can build such a system in Jinyun Mountain to save energy by using the model in figure 3. When life sewage is running into a septic tank, the farmers can screen the solid waste to dispose of as life garbage. Then the water runs into an ecological treatment area, which can be purified by the power of natural plants. This processing may eliminate the organic waste, which cannot be eliminated in the first step. Furthermore, these plants can get the nutrient substance in the water that is needed to grow. Through the ecological treatment area, the next step is that the soil treatment area further purifies the wastewater by the filtering of the soil. At last, the water can be used in the pond to keep fish or make other agricultural products, even watering the crops. This system is available and cheap for farmers to build.

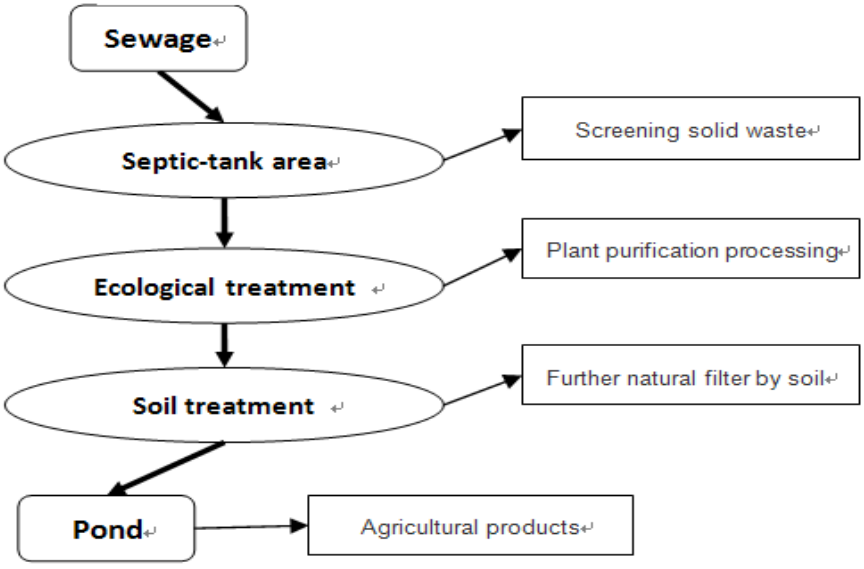

Fig 3. The natural purification processing in HF tourism

USE OF AGRICULTURAL PRODUCTS OR WASTES

How can agricultural products and wastes save energy in HF tourism? We can use them as materials to build houses or produce biogas. As we discussed above, the straws have been used in many ways such as burning, constructing the walls, making biogas, becoming handiworks and arts, forage etc. In addition, rich bamboo resources in Jinyun Mountain can be used to make items used daily like cups, bowls, stairs, houses, chairs, beds, buckets and so on. Beyond that, the poultry droppings are the best fertilizer for crops. All these "agricultural products or wastes can directly save energy." [13]

Tourism Environment Construction Planning: Ecological Enjoying

The beautiful natural environment and historical places are the two most attractive characteristics of Jinyun Mountain. Nowadays, the inclination of tourists is to breathe in the nature, to fall in the feelings of nostalgia and to dream of nirvana, and then all the fatigue and pressure will disappear suddenly. To construct the ideal ecological environment of tourism, the following principles may be obeyed: (1) be clear of what the aim is of ecological tourism in HF of Jinyun Mountain, (2) fully considering the carrying capacity of the ecological tourist attractions, [14] (3) designing all kinds of activities to let the tourists participate in, helping more tourists understand the mystery of ecological tourism through their actual personal experience and realizing that the ecological tourism is not just about enjoyment, but also the responsibilities of protecting natural resources and saving energy.

Implementation and Security of Tourism Planning: Government Supporting

Surely the government plays a very important role in this health tourism-saving energy strategic plan. The supporting system of government consists of many aspects, such as fund, law, policy, tax and so on (see table 1).

TABLE 1: THE CONSTRUCTION OF SUPPORT SYSTEM OF LOCAL GOVERNMENT

Items	Contents
Training	Provide the free training courses for the HF farmers who want to start the business.
Infrastructure	Build the infrastructures such as the road, electricity power network, running water etc.
Fund	Afford low-interest loans to farmers who are capital shortage.
Law	Formulate relevant laws and regulations to protect natural areas, tourists and HFs.
Technology	Provide technical support to HF that builds energy saving facilities.
Tax	Tax reduction or exemption on those better performance HF on energy saving.
Policy	Making related preferential policies to encourage the development of HF to save energy.
Assistance	Offer assistance to those suffering from natural disasters or accident risk when traveling here.
Management	Establish the professional team to lead and manage health tourism of HF

CONCLUSION

There is little literature discussing tourism saving energy in China, letting alone case studies on how to conserve power while traveling. "Actually, there is a close

relationship between tourism and energy saving if a reasonable plan is implemented."[15] Not only the theoretical illustration, but also practical use can bring more profits to HFs. HF tourism can be an interesting and environmental friendly recreational way which can be accepted by most of Chinese people. Based on the tendencies of the development of HF tourism, we can see the bright future of constructions of health tourism attractions in rural places especially in natural landscape areas. This "exercise tourism" has two functions: one is to improve the health level of tourists when they are traveling; the other is in favor of protecting the environment and saving energy directly or indirectly.

According to the case study, it's found that there are mainly two aspects of saving energy as widely as possible under the strategic plan following the concept of health tourism. On one hand, in the perspective of tourists, they are saving energy indirectly while traveling to rural places. If the attractions can urge tourists to stay in HF for weekends or other vacations, they would produce an unexpected effect in energy savings. Tourists would spend less time at home so that they may have less possibility to use household appliances, especially the computer and TV sets which use much energy. The purpose of building attractions in HF tourism area is to let people hold the interest in the entertainment while saving energy. On the other hand, in the perspective of HF stakeholders, they are saving energy directly while constructing the health tourism attractions. In this saving mode, farmers can use less power on residential buildings and using rural natural energy to protect the local natural environment by life rubbish treatment methods and sewage treatment systems instead. What's more, the use of agricultural products or wastes is even more energy-saving. It can help farmers in buying fewer industry products, which means less coal and gas would be used to make those products.

In sum, no matter what field it is, "energy savings is an engineering system which needs people to plan carefully." [16] Saving energy doesn't mean to stop the development of the economy and society to protect nature. In this low carbon period, we should consider a reasonable way to improve the economic and social developmental level with less waste of power and pollution of environment. By changing the traditional tourism mode of HF, the systematic design of health tourism can achieve such goals with less wasted energy and more income for local development.

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Cover page

Title: *A Study on the Microaerobic Removal of Hydrogen Sulphide in Biogas*

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ABSTRACT

In this paper rice straw was used as raw materials to produce biogas by anaerobic batch fermentation at 35℃. The hydrogen sulfide in biogas could be removed with micro-oxygenation technology (supplying limited oxygen to anaerobic fermentation tank). The control experiment (R0) and experimental groups (R1~R7) were carried out with the addition of 0 times, 0.5 times, 1.0 times, 2.0 times, 3.0 times, 4.0 times, 5.0 times, 10.0 times of theoretical oxygen demanded respectively. The results showed that after the desulfurization, the average hydrogen sulfide concentration in biogas produced from R3, R4, R5 were 314±111 mg/m^3, 250±84 mg/m^3, 166±100 mg/m^3 respectively and a removal efficiency higher than 92%. There were no obvious changes of the gas production level and methane concentration with adding oxygen. The oxygen residue in biogas was less than 0.5% basically [1-2].

1. INTRODUCTION

The resources of rice straw in china is very abundant. It used to be indispensable living resources to China's rural life, but the phenomenon of rice straw's surplus supply appeared with the improvement of rural energy structure and the using of alternative energy. It is forbidden to burn the rice straw away at the field, however the phenomenon happened from time to time which caused atmospheric environment pollution and resources waste at the same times. It is meaningful to use of rice straw as the raw materials for biogas production which avoid the environmental pollution and obtaining biogas energy.

Biogas is a kind of renewable clean energy which can replace other non-renewable energy to relieve the energy crisis and protect the natural environment. Biogas is a kind of mixed gas and the main ingredient are methane and carbon dioxide (60~70%, 30~40%, respectively). Nevertheless, it also contains many other impurities such as hydrogen sulfide. Biogas can be employed for power generation, as fuel for vehicles or incorporation into the natural gas pipe network and hydrogen sulfide concentration should meet the requirement of no more than 300 mg/m^3, 15 mg/m^3, 20 mg/m^3

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respectively. The concentration of hydrogen sulfide in the untreated biogas is between 1 and 12 g/m^3, so hydrogen sulfide must be reduced to acceptable levels before used.

Micro-oxygenation technology is an emerging desulfurization technology for biogas, which supplying oxygen to the anaerobic digester. The hydrogen sulphide concentration in the biogas can be reduced by the reaction between hydrogen sulfide and oxygen (the final product are elemental sulphur or sulphate). Micro-oxygenation technology shows competitive performance with lower cost and lower or none secondary pollution [3] compared with traditional biogas desulfurization technology. Although this technology had caused some scholars' (such as Diaz et al,2010)[4]) attention, the researchers of it were few. There are no scholars consider the influence of oxygen supply to oxygen residue in biogas, While the standard of oxygen residue is no more than 0.5% when biogas is employed as fuel for vehicles or incorporating into the natural gas pipe network. In this paper, micro-oxygenation technology was chosed as biogas desulfurization technology and rice straw was chosed as raw materials to explore the best technological parameters.

2. EXPERIMENTAL MATERIALS AND METHODS

2.1 Experimental Materials

The rice straw was collected from a local farmland, Yuyao, China. After being washed and dried, all of these rice straw was cut into sections at lengths of 2 cm to 3 cm, and the total solid (Ts), volatile solid (Vs) of it were 90.95%, 85.22% respectively.

The inoculum was biogas slurry which obtained from Zhengxing animal husbandry limited company, Hangzhou, China. The total solid (Ts), volatile solid (Vs) of it were 0.57%, 0.47% respectively.

2.2 Experimental Design

This experiment adopted batch fermentation as experimental method. The self-made experimental device constituted of a biogas fermentation bottle (500ml) with a working volume of 400ml, a biogas collecting bottle (1000ml) and a liquid collecting measuring cylinder. Putting experimental device into a water bath with the constant temperature of 35 ± 1℃. Hydrogen sulfide production was calculated according to the biogas production and hydrogen sulfide concentration. Daily theoretical oxygen demanded was calculated according to the hydrogen sulfide production.

The control experiment (R0) and experimental groups (R1~R7) were designed with the addition of 0 times, 0.5 times, 1.0 times, 2.0 times, 3.0 times, 4.0 times, 5.0 times, 10.0 times of theoretical oxygen demanded respectively to explore the influence rules on desulfurization efficiency and biogas production. Setting three repeats of the experiments with a fermentation period of 40d .The specific details of parameters are given in Table 1.

TABLE1 EXPERIMENTAL CONDITIONS FOR THE MULTIPLES OF THE THEORETICAL OXYGEN DEMANDED SUPPLIED

The serial number of fermentation device	R0	R1	R2	R3	R4	R5	R6	R7
The oxygen supplied (the multiples of theoretical oxygen demanded)	0	0.5	1.0	2.0	3.0	4.0	5.0	10.0

2.3 Experimental Analytical Methods

Biogas was collected via drainage and the daily biogas production equal to the daily drainage. Ts and Vs were measured by the gravimetric method. Biogas composition such as oxygen, methane was measured by gas chromatography (KexiaoGC-1690) with thermal conductivity detector (TCD). Hydrogen sulfide concentration in biogas was measured by 《Natural gas-Determination of sulfur compound》(GB/T11060.2-2008).

3. RESULTS AND DISCUSSION

3.1 Removal Efficiency of Hydrogen Sulfide and the Oxygen Residue

The removal efficiencies of hydrogen sulfide under various oxygen supplied were showed in Fig.1. The hydrogen sulfide concentration in the biogas of R0 showed small variances during the experiment and the average concentration was 3235±185mg/m^3, which could be used as a base to calculate the desulfurization efficiency. Fig.2 showed oxygen residues in biogas under various oxygen supplied. Oxygen residue of R0 was below 0.1% which illustrated that original oxygen residue in biogas was very low.

When the oxygen supply was insufficient (such as R1), the average hydrogen sulfide concentration was 2220±260 mg/m^3 and the desulfurization efficiency was very low (30.6%). The average hydrogen sulfide concentration (800±180 mg/m^3) decreased and the desulfurization efficiency increased rapidly (72.3%, still relatively low) even when the oxygen supply reached the theoretical oxygen demanded (such as R2). The reason is that part of the oxygen was left in biogas and on the other hand oxygen may consumed by the over oxidation of sulfide or by deputy reactions.

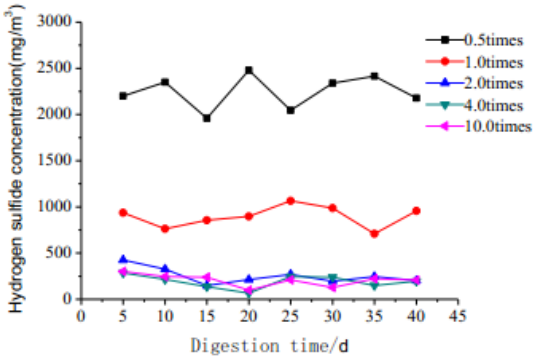

Fig.1. Removal efficiency of hydrogen sulfide under various oxygen supplied

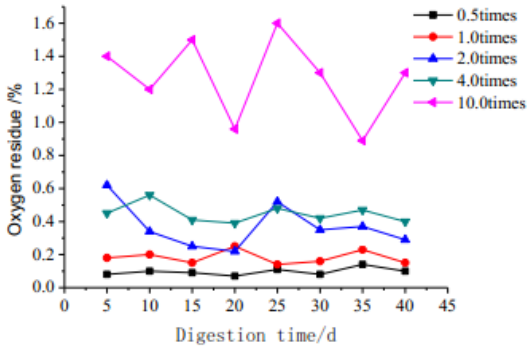

Fig.2. Oxygen residue under various oxygen supplied

When oxygen was over supplied till 4 times theoretical oxygen demanded. The average hydrogen sulfide concentration of R3~R5 were 314±111 mg/m^3, 250±84 mg/m^3, 166±100 mg/m^3 and the average removal efficiencies were 92.0%, 92.6% and 94.1%, meanwhile the oxygen residues were all below 0.5%. The result shows the proper oxygen supply level. If the oxygen supply increased farther (such as R6, R7) the average hydrogen sulfide concentration (218±72 mg/m^3, 199±100 mg/m^3 respectively) or the average removal efficiencies (93.8%, 93.8% respectively) did not changed obviously. However the oxygen residues in biogas were higher than 0.5%.

3.2 Biogas Production and Methane Concentration

3.2.1 DAILY BIOGAS PRODUCTION AND CUMULATIVE BIOGAS PRODUCTION

Fig.3, Fig.4 showed the variations of daily biogas production and cumulative biogas production under various oxygen supplied respectively. The curves for biogas production of R0 and R1~R7 had similar trends. At earlier stage daily biogas production was low, during which hydrolysis, hydrogen-producing and acetic acid-production phase had absolute predominance. The daily biogas production increased gradually and reached a gas peak at middle stage when the fermentation gave priority to methane-producing phase. At later stage, the daily biogas production was reduced gradually and tended to zero as the raw materials were fermented completely and the activity of methanogenus were attenuated.

The Fig.4 showed that the addition of oxygen could lead to a certain increase of total biogas production (R1~R7) compared with R0 (without addition of oxygen) and the average yield of rice straw's anaerobic fermentation could be 250mL/g rice straw. These experimental results indicated that the supply of limited oxygen did not reduce the production of biogas at least. The reason could be that limited oxygen supplied caused facultative anaerobic bacteria using oxygen for hydrolysis [6].

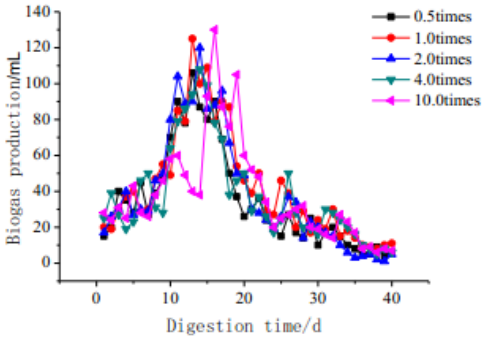

Figure 3. Daily biogas production under various oxygen supplied

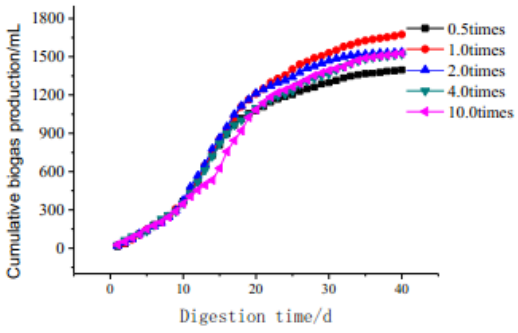

Figure 4. Cumulative biogas production under various oxygen supplied

3.2.2 METHANE CONCENTRATION

The Fig.5 showed the variations of methane concentration under various oxygen supplied and the curves for methane concentration of R0 and R1~R7 had similar trends. Methane concentration increased at earlier stage when the fermentation gave

priority to hydrolysis, hydrogen-producing and acetic acid-production phase. At middle stage, methane concentration reached to the maximum on account of absolute predominance of methane-producing phase. Methane concentration tended to be stable and remained at about 60% as the fermentation were carried out at later stage. We could learned that the methane concentration of R4~R7 was even higher than R0 as showed in Fig.5. The most possible explanation was because of the higher desulfurization efficiencies of R4~R7 and lower hydrogen sulfide concentration could reduce the toxicity to the methanogenus and increase the methane production[7].

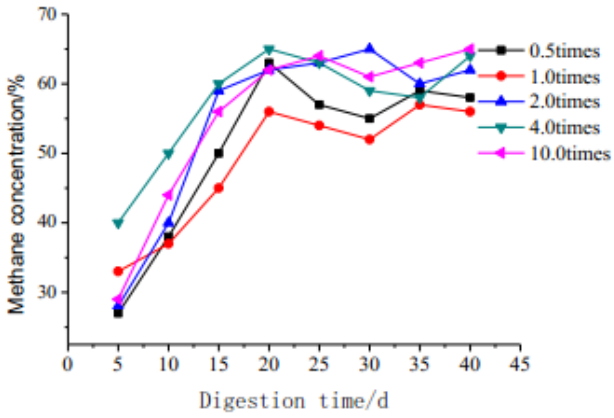

Figure 5. Methane concentration under various oxygen supplied

4. CONCLUSIONS

In this paper the rice straw was used as raw materials to produce biogas by anaerobic batch fermentation at 35℃ and hydrogen sulfide in biogas was removed with micro-oxygenation technology. The following conclusions could be drew.

(1) There were no obvious changes of the biogas production level and methane concentration with the addition of limited oxygen.

(2) When the oxygen supply was insufficient or just reached the theoretical oxygen demanded, the oxygen residues could be controlled below 0.5% whereas the desulfurization efficiencies were not high (30.6%, 72.3%). When the oxygen supply was far more than theoretical oxygen demanded (such as R6, R7), the average hydrogen sulfide concentration (218±72 mg/m^3, 199±100 mg/m^3 respectively) or the average removal efficiencies (93.8%, 93.8% respectively) did not changed obviously, while the oxygen residues in biogas were higher than 0.5%.

(3) The oxygen residues could be controlled below 0.5% and the average hydrogen sulfide concentration were 314±111 mg/m^3, 250±84 mg/m^3, 166±100 mg/m^3 respectively with the desulfurization efficiency higher than 92% when the oxygen supplied were 2.0 times, 3.0 times, 4.0 times of theoretical oxygen demanded.

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Cover page

Title: *Nitrogen, Phosphorus and COD Losses from Paddy Fields under Different Water and Fertilizer Management Regimes*

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ABSTRACT

The objective of this study was to quantify the impacts of water and N-fertilizer management on the quality of surface and percolating water from paddy fields. The experiments were carried out for middle-season rice in Tuanlin, Hubei province in 2009, and for late rice in Yongkang, Zhejiang Province in 2007. At both sites there were 2 water treatments (AWD, alternate wetting and drying irrigation; CF, continuous flooding). At Tuanlin there were 3 N-rate fertilizer treatments (N1=180kg N/ha, farmers practice; N2 = 270kg N/ha; N3 = 360kg N/ha) in sub plots. At Yongkang, there were 2 N-rates (N0=0; N1=230 kg N/ha, farmers practice), and 3 N-application time treatments (F0= control; F1 = 2 splits, as farmers practice; F2 = 3 splits; F3=4 splits). Compared with CF, AWD reduced losses of total N and P (TN, TP), and chemical oxygen demand (COD), significantly. The TN losses in Tuanlin were reduced by 48%, 30% and 29% in N1, N2 and N3, respectively. Also with AWD, TP loss was reduced by 7% in N3, but no change was observed in N1 and N2. Using farmers' N practice in Yongkang, AWD reduced TN and TP losses, and COD, by 46%, 26%, 36% respectively, averaged over N splits. Using farmers' N practice and AWD, compared with 2 splits, 3 splits reduced the loss of TN and TP by 55% and 43%, respectively, while COD was only reduced by 4%. AWD reduced TN and TP losses and COD because it reduced surface water drainage and deep percolation in the paddy field. P pollution from paddy fields is very small. The results of this work show that water saving combined with N-fertilizer management can not only increasing water and fertilizer use efficiency but also greatly reduce pollution from paddy fields.

INTRODUCTION

Rice is one of the most important food crops in China, and is a major user of water and fertilizer. Rice accounts for 27.4% of China's grain area and 36.3% of China's total output of food crops, and for 60% of China's total agriculture water use [1].The average N use in paddy fields in China is around 180kg/ha, significantly higher than the world average. Nitrogen use efficiency is low at 28.3%, compared

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with attainable levels of 30%~50%. Phosphorus use efficiency is only about 13% [2].

Over the past 20 years, China and other Asian countries have put considerable effort into the development and dissemination of technologies for increasing yield, improving fertilizer use efficiency and saving irrigation water for rice [3,4]. However, studies on reducing pollution of surface and ground waters from rice are lacking. In rice growing areas, drainage water from paddy fields contaminated with N and P is thought to be the main cause of agricultural non point source (NPS) pollution. Water saving irrigation (WSI) technologies such as alternate wetting and drying (AWD) can significantly reduce the amount of surface and deep (beyond theroot zone) drainage from paddy fields[3,5]. This in turn may reduce pollution from N and P in the drainage water and improve fertilizer use efficiency [5,6]. Therefore experiments were conducted to quantify the effects of water and fertilizer management on pollution of surface and deep drainage water.

MATERIALS AND METHODS

Experiment Sites

Two experiments were carried out. One was at Tuanlin Irrigation Experimental Station (30°50'N, 111°15´E, 100 m ASL), Hubei province, China, during the middle rice season of 2009. The other experiment was at Yongkang (28°42'N, 120°12'E, 85.4m ASL), Zhejiang province, China, during the late rice season of 2007.

At Tuanlin, the soil texture is loam and clay loam. The climate belongs to subtropical monsoon; the average annual rainfall is 700~1100 mm, with 1300~1800 mm evaporation (20 cm pan) and annual average sunshine time 1300~1600 h. At Yongkng, the soil texture is sandy clay. The groundwater depth during the irrigation period is usually below than 0.5 m. Climate in Yongkang also belongs to subtropical monsoon with an annual average rainfall of about 1483.2 mm, 919.6 mm of evaporation (E601 pan), annual average sunshine time 1909 h.

Experimental Design

At both sites, the experiment was conducted in a farmer's field in a split-plot design with 3 replications. At both sites the main plots were two water treatments: CF = continuous flooding, and AWD =alternate wetting and drying (Table 1). At Tuanlin, the subplots were three N-rates (N1=180 kg N/ha, farmers practice; N2= 70 kg N/ha; N3 = 360 kg N/ha). Plot size was 4 m×15 m. At Yongkang, the subplots were two N-rates (N0=0; N1=230 kg N/ha, farmers practice), and three N-application split treatments (F1=2 splits, farmers practice; F2=3 splits; F3=4 splits), and plot size was 8 m×12.5 m. At Tuanlin, Mid-rice Liangyouxiang No.4 was transplanted on 27th May 2009, and harvested on 4th September. At Yongkang, late-rice Zhongzheyou No.1 was transplanted on 4th July, and harvested on 14th October.

At both sites, plant spacing was 20 cm x 20 cm, and all the P and K fertilizer (40 kg P/ha, 70kg K/ha) was broadcast onto the puddled soil 1 d before transplanting.

TABLE 1 WATER AND N-FERTILIZER TREATMENTS

Station	Treatment	N (kg N/ha)	Basal	DAT	Early Tillering	Heading
Tuanlin 2009	CF N1	180	50%N	50%N		
	CF N2	270	50%N	50%N		
	CF N3	360	50%N	50%N		
	AWD N1	180	50%N	50%N		
	AWD N2	270	50%N	50%N		
	AWD N3	360	50%N	50%N		
Yongkang 2007	CF N0	0				
	AWD N0	0				
	CF N1F1	230	50%N	50%N'		
	AWD N1F1	230	50%N	50%N		
	AWD N1F2	230	50%N	30%N	20%N	
	AWD N1F3	230	50%N	25%N	15%N	10%N

AWD-Alternate wetting and drying; CF-Continuous flooding; Basal-Basal fertilizer, applied 1 day before transplanting; DAT-Top dressing, applied 10 days after transplanting; Early Tillering fertilizer applied 5days before early tillering; Heading fertilizer, applied during the Heading and Flowering growth period.

Measurements

The amounts of irrigation, surface drainage and percolation were measured for each main plot, and rainfall was measured at the site. Water depth was measured daily in perforated PVC pipes (40 cm long, 20 cm diameter) inserted to a depth of 25 cm in each subplot. Soil inside the pipes was dug out to a depth of 25 cm. The water depth in the percolation tubes (5 cm diameter, 65 cm long, installed at 40 cm depth) was measured every 3 days.

Water samples were collected in each growing stage, during surface drainage and top-dressing, and analyzed for total N (TN), NO_3^--N, NH_4^+-N and total P (TP).

Evapotranspiration was computed from climate data at a nearby meteorological station, using the Penman-Monteith method.

RESULTS AND DISCUSSIONS

Drainage and Percolation at Tuanlin

Drainage and percolation were significantly ($p<0.05$) reduced by averages of 14% and 13%, respectively, with AWD (Table 2).

Compared with CF, AWD plots can retain more rainfall, due to the generally lower water depth. The rainfall utilization efficiency (proportion of rainwater capture) over the whole season was 34.2% in AWD and 23.3% in CF. Both treatments had low rainfall use efficiency because there were 3 heavy rains of 65-74 mm. The lower percolation with AWD was due to the fact that there were many periods when the soil was not ponded.

TABLE 2 DRAINAGE AND PERCOLATION OF MID-SEASON RICE (TUANLIN, 2009)

Water Source	N	CF	AWD	Difference
Drainage (mm)	N1	306	245	
	N2	281	225	
	N3	280	274	
	Average	289	248	*
Percolation (mm)	N1	162	145	
	N2	170	143	
	N3	166	148	
	Average	166	145	*

ns = not significant at 5% level by LSD; *= significant at 5% level by LSD.

N and P losses at Tuanlin

AWD reduced TN losses compared with CF, by 48%, 30% and 29% in N1, N2 and N3, respectively. TN and Ammonium N (NH_4^+-N) were significantly ($P<0.05$) reduced by AWD in N1 (Table 3), the differences in N2 and N3 were, however, not statistically significant at the 5% level. There were no significant effects of AWD on Nitrate N (NO_3^--N) and TP losses, except in N1 level AWD reduced 34% of nitrate N, which were very low in all treatments. This means ammonium N is the main pollution losses. There was not significant interaction between water management and N rate on pollutants (Table 4).

TABLE 3 TOTAL N AND P LOSS FROM PADDY FIELD (TUANLIN, 2009)

N	CF		AWD		Difference	CF		AWD		Difference
	TN(kg/ha)					NH_4^+-N(kg/ha)				
N1	18.11	a	9.44	a	*	23.92	a	8.77	a	*
N2	14.68	a	10.31	a	ns	20.57	a	13.63	a	ns
N3	20.92	a	14.88	a	ns	27.13	a	26.91	a	ns
	NO_3^--N(kg/ha)					TP(kg/ha)				
N1	0.96	a	0.63	b	ns	0.4306	a	0.4229	a	ns
N2	1.11	a	1.10	a	ns	0.4073	b	0.4137	a	ns
N3	1.27	a	1.12	a	ns	0.5273	a	0.4911	a	ns

In a column and the same pollution and water treatment, means followed by the same letter are not significantly different at the 5% level by LSD; * =significant at 5% level by LSD; ns= not significant at 5% level by LSD.

TABLE 4 SIGNIFICANCE TEST OF N AND P LOSS FROM PADDY FIELD (TUANLIN, 2009)

Factors	TN			NO_3^--N			NH_4^+-N			TP		
	F	P	Sig	F	P	Sig	F	P	Sig	F	P	Sig
Water	8.191	**0.019**	*	0.651	0.440	ns	7.231	**0.043**	*	0.101	0.758	ns
Fertilizer	2.297	0.156	ns	1.228	0.338	ns	3.547	**0.087**	ns	2.517	**0.135**	ns
Water*Fertilizer	0.311	0.74	ns	0.2	0.823	ns	1.031	0.395	ns	0.109	0.898	ns

*-significant,at 5% level by F-test; ns-not significant at 5% level by F-test

There was no relationship between TN concentration, water and N-rate treatments in the surface drainage and percolation water. TP concentration was not affected by the water and N-rate treatments.

Nitrogen Concentration Trends During the Growth Period at Tuanlin

N concentrations were the highest in both the percolation and surface drainage water shortly after transplanting, with peak values of 20~30 mg L^{-1} of TN in N1 around 2-4 DAT which declined to less than 5mg L^{-1} by about 15-18 DAT (Figs 1 and 2). There was also a small peak at the milk stage, maybe it was because at the ripening stage, the fertilizer absorption rate decreased and the old leaves came to release its nutrition.

The TN loss between transplanting and late tillering was significantly larger than during later periods. In CF/N1, around 85% of the loss as TN, Nitrate N (NO3-N) and Ammonium N (NH_4^+-N)occurred during this period, while in AWD/N1 the proportion of TN and NO_3^--N lost during this period was less (68%). The reason for the higher losses is because the first few weeks after transplanting was the high N concentration during this period as a result of the basal and topdressing N applications.

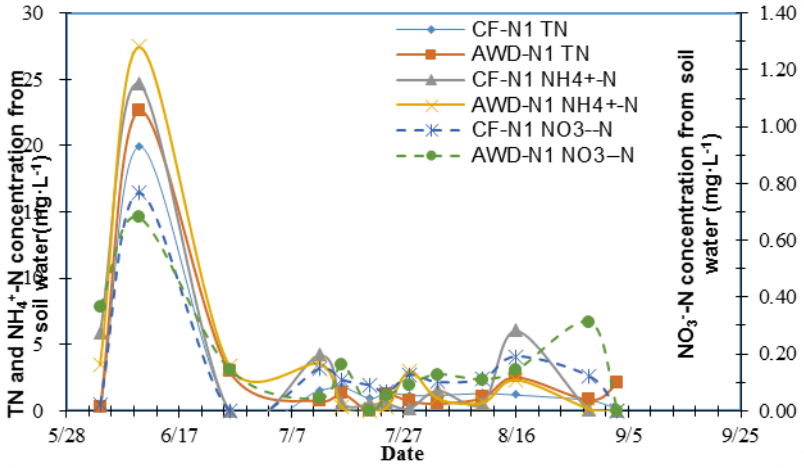

Fig. 1 N concentration trends from percolation water (Tuanlin, 2009)

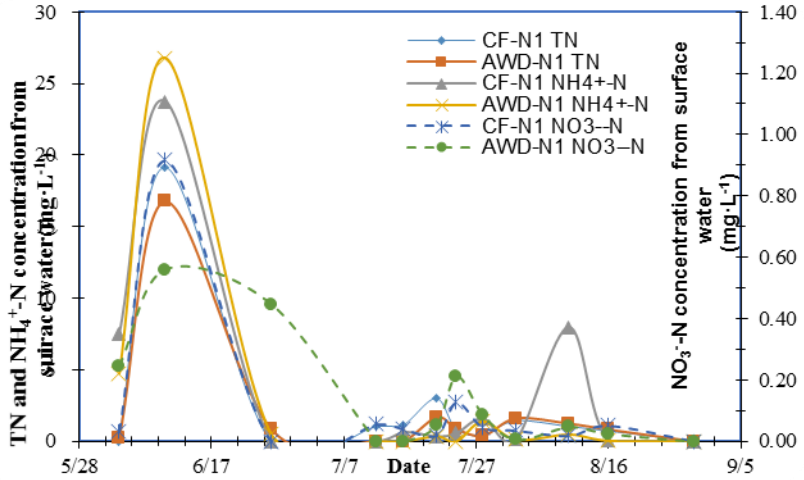

Fig. 2 N concentration trends from surface water (Tuanlin, 2009)

TN, TP and COD Losses at Yongkang

At Yongkang, AWD reduced surface drainage from 274 to 183mm, and percolation from 45 to 27 mm. The losses of TN and TP, and the COD, were much higher in the surface drainage water than in the percolation water (Table 5). Compared with CF, AWD significantly reduced TN and TP losses, and COD, by 46%, 26% and 36%, respectively.

TABLE 5 TN AND TP LOSS AND COD OF SURFACE DRAINAGE AND PERCOLATION WATER UNDER DIFFERENT TREATMENTS OF LATE RICE (YONGKANG, 2007)

pollutant	Water Source	CF	AWD	Difference	Reduction Rate(AWD to CF)
TN（kg/ha）	Drainage	12.36	6.48		
	Percolation	0.62	0.54		
	Total	12.98	7.02	*	45.90%
TP（kg/ha）	Drainage	0.27	0.2		
	Percolation	0.04	0.02		
	Total	0.31	0.23	*	26.30%
COD（kg/ha）	Drainage	42.42	33.23		
	Percolation	20.97	7.28		
	Total	63.4	40.5	*	36.10%

*-significant at 5% level by LSD; ns-not significant at 5% level by LSD

Fertilizer Application Splits in Affecting NPS Pollution at Yongkang

Compared with 2 splits of N-fertilizer, 3 splits reduced TN and TP losses significantly by 55 and 43%, but COD was not significantly reduced, respectively with AWD water management. However there was little further reduction with 4 splits (Table 6).

TABLE 6 POLLUTANTS LOSS UNDER DIFFERENT N-FERTILIZATION APPLICATION (YONGKANG, 2007)

Treatment	TN	TP	COD
	（kg/ha）	（kg/ha）	（kg/ha）
AWDN1F1	10.94 b	0.27 b	40.46 a
AWDN1F2	4.97 a	0.15 a	38.75 a
AWDN1F3	5.14 a	0.16 a	42.30 a
Reduction Rate(AWDN1F2 to WAWDN1F1)	54.6 %	43.3 %	4.2%
Reduction Rate(AWDN1F3 to AWDN1F1)	53.0 %	40.6 %	-4.5 %

In a column and the same pollution, means followed by the same letter are not significantly different at the 5% level by LSD

CONCLUSIONS

(1) The results suggest that the key way to reduce pollution from paddy fields is to reduce surface drainage, as the volume of drainage water was higher than of percolation water, while the average concentration of pollutants in the drainage

water was similar to that in the percolation water. This is especially the case if there is heavy rain during the first few weeks after transplanting when there is a lot of N in the floodwater and topsoil as a result of basal and top dressing N applications. Thus improved water and fertilizer management during this period are important to reduce pollution losses.

(2) Compared with CF, AWD reduced N and P losses, and COD, significantly. In Tuanlin, AWD reduced TN losses from mid-season rice by 48% using the farmer fertilizer N rate. However, P losses were only reduced by 2%. In Yongkang, AWD reduced TN and TP losses from late rice by 46 and 26%, respectively, and COD by 36%. Our experiments show that P pollution from paddy fields is very small.

(3) Increasing the number of N-fertilizer splits significantly reduced TN and TP losses, by 55 and 43% respectively, however the effect on COD was small. From 3 to 4 application splits of N-fertilizer the reduction effect is not obvious.

(4) AWD can decrease N and P losses, and COD, mainly because both surface drainage and percolation are reduced due to generally lower water depth and periods of non-ponding. Neither AWD nor CF has a significant effect on reducing the concentration of pollutants in the surface drainage or percolation water.

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Title: *DSC and XRD Analysis of (A384.1)(1-x)[(MgO)p]x Composites*

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ABSTRACT

MgO reinforced Al/Al Alloy based MMC's presents synthesis and characterization of (A384.1)(1-x)[(MgO)p]x based MMCs. This work has been undertaken to investigate and improve mechanical and physical properties of A384.1 Al Alloys by the addition of MgO particulate reinforcements. During the X-Ray Diffraction the intensities of the peaks corresponding to Al, Si and MgO are different in different samples; none of the MMC samples exhibited any proof of existence of any other impurity phase. This second endothermic peak is followed by a single exothermic peak in case of x=0.0 sample for differential scanning calorimetry studies, within temperature range of 450^0C to 520^0C. This effect becomes more prominent as MgO doping increase. For x=0.20 reinforced sample, two distinguishable exothermic regions have been observed, one from 370^0C to 450^0C and formation of two separate exothermic peaks. This effect may be attributed to dissolution of semi coherent inter-metallic phases and the evolution of $MgAl_2O_4$ second phase respectively.

INTRODUCTION

Magnesium is the lightest structural (A384.1)(1-x)[(MgO)p]x A 384.1 Al alloys based Composites using MgO doping metal with a specific density of 1.74 g/cm3 which is about two third of density of aluminum (2.7 g/cm^3) and one quarter of iron (7.874g/cm^3). One of the earliest known applications of Mg is in compound form as magnesium sulphate for medicinal purposes known commonly as Epsom salts in the late 1600s to early 1700s. Today, major automotive manufacturers are researching into the applications of Mg alloys in engines and car parts in order to reduce the weight and environmental impact due to gas emission [1-2].

The limit on electromagnetic radiations regulations also prompted manufacturers of electronic devices to use magnesium alloys owing to its excellent resistance to electromagnetic radiation such as in mobile phones, cameras (A384.1)(1-x)[(MgO)p]x and laptops. In the present study, an attempt has been made to reinforce MgO particulate in Al Alloy (A 384.1) to improve micro-structural characteristics, mechanical properties and oxidation resistance of metal matrix A 384.1 Al alloy. The

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Stir Casting technique followed by extrusion has been used to synthesize these materials. All the extruded samples were then characterized using density/porosity measurements, XRD, DSC and mechanical testing [3,4,6].

MATERIAL SYNTHESIS

The un-reinforced and MgO reinforced composites were successfully synthesized by using stir casting. The reinforced MMC's with nominal composition (A384.1)(1-x)[(MgO)p]x were fabricated by using A384.1 Al Alloy as matrix and MgO with 0.053, 0.106 and 0.220 μm particle sizes as reinforcement in varying amounts (x=0.0 to 0.20) followed by extrusion. For structural, micro-structural and mechanical characterization, sample preparation was done, first by polishing the sliced samples with emery paper up to 1200 grit size, followed by polishing with MgO suspension on a grinding machine using velvet cloth. Finally, the samples were polished with 0.5 μm diamond paste.

MATERIAL CHARACTERIZATION TENSILE CHARACTERISTICS

The samples with x=0.05 exhibited highest values of 0.2% proof stress and UTS among all the samples synthesized with particle sizes 0.053, 0.106 and 0.220 μm. The highest values of 0.2% proof stress and UTS is observed in samples with particle size 0.106 μm and 0.053 μm respectively. A steep increase in the values of 0.2% proof stress and UTS are observed till x=0.05, but with the further increase in doping and change in particle size the value of 0.2% proof stress and UTS decline as can be clearly seen in Figure-1 and Figure-2. A fall in the values of all the samples is observed continuously upto x=0.20. The reduction in 0.2% proof stress and UTS is due to porosity enhancement with increasing doping 'x'. The porosity is found to be high near the MgO particles in Al alloy matrix, due to which some cracks in the local regions propagate at the interface between the matrix and particle. It is due to the high thermal stresses generated at the interface during cooling as a result of the large difference in the coefficient of thermal expansion between the matrix and the particles. This may lead to decohesion of particles at the particle-matrix interface i.e early failure and hence poor tensile properties. Therefore, in order to realize the strengthening effect of the reinforcement, the interfacial bonding between the particle and matrix must be strong. It has been established in the previous studies that second phases and grain boundaries location inhibit dislocation movement [5] and sliding [7], leading to an increase in strength as observed in case of x=0.05 samples. An increase in failure strain is exhibited by Al Alloy-MgO formulations corresponding to these samples. This observation is similar to the findings of other researchers. The increase in failure strain with an increase in the amount of Mg can be attributed to the increasing amount of second phase in the microstructure. It may be noted that the presence of harder second phase leads to plastic incompatibility with the matrix during tensile loading, leading to early crack nucleation, at the porosity present near the MgO particulate. The increase in 0.2% proof stress and ultimate tensile strength of the composites due to the addition of MgO can be attributed to solid solution strengthening [5][6], the presence of reasonably distributed (predominantly at grain boundaries) harder second phase $MgAl_2O_4$ [7] and an increase in the amount and second phase [7].

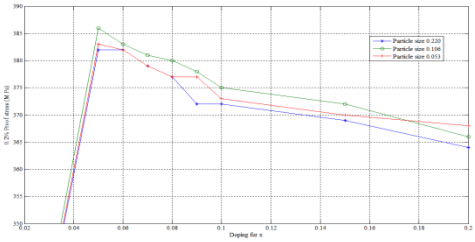

Figure-1; Figure-5; 0.2% proof stress with respect to doping of ‘x’ for tensile properties

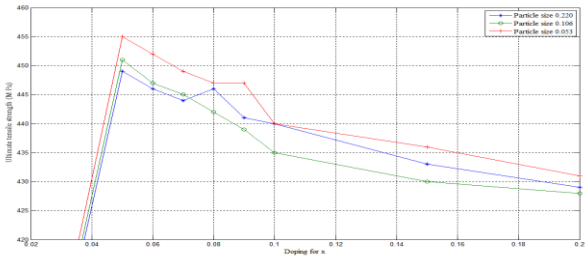

Figure-2; ultimate tensile strength with respect to doping of ‘x’

COMPRESSIVE CHARACTERISTICS

The composites showed 404 MPa as the highest values of 0.2% proof stress at x=0.06 and particle size 0.106 µm. The highest value of compressive strength 787 MPa is exhibited by MMC with x=0.10 and particle size 0.220 µm. Figure-3 shows that data graphically, from where it is clearly observed that the values are steady up to x=0.08 and after that there is a sudden fall in the values of 0.2% proof stress. The sample with 0.053 µm particle size exhibits a lower value of 0.2% proof stress at x=0.20 as compared to its other counterparts.

In general, an increasing trend in the compressive properties is observed in all the samples as shown in the Figure-4. This can be explained in the light of integrity of microstructure vis-a-vis porosity present. The interface between the reinforcement and matrix plays a determining role on compressive strength of composites. Main strengthening in composites comes from effective transfer of load from the matrix to the particle via the interface [7-8]. The increase in compressive strength of MgO based composites can primarily be attributed to Significant grain refinement [7], the presence of reasonably distributed harder particulates/second phases [8], dislocation generation due to elastic modulus mismatch and coefficient of thermal expansion mismatch between the matrix and reinforcement [8-9] and load transfer from matrix to reinforcement.

Clearly, the MMC's synthesized in the present study exhibit a significant enhancement of compressive strengths coupled with the reduction of failure strain. This enables these materials to absorb large amount of energy up to fracture, corresponding to the large area under engineering stress - strain curve [7-8] clearly reveal the enhanced damage tolerance capability of these materials.

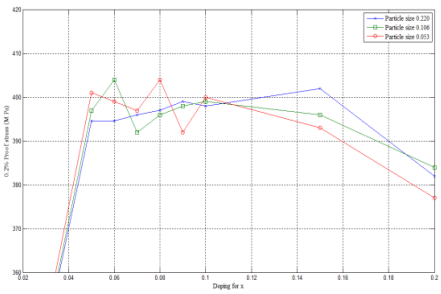

Figure-3; 0.2% proof stress with respect to doping of ‘x’

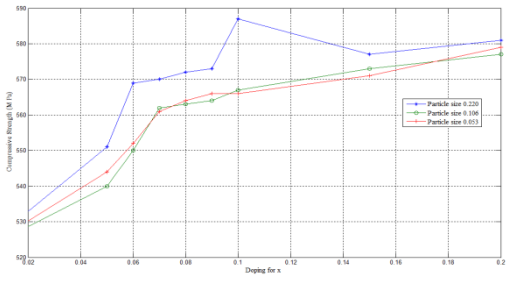

Figure-4; compressive strength with respect to doping ‘x’

X-RAY DIFFRACTION

The XRD micrographs of the samples at the different value of 'x', and its composite counterpart with varying particle sizes, from 0.053, 0.106 and 0.220 um , are shown in Figure-5, Figure-6 and Figure-7 respectively. The unreinforced sample exhibited the characteristic prominent peaks of (A384.1)(1-x)[(MgO)p]x A 384.1 Al alloys based Composites using MgO doping Al and Si. As the doping 'x' increases, additional peaks corresponding to MgO and $MgAl_2O_4$ start emerging. The intensity of these peaks increases with increasing amount of doping 'x'. The MMC's with particle size 0.053 μm, show a poor dependence of change of the heights in MgO and MgAl2O4 peaks as a function of doping 'x'. The height of MgO peaks almost becomes constant beyond x=0.10. However, for 0.106 μm particle size, the MgO and MgAl2O4 peaks are found to increase continuously with doping. The composites with particle size 0.220 μm do not show any such trend [10-11]. Although the intensities of the peaks corresponding to Al, Si and MgO are different in different samples, none of the MMC samples exhibited any proof of existence of any other impurity phase.

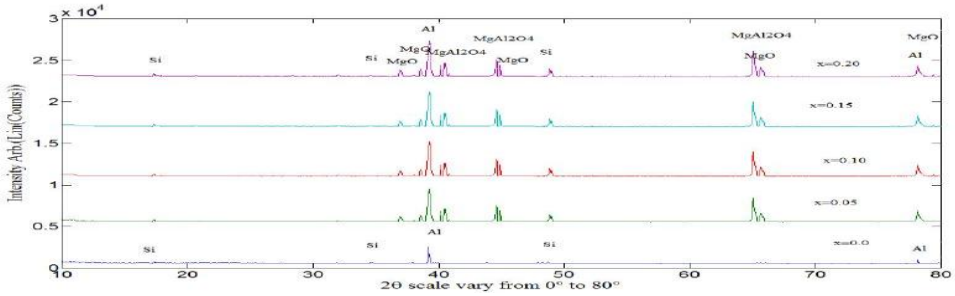

Figure-5;XRD for particle size 0.053 with respect to x

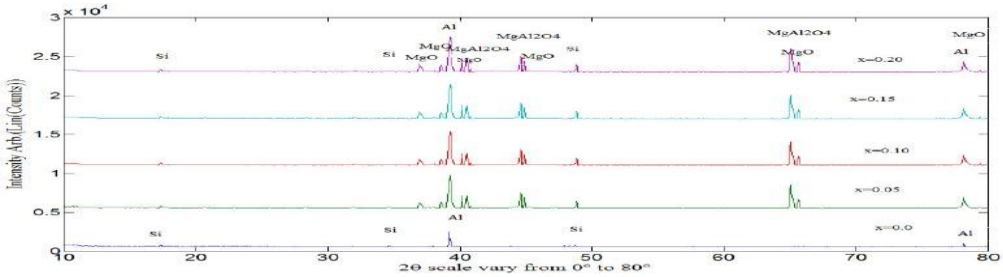

Figure-6; XRD for particle size 0.106 with respect to x

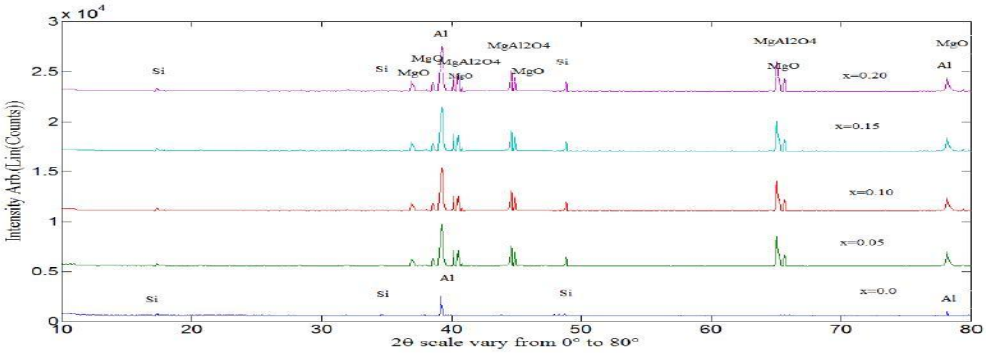

Figure-7; XRD for particle size 0.220 with respect to x

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

The Differential Scanning Calorimetric (DSC) was performed on the different composites of MgO at the rate of 2^0C per minute and the results are shown in Figure-8. These investigations are done to evaluate the performance of the fabricated MMC samples under actual service conditions and to know the application potential of the fabricated Al/Al Alloys matrix-ceramics particulate composite in technological applications, to determine the phase stability, transformations and to study precipitation in these materials. The DSC traces showed two major endothermic peaks followed by compensatively weak exothermic peaks. The qualitative interpretation of DSC plots occurring in A384.1 reinforced with MgO in terms of the two phase transformations/development is as follows [10]:

1. Cu and Mg in Al rich GPS Zones containing incoherent inter-metallic phases.
2. Advent of fully ordered and coherent inter-metallic phases.

Followed by oxidation of matrix alloys through the formation of second phase of magnesium aluminate ($MgAl_2O_4$). The first endothermic peak at about 170^0C in unreinforced sample may be ascribed to the localized effect of formation of powdered inter-metallic phase and thus formation of Cu and Mg containing Guinier-Preston zones (GPB) in the Al rich matrix. It is evident from the thermogram that tendency of evolution of this less ordered phase becomes more prominent with the increasing doping of MgO. Followed by the endothermic peak, is a less prominent exothermic peak nearly at 245^0C in unreinforced alloys that can be attributed to the dissolution GPB zones phase. The dissolution temperature is found to be critically dependent on the MgO doping, is as evident from the sharp exothermic peak at 220^0C in x=0.20 MMC samples.

This effect may be attributed to dissolution of semi coherent inter-metallic phases (described above) and the evolution of $MgAl_2O_4$ second phase respectively. The detection of $MgAl_2O_4$ in XRD investigations also supports this point [12]. As precipitation dissolution effects and phase evolution are thermally activated processes, the various thermal events observed in Figure-8 have explicit heating rate dependence. The lower heating rate ensures smooth commencement of the thermal events, the higher heating rate is found to shift the various events to higher temperatures [13-14].

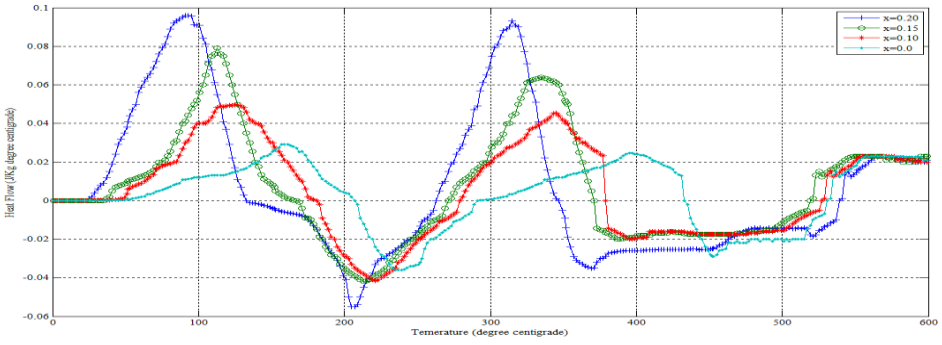

Figure-8; DSC curves of the composites with respect to 'x'

CONCLUSIONS

The disintegrated melt deposition technique coupled with hot extrusion can be used to synthesize (A384.1)(1-x)[(MgO)p]x based formulations. Increasing the presence of MgO in composites leads to a significant improvement in hardness, 0.2% proof stresses, ultimate tensile strength and compressive strength. Excellent oxidation resistance of composite formulations was realized and attributed to the presence of MgO in temperature range 300^0C -500^0C. This effect may be attributed to dissolution of semi coherent inter-metallic phases (described above) and the evolution of $MgAl_2O_4$ second phase respectively. The lower heating rate ensures smooth commencement of the thermal events, the higher heating rate is found to shift the various events to higher temperatures.

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Cover page

Title: *Morphological Responses of Seed Germination and Seedling Growth of Three Platycodon Grandiflorum Varieties to Temperature Stress*

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ABSTRACT

The effects of culture temperature on seed germination and seedling growth of three *Platycodon grandiflorum* varieties from Shangzhou, Shaanxi (culture-type), Chifeng, Inner Mongolia (culture-type), and Zhashui, Shaanxi (wild-type), respectively were investigated. Results showed that culture temperature significant affected the characteristics of seed germination and seedling growth of the three *P. grandiflorum* varieties. The lower and upper temperature limits for germination of *P. grandiflorum* seeds were 5 ℃ and 40 ℃, respectively. The optimal temperatures of seed germination were 25 ℃ for the Shangzhou and Chifeng varieties and 20 ℃ for the Zhashui varieties; the optimal temperature of seedling growth was 25 ℃ for all of the three varieties. Excessively high and low temperatures both negatively affected the seed germination and seedling growth processes of *P. grandiflorum*, possibly through different inhibitory mechanisms. At the low temperature (10 ℃), the germination starting time of *P. grandiflorum* seeds was significantly delayed; as the culture temperature increased, the germination percentage, germination vigor, and germination index of *P. grandiflorum* seeds as well as the fresh weight, dry weight, root length, shoot length, and root/shoot length ratio of *P. grandiflorum* seedlings showed increases followed by decreases for all of the three varieties. Low temperature mainly imposed inhibitory effects on *P. grandiflorum* by delaying the germination starting time of seeds and slowing down the growth of roots, whereas high temperature was mainly harmful to *P. grandiflorum* by reducing the germination percentage of seeds, slowing down the growth of roots, and promoting the over growth of leggy shoots.

Keywords: *Platycodon grandiflorum*, temperature stress, seed germination, seedling growth

INTRODUCTION

Platycodon grandiflorum (Jacq.) A. DC, the only species of the genus *Platycodon*, family *Campanulaceae*, is a species of herbaceous perennial

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dicotyledonous dual-purpose plant with medicinal, edible, ornamental values. In recent years, the effects of *P. grandiflorum* in anti-aging, tumor suppression, insect sterilization, and odor eliminating agent have been continuously discovered. Due to the rapid growth of demand, wild resources alone can no longer meet the demand for raw materials of *P. grandiflorum* by the society. In this context, studies on the germplasm resources and genetic breeding of wild-type *P. grandiflorum* are widely carried out(Liu et al., 2006).

P. grandiflorum have small seeds which weigh approximately 1 g per thousand grains(Liu et al., 2009). Under normal circumstances, the field germination percentage of *P. grandiflorum* seeds is approximately 1% only, which can easily causes the formation of seedlingless ridges and serious loss of production. Genetic analysis of plant traits and breeding of new varieties both require a certain population size. Therefore, the low germination percentage of *P. grandiflorum* seeds becomes one of the limiting factors for expanding the breeding population. This situation not only increases the difficulty in seed selection of excellent new varieties but also significantly affects the process of *P. grandiflorum* breeding as a whole.

Research on the germinating capacity of *P. grandiflorum* seeds shows that under artificial conditions(25℃, RH 65%), the germination percentage and germination vigor of mature *P. grandiflorum* seeds are 93% and 78%, respectively(Liu et al., 2009). These findings indicate that *P. grandiflorum* seeds have potentially high germinating capacity. As compared with the artificial germination conditions, field temperature and moisture conditions may be the main causes for the low field germination percentage of *P. grandiflorum* seeds. In North China, *P. grandiflorum* is commonly cultivated by seeding in mid-to-late March, during which seed germination and seedling emergence are easily affected by low temperature. Thereafter, the temperature rises quickly; thus, the growth of *P. grandiflorum* seedlings is easily affected by high-temperature stress. Therefore, research on the patterns of seed germination and seedling growth of *P. grandiflorum* under temperature stress is of practical significance to the production of *P. grandiflorum* and construction of its genetic breeding population.

In addition, China is on of the major distribution areas of *P. grandiflorum*. Due to its overuse over the years, the wild resources of *P. grandiflorum* in Qinling Mountains, Shangluo have been endangered. At present, the protection of wild resources of *P. grandiflorum* and ecological restoration of its population quantity are urgently needed. The stages of seed germination and seedling growth are critical periods in which a population can be planted in a specific environment. Under field conditions, temperature effect has important ecological indication for the germination time of seeds(Khan M A et al., 2003). Therefore, studying the effects of different temperature conditions on the processes of seed germination and seedling growth of different *P. grandiflorum* varieties is of great biological and ecological significance(Al-Khatecb S A et al., 2006).

Presently, research of *P. grandiflorum* seeds has primarily focused on the aspects of maturation, dormancy, and storage methods. Liu et al. investigated the changes in seed vigor and associated physiological dynamics during the maturation of *P. grandiflorum*. Yan et al. (Yan et al., 2007) analyzed the relationship between the seed quality and seedling emergence of *P. grandiflorum*. Zhao et al.(Zhao et al., 2000) found that endogenous inhibitory substances are the main causes for

dormancy of *P. grandiflorum*. Liu(LIU 2009) studied the method for breaking seed dormancy and demonstrated that the treatment of soaking with 0.5% KNO_3 and 250 mg/L GA_3 for 24 h significantly promoted seed germination. In addition, Guo et al.(Gou et al., 2006) optimized the culture conditions for germination of *P. grandiflorum* seeds. However, there remains a lack of knowledge regarding the response mechanism of seed germination and seedling growth of different *P. grandiflorum* varieties to temperature stress and the germination strategy of *P. grandiflorum* seeds under extreme temperature conditions.

Temperature is an important eco-factor of seed germination(Zhu et al., 2011, ZHANG et al., 2010). The germination of seeds of different plant species and even different eco-types of the same species may show different responses to temperature stress(Liu et al., 2011). The questions are: How does temperature stress impact seed germination and seedling growth of *P. grandiflorum*, and are there differences in the effects of temperature stress between seeds of different varieties or not? Can *P. grandiflorum* seeds take specific strategy and what kind of strategy they will take to respond to temperature stress?

To solve the above questions, we conducted an experimental study on the effects of temperature stress on seed germination and seedling growth of *P. grandiflorum* with three different varieties. The results will provide reference data for construction of *P. grandiflorum* culture population as well as protection and restoration of its wild population.

MATERIALS AND METHODS

Plant Materials

Platycodon grandiflorum seeds of three varieties were used in the present study, including the Shangzhou variety (cultured-type) from a cultivated population in Zhangcun Township, Shangluo City, Shaanxi Province; the Chifeng variety (cultured-type) from a cultivated population in Malin Township, Chifeng City, Inner Mongolia; and the Zhashui variety (wild-type) from a wild population on the slope land surrounding the Hougou Village, Zhashui County, Shaanxi Province. The seeds were collected in October 2007 and stored in a refrigerator at 4 ℃. The seeds were sowed in a Nursery Garden of *Platycodon grandiflorum* Germplasm Resources in Shangluo College in March 2008. Then, mature seeds were harvested from the three *P. grandiflorum* populations in October 2009. All seeds were dried in oven-dried and then stored in a refrigerator at 4 ℃ before use.

Analytical Methods

High-quality seeds were chosen and treated with a 0.4% $KMnO_4$ solution for surface disinfection, then rinsed with distilled water. Two layers of moist filter paper were placed in a Petri dish as the germinating bed. The disinfected seeds were evenly placed on the germinating bed (100 grains each), covered with a lid, and then cultured in an incubator at 10–35 ℃ with 5 ℃ increments under light. Each treatment was repeated three times.

During the culture period, the germination of seeds was checked and recorded daily; distilled water was added to the original level by weight. An equal length of

the radicle to the seed was taken as the criterion for seed germination. After the completion of germination, the germination vigor, germination percentage, and germination index, and vigor index of seeds were calculated; the lengths of radicle and germ were measured; fresh and dry seedlings were weighed. The parameters of germinating seeds were calculated as follows:

(1) Vigor index of seeds:

Germination percentage (%) = $n / N \times 100$;

Germination index (GJ) = $\Sigma Gt / Dt$; and

Vigor index (VI) = germination index (GJ) $\times S$

where n is the total number of seeds normally germinating at the end of the germination stage, and N is the total number of seeds tested; Gt is the number of germinating seeds on day t, and Dt is the corresponding number of days; S is the average fresh weight of radicle.

(2) Average length of radicle and germ:

After seed germination for 12 d, 10 seeds were taken from each treatment for measurement of the radicle and germ lengths; the average values of the lengths were calculated. Additionally, the fresh and dry weights of seedlings were determined.

Data Analysis

The experimental data were processed and analyzed using SPSS15.0 software package.

RESULTS

Effects of Temperature on the Germination Starting Time of *P. Grandiflorum* Seeds

The germination starting time of *P. grandiflorum* seeds at the same temperature (except for 15 ℃) varied among different varieties (Table 1 and Fig. 1). Overall, the germination starting time of the Chifeng variety was shorter than those of the Shangzhou and Zhashui varieties($p<0.05$). For example, seeds of the Chifeng variety stared germinating on day 3, whereas those of the Shangzhou and Zhashui varieties stared germinating on days 4 and 5, respectively, showing significant differences between each two of the three varieties($p<0.05$)

As the culture temperature increased, the germination starting time of different varieties generally showed similar variation trends. The low temperature treatment (10 ℃) apparently prolonged the germination starting time; increasing temperature treatment shortened the germination starting time of all varieties, but excessively high temperature (30–35 ℃) again extended the germination starting time of the seeds.

Effects of Temperature on the Germination Course of *P. Grandiflorum* Seeds

During the culture period, the germination percentages of different varieties varied over time in an S-like shape (Fig. 1). The germination percentages of seeds

varied in a slow - fast - slow pattern, with slow increases at the initial stage of germination emergence, followed by rapid increases and subsequent stabilization. At 20℃ and 25℃, the rapid increasing period of germination percentage of the seeds started relatively early in all of the varieties; as the temperature increased or decreased, the rapid increasing period of germination percentage of the seeds was obviously delayed and the slope of its curve became significantly smaller.

Effects of Temperature on the Germination of *P. Grandiflorum* Seed

The germination percentage of *P. grandiflorum* seeds reflects their germinating capacity under appropriate conditions, and the germination index is a comprehensive reflection of the level of seed vigor(Zhang et al., 2010, Liu et al., 2011). As the culture temperature increased, the germination percentage, germination vigor, and vigor index of *P. grandiflorum* seeds all showed increases followed by decreases. With increasing temperature in a low temperature range, the germination indices of seeds gradually increased and peaked; further increasing the temperature led to significant inhibition of seed germination (Table 1).

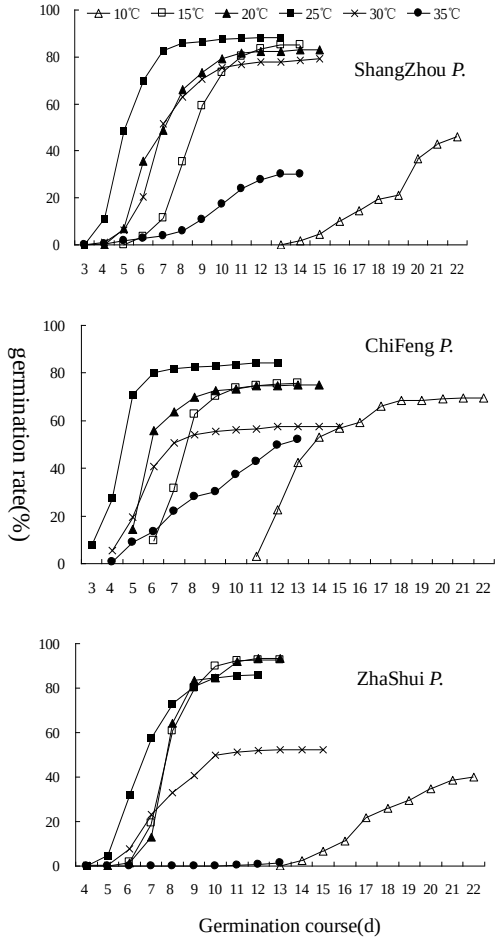

Fig 1 Effects of culture temperature on seed germinationcourse of tree variety of *Platycodon*

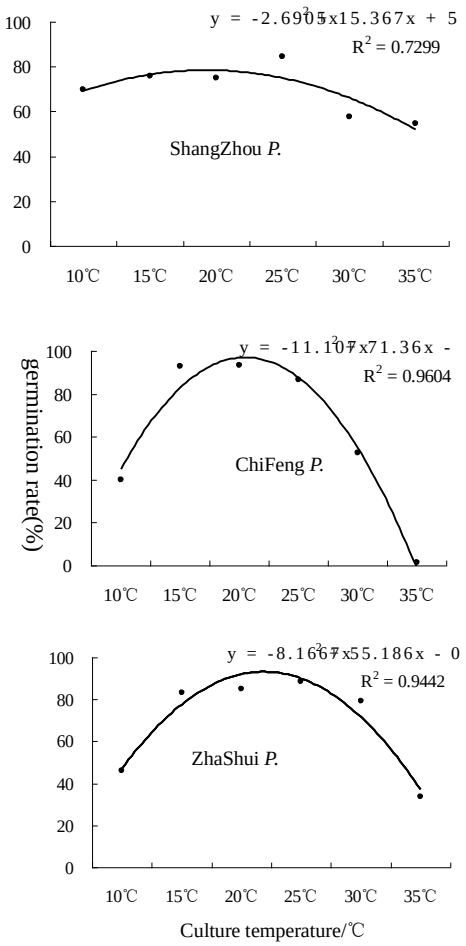

Fig 2 Relationship between the culture temperature and the seed germination percentage of three variety of *Platycodon*

Additionally, the optimum temperature range of seed germination varied among three varieties, 15–30 ℃ for the Shangzhou variety and 15–25 ℃ for the Chifeng and Zhashui varieties. The optimum temperatures of seed germination were 25 ℃ for the Shangzhou and Chifeng varieties and 20 ℃ for the Zhashui variety (Table 1). Under the above optimum temperature conditions, the germination percentage, germination vigor, and vigor index of *P. grandiflorum* seeds reached their maximal levels. Generally, seed germination of the Chifeng variety was subjected to low negative effects by the extreme temperature, whereas that of the Zhashui variety favored relatively cold (20 ℃) environment and was sensitive to temperature changes. In particular, the seed germination process of the Zhashui variety showed intense responses to the extremely high temperature stress and was almost completely inhibited by the high temperatures of 35 ℃.

TABLE 1 EFFECTS OF CULTURE TEMPERATURE ON SEED GERMINATION OF TREE VARIETY OF *PLATYCODON*

Temperature	The initiatory time of seed germination			Germination percentage/%			Germination vigor/%			Vigour index		
	A	B	C	A	B	C	A	B	C	A	B	C
5℃	-	-	-	-	-	-	-	-	-	-	-	-
10℃	14.3a	11.0c	14.0a	46.0hi	69.7ef	40.0ij	0.0h	42.3cd	0.0h	2.50g	5.19f	2.29g
15℃	6.0d	6.0d	6.0d	83.0abcd	75.7cde	92.7a	35.3de	62.7b	60.7b	9.78d	9.99d	11.36cd
20℃	5.0e	5.0e	6.0d	85.0abcd	75. 0de	93.3a	48.7c	63.7b	63.0b	11.93c	12.20c	16.45b
25℃	4.0f	3.0g	5.0e	88.3ab	84.3abcd	86.3abc	70.0b	80.0a	32.0e	16.28b	18.00a	12.44c
30℃	4.0f	4.0f	6.0d	79.3bcde	57.7g	52.3gh	20.3f	40.7cde	7.7gh	11.19cd	9.90d	6.74e
35℃	4.7ef	4.7ef	13.0b	33.7j	54.3gh	1.3 k	2.7h	13.3fg	0.0h	3.48g	6.91e	0.11h
40℃	-	-	-	-	-	-	-	-	-	-	-	-

Note: Different letters in the same column indicate statistically significant differences among the he temperature treatments at the $P<0.05$; the same as below. A: Shangzhou variety (cultured-type); B: Chifeng variety (cultured-type); C: Zhashui variety (wide-type).

Effects of Temperature on the Growth of *P. Grandiflorum* Seedlings

Temperature stress had significant effects on the fresh weight of *P. grandiflorum* seedlings (Table 2). As the temperature increased, the fresh weight of seedlings showed generally consistent variation trends (unimodal curve) in different varieties (Fig. 2). Within the low-to-medium temperature range, the fresh weight of seedlings significantly increased with increasing temperature and reached the maximum level at 25 ℃ (Shangzhou and Zhashui varieties) or 30 ℃ (Chifeng variety); thereafter, increasing temperature led to decreases in the fresh weight of seedlings. Likewise, the dry weight of seedlings slightly varied in a unimodal curve with increasing temperature; however, the effects of temperature or variety on the dry weight of seedlings were not statistically significant.

As the temperature increased, the root length, shoot length, and root/shoot ratio of *P. grandiflorum* seedlings all showed increases followed by decreases (Table 2). The shoot lengths of all seedlings were longest at the culture temperature of 30 ℃, whereas the root length and root/shoot ratio reached the maximum level at 25 ℃. These results indicate that the culture temperature of 25 ℃ is conducive to root elongation, whereas the 30 ℃ treatment significantly promotes shoot elongation and

inhibits young root elongation. During this period, the height of seedlings increased mainly due to the elongation between shoot internodes, showing obvious characteristics of leggy shoots.

TABLE 2 EFFECTS OF CULTURE TEMPERATURE ON SEEDING GROWTH OF THREE VARIETY OF *PLATYCODON*

Temperature	Fresh weight of seeding/g			Dry weight of seeding/g		
	A	B	C	A	B	C
10℃	0.116i	0.193h	0.113i	0.032a	0.035a	0.030a
15℃	0.281fg	0.277fg	0.341ef	0.045a	0.037a	0.126a
20℃	0.403de	0.436d	0.258gh	0.041a	0.042a	0.026a
25℃	0.510bc	0.545ab	0.588a	0.053a	0.056a	0.063a
30℃	0.503bc	0.554ab	0.461cd	0.032a	0.032a	0.111a
35℃	0.229gh	0.280fg	0.096i	0.034a	0.038a	0.041a

Temperature	Root length/cm			Shoot length/cm			Root/shoot length ratio		
	A	B	C	A	B	C	A	B	C
10℃	5.1g	10.6f	5.5 g	1.6g	5.1de	3.6def	3.31d	2.08e	1.55ef
15℃	10.4f	13.6ef	13.0 ef	3.1fg	3.9def	3.5def	3.37d	3.49d	3.68d
20℃	20.3cd	23.5bc	23.9 bc	3.4ef	3.3ef	3.5def	6.09c	7.17b	6.82b
25℃	24.0b	29.5a	31.2 a	3.4ef	3.5def	3.6def	7.15b	8.35a	8.75a
30℃	14.4e	14.4e	19.1 d	25.4a	24.4ab	22.9b	0.57g	0.59g	0.83fg
35℃	4.4gh	3.7gh	1.4 h	10.7c	10.9c	5.3d	0.41g	0.34g	0.30g

DISCUSSION AND CONCLUSIONS

Temperature is one of the important factors that affect seed germination (Huang et al.,2003; Benech-Arnold et al., 2000; Bouma et al., 2001) , which plays an important role in water uptake of seeds, sprouting, radicle and germ elongation, and even the whole formation process of seedlings (Winkler et al., 2002; Zhou et al., 2006; Li et al., 2007). Temperature may play a dual role in seed germination by directly affecting this process or through indirect regulation of seed dormancy (Wang et al., 2006; Ma et al., 2007; Khan et al., 2003; Feng et al., 2010). For the Zhashui variety, the seeds germinated at 10–30 ℃ but were almost completely inhibited at 35 ℃; for the Shangzhou and Chifeng varieties, the seeds germinated at a wider temperature range of 10–35 ℃. The processes of seed germination and seedling growth of *P. grandiflorum* were significantly inhibited at both low and high temperature extremes. However, excessively low and high temperatures caused different harms to seed germination and seedling growth of *P. grandiflorum*. The excessively low temperature mainly imposed an inhibitory effect by delaying seed germination and young root elongation, whereas excessively high temperature was harmful to *P. grandiflorum* mainly through inhibiting the germination of seeds, delaying the elongation of young roots, and promoting the overgrowth of leggy shoots.

The optimal temperature of seed germination for the Shangzhou and Chifeng

varieties was 25 ℃. By comparison, the optimal temperature of seed germination for the Zhashui variety was relatively low, 20 ℃. The seeds of Zhashui variety preferred to germinate in a cold environment, possibly through their eco-adaption to the cold alpine habitats. Due to over-harvesting of wild *P. grandiflorum* over the years, the population size of this species has decreased rapidly, and the existing wild resources of *P. grandiflorum* have been limited to certain sparsely populated, alpine cold regions. The processes of seed germination and seedling formation are critical stages in the population construction of a species. Under the long-term effects of alpine cold habitat, the seeds of wild *P. grandiflorum* have gradually adapted to the ecological requirement for germination at low temperature, allowing the alpine wild *P. grandiflorum* population to pass through the critical stage of population construction. On the other hand, the two cultivated varieties of *P. grandiflorum* are mainly distributed in plain lands or low-altitude hillsides, where the temperature rises rapidly to relatively high levels in the germination stage of *P. grandiflorum* seeds. In the long process of domestication, the cultivated varieties of *P. grandiflorum* have been gradually adapted to the field habitats.

Temperature has significant effects on the seedling growth and morphogenesis of *P. grandiflorum* (Zhang et al., 2010; Tao et al., 2011; Ma et al., 2008). At 20–25 ℃, the root/shoot ratios of different *P. grandiflorum* varieties were all greater than 6.09, clearly favoring the root growth of seedling. At ≥30 ℃, the growth of young roots was significantly inhibited while shoot growth was significantly promoted; as compared to that at 25 ℃, the shoot length increased by more than 4.8-time while the root length decreased by 1.6-time. These morphological changes can explain that in cultivation and production of *P. grandiflorum*, a large number of seedlings may die at the stages of seedling emergence and growth in continuous sunny hot days, even under water filling conditions. Due to direct solar radiation, the surface soil temperature increases rapidly after the noon. In the seedling emergence period of *P. grandiflorum* (mid-late April to early May in South Shaanxi), the surface soil temperature can easily approach or exceed 30 ℃. Under high-temperature conditions, the shoot growth rate of *P. grandiflorum* significantly increases and allows the seedlings rapidly come up out the ground and further elongate. In this stage, the growth of young roots is significantly inhibited. The young roots that are relatively short are unable to rapidly grow into deep soil for absorbing soil moisture and thus cannot meet the substantial water demand of high transpiration by the above-ground seedling. This will eventually lead to physiological water deficiency of seedlings and may cause their death.

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Cover page

Title: *Gene Divergence of Interferon Regulatory Factor (IRF) 4, 8, 9 and 10 in Teleost fish*

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ABSTRACT

IRF4, IRF8, IRF9 and IRF10 belong to IRF4 subfamily in interferon regulatory factor family. The Phylogenetic tree, identity and evolutionary rate of IRF4, IRF8, IRF9 and IRF10 were compared in the study, which would elucidate the conservation and diversification in evolution about the four genes in bony fishes. Not only the gene number changed from one to three, but also the genomic organization varied in different bony fishes. In protein sequence similarity, IRF8 showed the highest identity between different teleosts (60%-87%), followed IRF4a (54-88%). The identity between different IRF9 proteins was lowest, value of 35-71%. Evolutionary rate was examined by estimating the proportion of nonsynonymous to synonymous substitution rates (Ka/Ks). The average Ka/Ks ratios of five genes in IRF4 subfamily among different teleosts were all lesser than 1, indicating they were under purifying selection. However, evolutionary rate between the five genes was different. The Ka/Ks ratio was about 0.5, 0.3, 0.20, 0.25 and 0.15 for IRF9, IRF10, IRF4a, IRF4b and IRF8, respectively. All these data proved IRF8, IRF4a and IRF10 showed higher conservation while IRF4b and IRF9 presented higher diversification in bony fish.

INTRODUCTION

IRF family members play a crucial role in both the innate and adaptive responses [1]. Today, most documents reported the family include IRF1 to IRF10 in vertebrates and it is divided into four subfamilies: IRF1 subfamily, IRF3 subfamily, IRF4 subfamily and IRF5 subfamily, in which IRF4, IRF8, IRF9 and IRF10 belong to IRF4 subfamily [2].

Although IRF4, IRF8 and IRF10 are in the same subfamily and are all capable of repressing the ovalbumin gene in chicken, these factors differ in constitutive and inducible expression as well as function [3-7]. Although IRF10 and IRF8 are induced by IFN1 and IFN-γ in primary fibroblasts, induction of IRF8 is rapidly induced while the expression of IRF10 is induced relatively late and is dependent on protein synthesis. In contrast to IRF8 and IRF10, IRF4 expression is not detected in response to treatment with IFN (both types). IRF10, like IRF4, is induced by ConA in splenic cultures but surprisingly, IRF8 expression is unresponsive to ConA

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treatment [4]. Moreover, expression of these IRFs is repressed by estrogen [3]. Although IRF10 is up-regulated by IRF4 and its expression elevated significantly by the combination of IRF4 and v-Rel in chickens [4], their function differ [8,9].

Although the function on IRF4, IRF8, IRF9 and IRF10 was not studied in detail in teleosts, IRF4, IRF8, IRF9 and IRF10 had been proved that they all involved in the antiviral response of bony fish [10,11]. In the study, the genomic organization, identity, conserved domain and evolutionary rate on IRF4, IRF8, IRF9 and IRF10 in the bony fish were analyzed, which would further shed new light on the conservation and diversification in evolution of genomes and proteins and help to clarify the differentiation of function.

METHODS

Genomic sequences were downloaded from the Ensembl Database and NCBI database. All predicted IRF protein sequences were verified by BLASTP in the NCBI database. And the SIM4 program was used to reconstruct the exon-intron structure.

For molecular phylogenetic analysis, protein sequences were first aligned using ClustalW. Phylogenetic tree was constructed using the neighbor-joining method within the Mega 5.0 program. Data were analyzed using Poisson correction, and gaps were removed by pairwise deletion. The degree of confidence for each branch point was determined by bootstrap analysis (1,000 times). Accession numbers used were listed following the genes in the phylogenetic tree.

Multiple sequence alignment of CDS was performed using ClusterW (Codons) in Mega 5.0. The value of substitution per nonsynonymous site (Ka) and substitution per synonymous site (Ks) were calculated using K estimator 6.0.

RESULTS

Based on a Clustal W alignment using the MEGA 5.0 method, the phylogenetic tree of IRF proteins in IRF4 subfamily was constructed. As shown in Figure 5, the tree was separated into four clades, with each clade including one kind of IRF. However, IRF4 was further divided into two branches. One branch included IRF4a gene and the other included IRF4b gene. Flounder IRF4 gene clustered together with IRF4b. These data show that IRF4 duplicate twice and then formed two IRF4 genes in many teleosts. But there were three IRF4 genes in zebrafish and only one gene in flounder. Among the four kinds of IRF, IRF4 exhibited the closest relationship to IRF10 and they were clustered into one bigger group. And then was clustered with IRF8 and IRF9 (Figure 1).

To assess further the divergence degree of four genes in IRF4 subfamily, we examined the evolutionary rate, which is measured by Ka/Ks, the ratio of the rate of nonsynonymous to synonymous change in their coding sequences. Ka/Ks values of IRF4 in different teleosts was lesser than 1, indicating IRF4 genes in teleosts are under purifying selection. And the Ka/Ks between zabrafish IRF4a and other IRF4a was the smallest, average value of 0.13. The value of Ka/Ks between medaka IRF4a and other IRF4a is the largest, average of 0.27 (Table 1).

However, the value of Ka/Ks between zebrafish IRF4b1 or IRF4b2 and other species IRF4b was higher, 0.33 for IRF4b1 and 0.41 for IRF4b2 (Table 2). On the contrary, the Ka/ks between medaka IRF4b and other IRF4b was the smallest with the average of 0.20 (Table 2). In the four genes, the Ka/Ks value of IRF8 was the lowest in all teleost fish, which indicated that the evolutionary rate IRF8 was slowest in teleosts (Table 3). In spite of this, the Ka/Ks between coelacanth IRF8 and other most IRF8 genes could not count with the common model (Table 3). Contrary to IRF8, the Ka/Ks between IRF9 genes was greatest, most of them were about 0.5 (Table 4). Moreover, Ka/Ks between fugu IRF9 and cod IRF9, tetraodon 9-8 and tetraodon 9-5 were greater than 1, indicating the four IRF9 genes were under positive selection. The Ka/Ks between IRF10 genes was about 0.3 (Table 5).

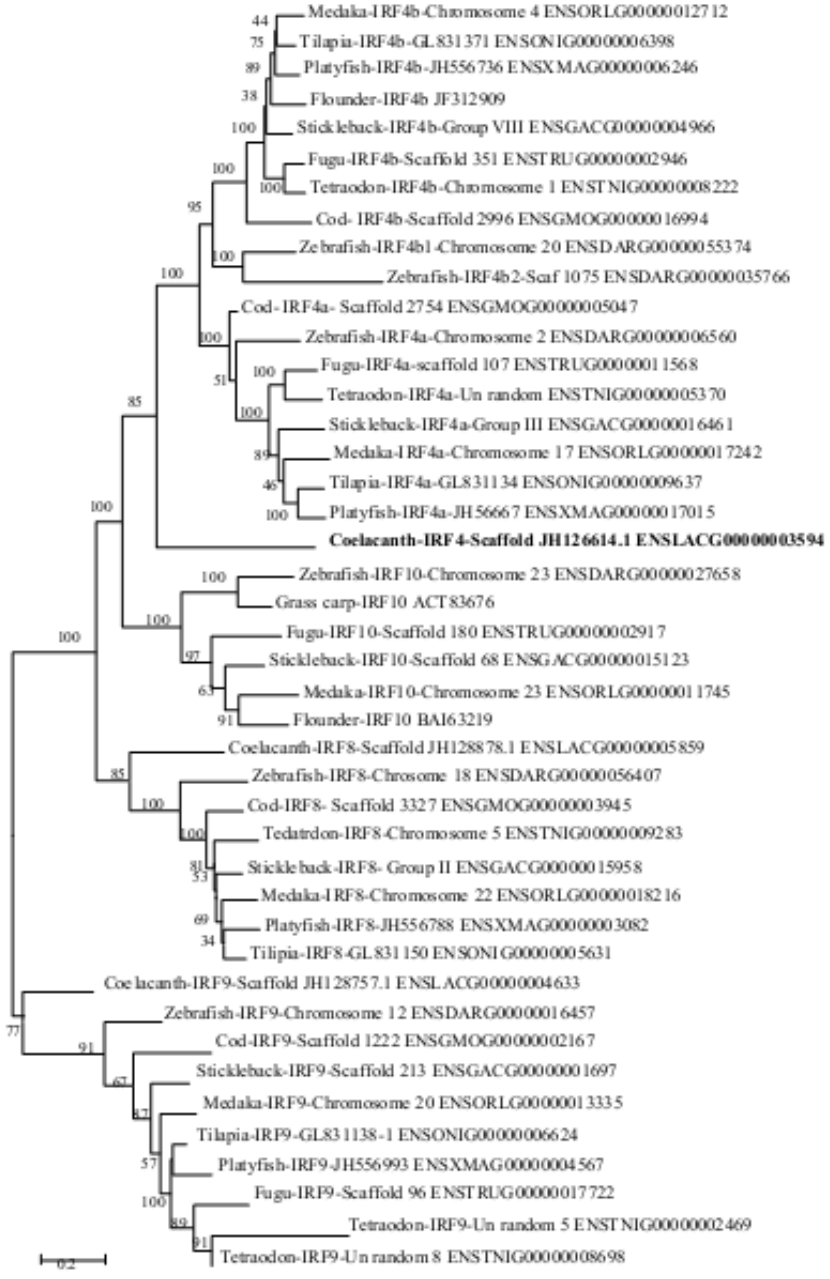

Fig.1 Phylogenetic tree with IRF4, IRF8, IRF9 and IRF10 in teleosts

TABLE 1 THE KA/KS RATIO OF IRF4A IN TELEOSTS

	Medaka	Fugu	Platyfish	Stickleback	Tetraodon	Tilapia	Zebrafish	Cod
Fugu	0.33							
Platyfish	0.28	0.18						
Stickleback	0.27	0.25	0.20					
Tetraodon	0.27	0.28	0.17	0.18				
Tilapia	0.26	0.16	0.13	0.11	0.19			
Zebrafish	0.34NA	0.15	0.10	0.12	0.14	0.09		
Cod	0.17	0.23	0.20	0.19	0.19	0.14	0.17	
Average	**0.27**	**0.23**	**0.18**	**0.19**	**0.20**	**0.16**	**0.13**	**0.18**

NA stands for the null data with uncommon model and the null data did not count in the average, it is same in the following tables.

TABLE 2 THE KA/KS RATIO OF IRF4B IN TELEOSTS

	Medaka	Fugu	Platyfish	Stickleback	Tetraodon	Tilapia	Zebrafishb1	Zebrafishb2	Cod
Fugu	0.18								
Platyfish	0.15	0.24							
Stickleback	0.14	0.29	0.15						
Tetraodon	0.17	0.30	0.22	0.21					
Tilapia	0.15	0.31	0.12	0.16	0.23				
Zebrafishb1	0.22	0.31	0.27	0.31	0.35	0.32			
Zebrafishb2	0.41	0.43	0.42	0.36	0.41	0.32	0.49		
Cod	0.19	0.30	0.21	0.31	0.29	0.26	0.37	0.44	
Average	**0.20**	**0.30**	**0.22**	**0.24**	**0.27**	**0.24**	**0.33**	**0.41**	**0.30**

DISCUSSION

The gene of IRF4 in the teleosts was quite complex and there are 1-3 genes in teleosts. Phylogenetic tree showed all IRF4 was further sub-clustered into two distinct clades, one is called IRF4a and the other is called IRF4b. IRF4a and IRF4b existed in almost all teleosts. Only one IRF4 gene has been reported in the mammalian, birds and frogs, they belong to IRF4b [11], suggesting that in teleost fish, especially in zebrafish tandem gene duplication or additional independent chromosome duplication might have occurred. Moreover, some IRF4 genes have several transcripts. For example, there have two, seven and two transcripts in zebrafish IRF4b, fugu IRF4a and rainbow trout IRF4 [12]. Almost all IRF4 contain an DNA binding domain and IRF association domain and the latter mediates formation of homo- or hetero-dimers with other transcription factors, which are required for accurate promoter targeting and regulation of transcription [13].

The identity between IRF8 proteins in teleost fish reached at 60-87%. A very low Ka value (<0.23) and Ka/Ks ratio (<=0.18) between different species (Table 3), further proved. The IRF8 CDS shows a highly degree of sequence conservation within teleosts. The nonsynonymous substitution rate of IRF8 gene is very low compared to other three genes. For example, the maximum Ka for IRF8, IRF9, IRF10, IRF4a and IRF4b was 0.23, 0.81, 0.40, 0.32 and 0.60 respectively. Although

IRF4 has diverged to 1-3 genes, IRF4a has a relative high identity between bony fishes (54-85%) and the Ka and Ka/Ks ratio was low in most species. Most of Ka value and Ka/Ks ratio between IRF4a was about 0.20. These results further demonstrated that IRF4a was also high conserved. By comparison, IRF9 genes had a much higher Ka value and higher Ka/Ks ratio (Table 4). Most Ka value was 0.20-0.90 while many Ka/Ks ratio was 0.20-0.80. Further, the Ka and Ka/Ks between fugu IRF9 and cod IRF9 was 0.68 and 1.04, showing the gene between the two species had a great divergence. The Ka value between the two genes of tetraodon IRF9 was only 0.0125, but the Ka/Ks was 1.24. The x value (Ka/Ks ratio) has a straight forward measurement of selective pressure at each codon of protein-coding [14]. These showed that IRF9 was under great pressure in evolution and may evolve toward positive selection.

TABLE 3 THE KA/KS RATIO OF IRF8 IN TELEOSTS

	Medaka	Fugu	Platyfish	Stickleback	Tetraodon	Tilipia	zebrafish	cod	Coelacanth
Fugu	0.17								
Platyfish	0.14	0.16							
Stickleback	0.17	0.15	0.16						
Tedatrdon	0.18	0.16	0.16	0.16					
Tilipia	0.13	0.13	0.12	0.14	0.13				
Zebrafish	0.16	0.16	0.16	0.15	0.17	0.15			
Cod	0.14	0.13	0.12	0.12	0.14	0.08	0.12		
Coelacanth	0.40NA	0.38NA	0.37NA	0.33NA	0.38NA	0.39NA	0.16	0.35NA	
Average	**0.16**	**0.15**	**0.14**	**0.15**	**0.16**	**0.12**	**0.15**	**0.12**	NA

TABLE 4 THE KA/KS RATIO OF IRF9 IN TELEOSTS

	Medaka	Fugu	Platyfish	Stickleback	Tetraodon8	Tetraodon5	Tilapia	Zebrafish	cod	Coelacanth
Fugu	0.45									
Platysh	0.20	0.46								
Stickleback	0.50	0.78	0.42							
Tetraodon8	0.50	0.61	0.49	0.57						
Tetraodon5	0.53	0.65	0.52	0.61	1.24					
Tilapa	0.24	0.31	0.18	0.32	0.34	0.37				
Zebrafish	0.44NA	0.36	0.22	0.17	0.44NA	0.44NA	0.32NA			
cod	0.43	1.04	0.60	0.61	0.54	0.61	0.42	0.45		
Coelacanth	0.59	0.34	0.59	0.79	0.39	0.39	0.34	0.56	0.54	
Average	**0.43**	**0.56**	**0.41**	**0.53**	**0.58**	**0.62**	**0.31**	**0.35**	**0.58**	**0.50**

TABLE 5 THE KA/KS VALUES OF IRF10 IN TELEOSTS

	Medaka	Fugu	Stickleback	Zebrafish	Flounder
Fugu	0.29				
Stickleback	0.34	0.37			
Zebrafish	0.13	0.28	0.34		
Flounder	0.35	0.25	0.29	0.35	
Average	**0.28**	**0.30**	**0.33**	**0.28**	**0.31**

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Cover page

Title: *Soil Clustering Analysis for Soil Management in Pa Deng, the Adjacent Area of Kaeng Krachan Natural Park, Thailand*

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ABSTRACT

Soil clustering analysis for soil management in Pa Deng the adjoining Kaeng Krachan Natural Park, Thailand was aims to categorize soil properties in the Pa Deng area in order to apply as the database for appropriate soil management. The study was performed by hierarchical cluster analysis and spatial analysis using remote sensing and geographical information system techniques for soil mapping. Fifty-nine soil samples were collected for laboratory analysis of pH, texture, organic matter, phosphorus and potassium. The analytical results were subjected to hierarchical cluster analysis with average linkage and squares Euclidean distance in order to cluster soil properties and highlight critical parameters for each cluster. The clustering results showed four soil property typologies i.e. low fertile soil, moderately fertile soil, strongly acidic low nutrient soil, and high fertility soil. The analysis indicates that some soils at Pa Deng area are infertile and high level of acidic in soil, the area need suitable amendment by appropriate methods prior to renewed use in agriculture.

Keywords: Clustering soil properties; Clustering analysis; Soil management; Soil properties

INTRODUCTION

Soil is comprised of minerals, soil organic matter (SOM), water, and air. Soil quality is very important for agricultural area. Soil quality is essential for plant and animal health, maintenance of the environment, and sustained biological productivity [1].Then, the investigation of soil properties will become increasingly important in the future [2], [3], [4]. Land degradation is the reduction in the capacity of the land to provide ecosystem goods and services and assure its functions over a period of time for the beneficiaries of these. Land degradation is associated with poverty and drought.

Soil degradation has two major components: the loss of soil through erosion and the loss of soil fertility. Both components lead to progressively lower crop yields, increased costs of production, and may end up in land abandonment and desertification. The degradation may also result from inappropriate land use or from a process or combination of processes, including processes arising from human activities and habitation patterns [5].Land use is considered the major driving force of

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land degradation. Then, many studies indicate that land use and soil management practices greatly impact the direction and degree of soil quality changes [6], [7], [8], [9]. In Thailand, soil erosion by water has been considered as one of the most influential causes of land degradation due to losses of surface soil and plant nutrients [10].

In addition, soil conservation measures proposed by Land Development Department (LDD), the main agency for assessing soil degradation and setting soil conservation measures, were both mechanical measures and agronomic measures. However, in-deep approach and spatial analysis of soil properties of degraded site are not intensively conducted in Thailand, which causes that the soil conservation measures are not efficiently practiced in most areas in Thailand. For this reason, the soil degradation problem expands extensively in Thailand. So, soil properties of degraded areas must be investigated in detail in order to identify the critical parameters which influence soil quality of the site and which drive degradation and desertification processes. The objective of this research is to investigate and categorize soil properties in the selected study area using clustering analysis.

Pa Deng area was conducted under the project of Huay Sai Royal Development Study Centre and the study area is a part of Huay Sai Royal Development Study Centre. Therefore, the results of this study will be forwarded to Huay Sai Royal Development Study Centre and relevant agencies in the study area in order to apply as the database for setting and implementing soil conservation measures that are suitable for this site. Moreover, the results will be utilized for laying down mitigation measures for the soil degradation in the study area.

MATERIALS AND METHODS

Study Area Description

Pa Deng sub-District is located in Kaeng Krachan District, Phetchaburi Province in the watersheds of the Pranburi and Pranburi rivers. The study area, Pa Deng covering an area of approximately 41,780 hectares. Geographical location lies between the latitudes 99°20′E to 99°37′E and 12°33′N to 12°45′N at an altitude of 140 m above mean sea level. The Tanowsri Mountain Range, which runs from north to south, forms the western boundary of the site.

Pa Deng is a piedmont plateau, sloping gradually from west to east. The majority of the land is slope complex land with slopes greater than 35%. Due to their slope, soils in these areas have not yet been surveyed or classified. Overall, the terrain at Pa Deng has been classified as undulating and rolling terrain.

Some regions of Pa Deng lie within the perimeter of the national forest reserve forest and Kaeng Krachan National Park, comprising tropical rain forest. Pa Deng is surrounded by mountains with a plain at the center. The topsoil texture is sandy loam or loam with medium to high soil permeability. The main occupation of the majority of the population is agriculture, dominated by monoculture and livestock farming.

The mean annual temperature and rainfall are 27°C and 1,070 mm, respectively. The rainy season extends from May to November. The area faces increasing population pressure due to limited utilizable land: the central plain of Pa Deng which can be utilized for agriculture or housing represents only 12% of the total area, whilst

population growth is 2.80% p.a. For this reason, it may lead to the expansion of agricultural area and the invasion of forest and foothill area by agricultural area. This may cause that the soil erosion in Pa Deng will become more stressful [11].

Soil Properties Investigation

The preliminary studies of topographic map (1:50,000) and field survey, using GPS, studying location was appointed. For Laboratory analysis, fifthly nine soil samples were collected on a 1x1 km grid plotted from different horizons of 15 soil profiles located in Pa Deng area. Since the majority of Pa Deng sub-district lies within the national forest reserve and Kaeng Krachan National Park area, the investigation of soil properties of Pa Deng was conducted only for the plain area of Pa Deng which can be utilized for cropping. The soil properties examined included the pH, soil texture, organic matter (OM), phosphorus (P) and potassium (K).

Soil Mapping

The spatial data and attribute data of soil texture and soil fertility were individually prepared in shapefile format for input into ArcGIS software. The soil properties map of the study area was created by overlaying the prepared shapefiles using ArcGIS.

Soil Properties by Cluster Analysis

The results of soil properties analysis were subjected to hierarchical clustering analysis with average linkage and squares Euclidean distance in order to cluster soil properties and highlight critical parameters for each cluster. The conceptual framework is shown in Figure 1.

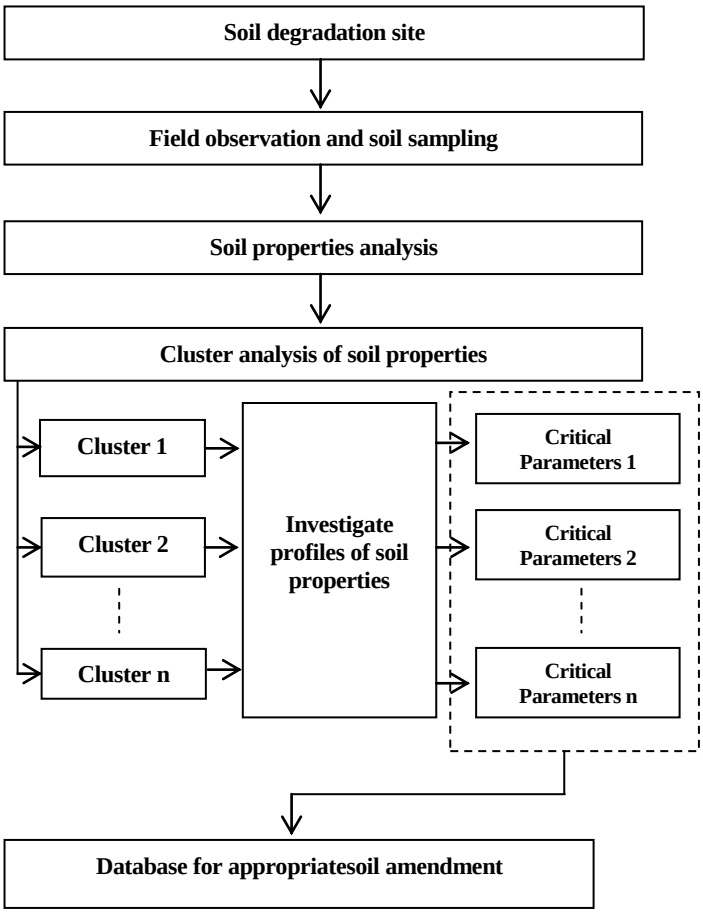

Figure 1. Conceptual frameworks for the research

RESULTS

Soil Properties

Analysis of soil texture and field observation indicated that approximately 85% of the total area of Pa Deng comprises slope complex land with slopes exceeding 35%. The dominant soil texture within the plain area is sandy loam, followed by silt loam and loamy sand, respectively. The analysis results of soil properties are summarized in Table I.

TABLE I. OVERVIEW OF ANALYSIS OF SOIL PROPERTIES

Parameter	Unit	Min.	Max.	Mean	Mode
%Sand	%	26.0	84.0	50.6	41.0
pH	-	4.0	7.4	6.4	7.4
Organic matter (OM)	%	0.5	4.6	1.9	1.6
Phosphorus (P)	mg/kg	1.0	1,204.0	56.1	2.0
Potassium (K)	mg/kg	20.0	740.0	174.6	130.0

The laboratory analysis results of soil samples collected from the plain area of Pa Deng revealed soil pH ranging from 4.0 to 7.4, organic matter ranging from 0.5% to 4.6%, available phosphorus ranging from 1.0 to 1,204.0 mg/kg, exchangeable potassium from 20.0 to 740.0 mg/kg.

The analytical results showed that most soil of study area is slightly acid with moderate OM and nutrient.

Soil Map

The fertility rate of the soils was varied; ranging from very low fertility to very high fertility. Drainage ability was between not good and good. There are sloping complex areas with a gradient of less than 6 – 35%. There are also including piedmont areas with a gradient of higher than 35% located in Kaeng Krachan National Park and National Reserved Forest areas. The soil depth is ranging from shallow soils (less than 25 cm) to deep soils (deeper than 100 cm).

Soil texture and soil fertility in the study area was investigated by applying the GIS technique. Figure 2 illustrates the soil texture and soil fertility of Pa Deng Area.

Clustering of Soil Properties

Figure 3 illustrates the resulting dendrogram obtained by clustering soil properties data, which gives four property typologies (TABLE II).

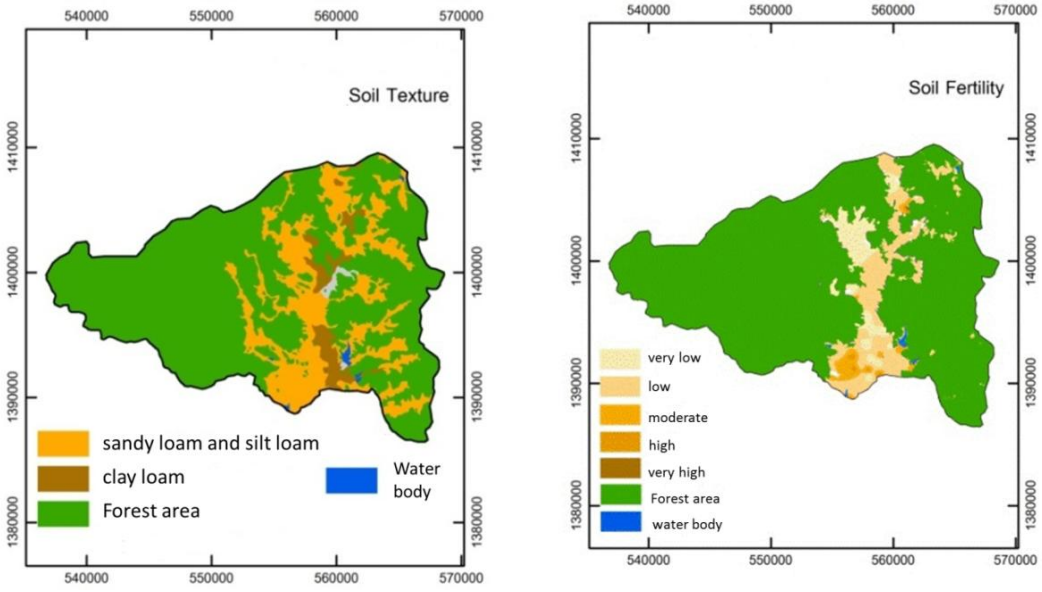

Figure 2. Soil texture and soil fertility of Pa Deng Area

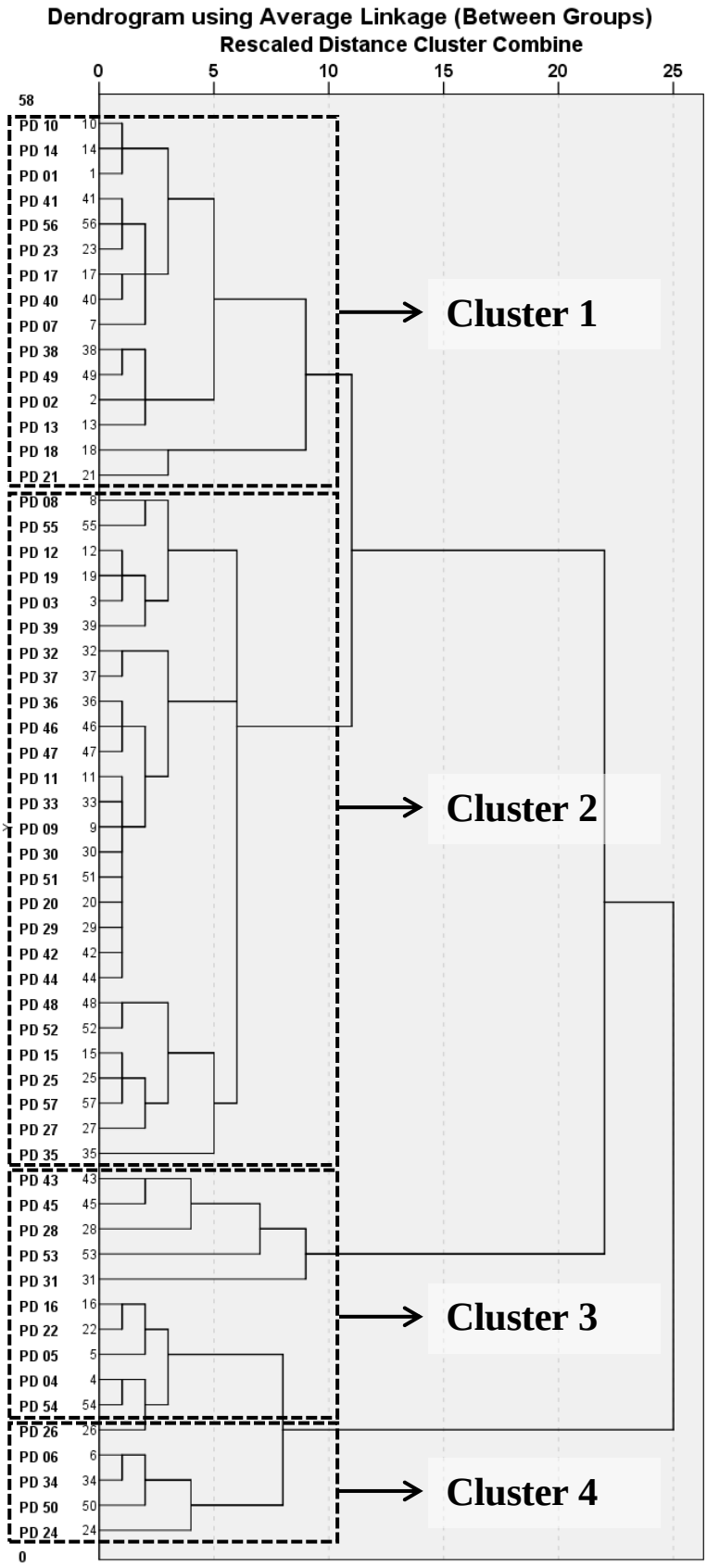

Figure 3. Clustering analysis of soil properties

TABLE II. DESCRIPTION OF EACH SOIL CLUSTER

Cluster	Descriptions
1	Low fertile soil
2	Moderately fertile soil
3	Strongly acid soil
4	Fertile soil with high organic matters

Histograms of soil properties of the five parameters and four soil groups obtained from cluster analysis were plotted in order to investigate the profiles of soil properties of each soil groups (Figure 4). As can be seen in Figure 4, soil Clusters 1, 2 and 3 have similar nutrient levels. Soil in Cluster 3 is strongly acid meanwhile soil in Cluster 2 is characterized by moderate organic matters. Cluster 4 differs in that it has more fertile soil than the other clusters.

DISCUSSION AND CONCLUSIONS

Pa Deng area has faced soil degradation due to expansion of agriculture land with monoculture and over-cultivation without any consideration of soil conservation. The change of land use and land cover had a significant influence on risk of soil degradation [12], [13], [14]. Cluster analysis of soil properties highlighted that soil cluster 1 and 3 have the problem. The soil cluster 1 has low fertile soil which can be improved by adding organic fertilizers. Meanwhile, soil cluster 3 has strongly acid soil and sandy loam soils with low fertile soil were identified as critical problem. Soil acidification will have a major impact on agricultural productivity and sustainable agriculture and it can also extend into subsoil layers posing serious problems for plant root development and remedial action [15]. Moreover, soil acidification impact on temperate ecosystems and reduction in plant biodiversity [16]. Some plants have difficulty absorbing any nutrients at all in highly acidic soils [17]. Soil acidification has a negative impact upon a range of natural resource functions; there is an additional loss in soil structure due to the loss of fine clay fractions [18].

The significant problems that effect to soil quality of Pa Deng area are the monocropping with very chemical intensive that can stress the fertile topsoil and reduce genetic diversity. Moreover, the expansion of agricultural area with this pattern of cropping show a high risk of soil erosion due to the absence of soil conservative measures, both mechanical and agronomic measures, and its rolling terrain [11]. The soil properties that affect land management, clustering soil properties can indicate the critical soil problems affecting each zone; obtaining these data is an important prerequisite for selecting appropriate soil amendment methods. Soil properties should be regularly monitored for inclusion in the land development database of Huay Sai Royal Development Study Centre, the conducted organization of Pa Deng area.

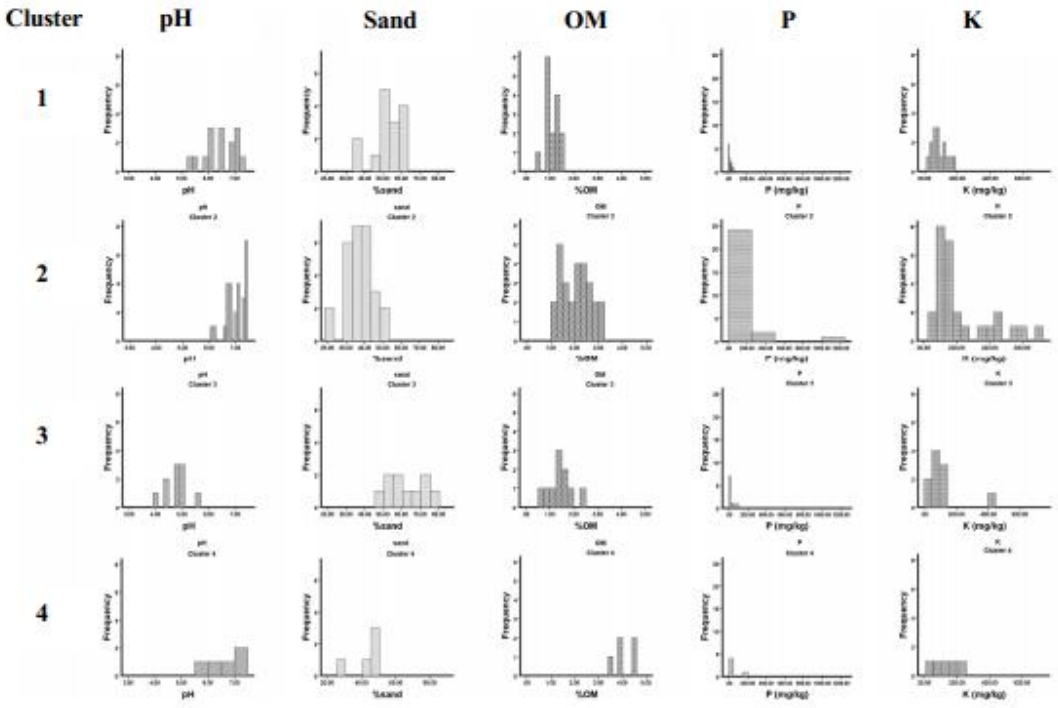

Figure 4. Profile of soil properties of each soil group

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Cover page

Title: *Biochar Production for Soil Amendment at Huay Sai Royal Development Study Center and Pa-deng Biochar Research Center (PdBRC), Petchburi Province, Thailand*

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ABSTRACT

Use of biochar produced from crop wastes as a carbon sink was one of several climate change mitigation measures studied at the Huay Sai Royal Development Study Center and Pa-deng Biochar Research Center (PdBRC), Petchaburi Province, Thailand. Biochar is produced using a controlled-temperature biochar retort using slow pyrolysis. The current research aimed to develop an inexpensive biochar retort that agriculturists can build themselves, enabling them to produce biochar following guidelines issued by the Food and Agriculture Organization (FAO), and in so doing contribute to reduction of GHG emissions. Six types of biochar feedstocks were tested, including corn cobs and 5 types of locally available wood. The controlled temperature in the retort was set in the range of 500-600^{o}C. The biochar produced was subjected to analysis of C-H-N, calorific value, surface and interface structure and water holding capacity. Preliminary results show differences in the suitability of the biochars tested, with *Samanea saman* and *Leucaena leucocephala* offering the most optimal combination of matrix surface area and water-holding capacity. Such materials are likely to be suitable for use in soil amendment, combined with organic fertilizer. Further fieldwork is needed to test the utility of biochar for different types of soil and crops.

Keywords: Biochar, climate change, mitigation, carbon capture, Thailand

INTRODUCTION

Rain-fed agriculture is commonly practiced in many areas of Thailand, but in recent years the increasing frequency of extreme climate events such as floods, heat waves and droughts have impacted on agricultural production, and thus the economic well-being of resource-poor farmers. It is therefore important that Thailand develops strategic measures for climate change adaptation and to undertake mitigation measures that also support the country's development goals. Despite the severity of the situation, the level of understanding of climate change, its origins and implications are generally not well understood by the Thai government or individual farmers. Although the government has established an agency - the Thailand Greenhouse Gas Management Organization (TGO), this agency focuses on policies rather than implementation. Most farmers are unaware that their own activities result in GHG emissions, and still follow their traditional practice of burning crop wastes in the open field, resulting in air pollution and GHG emissions. At the same time, soil fertility has deteriorated in

many areas due to intensive use of pesticides and chemical fertilizers; soils in the current study area are in urgent need of improvement to improve structure, fertility and water-holding capacity. Several studies researches have already been conducted, but the utility of biochar as a soil amendment has yet to be investigated. Huay Sai Royal Development Study Center and Pa-deng Biochar Research Center (PdBRC) were established to seek new practical approaches to reduce GHG emissions resulting from agriculture, particularly the use of biochar as a soil amendment. It is envisaged that should the experimental findings support the utility of biochar as a carbon sink; the concept will be introduced to farmers in the area. At this stage, the effect of biochar on crop yield and its suitability to area's soil types is under investigation.

MATERIALS AND METHODS

Experimental sites

The research was conducted in 2 locations: Huay Sai Royal Initiative Development Project in Amphoe Cha-am [1,2], and Pa-deng Biochar Research Center (PdBRC) [3], Tambon Pa-deng, Amphoe Kaeng Krajan, both in Petchburi Province, southern Thailand. These 2 areas are about 200 and 250 kilometers from Bangkok, respectively. The sites were selected because of serious soil degradation problems and frequent droughts in both areas almost every year [1,2]. The laboratory analysis was conducted at the experiment sites, while some samples were sent to Chulalongkorn University in Bangkok for more detailed analysis [3].

Research Methodology

The study began by designing a biochar retort that could be constructed easily from low-cost, locally-available components by local farmers, even in remote areas. Most important, the retort had to be easy to operate to obtain biochar with the appropriate characteristics for soil amendment. The retort was designed and constructed to produce biochar with suitable characteristics according to the FAO guidelines [4]. The retort's operating temperature was controllable in the range of 500-600^{o}C, and designed for slow pyrolysis. The retort was constructed using a readymade concrete cylinder for the kiln (these cylinders are widely sold for use as septic tanks). The kiln has a diameter of 1.0 m with height of 0.40 m. The retort itself is positioned inside the kiln, and uses a regular 200 L drum. The retort temperature is controlled by varying the size of holes made at the lower level of the outer concrete cylinder. The size of the holes is critical and the ideal size depends on several factors such as wind direction and velocity. After a development period of more than a year, the biochar retort design was successfully finalized. Air circulation inside the kiln was tested to control combustion temperature.

The volume of biomass required as feedstock for biochar production and biomass to fuel the process must also be considered. For the experiment, the volume of biomass for biochar and biomass for fuel is in the ratio of 1.0:0.6 or using fuel about 60 percent of the total biomass used for biochar production which will get about 30 % of the biomass. Biochar produced by slow pyrolysis (24 hours at 500-600^{o}C) using this retort was applied to experimental plots of local crops in farmers' fields (sorghum, corn, rice, chili, pineapple and mushrooms).

Biomass Used for Biochar Experiments

Six sources of biomass were tested: 1). *Samanea saman* (Jacq.) Merr. Fabaceae (Leguminosa-Mimosoideae), Rain tree or Chamchulee 2) *Leucaena leucocephala* (Lam.) de Wit Fabaceae (Leguminosae-Mimosoideae), Horse Tamarind or Kathin 3) *Blachia siamensis* Gagnep. Euphorbiaceae or Krachid 4) *Getonia floribunda* (Roxb) Lam. or Sakaewan 5) *Albizzia myriophylla* (Leg.) or Cha-em; and 6) Maize cobs.

RESULTS

The characteristics of biochar produced by the retort are summarized below.

CHN Analysis

The results of the CHN analysis are tabulated in Table I below.

TABLE I. C-H-N ANALYSIS

Biochar sample	Biomass dry weight (av.)			Biochar (av.)		
	%C	%H	%N	%C	%H	%N
1. *Samanea saman*	42.69	6.21	0.0	84.78	2.43	0.64
2. *Leucaena leucocephala*	43.76	6.17	0.15	84.61	2.47	1.03
3. *Blachia siamensis*	43.11	6.57	0.16	78.88	1.38	0.78
4. *Getonia floribunda*	44.44	6.69	0.0	74.56	3.23	1.20
5. *Albizzia myriophylla*	42.35	6.36	0.35	66.64	3.77	1.00
6. Maize cobs	41.66	6.84	0.74	81.35	2.42	1.00

In terms of proximate analysis, biochar from *Samanea* and *Leucaena* had the highest carbon content (84.78% and 84.61%, respectively), and *Albizzia* the lowest (66.64%). Carbon content of maize cob biochar was intermediate (81.35% C). However, nitrogen levels in *Leucaena* were higher than for *Samanea* (1.03, 0.64%, respectively), which could significantly influence soil fertility.

Calorific Value

Analysis indicated that with the exception of *Albizzia*, calorific values of biochar (calories per gram) were all similarly high, indicating its suitability both as a soil amendment and also for cooking or heating.

TABLE II. BIOCHAR CALORIFIC VALUE

Biochar sample	Heating Value (cal/g)		
	1st Experiment	2nd Experiment	Average
1. *Samanea saman*	7,567.9	7,633.8	7,600.9
2. *Leucaena leucocephala*	7,341.5	7,339.8	7,340.7
3. *Blachia siamensis*	6,890.4	6,907.5	6,899.0
4. *Getonia floribunda*	7,765.2	7,716.1	7,740.7
5. *Albizzia myriophylla*	5,955.2	5,832.2	5,893.7
6. Maize cobs	7,051.7	7,091.6	7,071.1

In terms of calorific value, the average of two experiments showed that *Getonia* had the highest heating value (7,740.7 cal/g), followed by *Samanaea* and *Leucaena* (7,600.9 and 7,340.7 cal/g, respectively). *Albizzia* biochar had the lowest calorific value (5,893.7cal/g)), with maize cobs at an intermediate level (7,071.1 cal/g).

Surface Area and Pore Size

Surface area and pore size distribution are important attributes of biochar, influencing the material's capacity to retain and release nutrients and water. The multipoint Brunauer, Emmett and Teller (BET) method (1938) [5] was used to calculate the matrix surface area of the six types of biochar. Total pore volume and pore diameter was also calculated (Table III).

TABLE III. SURFACE AREA AND PORE SIZE

Biochar sample	Multipoint BET (m^2/g)	Total pore volume (cc/g)	Average pore diameter (Å)
1. *Samanea saman*	45.62	0.0372	32.61
2. *Leucaena leucocephala*	42.08	0.0374	35.53
3. *Blachia siamensis*	137.91	0.0977	28.34
4. *Getonia floribunda*	2.92	0.0104	143.1
5. *Albizzia myriophylla*	3.67	0.0150	163.1
6. Maize cobs	56.35	0.0405	28.72

The results indicate that biochar from *Blachia siamensis* was far superior to all other materials in terms of matrix surface area ($137.91 m^2/g$), and had the smallest average pore diameter. Maize cobs followed ($56.35 m^2/g$) and also had smallest pore diameter. Biochar from *Getonia* and *Albizzia* on the other hand had the largest pore diameter and the smallest surface areas per gram. *Samanea* and *Leucaena* biochar were intermediate in both characteristics.

Water Holding Capacity

Water-holding capacity is a key attribute for biochar in influencing overall soil properties. Significant differences in WHC were found among the six types of biochar, as shown in Figure 1.

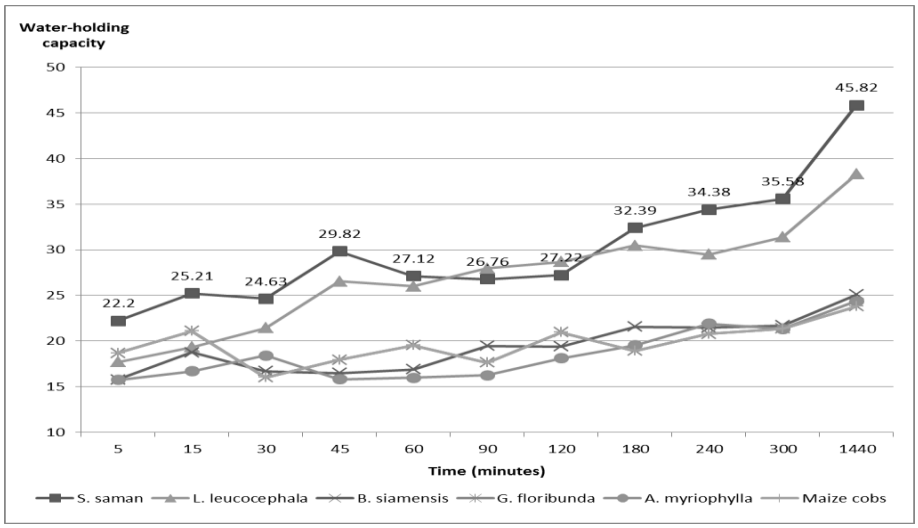

Figure 1. Water-holding capacity

The data show the superior water-holding capacity of *Samanea* and *Leucaena* over the other biochars. Surprisingly, the water-holding capacity of *Blachia* was unexpectedly low, considering its high matrix surface area and total pore volume.

DISCUSSION AND RECOMMENDATIONS

These preliminary experimental findings indicate the variability in characteristics among biochars derived from different types of biomass. The data indicate the superiority of *Samanea* and *Leucaena* over other types, due to their superior water-holding capacity. *Leucaena* biochar enjoys two additional advantages in terms of its higher nitrogen content, and its rapid growth on a range of soil types. However, the process of retort development also highlighted the influence of retention time and pyrolysis temperature on the characteristics of the biochar produced. Further research should examine these interactions, and also consider the most appropriate sources of biochar in view of local availability of biomass sources as feedstock.

The next phase of this investigation will examine the field performance of the six biochar types as a soil amendment in local crops such as sorghum, corn, rice, chili, pineapple and mushrooms. It will also be important to generate wider understanding among farmers of the climate change impacts of their activities, and to promote adoption of GHG mitigation measures. Biochar can contribute to this process, as well as to reducing the serious air pollution caused by annual burning of crop stubble. Altogether, promotion of biochar is in line with national strategy to introduce adaptation and mitigation measures in order to minimize extreme climate events and ensure food security.

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Cover page

Title: *Geneome Arrangement Comparative of Interferon Regulatory Factors in Bony Fish*

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ABSTRACT

IRF4, IRF8, IRF9 and IRF10 belong to IRF4 subfamily in interferon regulatory factor family. The genomic organization, identity and conserved domain of IRF4, IRF8, IRF9 and IRF10 were compared in the study, which will elucidate the conservation and diversification in evolution about the four genes in bony fishes. In genomic structure, IRF8 and IRF10 were quite comparable in all studied teleosts, showing a higher degree of conservation than those of IRF4 and IRF9. The size of genomic structure of IRF9 varied from 888 to 22672 bp. Tetraodon have two IRF9 genes and the exon-intron arrangement is totally different from IRF9 in other species. IRF4 was the most complex gene in the IRF4 subfamily. Not only the gene number changed a lot, but also the genomic organization varied in different teleost. So far, three IRF4 genes were found in zebrafish but two IRF4 genes (IRF4a and IRF4b) in other model teleosts, which showed that IRF4 duplicate twice in many fishes and then formed two IRF4 genes in many teleosts.

INTRODUCTION

IRF family members play a crucial role in both the innate and adaptive responses [1]. Today, most documents reported the family include IRF1 to IRF10 in vertebrates and it is divided into four subfamilies: IRF1 subfamily, IRF3 subfamily, IRF4 subfamily and IRF5 subfamily, in which IRF4, IRF8, IRF9 and IRF10 belong to IRF4 subfamily [2]. Recently, IRF11 in fish has been reported and it is similar to IRF1 and IRF2 [3,4].

Teleost fish are a large group in vertebrates. Several teleost fish are important in many areas. Zebrafish *Danio rerio* and medaka *Oryzias latipes* are widely used as ‘piscine mice’ in developmental biology for the study of different aspects of vertebrate organogenesis [5]. Platyfish *Xiphophorus maculatus* is an important model for the study of the development of pigment cells and melanoma [6]. Fugu *Takifugu rubripes* and tetraodon *Tetraodon nigroviridis* are pure genomics models for the compactness of their genome [7]. Stickleback *Gasterosteus aculeatus*, from the great lakes of East Africa, is a traditional model to study the molecular basis of speciation and the evolutionary mechanisms [8]. Furthermore, several other teleost

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fish such as tilapia *Oreochromis niloticus*, flounder *Paralichthys olivaceus* and grass carp *Ctenopharyngodon idella* are particularly studied at the genetic and genomic levels for at least partially an economical motivation.

IRF4, IRF8, IRF9 and IRF10 in the bony fish are analyzed in the study, which will shed new light on the conservation and diversification in evolution of genomes and proteins and will also help to clarify the differentiation of function.

METHODS

Genomic sequences were downloaded from the Ensembl Database for zebrafish, stickleback, medaka, fugu, tilapia, platyfish, tetraodon and from the National Center for Biotechnology Information (NCBI) database for flounder and grass carp. All predicted IRF protein sequences were verified by BLASTP in the NCBI database. The DNA region covering the query result of each predicted IRF protein coding region and the corresponding transcripts accessed by Ensemble database were extracted and the SIM4 program (http://pbil.univ-lyon1.fr/sim4.php) was used to reconstruct the exon-intron structure and calculate the intron phase.

RESULT

IRF4 was identified in all teleost fish examined in the present study. Interestingly, three IRF4-like genes were identified in zebrafish, which were named as IRF4a, IRF4b and scafNA1075-seg. However, two IRF4 genes were observed in medaka, fugu, tetraodon, stickleback, tilapia and platyfish and only one IRF4 gene was found in flounder. The genomic size of IRF4 varied from 3034 to 25107 bp, with the longest observed in zebrafish IRF4a, and shortest in fugu IRF4a. The exon-intron arrangement has a lot of difference in teleosts. The first IRF4 gene has eight exons and the second one has nine exons in zebrafish, fugu and medaka. It is interesting to note that IRF4a and IRF4b in platfish have the similar size and the same position for each homologous exons and introns. Although IRF4 genes had different exon-intron structure in genome, it had same exon size in most genes with the first exon of 198 bp, the second exon of 187 bp and the second exon from the bottom of 113 bp (Fig.1).

The IRF8, in general, had a similar exon-intron organization in teleost fish, with eight exons and seven introns. It is also interesting to note that all exons except for the third exon have the same size in stickleback, fugu, tetraodon and platyfish. In tilapia, the lenght of all exons is the same to that of the above four species except the third exon and eighth exon. On the contrary, zebrafish and medaka change a lot in most exons size and only have the same size to other species in second exon and the seventh exon. The intron sizes are comparable in different fishes, with the shortest in tetraodon (2115 bp) and the longest in zebrafish (5410 bp) (Fig.2).

The size of genomic structure of IRF9 changed from 888 to 22672 bp, with the longest found in zebrafish, and shortest in tetradon. The exon-intron organization varied between different species. In zebrafish, stickleback, platyfish and tilapia, the IRF9 had nine exons and eight introns and the first three exons have the same size except for the third exon in platyfish. Although there are only eight exons in fugu and medaka, the first, second, third and sixth exon has the same size to that in

zebrafish. Further compared with IRF9 in zebrafish, IRF9 may lack the last exon in medaka. The genome of IRF9 in tetraodon showed marked diversification when compared with IRF9 in other fishes. Surprisingly, tetraodon has two IRF9 gene and the exon-intron arrangement is totally different from IRF9 in other species. The one is only 888 bp and the other is 1711 bp. Even so, the first three exons have the same size with IRF9 in the other species (Fig.3).

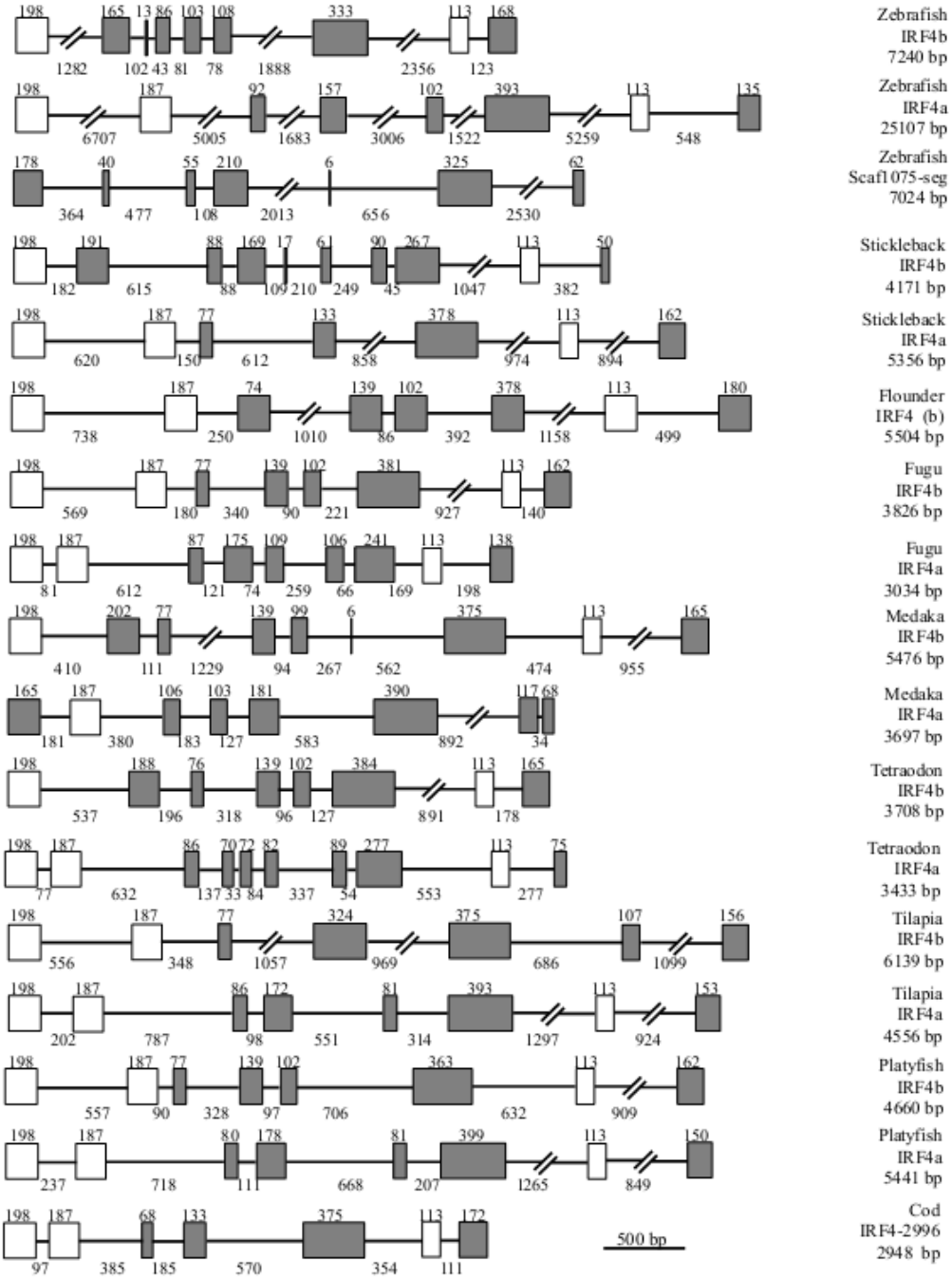

Figure 1 Schematic diagram of exon-intron arrangement of IRF4 genes in teleosts. Boxes represent exons and lines adjacent to exons represent introns. Unconservative exons and conservative exons are shown as black boxes and white boxes, respectively. The number of nucleotides in each exon and intron is, respectively, shown above and below the corresponding element. The following figures are same to figure 1.

The IRF10 generally had a similar exon-intron organization in teleost fish except for fugu, with eight exons and seven introns (Fig.4). The second and seventh exon have the same length in all species. Moreover, the first exon also has same size in zebrafish, medaka and grass carp. Compared with IRF4 and IRF9, the intron sizes are similar. And the size of genomic structure of IRF10 varied from 3073 bp in grass carp to 8638 bp in zebrafish (Fig.4).

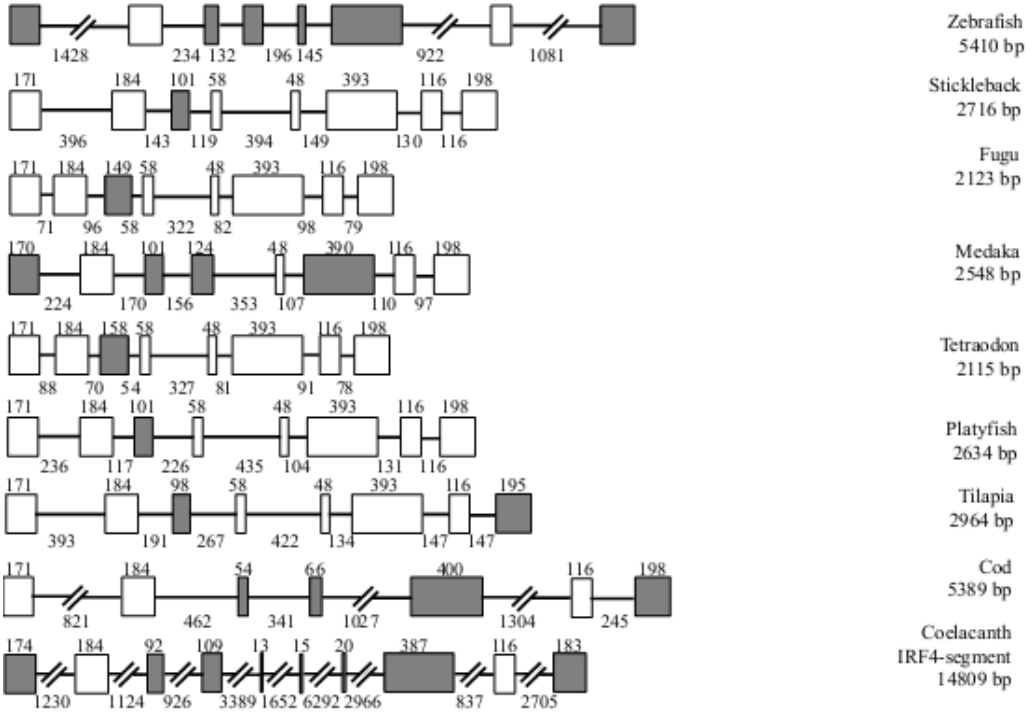

Figure 2 Schematic diagram of exon-intron arrangement of IRF8 genes in teleosts.

DISCUSSION

The gene of IRF4 in the teleosts was quite complex and there are two genes in some teleosts and three genes in zebrafish. Phylogenetic tree showed all IRF4 was further sub-clustered into two distinct clades, one is called IRF4a and the other is called IRF4b. IRF4a and IRF4b existed in almost all teleosts. To our knowledge, only one IRF4 gene has been reported in the mammalian, birds and frogs, they belong to IRF4b [9], suggesting that in teleost fish, especially in zebrafish tandem gene duplication or additional independent chromosome duplication might have occurred [10]. Moreover, some IRF4 genes have several transcripts. For example, there have two, seven and two transcripts in zebrafish IRF4b, fugu IRF4a and rainbow trout IRF4 [11]. IRF4 and IRF8 are two distinct members of the IFN family that are highly homologous to one other.

IRF9 was previously known as ISGF3γ, is active only in the presence of STAT1 and STAT2. Whereas exposure of cells to type I IFNs leads to the phosphorylation and dimerization of STAT1 and STAT2, expression of IRF9 itself is induced by IFN-γ. The trimeric complex IRF9–STAT1–STAT2 translocates to the nucleus, where it binds the ISRE of many genes, including IRF7 [1]. Furthermore, IRF9 plays an important role in IFN-a signalling. Increasing the

amount of IRF9 by over-expression or prestimulation of cells with IFN-γ or interleukin-6 results in higher levels of transcription of IFN-stimulated genes and an augmented antiviral response [12,13]. IRF10 was first cloned from chickens in 2002 and the closest relative to chicken IRF10 is IRF4 [14]. And the major function is that it can regulate the expression of guanylate-binding protein (GBP) and MHC class I by type I and II IFN stimulation [14]. Recently, IRF10 was reported in flounder and it played a vital role in not only virus infection but also bacterial infection [15]. There has eight exons and most exons have the same length in the seven teleosts. The identity between IRF8 proteins in teleost fish reached at 60-87%. These proved that IRF8 and IRF10 were very conservative.

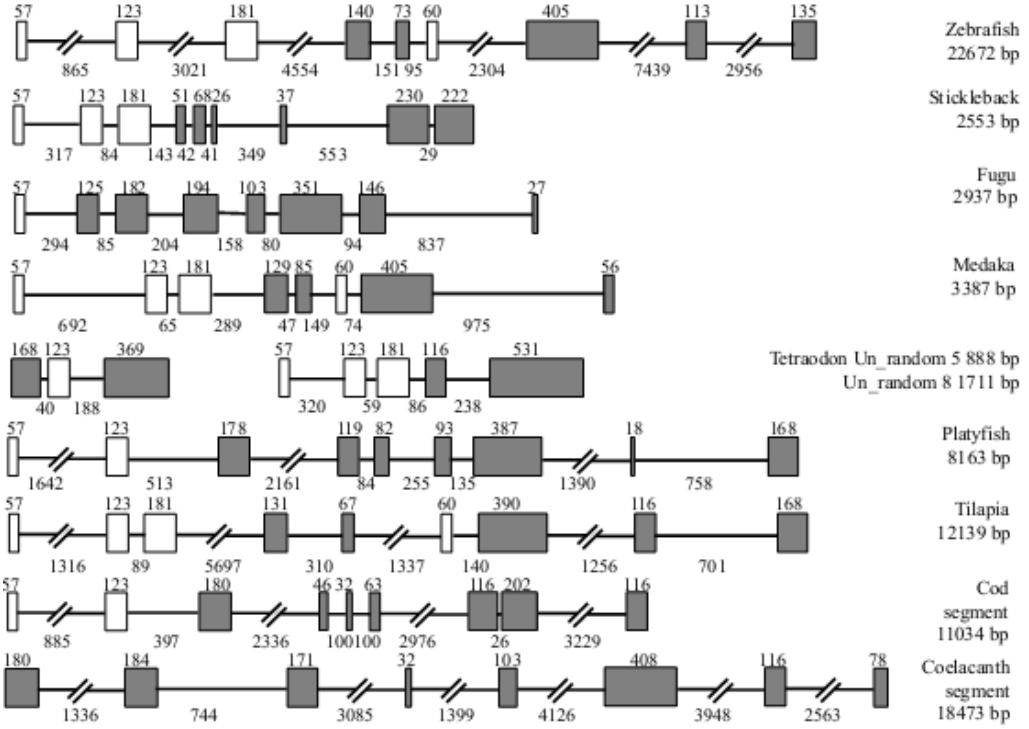

Figure 3 Schematic diagram of exon-intron arrangement of IRF9 genes in teleosts

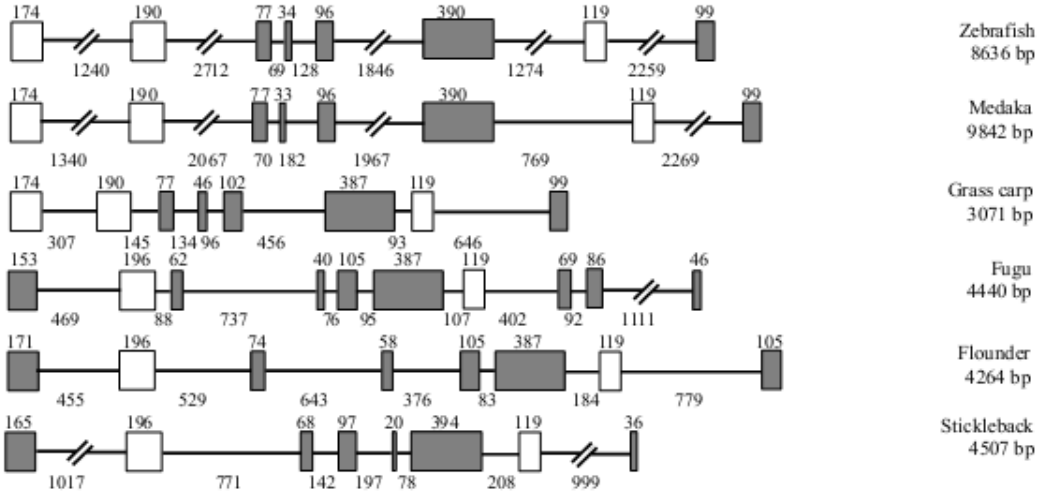

Figure 4 Schematic diagram of exon-intron arrangement of IRF10 genes in teleosts

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Cover page

Title: *Research on the Evaluation of Ecological Civilization Construction in Dian-zhong Urban Agglomeration*

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ABSTRACT

The report of 18th National Congress of CPC clearly pointed out that put resource consumption, environmental damage and ecological benefits into economic and social development evaluation system, establish the target system, assessment methods, incentive mechanisms to reflect the requirement of ecological civilization. This paper constructs ecological civilization evaluation index system; establish the level of development index model; attempt evaluation the ecological civilization construction of Dian-zhong Urban Agglomeration, and provide guidance for promoting the construction of ecological civilization.

1. INTRODUCTION

In order to promote ecological civilization construction (ECC), national and local have issued a series of specific measures, such as the ecological county (District, city) construction, make energy-saving and emission reduction targets, agricultural pollution control plan etc. Although these measures have certain effect, but the ECC often has certain blindness and randomness because of the lack of scientific evaluation system and method, on the importance of environmental protection is not enough. The "China Green National Accounting Study Report 2008 (public version)" published by the Ministry of environmental protection in 2010 October pointed out the cost of environmental pollution caused by economic development sustained growth, environmental pollution and ecological destruction pressure increases, the cost of environmental degradation increased from ￥5118.2 Billion to ￥8947.6 Billion, up 74.8%; virtual governance cost from ￥2874.4 Billion to ￥5043.1 Billion, up 75.4%; environmental degradation costs accounted for the proportion of GDP is about 3% in the past 5 years. Therefore, the report of 18th National Congress of CPC clearly pointed out that put resource consumption, environmental damage and ecological benefits into economic and social development evaluation system, establish the target system, assessment methods, incentive mechanisms to reflect the requirement of

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ecological civilization [1]. Therefore, this paper constructs the evaluation index system of ECC, establishes the evaluation model of the development level, and provides guidance for promoting the ECC of Dian-zhong urban agglomeration.

2. ESTABLISH THE EVALUATION SYSTEM OF ECC

2.1 The Evaluation Index System

Ecological civilization, also known as "green civilization", is to respect the laws of nature and protect nature as the premise, resources and environment carrying capacity as the basis, to create sustainable industrial structure, mode of production and consumption patterns as the main content, realizes the civilization development form that the human and natural environment of interdependence, mutual promotion and coexistence [2]. The report of 18th National Congress of CPC put forward the important goal of ECC, that is : " the construction of resource conserving and environment-friendly society has made significant progress, the main function area layout is formed basically, the resource recycling system is preliminary established; energy consumption per unit of GDP and CO2 emissions dropped significantly, the total discharge of major pollutants decreased significantly; the forest coverage rate is increased, the stability of the ecological system is enhanced, the living environment improved significantly ".

ECC is a complex system, can be divided into three layer structure with total target, sub target (subsystem) and the index according to the connotation of ecological civilization. The index weight was determined with AHP method. The metric value of each index was determined according to the international standard, the United States and other developed countries (see Table I).

TABLE I. The EVALUATION INDEX SYSTEM OF ECC

Target Layer A	Sub Target B	Weight W_{bk}	Index C	Metric value	Unit	Weight W_{ci}
The level of development of ECC (A)	Economic development (B_1)	0.2669	Per capita GDP (C_1)	8	￥10000	0.458
			Two and three industrial added value accounted for the proportion of GDP (C_2)	98	%	0.196
			Per capita local financial revenue (C_3)	1	%	0.347
	Social progress (B_2)	0.1743	The urbanization rate (C_4)	80	%	0.488
			Per capita disposable income of urban residents (C_5)	7.5	￥10000	0.254
			Per capita net income of rural households (C_6)	5	￥10000	0.259
	Resource conservation (B_3)	0.1445	Water consumption unit GDP (C_7)	30	m^3/￥10000	0.413
			Energy consumption unit GDP (C_8)	0.3	t/￥10000	0.453
			Per capita city built area (C_9)	82.4	m^2/per	0.135
	Pollution control (B_4)	0.1725	Wastewater emissions unit GDP (C_{10})	4	t/￥10000	0.515
			SO_2 emissions unit GDP (C_{11})	1.1	Kg	0.299
			Unit output value of industrial solid wastes (C_{12})	0.2	t/￥10000	0.186
	Ecological environment (B_5)	0.1366	City air pollution index (C_{13})	1.73	Index	0.226
			Forest coverage rate (C_{14})	29.0	%	0.356
			Ecosystem service value (C_{15})	1	Billion/ha	0.417
	Ecological culture (B_6)	0.1052	Cultural industries accounted for area GDP (C_{16})	15	%	0.395
			Per capita public library collection (C_{17})	2.5	Book/ per	0.292
			High school enrollment rate (C_{18})	100	%	0.313

2.2 The Evaluation Index System

The development level of ECC reflects what stage the construction of ecological civilization reached. The evaluation system can be transformed into three evaluation stage model based on evaluation index system.

(1) The development level index of index (Lc). For a index C, C as observed values, C' as its metric value. When C is positive indexes: Lc=C'/C; when C is negative index: Lc=C/C'.

(2) The development level index of subsystem (Lb). The subsystem consists of 3 indexes; the index weight of subsystem is W_{ci}.

$$Lb_k = \sum_{i=1}^{n} (w_{ci} \times Lc_i) \tag{1}$$

(3) The comprehensive development level index of system (La). The evaluation system consists of 6 subsystems; the subsystem weight of system is W_{bk}.

$$La = \sum_{k=1}^{6} (w_{bk} \times Lb_k) \tag{2}$$

(4) The evaluation grade of development level. The development level is divided into 7 grades according to the score range of system development level index value, where A is the best, G is the worst (see Table II).

TABLE II. The EVALUATION GRADE OF DEVELOPMENT

Grade	A	B	C	D	E	F	G
Score	0.8<	0.65-0.8	0.55-0.65	0.45-0.55	0.35-0.45	0.2-0.35	<0.2
Classification	Quality	Good	Intermediate	Primary development	Mild backward	Moderate backward	Severe backward

3. STUDY AREA AND DATA SOURCE

Dian-zhong urban agglomeration consists of Kunming, Qujing, Yuxi, Chuxiong four city (state) administrative districts, has an area of 96000 square kilometers. This region rich in resources, solid industrial base, is the most important and vigorous area (total economic output accounted for the province's 60%), but also the most concentrated area of resource consumption and environmental impaction (such as the energy consumption accounted for the province's 51.5%, wastewater emissions accounted for the province's 52.4% at 2010) in Yunnan. The government of Yunnan announced the “Dian-zhong urban agglomeration planning (2009-2030)” in May 27, 2011. The total population size will reach 24 millions, and the level of urbanization will reach 75% by the end of 2030 according to the plan. Dian-zhong urban agglomeration is located in the western frontier of underdeveloped area, special geographical environment, ecological importance, many minorities, faced with double pressure of economic development and ecological protection. Therefore, scientific evaluation is of great significance to the construction of ecological civilization in the region.

This paper relates to the data from the "Statistical yearbook of Yunnan province", " The unit GDP energy consumption and other indicators bulletin of Yunnan province and the state (city)", "National economic and social development statistical bulletin" published by the Statistics Bureau of Yunnan province. PM_{10} (due to the lack of the $PM_{2.5}$ data), SO_2 and NO_2 were selected to calculate the index value of city air pollution index according to the "Ambient air quality standard". The unit ecosystem service value index was calculated according to "Chinese terrestrial ecosystem service value per unit area" based on the land survey data in 2008 [3].

4. RESULTS AND ANALYSIS

The results show that: the comprehensive development level of ECC in Dian-zhong urban agglomeration is increasing year by year (see Figure I).

After the "Eleventh" period of development, The ECC development level index value of Kunming rose from 0.421 in 2005 to 0.517 in 2010, up 22.8%, evaluation grades from "mild backward" phase to stride into the "primary development" stage. The development level of each subsystem compared: ecological environment (A)> ecology culture (C)> social progress (D)> economic development (C)> resource conservation (F)> pollution control (F). The development level of resource conservation and pollution control 2 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Kunming.

The ECC development level index value of Qujing rose from 0.334 in 2005 to 0.404 in 2010, up 21.1%, evaluation grades from "moderate backward" phase to stride into the "mild backward" stage. The development level of each subsystem compared: ecological environment (A)> ecology culture (E)> social progress (F)> economic development (F)> resource conservation (F)> pollution control (F). The development level of social progress, economic development, resource conservation, and pollution control 4 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Qujing.

The ECC development level index value of Yuxi rose from 0.443 in 2005 to 0.532 in 2010, up 23.4%, evaluation grades from "mild backward" phase to stride into the "primary development" stage; The development level of each subsystem compared: ecological environment (A) > ecology culture (D) > pollution control (D) > economic development (E) > social progress (F) > resource conservation (F). The development level of social progress and resource conservation 2 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Qujing.

The ECC development level index value of Chuxiong rose from 0.395 in 2005 to 0.460 in 2010, up 16.5%, evaluation grades from "mild backward" phase to stride into the "primary development" stage; The development level of each subsystem compared: ecological environment (A)> ecology culture (E) > pollution control (F)> social progress (F)> economic development (F)> resource conservation (F). The development level of pollution control, social progress, economic development and resource conservation, 4 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Qujing.

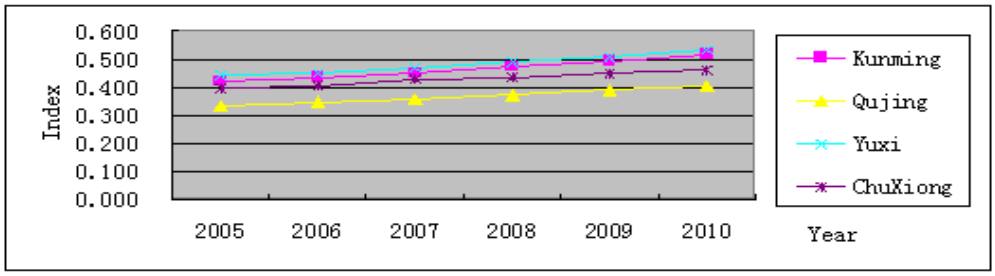

Figure 1. The development level of ECC in 2005-2010

5. CONCLUSION AND DISCUSSION

(1) The empirical results show, the development level of Dian-zhong urban agglomeration has been greatly improved in recent years, but the comprehensive level of development is still at a low stage. In addition to the ecological environment has a larger advantage, other subsystems development level is still a big gap. As the economic development growth rate much higher than the resource conservation and pollution control of Dianzhong urban agglomeration, resource demand and pollutant emissions will continue growing year by year.

(2) The construction of ecological civilization is complicated system engineering, not only the resource conservation and environmental governance, and relates to the construction of spiritual civilization and ecological system construction. The evaluation System does not consider these because of the difficulty of quantifying these indicators about spirit level and system level (such as ecological consciousness social justice, ecological democracy).

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The report of 18th National Congress of CPC clearly pointed out that put resource consumption, environmental damage and ecological benefits into economic and social development evaluation system, establish the target system, assessment methods, incentive mechanisms to reflect the requirement of ecological civilization. This paper constructs ecological civilization evaluation index system; establish the level of development index model; attempt evaluation the ecological civilization construction of Dian-zhong Urban Agglomeration, and provide guidance for promoting the construction of ecological civilization.

1. INTRODUCTION

In order to promote ecological civilization construction (ECC), national and local have issued a series of specific measures, such as the ecological county (District, city) construction, make energy-saving and emission reduction targets, agricultural pollution control plan etc. Although these measures have certain effect, but the ECC often has certain blindness and randomness because of the lack of scientific evaluation system and method, on the importance of environmental protection is not enough. The "China Green National Accounting Study Report 2008 (public version)" published by the Ministry of environmental protection in 2010 October pointed out the cost of environmental pollution caused by economic development sustained growth, environmental pollution and ecological destruction pressure increases, the cost of environmental degradation increased from ￥5118.2 Billion to ￥8947.6 Billion, up 74.8%; virtual governance cost from ￥2874.4 Billion to ￥5043.1 Billion, up 75.4%; environmental degradation costs accounted for the proportion of GDP is about 3% in the past 5 years. Therefore, the report of 18th National Congress of CPC clearly pointed out that put resource consumption, environmental damage and ecological benefits into economic and social development evaluation system, establish the target system, assessment methods, incentive mechanisms to reflect the requirement of

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ecological civilization [1]. Therefore, this paper constructs the evaluation index system of ECC, establishes the evaluation model of the development level, and provides guidance for promoting the ECC of Dian-zhong urban agglomeration.

2. ESTABLISH THE EVALUATION SYSTEM OF ECC

2.1 The Evaluation Index System

Ecological civilization, also known as "green civilization", is to respect the laws of nature and protect nature as the premise, resources and environment carrying capacity as the basis, to create sustainable industrial structure, mode of production and consumption patterns as the main content, realizes the civilization development form that the human and natural environment of interdependence, mutual promotion and coexistence [2]. The report of 18th National Congress of CPC put forward the important goal of ECC, that is : " the construction of resource conserving and environment-friendly society has made significant progress, the main function area layout is formed basically, the resource recycling system is preliminary established; energy consumption per unit of GDP and CO2 emissions dropped significantly, the total discharge of major pollutants decreased significantly; the forest coverage rate is increased, the stability of the ecological system is enhanced, the living environment improved significantly ".

ECC is a complex system, can be divided into three layer structure with total target, sub target (subsystem) and the index according to the connotation of ecological civilization. The index weight was determined with AHP method. The metric value of each index was determined according to the international standard, the United States and other developed countries (see Table I).

TABLE I. The EVALUATION INDEX SYSTEM OF ECC

Target Layer A	Sub Target B	Weight W_{bk}	Index C	Metric value	Unit	Weight W_{ci}
The level of development of ECC (A)	Economic development (B_1)	0.2669	Per capita GDP (C_1)	8	￥10000	0.458
			Two and three industrial added value accounted for the proportion of GDP (C_2)	98	%	0.196
			Per capita local financial revenue (C_3)	1	%	0.347
	Social progress (B_2)	0.1743	The urbanization rate (C_4)	80	%	0.488
			Per capita disposable income of urban residents (C_5)	7.5	￥10000	0.254
			Per capita net income of rural households (C_6)	5	￥10000	0.259
	Resource conservation (B_3)	0.1445	Water consumption unit GDP (C_7)	30	m^3/￥10000	0.413
			Energy consumption unit GDP (C_8)	0.3	t/￥10000	0.453
			Per capita city built area (C_9)	82.4	m^2/per	0.135
	Pollution control (B_4)	0.1725	Wastewater emissions unit GDP (C_{10})	4	t/￥10000	0.515
			SO_2 emissions unit GDP (C_{11})	1.1	Kg	0.299
			Unit output value of industrial solid wastes (C_{12})	0.2	t/￥10000	0.186
	Ecological environment (B_5)	0.1366	City air pollution index (C_{13})	1.73	Index	0.226
			Forest coverage rate (C_{14})	29.0	%	0.356
			Ecosystem service value (C_{15})	1	Billion/ha	0.417
	Ecological culture (B_6)	0.1052	Cultural industries accounted for area GDP (C_{16})	15	%	0.395
			Per capita public library collection (C_{17})	2.5	Book/ per	0.292
			High school enrollment rate (C_{18})	100	%	0.313

2.2 The Evaluation Index System

The development level of ECC reflects what stage the construction of ecological civilization reached. The evaluation system can be transformed into three evaluation stage model based on evaluation index system.

(1) The development level index of index (Lc). For a index C, C as observed values, C' as its metric value. When C is positive indexes: Lc=C'/C; when C is negative index: Lc=C/C'.

(2) The development level index of subsystem (Lb). The subsystem consists of 3 indexes; the index weight of subsystem is W_{ci}.

$$Lb_k = \sum_{i=1}^{n} (w_{ci} \times Lc_i) \tag{1}$$

(3) The comprehensive development level index of system (La). The evaluation system consists of 6 subsystems; the subsystem weight of system is W_{bk}.

$$La = \sum_{k=1}^{6} (w_{bk} \times Lb_k) \tag{2}$$

(4) The evaluation grade of development level. The development level is divided into 7 grades according to the score range of system development level index value, where A is the best, G is the worst (see Table II).

TABLE II. The EVALUATION GRADE OF DEVELOPMENT

Grade	A	B	C	D	E	F	G
Score	0.8<	0.65-0.8	0.55-0.65	0.45-0.55	0.35-0.45	0.2-0.35	<0.2
Classification	Quality	Good	Intermediate	Primary development	Mild backward	Moderate backward	Severe backward

3. STUDY AREA AND DATA SOURCE

Dian-zhong urban agglomeration consists of Kunming, Qujing, Yuxi, Chuxiong four city (state) administrative districts, has an area of 96000 square kilometers. This region rich in resources, solid industrial base, is the most important and vigorous area (total economic output accounted for the province's 60%), but also the most concentrated area of resource consumption and environmental impaction (such as the energy consumption accounted for the province's 51.5%, wastewater emissions accounted for the province's 52.4% at 2010) in Yunnan. The government of Yunnan announced the “Dian-zhong urban agglomeration planning (2009-2030)” in May 27, 2011. The total population size will reach 24 millions, and the level of urbanization will reach 75% by the end of 2030 according to the plan. Dian-zhong urban agglomeration is located in the western frontier of underdeveloped area, special geographical environment, ecological importance, many minorities, faced with double pressure of economic development and ecological protection. Therefore, scientific evaluation is of great significance to the construction of ecological civilization in the region.

This paper relates to the data from the "Statistical yearbook of Yunnan province", " The unit GDP energy consumption and other indicators bulletin of Yunnan province and the state (city)", "National economic and social development statistical bulletin" published by the Statistics Bureau of Yunnan province. PM_{10} (due to the lack of the $PM_{2.5}$ data), SO_2 and NO_2 were selected to calculate the index value of city air pollution index according to the "Ambient air quality standard". The unit ecosystem service value index was calculated according to "Chinese terrestrial ecosystem service value per unit area" based on the land survey data in 2008 [3].

4. RESULTS AND ANALYSIS

The results show that: the comprehensive development level of ECC in Dian-zhong urban agglomeration is increasing year by year (see Figure I).

After the "Eleventh" period of development, The ECC development level index value of Kunming rose from 0.421 in 2005 to 0.517 in 2010, up 22.8%, evaluation grades from "mild backward" phase to stride into the "primary development" stage. The development level of each subsystem compared: ecological environment (A)> ecology culture (C)> social progress (D)> economic development (C)> resource conservation (F)> pollution control (F). The development level of resource conservation and pollution control 2 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Kunming.

The ECC development level index value of Qujing rose from 0.334 in 2005 to 0.404 in 2010, up 21.1%, evaluation grades from "moderate backward" phase to stride into the "mild backward" stage. The development level of each subsystem compared: ecological environment (A)> ecology culture (E)> social progress (F)> economic development (F)> resource conservation (F)> pollution control (F). The development level of social progress, economic development, resource conservation, and pollution control 4 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Qujing.

The ECC development level index value of Yuxi rose from 0.443 in 2005 to 0.532 in 2010, up 23.4%, evaluation grades from "mild backward" phase to stride into the "primary development" stage; The development level of each subsystem compared: ecological environment (A) > ecology culture (D) > pollution control (D) > economic development (E) > social progress (F) > resource conservation (F). The development level of social progress and resource conservation 2 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Qujing.

The ECC development level index value of Chuxiong rose from 0.395 in 2005 to 0.460 in 2010, up 16.5%, evaluation grades from "mild backward" phase to stride into the "primary development" stage; The development level of each subsystem compared: ecological environment (A)> ecology culture (E) > pollution control (F)> social progress (F)> economic development (F)> resource conservation (F). The development level of pollution control, social progress, economic development and resource conservation, 4 subsystems backward is the main factor affecting the comprehensive level of ecological civilization construction in Qujing.

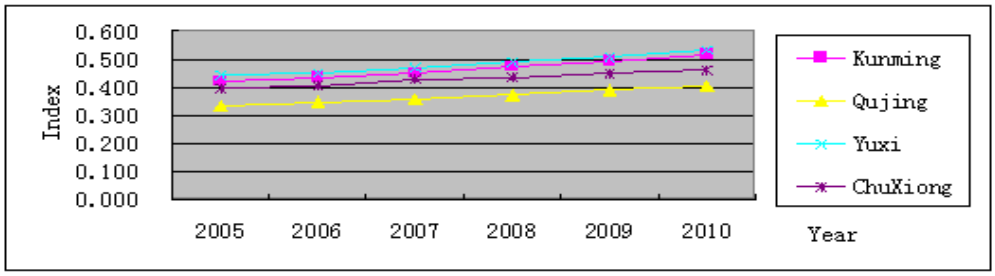

Figure 1. The development level of ECC in 2005-2010

5. CONCLUSION AND DISCUSSION

(1) The empirical results show, the development level of Dian-zhong urban agglomeration has been greatly improved in recent years, but the comprehensive level of development is still at a low stage. In addition to the ecological environment has a larger advantage, other subsystems development level is still a big gap. As the economic development growth rate much higher than the resource conservation and pollution control of Dianzhong urban agglomeration, resource demand and pollutant emissions will continue growing year by year.

(2) The construction of ecological civilization is complicated system engineering, not only the resource conservation and environmental governance, and relates to the construction of spiritual civilization and ecological system construction. The evaluation System does not consider these because of the difficulty of quantifying these indicators about spirit level and system level (such as ecological consciousness social justice, ecological democracy).

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Cover page

Title: *Prepared of Sericite with Higher Diameter Thickness Ratio from Tailings of Gold-Antimony*

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ABSTRACT

A technology of recovering sericite powder with higher diameter thickness ratio prepared from tailings was developed. The results indicate that the main component of tailings is SiO_2, Al_2O_3 and K_2O. By the process of classification, flotation and surface modification, a sericite powder with higher diameter thickness ratio can be obtained. The process is relatively simple, low cost and easy-to-production implementation.

1. INTRODUCTION

Tailings are the materials left over after the process of separating the valuable components from the uneconomic fraction (gangue) of an ore. In mining operations, significant quantities of tailings are produced during the processing of low-grade ores. In China, more than 300 million tons of tailings are produced each year [1]. But the tailings are still discarded as waste in large quantities. Large areas of land are needed to store these tailings, which reduces the area of usable farming land. The construction and maintenance of tailing disposal sites also increase the production cost of mining plants. In addition, tailings contain toxic and heavy metal ions[2-3],the release of heavy metals from these storage sites could cause serious environmental pollution in soil and water[4-5].

Although industrial waste, it is also a kind of potential resource because it contains SiO_2, Al_2O_3, CaO, K_2O, and etc. Sericite[6] is a phyllosilicate mineral that occurs as a gangue component in many base metal sulfide ore deposits around the world. Sericite has the general composition $KAl_2[Si_3AlO_{10}](OH)_2$, and its theoretical composition is SiO_2 45.3%, Al_2O_3 38.4%,K_2O 11.8%, and H_2O 4.5%. It has a hardness of 2~3 and a specific gravity of 2.8~2.9. Sericite with silky luster is a phyllosilicate mineral with two silicon-oxygen tetrahedral and one aluminum magnesium-oxygen octahedron constituting the unit crystal structure of sericite. The

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octahedron is located in the middle of the two silicon-oxygen tetrahedral[7]. Muscovite exhibits perfect cleavage along the {001} plane. Sericite is cryptocrystalline muscovite mica. Accordingly, properties of sericite are the same as those of muscovite mica. It is this cleavage property that is the basis for many of its industrial applications[8]. Sericite has the property of rich elastic, bendable, abrasion resistance and well wear resistance, insolublity in acid solution and chemical stability. Sericite is widely used in rubber, plastic, paint and other industries [9-11].

The aim of this work is to develop an economical and environmentally acceptable method to recover sericite powder with higher diameter thickness ratio from tailings. In the experiment, the tailings was classified，floated and surface modified in order to produce sericite powder with higher diameter thickness ratio. The filling of the rubber and plastic with surface modified sericite were studied by measuring the mechanical properties of the resulting composite material.

2. EXPERIMENTAL

2.1 Samples and Reagents

The tailings sample used in this study was taken from Shao Yang, Hunan Province, China. The chemical compositions of raw material were determined by X-ray fluorescence and the results were shown in Table 1. It can be observed that the raw sample was appeared to be a ready source of sericite. All reagent used in this study were analytical pure except for the Na_2SiO_3, which was industrial pure.

TABLE 1 CHEMICAL COMPOSITION OF SAMPLE (WT%)

Components	Al_2O_3	S	SiO_2	K_2O	Na_2O	TFe	MgO	CaO	Sb	Au (g/t)
Content (%)	13.04	0.20	64.71	2.10	0.67	1.94	1.09	0.33	0.22	0.25

2.2 Procedure

Fig.1 is the representative flowsheet of recovery sericite powder with higher diameter thickness ratio from tailings. The raw ore was classified at 37μ m and the coarse fraction was dropped. The -37μ m fraction became flotation feed. The milled slurry was transferred to a 3.0L laboratory XFG cell. The air flow rate was kept at 0.1Nm3/h monitored with a flow meter and impeller speed which was set at 1200 rpm. The flotation flowsheet and the reagent consumptions are shown in Fig.1.

The surface modification of sericite with silane was carried out by wet method. The silane coupling agent used was KH-570. A certain amount of silane/ethanol (1:10) solution was added into a suspension containing 50 gram of sericite powder and 250 mL of water. The mixture was stirred strongly in a high speed dispersing machine at a rotating rate of 1000 rpm. Then the suspension was filtrated followed by drying in an oven at a temperature of 100℃.

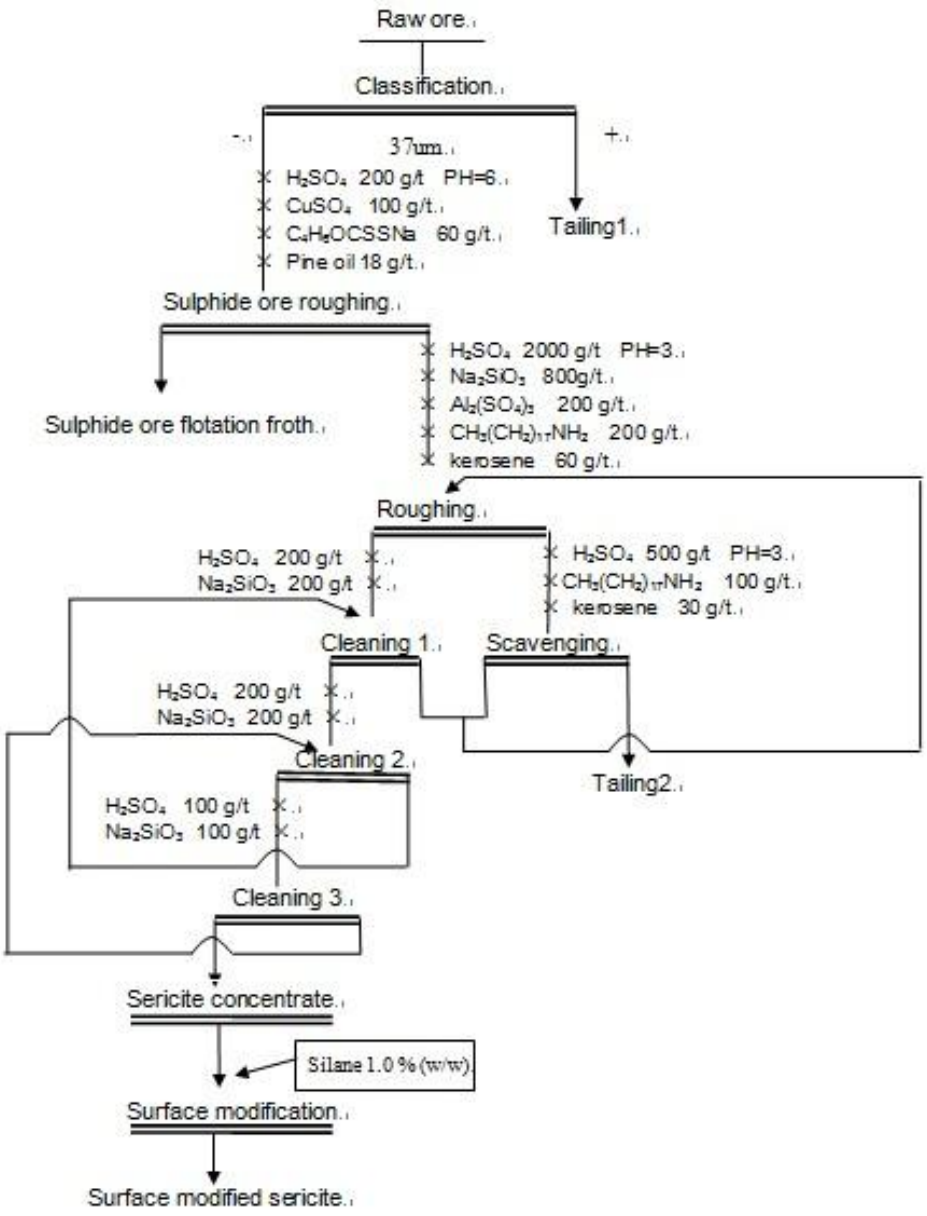

Fig.1 Flowsheet of recovery sericite powder from sample

3. RESULTS AND DISCUSSION

3.1 Classification

The classification result shows that the coarser the grain of tailings is, the lower distribution of sericite is. Due to sericite content and monomer dissociation rate of sericite of -37μm fine grained are high, it is unnecessary to grinding. Therefore it would be helpful to improve sericite content of raw materials and reduce grinding and flotation costs by hydrocyclone to remove coarse fraction. A particle size of 37μm was selected for the classification optimization. The results of classification process are shown in Table 2.

TABLE 2 RESULT OF CLASSIFICATION PROCESS (%)

Particle size interval (μm)	+147	-147+74	-74+37	-37+10	-10
Weight	15.48	24.15	12.73	19.50	28.14
distribution of sericite	8.0	19.0	5.5	7.2	60.3
Monomer dissociation rate of sericite	40	48	65	90	95

3.2 Flotation

A high specific surface area of the fine particles as well as the inevitable existing ions leads to flocculation in pulp. The zero point of charge of muscovite, deter-mined by Cases, is 0.95[12]. Nishimura et al. have also determined that the zeta potential is negative for all pH values greater than 2[13]. The pulp was regulated to a desired pH value using H_2SO_4.The effective and stable dispersion of pulp is the premise and basis of flotation. The sodium silicate is added to the pulp to improve the dispersion state of mineral particles and used as depressant of quartz. Aluminium is the most common activator proposed for the flotation of muscovite [14]. Aluminium sulfate is used as activator for the flotation of sericite. Flotation of both muscovite and sericite can be achieved with anionic or cationic collectors [15]. Because the surface of muscovite is negatively charged over the pH range of 2–12, the mineral is readily recovered using cationic collectors. Octadecyl amine is used as collector of sericite mineral.

In sericite processing, sulfide ore may be recovered in flotation concentrates through attachment to the valuable minerals as “slime coatings”. To improve quality of sericite, the sulfide ores were floated at first as “nuisance” mineral. Based on the optimized experimental conditions, results of flotation circuit which recovers sericite concentrate from tailings are listed in Table 3. It can be seen from table 3 that a sericite concentrate can be obtained by the process of classification and flotation.

TABLE 3 RESULTS OF ENTIRE FLOW CIRCUIT FOR RECOVERING SERICITE CONCENTRATE FROM TAILINGS (WT%)

Production	Yield	Al_2O_3 Grade	Al_2O_3 Recovery
Sericit concentrate	5.31	28.68	11.54
Sulphide ore flotation froth	1.05	15.65	1.25
Tailing2	36.41	16.13	44.52
Tailing1	57.23	9.84	42.69
Feed ores	100.00	13.19	100.00

3.3 Surface Modification

Surface modification of sericite powder was directly achieved by using a sericite concentrate which was obtained by flotation as raw materials by the process of wet method with the addition of 1.0 % (w/w) silane and drying at 100℃ for 3h.

Fig.3 shows the morphology of surface-modified sericite product prepared with the optimal experimental parameters. It can be seen from Fig.3 that the sericite

is in the shape of flakes with a diameter of 3.0-9.0 μm and a thickness of around 0.15 μm, giving an aspect ratio of 20-60. With careful observation, the sericite flakes appear to be quite loose, which revealing that the sericite has an excellent dispersion property. The particle size distribution of surface-modified sericite determined using a Malvern Instruments Mastersizer was –10um, with a D10 of 0.95μ m, a D50 of 2.01μ m, a D90 of 4.93μ m and an average diameter of 3.04μ m.

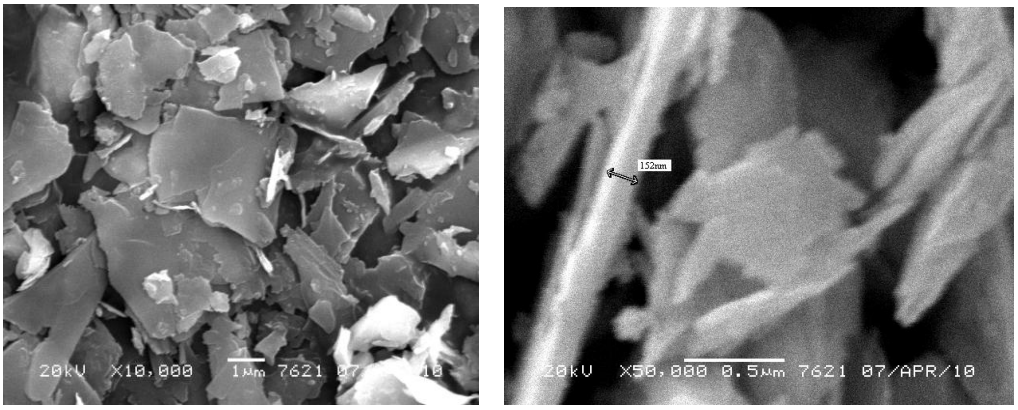

Fig. 3 SEM photographs of sericite sample with optimal experimental parameters

The result of chemical multi-element analysis of sericite is shown in Table 4. It can be seen from Table 4 that the content of Al_2O_3, K_2O and SiO_2 is 28.68%, 5.21% and 45.54%, respectively.

TABLE 4 THE MULTI-ELEMENT ANALYSIS RESULTS OF SERICITE (WT%)

Components	Al_2O_3	K_2O	Na_2O	TFe	SiO2
Content (%)	28.68	5.21	1.14	1.90	45.54

3.4 Applications of Surface Modified Sericite Powder

The mechanical properties of natural rubber and styrene-butadiene rubber filled with sericite were measured as shown in Table 5. The mechanical properties of the PP（polypropylene）plastic with sericite were measured as shown in Table 6.

It can be seen from Table 5~ 6 that surface modified sericite added to the rubber and plastic can raise the rubber and plastic product function and meet different demands for the mechanical properties of the material. Sericite is used as a reinforcing filler in the rubber and plastic industry with particle size of ultra-fine, the lamellar structure of two-dimensional, high aspect ratio and good surface activity. It is an effective method not only for its low cost but also for its broad applications.

TABLE 5 MECHANICAL PROPERTIES OF RUBBER FILLED WITH SERICITE

Formulation characteristics		natural rubber			styrene-butadiene rubber		
		sericite	calcium carbonate	general carbon black	sericite	calcium carbonate	general carbon black
Rubber performance							
Rheometer test of 153℃	maximum torque	28.9	23.0	31.8	39	32.7	36.7
	minimum torque	1.5	1.5	2.6	6.8	4.9	5.1
	T10	5′26″	5′	4′24″	8′48″	17′	13′24″
	T90	12′12″	12′24″	12′12″	15′48″	21′48″	19′21″
Rheometer conditions of 153℃/min		15	15	15	15	20	25
Shore A hardness		49	43	49	49	58	55
Tensile strength /Mpa		21.5	15.9	21.8	21.5	10.5	2.5
Elongation /%		614	807	564	614	842	448
300% stretch stress /MPa		4.8	1.6	6.3	4.8	3.4	1.8
500% stretch stress /MPa		13.7	3.5	16.7	13.7	4.3	--
Permanent deformation /%		49	32	26	49	48	8
Tear strength /KN/M		39	29	39	39	46	18
Elastic /%		59	55	58	59	50	51

TABLE6 MECHANICAL PROPERTIES OF THE SERICITE/PP COMPOSITE MATERIAL

filling ratio of sericite/%	Pure PP	10	20	30	40
Tensile strength/MPa	33.7	32.3	31.4	30.6	27.8
Bending strength/MPa	55.3	54.3	55.6	53.7	49.4
Impact strength/KJ/m2	15.9	13.2	13.9	12.8	10.7
distortion temperature(1800KPa)/℃	71	74	79	86	90

4. CONCLUSIONS

Recovering sericite powder with higher diameter thickness ratio from tailings can not only control the tailing pollution, but also supply new material for rubber and plastic industry. Considering its preferable social, economic and environmental benefits, a technology consisting of classification, flotation and surface modification was developed. The process is economical and environmentally acceptable, relatively simple and easy-to-production implementation, and the experimental results confirm its feasibility.

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Title: *Experimental Research of Filling Oxygen Ability of Ultramicro Bubble Generator*

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ABSTRACT

In order to test the oxygen transfer ability of ultramicro bubble generator, deoxidizer Na_2SO_3 and catalyst C_oCl_2 are added to the water to decrease dissolved oxygen of the water to zero, and then begin to aerate normally, record dissolved oxygen C_t concentration every minute until it increases to a level close to saturation. Aeration equipment oxygenation process belongs to the mass transfer process, so equation is deduced according to two-film theory, and it's conclude through the data into the equation calculation that the total ultramicro bubble generator oxygen transfer coefficient is 0.7264 min^{-1}, oxygen utilization ratio is close to 100%, oxygen filling capacity is 0.01048 kg/h, and aerobic power efficiency is 0.019 kg/(kw ħ).

INTRODUCTION

Oxygen transfer technology can accelerate reoxygenation by water body , make water self-purification process in aerobic condition, and enhance the vitality of aerobic microorganisms, improve the water effectively, increase water turbulence, is advantageous to the oxygen transfer, diffusion and liquid mixture[1]. Oxygen transfer process in the water fits two-film theory. There are two ways to improve the oxygen transfer efficiency in the water: One way is to increase the driving force of oxygen transfer, and another is the gas-liquid interface area. The first method are many, such as pure oxygen aeration method, deep aeration method, etc; another way, such as ultramicro bubble aeration method that become a research hotspot. The diameter of ultramicro bubble is under 50 microns, which has many unique advantages compared with ordinary bubble, mainly as follows: large surface area, rising slowly, large internal stress and rapid dissolving speed, and these advantages are beneficial to mass transfer from gas to liquid[2-4].

In recent years, researchers have developed all sorts of ultramicro bubble generator, Li Haipeng[5] has studied the jet aerator, Xing[6] has invented the distributor of hydrocyclone, Liu Chun[4] has researched hydraulic rotating shearing micro bubble generator. What they have done confirm ultramicro bubble aeration is reinforcement for oxygen mass transfer. In this paper, we study a kind of cycle type gas-liquid mixed

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ultramicro bubble generator[7],which provide guidance for the application of the device by testing oxygen filling ability.

INTRODUCTION OF ULTRAMICRO BUBBLE GENERATOR

Cycle type gas-liquid mixed ultramicro bubble generator(figure 1) is designed in accordance with the fluid mechanics structure. According to certain gas-liquid ratio, by self-priming gas-liquid mixing pump, gas-liquid mixed flow that is pumped into the cycle type gas-liquid mixed ultramicro bubble generator is formed. The mixed flow is high speed rotation under pressure (figure 2), and negative finale on which a gas or a liquid mixture of the gas outside are focused with its suction is formed in the middle of the generator . When high speed rotating liquid and gas spew from specially designed nozzles under the appropriate pressure, due to the vents mixture liquid of ultra high rotating speed and gas - liquid density ratio (1:1000) on the mechanics of multiplying effect, high speed, strong shear and high frequency pressure change on the gas-liquid contact interface to form artificial extreme conditions. Jet aerator on both sides to the water to form a large number of ultramicro bubble.

Fig.1.Ultramicro bubble generator[9]

Dissolution rate of ultramicro bubble in the water is over 85%, the dissolution of dissolved oxygen concentration can reach more than 10 mg/L, and ultramicro bubble is kept in the water for a long time with the form of bubbles (rising speed of 3 m/h) , and dissolved oxygen can be added to the water with consumption ,which for sewage microbial provide plenty of reactive oxygen species, strong oxidizing ionic group and ensure sufficient reaction time of the active oxygen. The water treated by the ultramicro bubble has much higher purification ability than the natural[8].

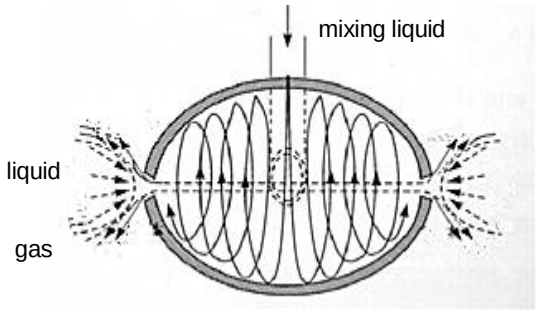

Fig.2. Ultramicro bubble principle[8]

The device provides a bubble generator which can be used for shellfish, oysters, pearl aquaculture industry, large reservoirs or lake water purification, the improvement of fishery waters, also in hot springs or beauty salon, home and business use , at the same time can be a pipe cleaning device of hot spring facilities or sewage treatment plant[8].

EXPERIMENT DEVICE

Experiment device diagram is shown in figure ,which is mainly composed of ultramicro bubble generator, water pump, flow meter, water tanks and other parts. Gas flow inside pipe can be regulated by adjusting the gas flow meter to adjust the amount of ultramicro bubble. All parts of the specific parameters are as follows:

(1) The tank: length*width * height = 50 cm * 30 cm * 30 cm (working volume is 29.27 L); (2) The water pump: type: 1ZDB-45, Q = 30L/min, H=45m; (3) The gas meter: Q_g = 0.6 L/min. (4) Aeration device: BT - 50.

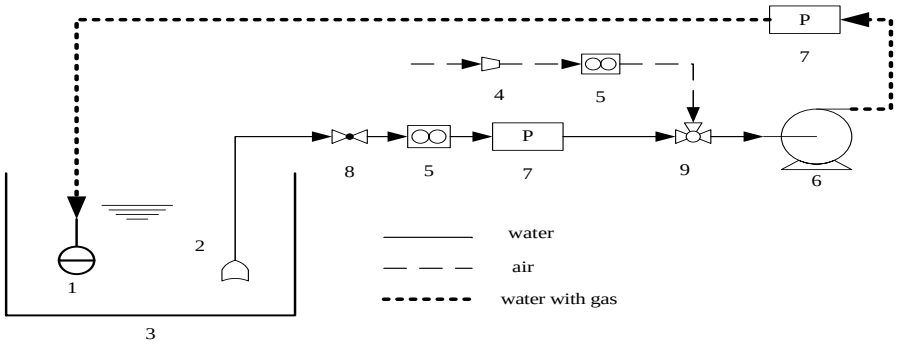

Fig.3. Experiment device

1-ultramicro bubble generator; 2-filter; 3-tank; 4-valve; 5-gas meter6-pump; 7-piezomete; 8- sphere valve; 9- union tee.

EXPERIMENTAL DATA

(1)The Cs is determined by empirical formula:

T is water temperature, $C_s = \dfrac{468}{31.6+T}$ (1)

(2) Calculating quantity of reagent [10]:

The amount of oxygen in the tank : $G = C_S \times V$ (2)

Deoxidizer Na_2SO_3 dosing quantity G_2: $G_2 = 1.5 \times 8G$ (3)

Catalyst $CoCl_2$ G3 dosing quantity: V is the volume of water in the tank,

$G_3 = 0.1 \times V$ (4)

EXPERIMENTAL CONDITIONS

T = 22.4 ℃, saturated dissolved oxygen C_s = 22.4 mg/L (measured), G_2 = 3056 mg, G_3 = 2.927 mg.

EXPERIMENTAL STEPS

Na_2SO_3 and $CoCl_2$ are dissolved with warm water and spread into the water evenly.Put probe of dissolved oxygen meter into the tank, and record the water temperature, dissolved oxygen concentration in the water. When the concentration of dissolved oxygen in water drops to zero, start to aerate. When air bubbles appear on the tank surface, start time and record dissolved oxygen C_t concentration every minute until it increases to a level close to saturation.

RESULTS AND DISCUSSION

Test results are shown in table 1.

TABLE 1 . AERATION TEST RESULTS

Time (min)	Test and calculate						
	C_t (mg/L)	C_s-C_t (mg/L)	(C_s-C_0)/ (C_s-C_t)	ln((C_s-C_0)/ (C_s-C_t))	2.303/ (t-t_0)	K_{La} (min^{-1})	Ln (C_s-C_t)
0	0	8.7	1.00	0			
1	2.2	6.5	3.94	0.595	2.30	1.371	1.8667
2	4.8	3.9	1.81	0.257	1.15	0.295	1.3524
3	6.3	2.4	1.38	0.138	0.77	0.106	0.8615
4	7.4	1.3	1.17	0.069	0.58	0.039	0.2364
5	8.0	0.7	1.08	0.035	0.46	0.016	-0.4055
6	8.4	0.3	1.03	0.014	0.38	0.005	-1.3218
7	8.6	0.1	1.01	0.003	0.33	0.001	-2.7081
8	8.9	-0.2	0.97	-0.012	0.29	-0.003	
9	9.0	-0.3	0.96	-0.016	0.26	-0.004	

The aeration performance of the aeration equipment is available from the following aspects to analyze [11].

(1) The total oxygen transfer coefficient K_{La}

Total oxygen transfer coefficient K_{La} is the number of oxygen transfer in unit volume water at unit time when the aerator is in the standard state (20 ℃, 1 atm) test conditions. K_{La} represents the total oxygen delivery. Oxygen transfer basic equation sees type: $-\frac{dc}{dt} = K_{La}(C_s - C)$ (5)

Cs stands for dissolved oxygen saturation concentration, mg/L; C is dissolved oxygen concentration, mg/L. $C = C_S(1 - e^{-K_{La}})$ (6)

The test results of ln (C_s-C)—t relationship curve is shown in figure 4: K_{La} = 0.7264 min^{-1} is obtained. Compared with the general aerator [12], at the same

temperature, ultramicro bubble generator has higher oxygen transfer efficiency.

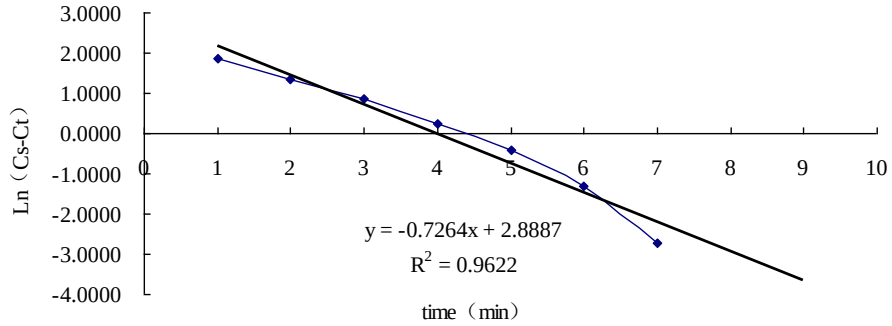

Fig.4. Aeration test results

Concentration of dissolved oxygen saturation , temperature, the nature and degree agitation of sewage factors have effect on rate of oxygen transfer, so in practice,we need to correct pressure and temperature in order to facilitate comparison.The nonstandard conditions K_{La} is converted into standard (20 ℃, 1 atm) under the condition of $K_{La\,(20)}$, following formula.

$$K_{La(20)} = K_{La(T)} \times 1.024^{(20-T)} \tag{7}$$

T is the temperature in testing, ℃; $K_{La\ (T)}$is the total transfer coefficient at T, min^{-1};$K_{La\ (20)}$ is the total transfer coefficient at 20 ℃, min^{-1}.

(2) Ability of filling oxygen OC

OC refers to the quality of oxygen transferred to water dissolved oxygen concentration is zero at unit time.

$$OC = K_{La(20)} \cdot [C_s - C]V \tag{8}$$

OC , kg/h; V is the aeration tank volume, m^3; Cs (standard saturated dissolved oxygen concentration is 8.7 mg/L; C is the actual concentration of dissolved oxygen in water at 20 ℃ , mg/L; In this experiment, it is zero. Therefore, OC = 0.01048 kg/h, aerobic capacity is relatively low because of the height of the pool [11].

(3) oxygen utilization ε

Oxygen transferred into the water accounts for a percentage of oxygen aerator in standard state and test conditions.It is a aeration device evaluation indicator that represent the utilization of the gas.

$$\varepsilon = \frac{OC}{0.28 \times \mathrm{q}} 100\% . \tag{9}$$

This calculation result is close to 100%,which is more than a hundred percent because of the low air flow [11], 0.6 L/min.

(4) Power efficiency of filling oxygen

$$N = \frac{H_b \cdot q_b \cdot v_b}{102 \times 3.6} \tag{10}$$

N is the theory of power, kW; H_b is reading of average barometric pressure gauge, m; ν_b is air density,and it is 1.205kg/m^3at standard state; Q_b is the actual flow of the gas, m^3/h. The power efficiency of filling oxygen:

$$E_P = \frac{OC}{N} \text{ (kW ħ).} \tag{11}$$

Calculated is 0.019 kg/(kW ħ).

CONCLUSION

Through the above of the tests of oxygen aeration equipment performance , the results of analysis show that the oxygen aeration device utilization rate is closed to 100%, total oxygen transfer coefficient K_{La} is 0.7264 min^{-1}, oxygen filling capacity is 0.01048 kg/h, and aerobic power efficiency is 0.019 kg/(kW ħ).

ACKNOWLEDGMENT

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Cover page

Title: *Synthesis of Selenium-Codonopsis pilosula Polysaccharide and Evaluation of Antioxidant Activity in vitro*

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ABSTRACT

The intent of the present investigation was to synthesize a new organic selenium compound, selenium-*Codonopsis pilosula* polysaccharide (SeCPP), and evaluate its antioxidant abilities on hydroxyl radicals and super oxide radicals *in vitro*. Sodium selenite and Codonopsis pilosula polysaccharide (CPP) were used to synthesize SeCPP by chemical method. The content of selenium was determined by inductively coupled plasma mass spectrometry (ICP-MS) and the result was 25.92mg/g. SeCPP was characterized by ultraviolet (UV) and infrared (IR) techniques. The results proved the successful synthesis of SeCPP. The antioxidant activities on hydroxyl radicals and super oxide radicals of SeCPP were investigated. The results showed that in the setting concentration range, the antioxidant activities increased with the elevation of concentration. The antioxidant activities of SeCPP were better than that of CPP. The clearance rates on superoxide anion radical of 2.0mg/mL CPP and SeCPP were 47.05% and 54.46%, respectively, while the scavenging rates on hydroxyl radical of 2.0mg/mL CPP and SeCPP were 50.20% and 61.75%, respectively. The results suggest that SeCPP should be applied as a novel selenium source in dietary supplements, with potent antioxidant properties.

INTRODUCTION

Codonopsis pilosula belongs to the family of Campanulaceae and its root usually applies to Chinese medicine. It is beneficial to the immune, digestive and hematopoietic systems, so that already applied for treatment of dyspepsia, poor appetite, fatigue and psychoneurosis [1]. It was reported that polysaccharide was one of the effective ingredients [2].

Selenium (Se) is an essential element for nutrition of a capital importance for humans [3]. It has been shown obvious anti-aging, anti-cancer and immunostimulatory effects. It is generally believed that organic selenium compounds are better and safer than inorganic selenium. Except some selenoproteins, natural or synthetic selenium

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polysaccharides compounds are explored as a novel selenium source in dietary supplements [4-5]. However, the study on selenium-*Codonopsis pilosula* polysaccharide (SeCPP) is limited.

Therefore, the objective of this study was to prepare SeCPP and evaluate its antioxidant abilities, including scavenging effects on hydroxyl radicals and superoxide radicals.

MATERIALS AND METHODS

Materials

Codonopsis pilosula polysaccharide (CPP) crude extract was purchased from XiAn TianYi Biotechnology Co. Nitro Blue tetrazolium(NBT), phenazine methosulfate(PMS), nicotinamide adenine dinucleotide-reduced(NADH), ethylenediaminetetraacetic acid(EDTA) were purchased from Sigma Chemical Co. All other chemicals used were of analytical grade and were obtained from Tianjin Kemio Chemical Co.

Purification of CPP

The crude polysaccharide was deproteined according to the Sevage method. Briefly, 10.0g CPP extract was dissolved in 100mL deionized water and then was treated with sevage reagent (1-butanol:chloroform=1:5) fully oscillated and centrifuged. The process was repeated until the supernatant was no free protein. The mixture was added into 3V ethanol in order to be precipitated at 4℃ over night. The precipitate was filtrated and lyophilized. Total sugar content of polysaccharide was quantified by phenol-sulphuric acid method, and glucose as the standard.

Synthesis of SeCPP

1.0g purified CPP was dissolved in 25mL deionized water and mixed with 1mL acetic acid. The solution was stirred until clear, then was added into 1.0g sodium selenite. After 48h at 50℃, the mixture was added into 3V ethanol in order to be precipitated. The precipitate was centrifugated and washed twice with ethanol. After dialysis, the production was obtained by lyophilization.

Property and Characterization of SeCPP

UV spectra were measured by a Lambda 25 UV-VIS spectrophotometer. IR spectra were measured by a 370 DTGS IR spectrometer with KBr disks. The content of selenium was determined by a 7700X Series inductively coupled plasma mass spectrometry (ICP-MS).

Scavenging Activity of Hydroxyl Radical and Superoxide Radical

The method of scavenging activity of hydroxyl radical and superoxide radical was according to Jin et al [6].

RESULTS AND DISCUSSION

Purification of CPP

Total sugar content of CPP was 78.95% quantified by phenol-sulphuric acid method and glucose standard curve.

Synthesis of SeCPP

SeCPP prepared was amorphous brown powder, odorless. The yield was 43.65% and the content of Se was 25.92mg/g.

Structure Analysis

The UV spectrum (Fig.1) showed that the optimum absorption peak turned from 277nm(CPP) to 279nm(SeCPP) and the peak of 225nm of CPP disappeared. The differences confirmed the structure changes between CPP and SeCPP. In the IR spectrum(as shown in Fig.2), compared with CPP, hydroxyl bending vibration absorption peak shifted from 1027 cm^{-1} to 1022 cm^{-1}. In addition, Se=O absorption peak was observed near 802 cm^{-1} in SeCPP IR spectrum. According to the results, Selenite was probably linked to C_6 hydroxyl group.

Scavenging Activity of Hydroxyl Radical

As seen in Fig.3, the scavenging effects of SeCPP and CPP on hydroxyl radicals were concentration related and the effect of SeCPP was better than CPP. For example, the scavenging effects of 2.0mg/mL CPP and SeCPP were 50.20% and 61.75%, respectively.

Vitamin C (V_C), a well-known antioxidant, was used in our assay as a standard and antioxidant activities of samples were compared with V_C. The results showed that the scavenging effect on hydroxyl radicals of Vc was better than that of SeCPP and CPP.

Scavenging Activity of Superoxide Radical

As shown in Fig.4, the inhibiting activities of SeCPP and CPP increased with increasing concentration from 0.125 mg/mL to 2.0 mg/mL. SeCPP showed better scavenging ability on superoxide radicals than CPP. At the concentration of 2.0mg/mL, the scavenging rate to superoxide radicals of SeCPP and CPP were 54.46%and 47.05%, respectively. Vc also indicated better scavenging activity. This was comparable to the scavenging activities on hydroxyl radicals.

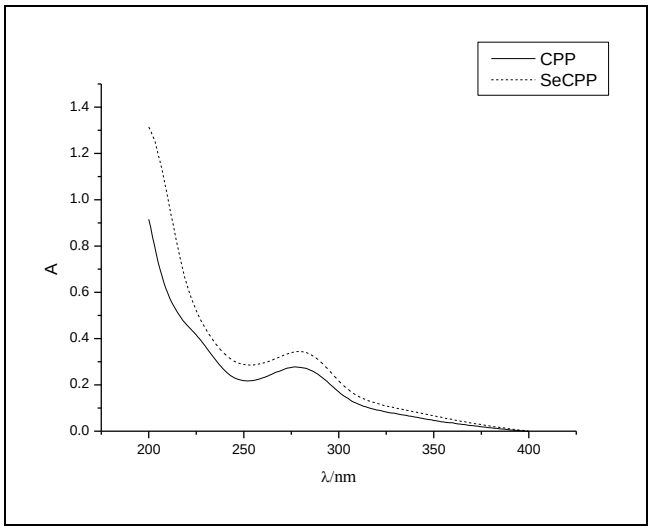

Figure 1. UV Spectrum of CPP and SeCPP

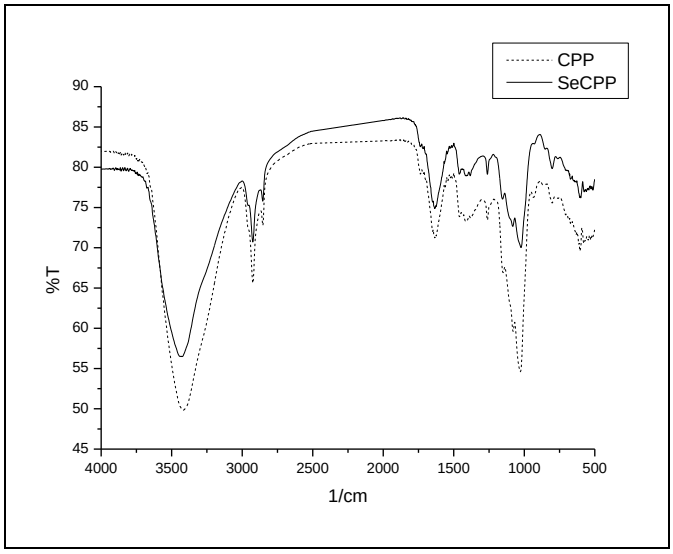

Figure 2. IR Spectrum of CPP and SeCPP

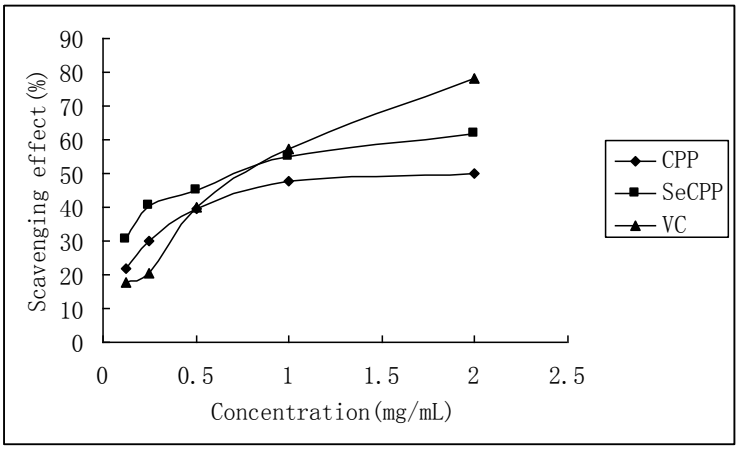

Figure 3. Scavenging effect on hydroxyl radicals of CPP, SeCPP and V_C

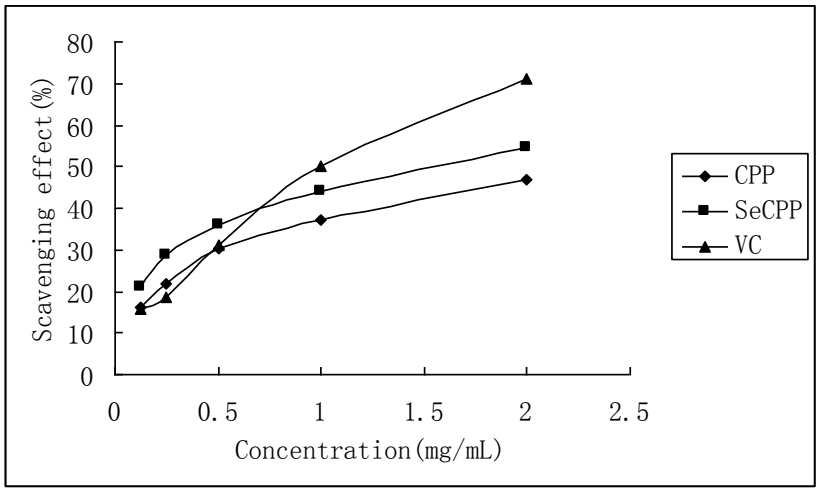

Figure 4. Scavenging effect on superoxide radicals of CPP, SeCPP and V_C

CONCLUSION

Diet is the major source of Se for general population and its bioavailability comes mainly from Se organic forms (generally more than 80%). In this paper, a kind of new organic selenium compound, SeCPP, was prepared successfully. It showed the concentration related scavenging ability on hydroxyl radicals and superoxide radicals which were better than that of CPP. The reason was probably due to the introduction of Se, which also had antioxidant ability itself. It is well known that Se is a cofactor of GSH-Px, which is involved in cellular protection from severe oxidation by free radicals.

Hydroxyl radicals and superoxide radicals are main reactive oxygen free radicals in living organisms, which could cause the general processes of aging and tissue damage. Although the scavenging ability on radicals of SeCPP is slightly lower than Vc, it is low toxic, biocompatible, fully biodegradable. SeCPP could potentially serve as a dietary supplement of Se, an antioxidant, or an ingredient for the formulation of nutraceuticals.

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Cover page

Title: *Establishment and the Early Growth of a Bio-Energy Plantation with Fast-Growing Salix Integra Thrub. Trees in Zhejiang (China)*

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ABSTRACT

Short Rotation Coppice was established with *salix Integra* Thrub branch cuttings on a former paddy field in Zhejiang (30 °126'N, 119 °847'E). The plots were planted in a completely randomized block design with cutting thickness. Ability to sprouting and rooting were investigated after 45 day's branch cutting. The result showed that sprout number, sprout diameter and sprout length increased with increasing cutting thickness. The thicker the cutting, the better total root length and worse average root diameter. With increasing cutting thickness, the shoot biomass and underground biomass increased significantly. The biomass of different modules among shoot-leaf and aboveground-underground was in a linear isogony growth.

INTRODUCTION

Fast-growing trees are one of the most promising alternatives for the production of biomass when planted in a Short Rotation Coppice (SRC) regime [1-2]. Willow (*Salix* spp.) are the most commonly used species for SRC in the subtropical region in China because of its high growth rate and biomass yield, its easy vegetative propagation from cuttings and coppice ability [3]. Numerous studies have been carried out to estimate the genetic adaptability, vegetative propagation, hybridization and growth and yield with commercial operations and large-scale tests of SRC in the mainly EU and America countries [4-6].

Willows are generally propagated and grown from hardwood cuttings with holding the genetic stability and consistency; going on with the propagate growth stage and shortening life cycle; lessening intraspecific competition and increasing the growth and yield [7-9]. In the present, the methods of hardwood cutting were mainly focused on survival rate, sprouting and rooting under the different cutting nutrients and thickness. One the one hand, use of small cuttings resulted in lower survival for lower N, P and K mineral element and carbohydrates during the early stages of plant development [10]. On the other hand, the thicker the hardwood cutting, the higher sprout number and sprout length and biomass [6]. In fact, willow sprouts originate from dormant buds and adventitious root from skin holes. As a result, the number of the sprout and adventitious root is probably affected by cutting

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thickness. Secondly, the nutrient competition between sprout and root may happen because of adhering to the same cutting. To test these hypotheses, we aimed to study the influence of cutting thickness on the process of sprouting, rooting and initial growth of *salix Integra* Thrub. plants, and also to estimate the growth relationship between sprouting and rooting.

MATERIALS AND METHODS

Plant Material and Plantation Establishment

The experimental site is located in Fuyang city (30°126'N，119°847'E), about 60 km from Hangzhou City at an altitude of 14.10 m above sea level. The long-term average annual temperature at the site is 6.2 ℃. Average annual precipitation is 1 452 mm. The region of the field site is pedologically described as a sandy red soil. The former land use was planted rice. Prior to planting, the seed bed was manufacture with 40 cm height, 200.00cm width and width between bed 50.0cm.

Hardwood cutting were obtained from a 3-year-old germplasm collection field of *salix Integra* Thrub. at the institute of subtropical forestry research of Chinese academy of forestry. Cutting thickness was determined with T_{C1}(0.5-1.4cm),T_{C2}(1.5-2.4cm）and T_{C3}(2.5-3.4cm). Completely randomized design was used in this experiment with 3 replicates. Each replicate includes 50 cuttings.

Measurements

At 45 days after planting, we surveyed sprout number (N_S), sprout length (L_S) and sprout basal diameter (D_S) in the field. Total root length (T_{RL}) and average root diameter (A_{RD}) were obtained by Win Rhizo root scanning (regent instruments, Québec, Canada). Root biomass (B_R), shoot biomass (B_S) and leaf biomass (B_L) were weighed after drying, respectively. Aboveground biomass (B_A) is summed with B_S and B_L and underground biomass (B_U) equals to B_R.

Statistical Analysis

Analysis of variance (ANOVA) and generalized linear regression model was used to analyze the influence cutting thickness. A *p*-value smaller than 0.05 was considered significant with least significant difference (LSD). The software package SPSS (SPSS Inc. Chicago, IL, USA) was used for all analysis.

RESULTS

Sprout Growth with Cutting Thickness and Underground Depth of Cutting

Sprout number, sprout basal diameter and sprout length were greatly influenced by cutting thickness ($p<0.01$). Sprout basal diameter, sprout number and sprout length increased with increasing cutting thickness (TABLE I). LSD showed that cutting thickness had the significant difference on sprout number (Figure 1-A), sprout basal diameter (Figure 1-B) and sprout length (Figure 1-C). Compared with

the T_{C1}, the sprout number with T_{C2} and T_{C3} increased 24.72% and 48.36, the sprout basal diameter with 2.19% and 38.25% and sprout length with 30.32% and 47.61%.

TABLE I. REGRESSION ANALYSIS OF N_S, L_S, B_S, B_L, B_A AND B_U OF *SALIX INTEGRA* THRUB. WITH T_C AND T_D.

Dependent Variable	Regression equation	Significance level	Correlation coefficient
N_S	$N_S=0.67\times T_C+7.70$	0.040	0.619
D_S	$D_S=0.07\times T_C+1.98$	<0.01	0.676
L_S	$L_S=0.78\times T_C+6.52$	<0.01	0.681
B_S	$Y=0.07\times T_C+0.29$	<0.01	0.824
B_L	$Y=0.10\times T_C-1.10$	<0.01	0.852
B_A	$Y=0.17\times T_C-8.37$	<0.01	0.868
B_U	$Y=0.009\times T_C+0.01$	0.011	0.712

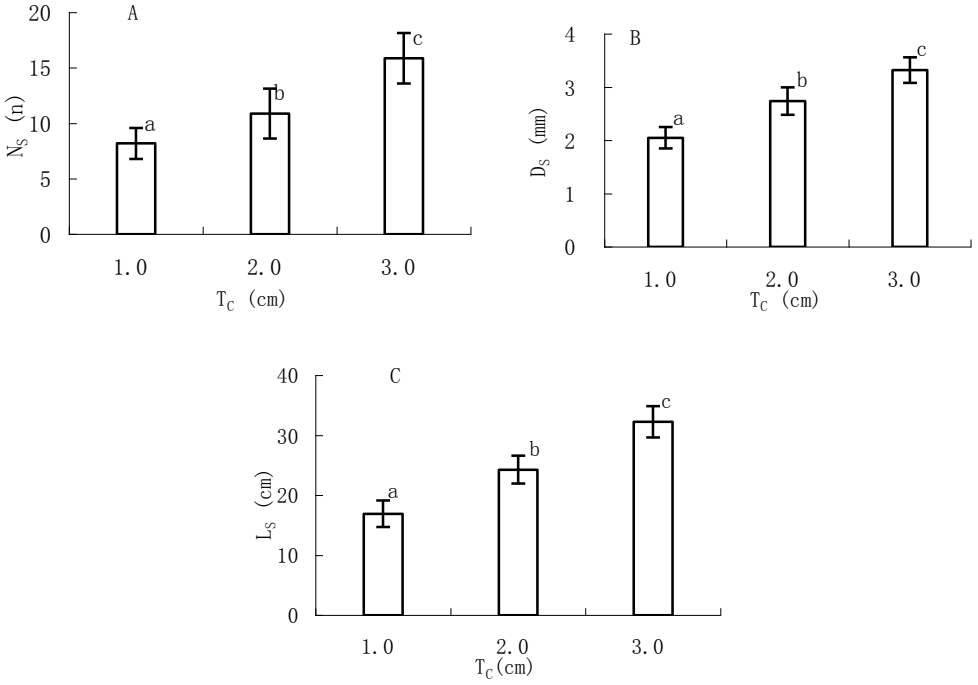

Figure 1. N_S, D_S and L_S of *salix Integra* Thrub. in the different T_C.

Root Growth with Cutting Thickness and Underground Depth of Cutting

The cutting thickness had greatly effect on T_{RL} and A_{RD} ($p<0.05$). The regression equation between cutting thickness and T_{RL} is $T_{RL}=1.442\times T_C+141.27$($R^2=0.42$), which mean that T_{RL} increased with increasing cutting thickness. The regression equation between cutting thickness and A_{RD} is $A_{RD}=-0.048\times T_C+7.877$($R^2=0.58$), which mean that A_{RD} decreased with increasing cutting thickness (Figure 2).

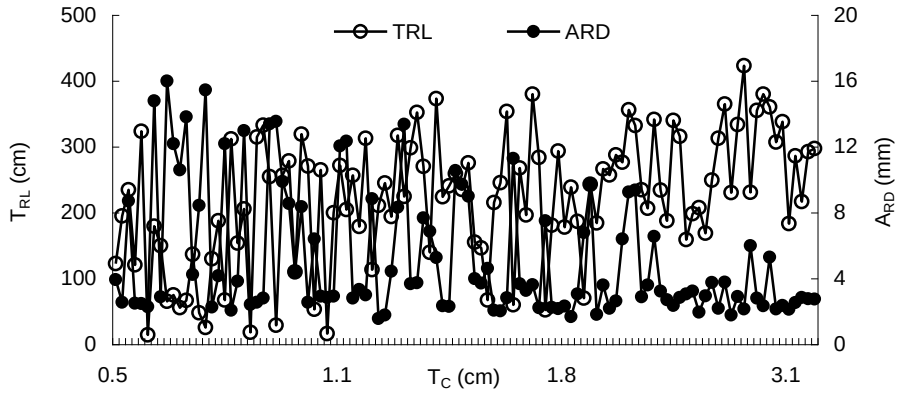

Figure 2. T_{RL} and A_{RD} under the different T_C.

Biomass Allocation

Shoot biomass, leaf biomass, aboveground biomass and underground biomass increased with increasing cutting thickness (TABLE II). In addition, the isogony growth relationship between aboveground biomass and underground biomass, and shoot biomass and leaf biomass existed based the linear regression equations (Figure 3).

TABLE II. BIOMASS ALLOCATION WITH T_C AND T_D OF *SALIX INTEGRA* THRUB. CUTTING (AVERAGE ± S.D.)

T_C (cm)	B_U (g)	B_S (g)	B_L (g)	B_A (g)
0.5-1.4	0.09^{a} ±0.07	0.38^{a} ±0.13	0.83^{a} ±0.29	1.21^{a} ±0.50
1.5-2.4	0.28^{b} ±0.02	1.20^{b} ±0.49	2.35^{b} ±0.48	3.55^{a} ±0.46
2.5-3.4	0.43^{c} ±0.08	2.05^{c} ±0.11	3.99^{c} ±0.23	6.04^{a} ±1.27

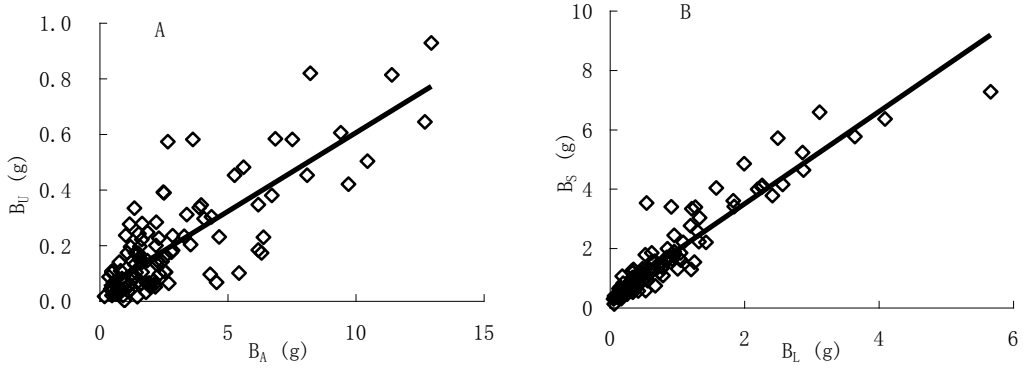

Figure3. Correlation between aboveground biomass and underground biomass, and leaf biomass and shoot biomass

RESULTS AND DISCUSSION

The dormant buds and lenticelles increased with increasing cutting thickness of *salix Integra* Thrub. Meanwhile, the thick cutting contained the high N, P and K contents and then has priority to supply the sprout growth [10]. That is why the thick cutting sprouted the thick sprout. Carbohydrate can guarantee energy source and is one of the most important structural material. The sprout can compound auxins and cytokines, which auxin can stimulate meristem rooting and promote root growth and cytokines can restrain adventitious root development [1]. As a result, the contents of auxin can exceed to some a threshold value can promote cutting rooting and root growth. In fact, the thicker cutting with the higher lignifications, the contents of carbohydrate before cutting dormancy, which can supply nutrient element and power during cutting rooting of *salix Integra* Thrub. The sprout number can be determined with cutting thickness. The more root number and the smaller average root diameter, the thicker cutting diameter, vice versa.

The more nutrient element with the thicker cutting can guarantee shoot, leaf biomass growth demand. Furthermore, the higher aboveground biomass can produce high content of auxin, which can stimulate cutting rooting and root growth. In addition, the positive linear correlation exists between aboveground biomass and underground, and between leaf biomass and shoot biomass, which indicates that the isogony growth happens with the different biomass components.

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Cover page

Title: *The Elemental Property of Whole Peptidoglycan Extracted from Lactobacillus. Paracasei Subp. Paracasei X12*

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ABSTRACT

In this study, whole peptidoglycan (WPG) was extracted from *Lactobacillus. paracasei subp. Paracasei* X12 strain and was estimated for the elemental property. SDS-PAGE analysis showed that the presence of whole peptidoglycan with dominant bands of approximately 14 kDa. Further analysis showed that amino acids present in the whole peptidoglycan consisted of alanine, glycine, glutamic acid and lysine. Scanning electron microscopy and transmission electron microscope investigation revealed the physical structure of whole peptidoglycan. The results suggested that whole peptidoglycan was unvaried during the isolation and chemical purification procedures and retained the shape of the whole cells completely.

INTRODUCTION

Lactobacillus, which is the most important [1] and probably the best studied microorganism among probiotics currently, in suppressing of tumors and enhancing immune response have been receiving increasing research attention [2]. The ability of several natural products (whole cells, heat-kill cells, cell walls, cytoplasm and DNA) from lactobacilli to inhibit cancers had been well established. Our group has previously suggested that the whole cells [3] and cell wall from *Lactobacillus. paracasei subp. Paracasei* X12 exerted anticancer activity against human colon cancer HT-29 cells. The gram-positive cell wall is mainly composed of peptidoglycan, the anticancer activity of cell wall of *Lactobacillus. paracasei subp. Paracasei* X12 strain might be attributed to peptidoglycan fractions. In this study, we presumed that anticancer activity of cell wall was contributed to whole peptidoglycan, and then investigated the elemental property of whole peptidoglycan of *Lactobacillus. paracasei subp. Paracasei* X12 strain.

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MATERIAL AND METHODS

Lactobacillus Strain and Culture Conditions

Lactobacillus. paracasei subp. Paracasei X12 strain was obtained from traditional cheese in Sinkiang, China [3]. The strain was cultured in de Man, Rogosa and Sharpe (MRS) (Difco) broth with 0.05 % (w/w) L-cysteine. The strains was subcultured twice before use and identified by 16S rRNA gene sequences [4].

Preparation of Whole Peptidoglycan from Lactobacillus Strain

Whole peptidoglycan was extracted from *Lactobacillus. paracasei subp. Paracasei* X12 strain according to the method of [5]. The protein content of whole peptidoglycan was determined by the coomassie brilliant blue method and whole peptidoglycan was stored at -80 ℃ until use.

SDS-PAGE Analysis of Whole Peptidoglycan

Whole peptidoglycan was suspended in 100 mL loading buffer. Gel electrophoresis with sodium dodecyl sulphate on 10 % polyacrylamide was carried out to compare the whole peptidoglycan. Bio-Rad SDS-PAGE broad-range molecular markers with molecular masses ranging from 14.4-116.0 kDa were used.

Amino Acid Composition Analysis of Whole Peptidoglycan

1.5 mL whole peptidoglycan at the concentration of 1mg mL^{-1} was mixed with 1.5 mL of 6 M HCl. After sealing the ampoule, the whole peptidoglycan was hydrolyzed under nitrogen at 110 ℃ for 24 h. The resulting solution was mixed with 1.5 mL of 6 M NaOH for neutralization and then adjusted to 5 mL with 0.02 M HCl. Amino acid compositions were measured via Hitachi L-8800 amino acid analyzer (Hitachi Corp., Japan).

Electron Microscopy Investigate the Morphology of Whole Peptidoglycan

Whole peptidoglycan was immersed in 2 % glutaraldehyde and was prepared for investigation using scanning electron microscopy (S-3400N, Hitachi Corp., Japan) [6]. Whole peptidoglycan was fixed with 2 % glutaraldehyde and was dehydrated by successive treatment with methanol and was investigated via Hitachi H-7650 transmission electron microscope (Hitachi Corp., Japan) [5] .

RESULTS AND DISCUSSION

The Protein Content of Whole Peptidoglycan

The protein content of whole peptidoglycan from *Lactobacillus. paracasei subp. Paracasei* X12 was determined by the coomassie brilliant blue method (Figure. 1). Calculated using the equation: Y= 0.0038X+0.0055. The protein content of WPG was 13.92 μg/mL. SDS-PAGE analysis revealed the presence of whole peptidoglycan and the major molecular masses were approximately 14 kDa (data was not showed).

Amino Acid Composition of Whole Peptidoglycan

Sekine et al (1985) suggested that whole peptidoglycan had a physically intact skeleton structure of cell wall. Whole peptidoglycan was composed of chains of peptidoglycan monomers cross-linked by short peptide bridge. The composition of the peptide bridge consists of several amino acid residues.

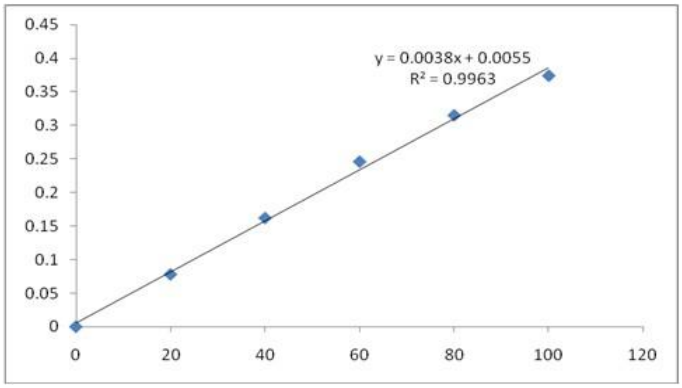

Figure 1. Concentration of Coomassie brilliant blue G-250 standard curve, Concentration of Coomassie brilliant blue G-250 (μg/mL).

Aiming to obtain further information, we analyzed the amino acid compositions of whole peptidoglycan of *Lactobacillus. paracasei subp. Paracasei* X12 strain. Results suggested that whole peptidoglycan contained alanine, glutamic acid, glycine and lysine, the approximate molar ratio of alanine, glutamic acid, glycine and lysine was 8:5:3:3 (Table 1). Generally speaking, Alanine, glutamic acid, glycine and lysine, which were common primary structure in peptidoglycan, were found in cell wall preparations from bacterias [7,8].

TABLE I. THE AMINO ACID COMPOSITION OF WPG PROTEINS.

Amino acid	X12 nmol
Asp	11.56
Thr	6.20
Ser	4.25
Glu	10.28
Gly	6.17
Ala[a]	16.07
Val[a]	4.07
Met[a]	5.44
Ile[a]	4.02
Leu[a]	6.68
Tyr	2.69
Phe[a]	2.37
Lys	6.86
NH3	11.55
His	2.12
Arg	2.96
Hydrophobic amino acids (%)	38.65

The Morphology of WPG

In our study, scanning electron microscopy (Figure. 2a and b) and transmission electron microscope (Figure. 3b) investigation revealed the physical structure of WPG was unvaried during the isolation and chemical purification procedures and retained the shape of the whole cells (Figure. 3a) completely. Similarly, Sekine et al (1985) suggested that whole peptidoglycan completely retained the intact cell wall structure.

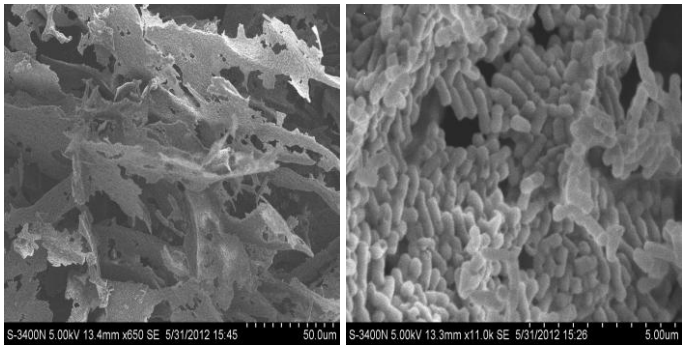

Figure 2.WPG extracted from X12 strain was investigated via scanning electron microscopy, original magnification 1000 × (a) and original magnification 11000 × (b).

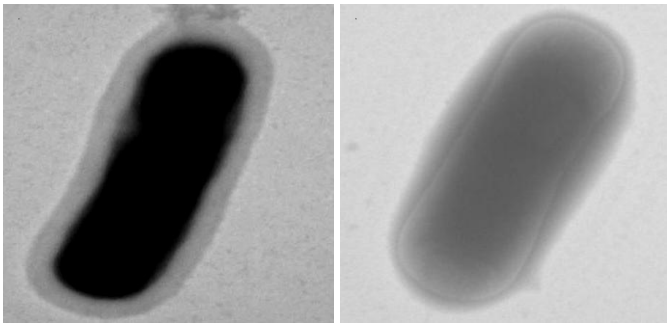

Figure 3. Transmission electron microscope investigated (original magnification 10000 ×) the whole cell (a) and WPG of X12 strain (b).

CONCLUSIONS

In this study, we investigated the elemental property of whole peptidoglycan of *Lactobacillus. paracasei subp. Paracasei* X12. Our study suggested that whole peptidoglycan had a physically intact skeleton structure of cell wall. Therefore, the anticancer activity of cell wall from *Lactobacillus. paracasei subp. Paracasei* X12 was contributed to whole peptidoglycan, which represent viable anticancer candidate for further investigation, currently in progress in our laboratory.

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Cover page

Title: *Planning Staff as an Actor of Geographic Information Systems (GIS) Translation in the Planning Departments of the Kuala Lumpur City Hall, Malaysia*

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ABSTRACT

The context of the planning organisation and the operation of political processes in the planning organisation have a substantial impact on the outcome of computerisation. However, there is also a need to consider the activities and characteristics of the individuals. The activity of individual planning staff can substantially affect the development and utilisation of computer-based systems. The aim of the paper is to identify the contributions of individual planning staff as an actor of GIS translation at the Department of Urban Planning (DUP) and the Master Plan Department (MPD) in the Kuala Lumpur City Hall (KLCH) Malaysia. The paper employed a semi-structured interview and observations of the activities of the department. The findings demonstrate that the skill, knowledge and training of the planning staff has made a highly significant contribution in obtaining the resources necessary for the translation of computer-based information systems in the MPD and the DUP. It is proved that the manner in which these factors interact with the GIS determines the processes which affect the utilisation process of automated systems. This means that the purchasing of GIS by the planning organisation is frequently associated with the awareness and readiness of the individual users of the planning staff. Consequently, each planning staff must possess the necessary ability, willingness and intimate knowledge to implement and use GIS successfully.

INTRODUCTION

Many researchers have highlighted issues in using information technology such as GIS, which are not only limited by hardware and software but depend largely on how individual users accept and utilise the technology [1], [2], [3], [4], [5]. Individual planning staff can perform an important role in the process of GIS translation in planning organisations. It is also important that the role played by the planning staff includes the necessary ability, willingness and awareness to implement and use GIS in the planning organisation. In many instances, key

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individuals often play an important role in both the initial acquisition of new technology and the subsequent processes of achieving effective utilisation [6].

The activity of individual planning staff can substantially affect the development and utilisation of computer-based systems. It is emphasised that individual staff members within planning organisations have different values and motivations, and that computerisation tends to challenge their interests; threatening some and offering opportunities to others. This suggests that individual planning staff can perform an important role in the process of GIS implementation in planning organisations. It is also important that the role played by the planning staff includes the necessary ability, willingness and awareness to implement and use GIS in the planning organisation. The interpersonal relationships between the users and the technical specialists have a significant impact on the development of information system [5], [7], [8]. This means that some planning staff with computer knowledge and skill in the planning organisation tends to be more interested in the design of GIS. As experts, they become involved in the implementation and utilisation processes of GIS in the planning organisation. They are more likely to act in accord with their perceptions of what the planning staff regards as desirable so as to avoid harming their career prospects.

METHODOLOGY

In total, 16 respondents were selected for the interview. They were planning officers and top managements officers involved in the development of GIS for the planning activities. They were selected based on their educational backgrounds and experiences in urban planning departments, and their roles in the planning departments concerning policy-making, management and research. This was undertaken in order to ensure that they have a relatively high level of knowledge and expertise in the field. At the same time, they are also involved in managerial and decision-making positions which indicate that they have influence over policy and/or practice on the ground. The analysis of data was conducted through a content analysis technique. In this study, data from the interviews were transcribed and the results are presented in the form of content analysis in order to support and provide more insight into the issues discussed. The 16 respondents interviewed include three planning directors (PD), two deputy directors (PDD), four senior town planning officers (STP), and seven planning officers (PO) from the planning departments and agencies. Each respondent was given a code according to their position. For example ‘PD1’ represents respondent number 1 of the three planning directors interviewed.

ANALYSIS AND FINDINGS

Perceptions of GIS Management

The management officers agreed that GIS plays a major role in planning processes. They suggested that GIS is the right tool for urban planning activities such as storing data, keying-in data, retrieving data, sharing data and processing planning applications. A Planning Director (PD2) states:

"Town planning by profession or nature of work is a multi-disciplinary activity; it is not a specialised job like engineering. To carry out town planning, you have to accommodate all disciplines in order to obtain the end-results. So, we often need to refer to other agencies just to come out with a decision on how to advise somebody. We need data from other agencies, and that is where we become dependent on other agencies' technology. If other agencies are using IT, we need to use technology (GIS) also; otherwise we cannot obtain the data (from other agencies)." PD2

PD2 further comments about the need of GIS in planning activities:

"…Basically it is a natural move from the ways of doing jobs manually to the digital approaches…so you know that…I do not have to get into details…because you know the benefits of digital technology…we are now in the IT era, so it is not a question of to consider or not to consider…it is just a natural step from preparing plans manually with the introduction of computer…so you obviously exploit this technology…and you exploit the technology of IT." PD2

The management officers believed that moral support obtained from the management level is a very important aspect that influences the utilisation of GIS in planning departments. The use of technology is strongly influenced by users' understandings of the properties and functionality of a technology [9]. This view has been strongly supported by the encouragement and support from officers within the departments. When questioned whether the mayor supports the application of GIS at the DUP, a Deputy Director from the department made a similar comment:

"…our director shows us the benefits of GIS for our planning activities." PDD1

Another senior town planning officer (STP2) made a similar comment:

"We have support from the top-management to use GIS." STP2

According to STP2 (a Senior Town Planning Officer), the supports that they had received are mainly from the planning director and the mayor. He adds that their Planning Director is aware of the importance of GIS for the planning works, especially in plan making.

Perceived Benefits of Using GIS

This section identifies the opinions of the management officers on the perceived benefits of using GIS in planning departments. With more than ten years in implementing and adopting GIS in the planning departments, the staff at the DUP, KLCH can eventually see the benefits of GIS. All the management officers indicated six main benefits of using GIS in the planning departments. They are time saving, ease of use, using GIS in decision-making processes, improved data management, improved data sharing and data standardisation and centralisation. The results presented here are in line with results of the questionnaire survey discussed above and studies by researchers on this subject [1], [5], [10], [11], [12].

All the management officers agreed that GIS has helped them to save time in processing planning applications, preparing and printing maps and plans, producing

reports, colouring plans and checking plans. A Planning Director (PD1) certainly agreed that GIS has helped planning staff to save time, for example, on the use of GIS to print plans. According to PD1:

"…definitely it (GIS) will save time" PD1

Other than GIS as a planning tool, most of the management officers agreed that GIS has assisted planning decision-making processes because of the quality of GIS data: it is accurate, useful, complete, reliable and current.

DISCUSSION

The relationship between individual planning staff and GIS suggests that all socio-technical relationships are products of both users and technology. The findings indicate that all planning staff at the MPD and the DUP view GIS as a means to accomplish tasks more quickly and easily, improve data management, improve data sharing, standardise and centralise data, save time, increase productivity effectiveness, improve decision-makings, reduce workloads, improve job performances and derive personal benefits in terms of improved professional performances and prestige. The findings show that users' satisfaction is somewhat different for direct and indirect GIS users. Indirect users are those who make use of the technology by relying on other members in the department. For direct GIS users, ease of use, time saving, exploitation of technology, data sharing, data management, improved decision-makings, trainings and documentations are all important for achieving satisfaction. As suggested by [4], and [13], regardless of the type of GIS use, quality, timeliness, accuracy, format, reliability, and completeness of the GIS products are of central concern in evaluating user satisfaction.

The results of the interview suggest that the Planning Director has made a highly significant contribution in obtaining the resources necessary for the development of computer-based information systems in the MPD and the DUP. A primary function of this leadership role is to set clear goals and objectives, to win acceptance among end users for such goals and objectives, and to provide the commitment which enables these goals and objectives to be realised in the utilisation process. The role of the Planning Director can be made easier if he/she receives support from the middle management. In addition, it is often found that the technical skills and interests of the Planning Director play an important role in encouraging the use of GIS among planning staff [2], [5], [8]. As GIS is rather new in the planning departments of the KLCH, the planning heads and officers need to gain more skills before they can provide leadership in promoting the use of GIS in their departments. They also need to have a generally good comprehension and appreciation of computer applications.

The emphasis that the Planning Director places on the role of GIS/information system in strategic planning processes is also significant. As a result, an information management strategy (the Development Control System within the DUP) has been developed which gives consideration to data accessibility and associated issues such as staff training. This in turn has encouraged the adoption of a centralised approach in the use of GIS in planning departments. Consequently,

these findings suggest that the activities of the Planning Director and supported by the Mayor, the Planning Officers, and all planning staff have been responsible for the creation of a favourable internal organisational context in which to utilise GIS in planning departments.

This study has revealed the relationship between the planning staff and GIS as mutually productive, where GIS will not only affect the working practices of the planning departments but where its adoption will impact upon GIS itself. It has been frequently argued that, as GIS becomes embedded in current practice, greater numbers of potential users will become more aware of it and its use will therefore become more widespread. Concurrently, this will promote investments and developments of the software and, as systems become refined and standardised as good practice, it will be increasingly difficult for users to circumvent the system.

CONCLUSION

The development of GIS has progressed dramatically in recent years and its use has proliferated in government planning departments. The findings of this study have profound implication for the designs, implementations and organisations of information systems. This study has identified that there is a strong contribution of the socio-technical factors to an understanding of GIS usage in the DUP and the MPD, KLCH between organisational contexts, people and technology. It has been proven that the manner in which these factors interact with GIS determines the processes which affect the utilisation process of automated systems. This study has demonstrated that a situation of mutual dependency between individual planning staff which influence the translation of computing technology and, at the same time the technology, has various impacts (benefits and problems) on planning departments and staff involved. Thus, the identification of the pertinent factors and the manner in which they interact enable greater understanding of the processes affecting the effective use of GIS in the DUP and the MPD, KLCH.

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Cover page

Title: *Studies on the Floral and Pollen Morphology of Agastache Rugosa*

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ABSTRACT

Agastache rugosa was used as Chinese traditional medicine for the treatment of cholera, vomiting and miasma, and seeds were its breeding material. However, the pollen characters of *A. rugosa* have not been studied before, and the available literature provided little information on the genus pollen. The pollen characters of *A. rugosa* were studied by using stereoscope and scanning electron microscope (SEM). The results showed, pollen grains were bilateral symmetric, heteropolar, subprolate in polar view, broadly elliptical in equatorial view, and belonged to medium size, P 32-38 μm × E 14-18 μm. Flowers in verticillasters, in terminal unbranched spikes; 1 or 2 basal verticillasters interrupted. Calyx tubular, 5-6mm long, glabrous. Floral and pollen micro-morphological features proved to be a good taxonomic marker between *A. rugosa* and another plant.

1. INTRODUCTION

Agastache rugosa (Fisch.et Mey.) O. Kuntze., a plant of the Lamiaceae family, is a perennial herb widely distributed in East Asian countries, such as China, Korea, Japan and so on. It is used as Chinese traditional medicine for the treatment of cholera, vomiting and miasma, and it has been reported to have antitumor, antifungal, antiatherogenic and cytotoxic activities [1-3]. This plant is increasingly cultivated in China to satisfy the rising demand for its essential oil by aroma therapists and herbalists. In Korean, it is also used as a wild vegetable and herbal drug for the treatment of anorexia, vomiting and other intestinal disorders [4-6].

Pollen structures were morphological attributes which can be used to distinguish related taxa or to integrate them into a common group [7]. So far, the pollen characters of *A. rugosa* have not been studied before. What's more, the available literature provided little information on the genus pollen. Therefore, we reported the flower and pollen morphology of *A. rugosa* as part of the programs of morphological investigation.

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2. MATERIALS AND METHODS

The flowers of *A. rugosa* were obtained from Xinyang City, Henan province, China. For stereoscope, the flowers of representatives were dissected and observed by a Nikon SMZ1500 stereoscope. For SEM, the flowers were freeze-dried with a Marin Christ ALPHA 1-4 LSC lyophilizer, at the temperature of -40℃ for 48 hours and vacuum inferior to 5×10^{-1} Torr. Then the lyophilized anthers were removed from filaments of stamen with the help of dissecting needles and then crushed to release pollen grains onto clean metallic stubs and sputter coated with gold-palladium by a Hitachi E-1010 ion sputter. Subsequently, the prepared pollen was examined and photo-graphed with a Hitachi S-4800 scanning electron microscope operated at 1.0 kV. The work was carried out in the Analytical and Testing Center of Hainan University. Descriptive terminology followed Erdtman [8], Kremp [9] and Punt et al. [10,11].

3. RESULTS

3.1 Description of Pollen Morphology

The scanning electron microscopy micrographs of *A. rugosa* were shown in Figure 1. The pollen grains were bilateral symmetric, heteropolar, subprolate in polar view, broadly elliptical in equatorial view, and belonged to medium size, P 32-38 μm × E 14-18 μm. In the same time, the statistical analysis was carried out with data of equatorial and polar diameter. Apertures six-colporate. Colpi usually narrow, long and sunken. colpus membrane covered with granules or crustate elements. Exine appeared as striato-reticulate type.

3.2 Description of Floral Morphology

The floral photographs of *A. rugosa* were shown in Figure 2. Flowers in verticillasters, in terminal unbranched spikes; 1 or 2 basal verticillasters interrupted. Calyx tubular, 5-6mm long, glabrous both inside and outside. Corolla 8-11mm long, upper lip 3-lobed, c.2mm long, lilac at margin; lower lip entire, c.1.0mm long, white; tube white outside; glabrous. Stamens 4, exserted, white; filaments 6-8 mm long, pale purple upside, white downside, adnate at different heights at the middle of corolla tube, glabrous at middle, puberulous at base. Pistil c.10mm long, pale purple upside, white downside; styles exserted, glabrous, white; stigma bifid, c.1mm long.

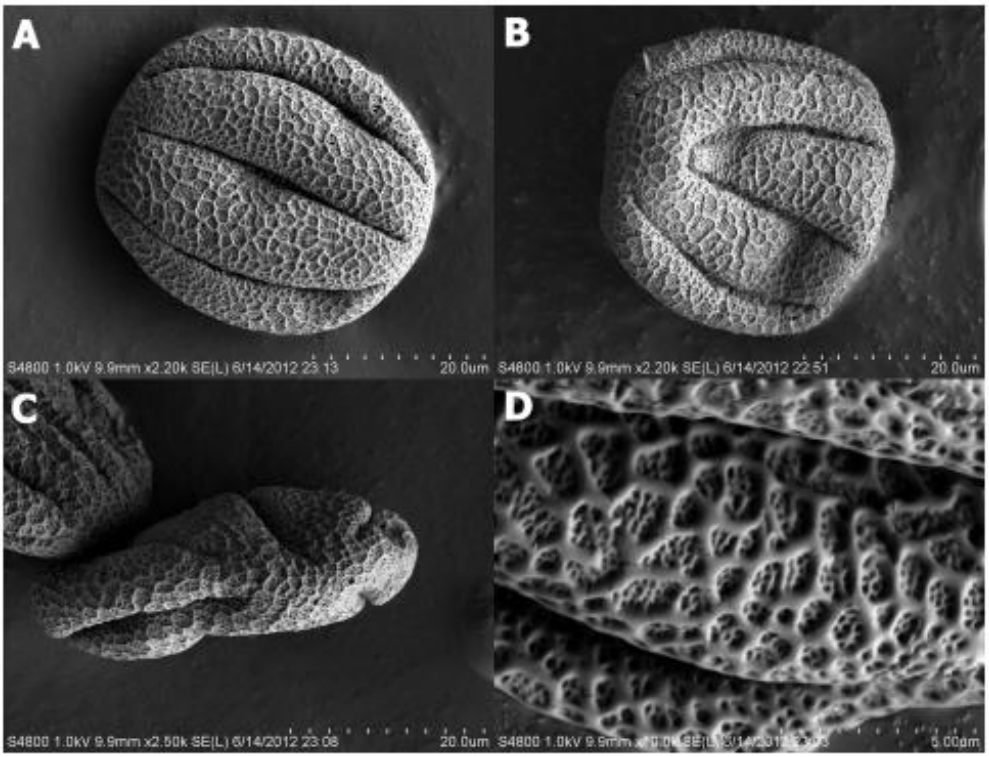

Figure1: Scanning electron microscopy micrographs of *A. rugosa* A. Equatorial view; B. Equatorial view; C. Polar view; D. Exine sculpturing

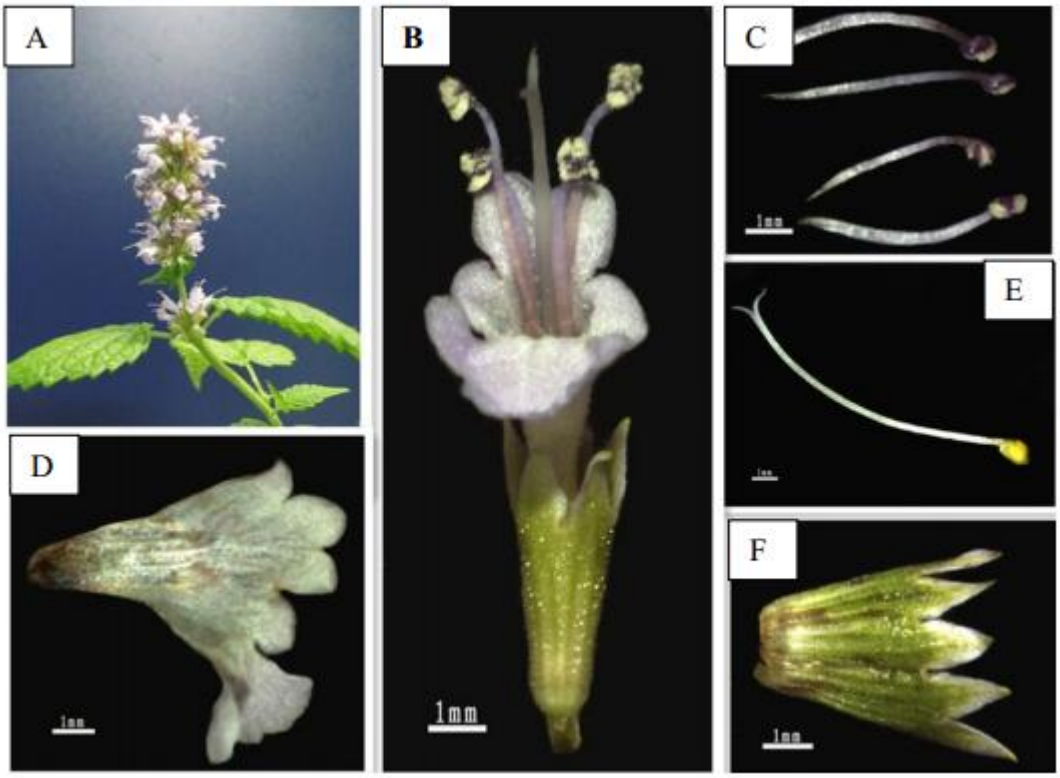

Figure 2: Floral morphology of *A. rugosa* under stereoscope A. Inflorescence; B. Flower; C. Stamens; D. Petal; E. Pistil; F. Calyx.

4. DISCUSSIONS

As a traditional Chinese medicine, *A. rugosa* is called HuoXiang whose chinese name is similar to that of *Pogostemon cablin* (Blanco) Benth. (GuangHuoXiang, often abbreviated to HuoXiang), so they are always confused in the herb market and the process of drug use [12]. Our own results, reported in previous studies, have demonstrated that the floral and pollen morphology of *P. cablin* [13]. There were some differences in the floral morphology of *A. rugosa* and *P. cablin*. As far as *P. cablin* was concerned, stamens were bearded, and the color of its calyx and corolla was bottle green. While *A. rugosa* stamens were noting, and the color of *A. rugosa* calyx and corolla was less thick than that of *P. cablin*.

Compared the pollen morphology of *A. rugosa* and *P. cablin*, there were also significant differences between them. First of all, the number of germinal aperture of *A. rugosa* was six, but *P. cablin* had three germinal apertures. Secondly, *A. rugosa* pollen belonged to the medium-size pollen, however *P. cablin* pollen was smaller than that of *A. rugosa*. Last but not least, *A. rugosa* pollen was heteropolar, but *P. cablin* pollen was isopolar. To the best of our knowledge, this was the first report on the differences in morphology of floral and pollen between *A. rugosa* and *P. cablin*. In conclusion, floral and pollen micro-morphological features proved to be a good taxonomic marker between *A. rugosa* and *P. cablin*.

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Cover page

Title: *Isolation and Identification of Autotoxic Compounds from Rhizosphere Soil of Pogostemon cablin*

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ABSTRACT

Autotoxicity has been reported to be one major problem hindering the consecutive cultivation of *P. cablin*. However, it still remained unknown in the case what were the potential autotoxic compounds produced by *P. cablin*. The autotoxic compounds from rhizosphere soil of *P. cablin* were isolated and identified by GC-MS in this paper. The result showed, a total of 11, 30 and 45 compounds were identified, accounting for 63.254%, 64.753% and 70.571% of extracts from control soil, 1 yr monoculture soil and 2 yr continuous monocultures soil, respectively. The autotoxic compounds from 2 yr monoculture soil were much more than those of 1 yr monoculture soil and control soil. The accumulation of autotoxic compounds from rhizosphere soil might be one of the main causes of continuous cropping obstacles of *P. cablin.*

1. INTRODUCTION

Pogostemon cablin (Patchouli), a plant of the Lamiaceae family, is extensively cultivated in Indonesia, the Philippines, Malaysia, China, India and Brazil [1-4]. The aerial part of *P. cablin* has been used for the treatment of the common cold, headache, fever, vomiting, indigestion and diarrhea as well as an antifungal agent in the medicinal materials of China and its surrounding regions[5]. Due to its vast cultivation in different localities in China under varying environmental conditions, *P. cablin* was divided into Paixiang (cultivated in Guangzhou city), Zhaoxiang (cultivated in Zhaoqing city), Zhanxiang (cultivated in Zhanjiang city) and Nanxiang (cultivated in Hainan province).

However, one major problem was that continuous monoculture of *P. cablin* in the same fields always resulted in a significant reduction in yield and quality. In order to address the continuous monoculture problems, farmers in the patchouli regions tended to increase fertilizer inputs to enhance crop yield. There has also been a rapid increase

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in pesticide use, leading to exacerbated soil environment with excessive pesticide residues. Therefore, it has become a matter of priority to study the mechanism of the continuous monoculture problems and provide a rational cropping system for *P. cablin*. So far, only one study has been reported on continuous monoculture problems of *P. cablin* [6]. However, it still remained unknown in the case what were the potential autotoxic compounds produced by *P. cablin*. In this study, we aimed to isolate and identify substances that contribute directly to the autotoxicity of *P. cablin.*

2. MATERIALS AND METHODS

2.1. Experimental Designs

Field trials were consisted of two treatments (1 and 2 yr continuous monocultures of *P. cablin*). Each treatment with three replications was set in a randomly arranged design, and the size of experimental plot was 4 m×5 m (20 m^2) in each treatment. *P. cablin* was planted in the plot at spacing of 1 m × 1 m. *P. cablin* in 1 yr monoculture plots was planted on September 18, 2010, harvested at the ripe stage, September 17, 2011, and thereafter kept uncultivated. *P. cablin* in 2 yr monoculture plots was planted on September 18, 2010, harvested at the ripe stage, September 17, 2011, and they were replanted on September 18, 2011 and harvested at the ripe stage, September 17, 2012 and thereafter soil samples were collected. In addition, three repeats of the control plots, i.e., fallow in whole year without *P. cablin*, were located adjacent to the treatment field.

2.2. Soil Samples

Soil samples were collected from the rhizosphere soils (adhering to the roots and rhizomes after shaking). The control samples were collected to the soil depth of 3-15 cm. Two treatment samples (1 and 2 yr continuous *P. cablin* cropping) were collected from five random sampling points for each plot. Fresh plants were carefully uprooted from the soil with a forked spade. Their roots, which grown at the soil depth of 3-15 cm were shaken to remove loosely attached soil. Fifteen root-adhered soil samples from each treatment and control samples were gathered and fully mixed, respectively. The soil samples were air-dried, sieved by 0.43 mm mesh and stored at room temperature in the laboratory according to the method of Hao et al [7].

2.3. Extraction and Isolation

Soil samples were soaked in water (m/v 1:10) for 24 h at room temperature. This was repeated three times. Aqueous extracts were concentrated under vacuum (45℃) to approximately one-third of the original volume. This was then partitioned with ethyl acetate. The ethyl acetate extracts were concentrated under vacuum and freeze dried by freezer dryer.

2.4. Trimethylsilylation and Identification of Autotoxic Chemicals

Trimethylsilylation was processed according to the reference of Hao et al [7]. Identification of autotoxic chemicals was performed by GC-MS [8].

3. RESULTS

A total of 11, 30 and 45 compounds were identified, accounting for 63.254%, 64.753% and 70.571% of extracts from control soil, 1 yr monoculture soil and 2 yr continuous monocultures soil, respectively.

The main constituents of water extracts from control soil were Hexadecane, 2-methyl- (5.582%), Octacosane (1.922%), Z-14-Nonacosane (1.714%), Tetradecane

(1.599%), Nonadecane, 1-chloro- (1.424%), Triacontane (1.129%), and 2-Phenanthrylamine,9,10-dihydro-3,7-dinitro- (1.135%).

In 1 yr monoculture soil, more than 1% of the ingredients included Benzyloxyamine-N,N-ditms (1.065%), [1,2'-Binaphthalene]-5,5',8,8'-tetrone,1',4-dihydroxy-2,3'-dimethyl- (2.095%), Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)- (1.307%), Docosane (1.708%), Tricosane (2.803%), Tetracosane (3.870%), Pentacosane (2.342%), Octacosane (1.294%), Heptacosane,1-chloro- (2.208%).

The varieties of components from 2 yr monoculture soil were much richer than those of control soil and 1 yr monoculture soil. Nonahexacontanoic acid (6.053%), Hexacosane (4.373%), Pentacosane (3.389%), Nonacosane (3.325%), Tricosane (2.959%), Tetrapentacontane,1,54-dibromo- (2.945%), Tetracosane (2.933%), Heptacosane, 1-chloro- (2.791%), 1-Bromodocosane (2.607%), [1,2'-Binaphthalene] -5,5',8,8'-tetrone, 1',4-dihydroxy-2,3'-dimethyl-, (2.388%), 1-Hexacosene (2.167%), 9-Methyl-Z-10-tetradecen-1-ol acetate (1.849%), Docosane (1.843%), Z-12-Pentacosene (1.326%), E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate (1.241%), Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)- (1.117%), Heneicosane (1.090%), E-9-Methyl-8-tridecen-2-ol, acetate (1.068%), and Dibutyl phthalate (1.030%) were the main components from 2 yr monoculture soil.

TABLE 1. THE COMPONENTS OF WATER EXTRACTS FROM CONTROL, 1YR MONOCULTURE AND 2YR MONOCULTURE SOIL FROM *P. CABLIN*

Compound type	Components	Relative contents (%)		
		Control soil	1 yr monoculture soil	2 yr monoculture soil
Aliphatic acids	Propanoic acid	0.950±0.001[a]	-[b]	-
	Malonic acid*	-	-	0.161±0.001
	Tetradecanoic acid*	-	-	0.089±0.001
	Hexadecanoic acid*	-	0.426±0.001	-
	Nonahexacontanoic acid	-	-	6.053±0.010
Alcohols	Hexadecanol	-	0.265±0.001	-
	1-Decanol, 2-hexyl-	-	0.189±0.000	-
	4-Tridecanol	-	-	0.289±0.000
	4-hexadecanol	-	-	0.260±0.001
	1-Pentacosanol	-	-	0.798±0.002
	1-Hexacosanol	-	0.109±0.001	-
	1-Hentetracontanol	-	-	0.235±0.001

Esters	Acetic acid, octadecyl ester	-	0.025±0.001	0.031±0.001
	Hexadecanoic acid, 2-hydroxy-, methyl ester	-	0.216±0.001	0.569±0.002
	Dibutyl phthalate*	-	0.689±0.001	1.030±0.002
	7-Methyl-Z-tetradecen-1-ol acetate	-	0.195±0.001	-
	E-11,13-Dimethyl-12-tetradecen-1-ol acetate	-	0.617±0.001	0.826±0.001
	Methanesulfonic acid, 2,7-dioxatricyclo [4.3.1.0(3,8)]dec-5-yl ester	-	0.199±0.001	-
	E-9-Methyl-8-tridecen-2-ol, acetate	-	-	1.068±0.002
	9-Methyl-Z-10-tetradecen-1-ol acetate	-	-	1.849±0.001
	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	-	-	1.241±0.01
	1,2-Benzenedicarboxylic acid, dinonyl ester*	-	-	0.124±0.001
Benzoic Acids	Benzeneacetic acid*	-	-	0.893±0.002
Aldehydes	Octadecanal	-	-	0.595±0.001
Ketones	2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (R)-	-	-	0.420±0.001
Benzenes	Benzyloxyamine-N,N-ditms	-	1.065±0.000	-
	[1,2'-Binaphthalene]-5,5',8,8'-tetrone, 1',4-dihydroxy-2,3'-dimethyl-	-	2.095±0.010	2.388±0.010
Sulfides	1-Octadecanethiol	-	0.031±0.001	0.05±0.001
Alkanes	Tetradecane	1.599±0.001	-	-
	Hexadecane, 2-methyl-	5.582±0.010	-	-
	Heptadecane	-	-	0.102±0.001
	Octadecane*	-	0.152±0.001	0.282±0.001
	Nonadecane*	-	0.452±0.001	0.532±0.000
	1,11-Dibromoundecane	-	0.249±0.001	0.391±0.001
	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-	-	1.307±0.001	1.117±0.001
	1-Octadecene	-	-	0.393±0.001
	1-Nonadecene	-	0.121±0.001	-
	Eicosane	0.873±0.001	-	0.895±0.001
	Heneicosane*	-	-	1.090±0.002
	Octadecane, 1-chloro-	-	-	0.548±0.002
Alkanes	Nonadecane, 1-chloro-	1.424±0.001	0.403±0.001	0.997±0.002
	Docosane	-	1.708±0.002	1.843±0.005
	Tricosane*	-	2.803±0.020	2.959±0.010
	Tetrapentacontane, 1,54-dibromo-	-	-	2.945±0.001
	Z-12-Pentacosene	-	-	1.326±0.010
	1-Bromodocosane	-	-	2.607±0.010
	Tetracosane	-	3.870±0.010	2.933±0.001
	Pentacosane*	-	2.342±0.010	3.389±0.001
	Hexacosane	-	-	4.373±0.001
	1-Hexacosene	-	0.180±0.001	2.167±0.001
	Octacosane*	1.922±0.001	1.294±0.000	-
	Nonacosane	-	-	3.325±0.001
	Z-14-Nonacosane	1.714±0.001	0.309±0.001	0.480±0.000
	Heptacosane,1-chloro-	-	2.208±0.000	2.791±0.000
	Triacontane	1.129±0.001	-	-
Others	2,5-Furandione,3-(2-dodecen-1-yl)dihydro-	-	0.254±0.001	0.353±0.001
	2-Phenanthrylamine, 9,10-dihydro-3,7-dinitro-	1.135±0.001	-	-
	Silanol, trimethyl-, phosphate (3:1)	40.356±0.80	35.910±0.20	12.012±0.60
	Pyridine	6.570±0.020	5.070±0.010	1.752±0.002
Total identified compounds		63.254	64.753	70.571

Note: [a] showed mean value and standard deviation (n=6). Every sample was processed in duplicate, and the measurements of final extracts were repeated three times. [b] represented not detected. * represented allelochemicals reported by others.

4. DISCUSSIONS

Continuous cropping obstacle, or replanting disease in cropping sequence, has been recognized as a major problem as early as 300 B.C. in various plant species such as food crops, oil crops, medicinal plants, and horticultural crops, etc [9]. The harmfulness caused by continuous cropping obstacle includes the deterioration in soil physicochemical characteristics, increase in pest and disease, and reduction in crop yield and its quality, which significantly restrict the sustainable development of agricultural productivity [10-11]. These components (Nonadecane, Heneicosane, Tricosane, Pentacosane, Octacosane, 1,2-Benzenediol, Dibutyl phthalate, Tetradecanoic acid, Heptadecane, Octadecane, Hexadecanoic acid, Benzeneacetic acid, n-Pentadecanoic acid, 1,2-Benzenedicarboxylic acid dinonyl ester, Phenol, 2-methoxy-4-(1-propen-1-yl)-, *P*-Hydroxybenzoic acid, Vanillic acid, Malonic acid, Salicylic acid, Succinic acid) were reported to be autotoxic compounds [12,13]. As was shown in Table 1, autotoxic compounds could not be found in control soil. The components of water extracts from 2 yr monoculture soil were much richer than those of 1 yr monoculture soil and control soil. What's more, most of these autotoxic compounds reported by others were found in the 2 yr monoculture soil. Several autotoxic compounds were found in the 1 yr monoculture soil. The results indicated that there were some increase in the varieties and contents of autotoxic compounds from rhizosphere soil with the growth of continuous monoculture, which was consistent with other reporters [12-13]. Therefore, we concluded that the accumulation of autotoxic substances from rhizosphere soil might be one of the main causes of continuous cropping obstacles of *P. cablin*.

ACKNOWLEDGMENTS

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Title: *Adsorptive Removal of Cd(II) from Aqueous Solution Using Pine Cone and H_2so_4 Modified Pine Cone*

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ABSTRACT

In this study, the raw pine cone and H_2SO_4 modified pine cone were tested to remove Cd(II) ions from water. The results showed the Cd(II) adsorption capacity of the pine cone and H_2SO_4 modified pine cone were 14.96 and 74.94 mg/g, respectively. The modified pine cone had higher adsorption capacities than raw pine cone, which can be attributed to the surface structural changes by the acid treatment. Equilibrium adsorption data are more consistent with the Langmuir isotherm equation than with the Freundlich equation. The Cd(II) adsorption on the two adsorbents tends to increase with increasing solution pH under acidic conditions (pH 2.0 -6.5) . The optimum pH for Cd(II) adsorption is 6.0. The desorption ability of Cd(II) by 0.1 M HCl solution was found around 96.7–99.1% .

INTRODUCTION

Heavy metal remediation of aqueous streams is of special concern due to persistency of heavy metals in environment [1-2]. Conventional treatments technologies for the removal of these toxic heavy metals are not economical further resulting in generate huge quantity of toxic chemical sludge. Adsorption is as a potential alternative to the existing conventional technologies for the removal and/or recovery of metal ions from aqueous solutions [3]. The major advantages of adsorption over conventional treatment methods include: low cost, high efficiency, minimization of chemical or sludge, regeneration of adsorbents and possibility of metal recovery [4]. Cadmium is a highly toxic element affecting the environment and considered to be carcinogenic. The discharged in effluent will be absorbed and accumulated by microorganisms. Also, cadmium will be transferred to humans via the food chain and then cause serious damage to kidney and bones. That is why we need consider cadmium pollution. The most representative forest in Korea is the coniferous forest. Coniferous forests account for 52% of the total forest land, the majority of the tree species of Korea's forests are the red pine and white pine. In the recent years pine cone has been tried with varying success for heavy metal removal [5-7] and dye [8]. Large quantities of cones in Korea were regarded as Forestry

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waste and thus there is a potential to be used as adsorbent for removing heavy metals. The objective of the present work was to investigate the feasibility of producing adsorbent from the pine cone of Korea by H_2SO_4 modification and their ability to remove Cd (II) ions from aqueous solution.

2. Methods

2.1. Materials

The raw pine cone (PC) used in the present experiments were collected around University of Ulsan located in an urban residential area in Ulsan, Korea. The collected cones were washed to remove impurities such as sand, leaves and soils. Pine cones were then dried at 90 ℃ for 12 hours. The resultant powder was sieved. The sieved particles about 150μm to 200μm were collected, preserved at room temperature in an airtight plastic container and used for analysis as well as adsorption experiments. The chemical composition of the pine cone is presented in **TABLE I**. The modified pine cone sample was prepared by mixing 10 g of raw pine cone powder with 100 ml of 0.5 M H_2SO_4 solution. The whole reaction mixture was stirred in a magnetic stirrer at 25^{o}C for a period of 12h and then the powder was filtered and repeatedly washed with distilled water. The washed powder was then oven dried overnight at 50 ℃ and used for adsorption and characterization.

TABLE I. CHEMICAL COMPOSITION OF PINE CONE RAW

Cellulose	46.7
Lignin	24.9
Hemicelluloses	23.6
extractives	4.8

2.2. Chemicals and Analytical Instruments

The Cd(II) stock solution containing 1000 mg Cd/L was prepared by dissolving cadmium nitrate ($Cd(NO_3)_2$) powder (analytical reagent grade) in distilled water. Cd(II) working solutions in different concentrations were prepared by diluting the Cd(II) stock solution with distilled water. The pH measurements were done with a Digital pH meter Orion 5Star and pHs of solutions were adjusted to the required value by using 0.1M NaOH and 0.1 M HNO_3. Concentrations of Cd(II) were measured using atomic absorption spectrophotometer VARIAN AA240. The scanning electron micrographs (SEM) were obtained on Hitachi 4700 microscope to identify morphology information on the PC and H_2SO_4 modified PC.

2.3. Effect of solution pH on Cd(II) adsorption

Batch experiments of adsorption were performed in 250 mL Erlenmeyer flasks. Flasks were being agitated on Shaking water bath HST -205SW at 120 rpm for specified time intervals. The effect of pH on Cd(II) adsorption was examined maintaining pHs at different values between 2 and 6.5. Concentration of Cd(II)

solution for the study of solution pH effect was 100 mg/L and adsorbent dose was 0.1g at 25 ^{o}C.

2.4. Study of Adsorption Isotherms

Cd(II) adsorption isotherm study was carried out with different initial concentrations of Cd(II) and a fixed concentration of the adsorbents at room temperature (25℃). Six levels of initial Cd(II) concentrations (50, 100, 200, 300,400 and 500 mg/L) were used. The pH of the solution was maintained at an optimum pH and reaction time was 60 min. At the end of the adsorption period, the solution was filtered through a 0.45 μm membrane filter and then analyzed for Cd(II). The quantity of adsorbed Cd(II) (adsorption capacity) was calculated from the decrease in the Cd(II) concentration of interest solution. The isotherm data on Cd(II) adsorption were fitted to Langmuir and Freundlich equations.

2.5. Desorption Studies

To evaluate Cd(II) desorption from the samples, the residual solids retained on the filter paper were collected in a 250-mL Erlenmeyer flask after filtration of the suspension from an adsorption test solutions. To each flask 100 mL of 0.1 M HCl solution was added. The flask was covered during magnetic stirring at 120 rpm for 12h while pH was maintained at the same value as in the desorption experiment. The suspension solution was filtered and then analyzed for Cd(II) concentration in a similar way to described previously. The quantity of desorbed Cd(II) was determined by the amount of Cd(II) in solution after the desorption experiment.

3. RESULTS AND DISCUSION

3.1. Scanning Electron Microscope (SEM)

The microstructures of pine cone before and after the acid treatment were observed by scanning electron micrographs. The SEM shown in Fig.1 enables the direct observation of the surface microstructures of pine cone and H_2SO_4 modified pine cone.

Micrographs show considerable changes in morphology of the PC after the acid treatment with increased number of pores on adsorbent surface which can be utilized for more sorption potential of Cd(II) ions in aqueous solution.

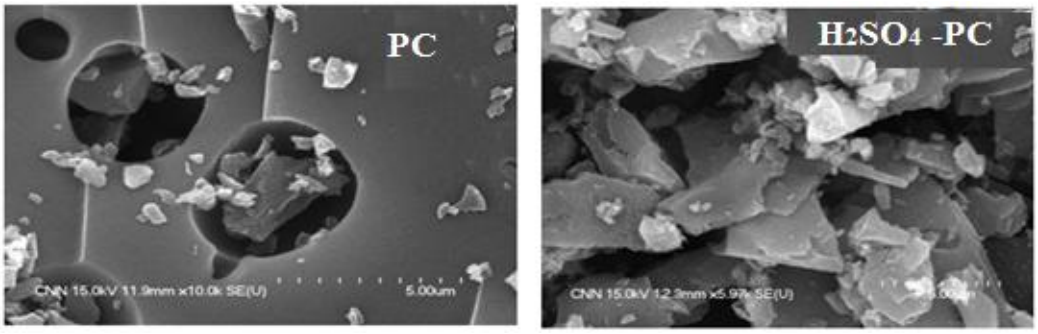

Figure 1. Scanning electron microscope of PC and H_2SO_4 modified PC

3.2. Effect of pH on Cd(II) Adsorption

The effect of initial pH on the Cd(II) adsorption by pine cone and H_2SO_4 modified pine cone were shown in Figure. 2. The initial pH effect at strong acidic condition , pH 2.0 -3.0 was much lower than at weak acidic conditions , pH 5.0-6.0. This is because there is more competition between H^+ ions and Cd(II) ions for adsorption sites of the pine cone and H_2SO_4 modified pine cone at lower pH conditions. The maximum adsorption of Cd(II) was observed at pH 6.0. The pH above 6.5 for Cd(II) was not used in order to avoid the precipitation of metal ions in the form of their hydroxides [9]. This phenomenon can be explained by the surface charge of the adsorbent and the H^+ ions present in solution. At high pH values, presence of H^+ ion in solution decreased and the surface of the adsorbent has a higher negative charge which results in a higher attraction of Cd(II) ions.

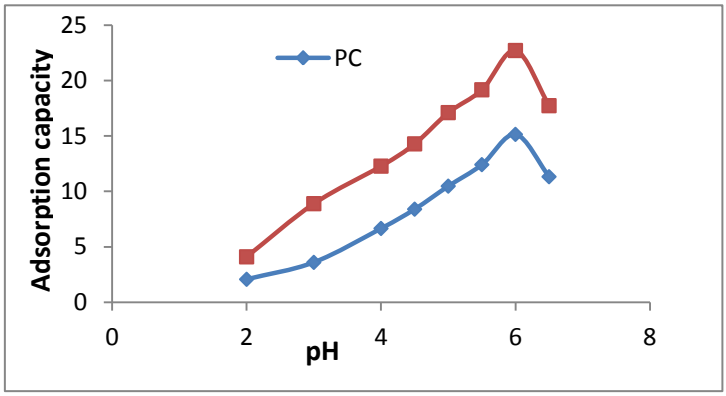

Figure 2. Efect of solution pH

3.3. Cd(II) Adsorption Isotherms

Figure.3 shows the equilibrium adsorption uptake of Cd(II) ions by the adsorbents at different initial concentration Cd(II) . The increase initial concentration of Cd(II) greatly increases the equilibrium adsorption uptake of Cd(II) . The adsorption capacities of Cd(II) tend to get a constants value. This mean that there is a maximum or limit value available for active adsorption of Cd(II), due to the saturation of the sorbent sites by Cd(II) ions. The maximum adsorption of the pine cone and H_2SO_4 modified pine cone were 14.96 and 74.94 mg/g, respectively. Two typical isotherms, as described below in Eqs. (1) and (2), were used for fitting the experimental data:

Equation(1) : Freundlich equation: $q=KC1/n$

Equation(2) : Langmuir equation: $q =qmbC/(1+bC)$

where q is the amount adsorbed at equilibrium (mg/g), and C is the equilibrium concentration (mg/L). The other isotherm parameters can be determined by regression of the experimental data using each isotherm equations shown in Fig 3(A) and (B). The isotherm data showed Figure. (3A) and (3B) were fitted with two models. Table 2 shows adsorption model parameters, including correlation coefficients (R^2) estimated from each isotherm.

TABLE II. LANGMUIR AND FREUNDLICH ISOTHERM CONSTANTS

Adsorbent	Langmuir isotherm			Freundlich isotherm		
	q_{max} (mg/g)	b	R^2	K_f	1/n	R^2
PC	14.96	0.0141	0.961	10.69	0.3239	0.894
H_2SO_4 modified PC	74.94	0.0095	0.990	11.96	0.3083	0.871

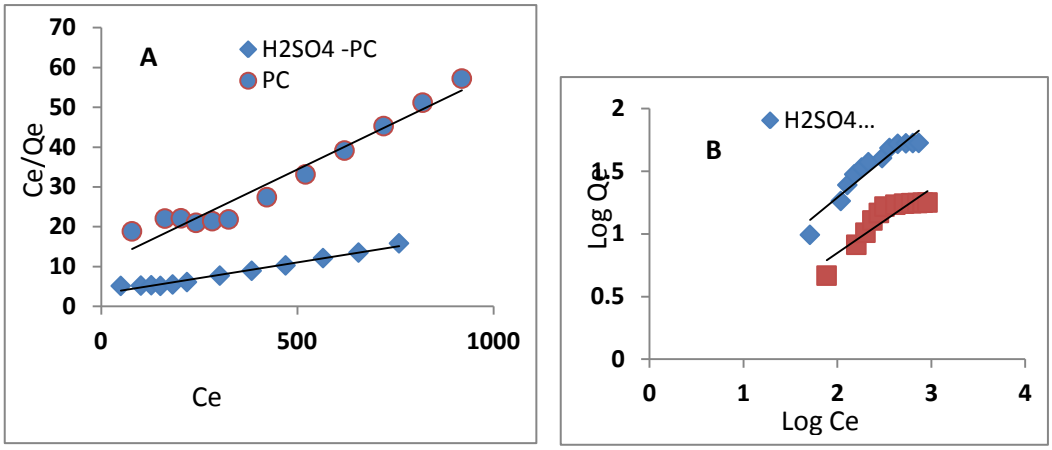

Figure 3 (A): Langmuir isotherm and 3(B): Freundlich isotherm

3.5. Desorption Studies

The adsorption of Cd(II) on pine cone and H_2SO_4 modified pine cone is highly pH dependent, hence its desorption may also controlled by adjusting the pH of the interest solution. Hydrogen ions may replace the Cd(II) ions on the metal loaded adsorbent thus functioning as cation exchanger [10]. The tests of Cd(II) desorption were conducted with three initial concentrations of Cd(II) (50, 100, and 500 mg/L). **TABLE.III** shows desorption data of Cd(II) adsorped by the PC and H_2SO_4 modified PC .

TABLE III. DESORPTION OF Cd(II) IONS

Initial Conc. (mg/L)	**Sample**	**Adsorbed Cd(II) (mg/g)**	**Desorbed Cd(II) (mg/g)**	**Desorbability (%)**
50	PC	6.21	6.11	98.4
	H_2SO_4 -PC	14.23	13.84	97.3
100	PC	9.96	9.87	99.1
	H_2SO_4 -PC	23.39	22.61	96.7
500	PC	14.58	14.17	97.2
	H_2SO_4 -PC	74.41	73.36	98.6

The identified desorbability of Cd(II) ranged from 96.7 to 99.1 %. The elution of the adsorbed Cd(II) solution allows collection of Cd(II) ions and concentrating solution which could be suitable for recovery of the cadmium. Also the adsorbents used for Cd(II) removal can be regenerated through proper procedures and then the regenerated adsorbent may be reused for Cd(II) removal again.

4. CONCLUSIONS

In the adsorption and desorption study of Cd(II) ions in aqueous solution using pine cone and H_2SO_4 modified pine cone, the followings can be concluded

1. The identified adsorption capacity of Cd(II) ions by the PC and H2SO4 modified PC were 14.96 and 74.94 mg/g , respectively.

2. The adsorption of Cd(II) by the both adsorbents well fitted with Langmuir isotherm equation ($R^2 \geq 0.961$, 0.990) rather than Freundlich isotherm equation.

3. The adsorbed Cd(II) easily desorbed by 0.1M HCl solution with desorbability of 96.7 – 99.1%.

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Cover page

Title: *An Simulation of Farm Tractor Reliability with Different Distribution Assumption.*

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ABSTRACT

When design a farm tractor, the reliability sketch is very important for the project. The paper give several examples with different reliability assumption, or different matching. From comparing, some optimum plan appear for 500-600 hours live with reliability of 0.37 by economic components. This result thanks to the reliability forecast skill, especially the Weibull distribution.

FRAM TRACTOR ARCHITECTURE COMPARING

Commonly, the farm tractor is consist of the engine, the chassis, the electrical appliances and the body [1], as Fig.1. When carrying out a design, the above view of architecture for a farm tractor would be so unfavorable for engineering, that it would impede the partition of function unit, which would complicate the design of the reliability and the maintenance. The farm tractor has a simple architecture comparing with other farm machines, to accept and introduce this architecture as Fig. 2 might be a good idea.

FOUR EXAMPLES OF DIFFERENT DISTRIBUTION

From the view of reliability and maintenance, to set up a suited architecture of the tractor according to the assembly process so as the maintenance rules firstly, would be the essential task. This technology combined with other reliability tools, is more appropriate and conducive to the develop of the whole reliability design.

There is a simulating sketch plan of a farm plan in simulation, which is of a two variable Weibull distribution of live, of which the distribution is described by formula1. The parameter of the distribution is that, the live parameter A is 30 thousand hours. And the shape parameter of example 1 is B=6.0, which of the example 2 is B=3.0, that of the example 3 is B=1.0, the example 4 is B=0.5. So the cumulated

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failure probability corresponding to the live and the distributions are listed in the Tab.1 and Fig.3. The data show that, the earlier fault would increase when the shape parameter decrease from 6 to 0.5[2], and at the same time the maxim life would be increase. Which distribution would be more perfect? Nearly all the engineers would consider the market profits of example 3[3-4]. The reason is that the maintenance cost would greatly change many things.

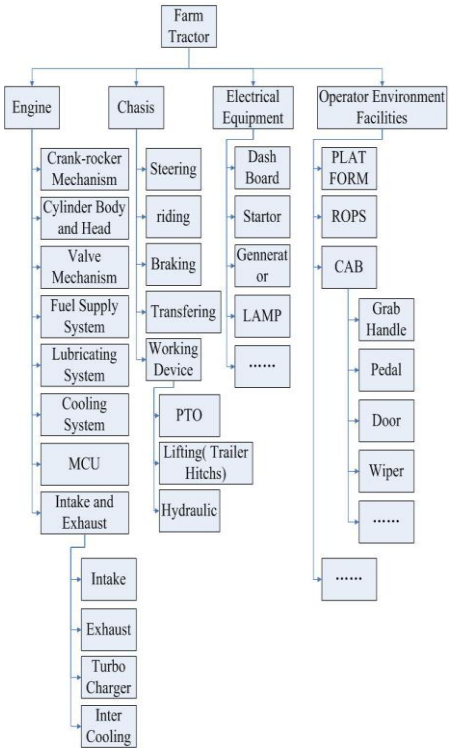

Figure 1. custom architecture of farm tractor

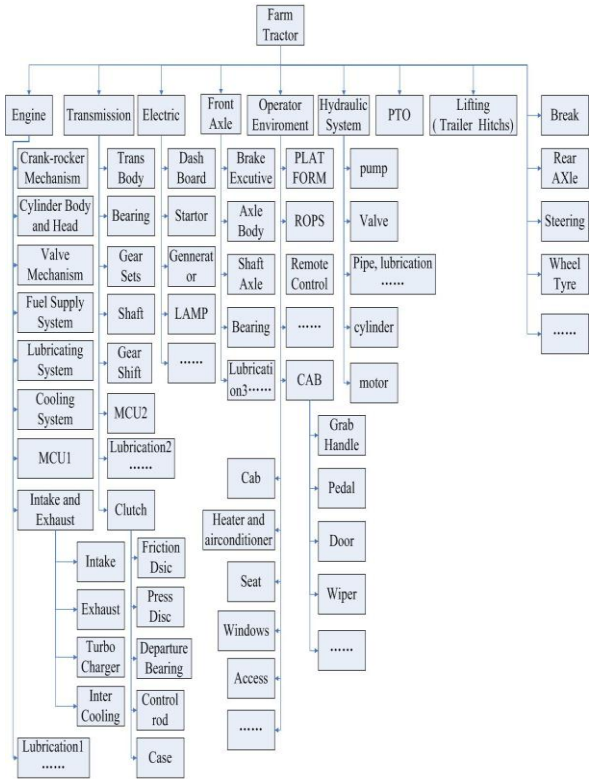

Figure 2. new architecture of farm tractor

$$F = 1 - e^{-(t/A)^B} \tag{1}$$

A RELIABILITY SKETCH OF THE FARM TRACTOR

If an engineer meet a project similar to the example 3 in table1, what he would confront is that the tractor would often operate in a non-reliability state exceed user expectation. The reason is as follow[5].

TABLE 1. A LIFE DISTRIBUTION OF 4 FARM TRACTOR RELIABILITY SKETCH

$L_R(10^3\ Hour)$											
No.	1	2	3	4	5	6	7	8	9	10	11
F (t)	1e-6	1e-5	1e-4	1e-3	3e-3	0.01	0.05	0.1	0.5	0.63	0.9
Ex 1	3.0	4.4	6.5	9.5	11.1	13.9	18.3	20.6	28.2	30	34.5
Ex 2	0.3	0.65	1.4	3.0	4.1	6.5	11.2	14.2	26.6	30	39.6
Ex 3	3e-5	3e-4	3e-3	0.03	0.08	0.3	1.5	3.16	20.8	30	69.1
Ex 4	-	-	-	3e-5	2e-4	3e-3	0.08	0.33	14.4	30	159

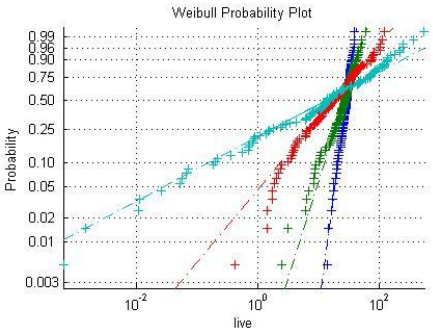

Figure 3. the Comparing of Tractor Live

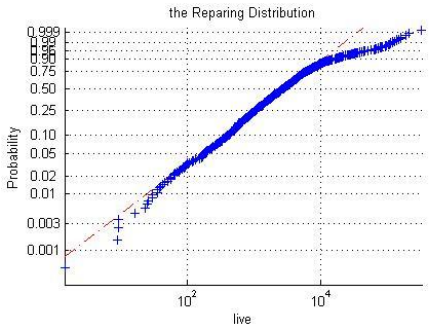

Figure 4. the Repairing Distribution of the tractor

To ensure the MTBF over 350 hours in example 3, the reliability of all the assembly would increase to a high level. The assumption is that, the distribution of all the assembly is the same distribution as the tractor itself, and the weight of all the assembly is equal to each other. So the formula would change to a simple form as following expression Formula 2. The match use the old architecture as Fig.1, in which the system would looks more simple than the newly introduced one.

TABLE 2 THE MTTF OR THE MTBF MATCHING OF ASSEMBLY AND TRACTOR

	Ex 3		*Ex 2*	
Item	MTBF	Reliability at 350h	MTBF	Reliability at 350h
Power (h)	4000	0.92	4000	0.9993
Tran (h)	4000	0.92	4000	0.9993
Steering (h)	3000	0.89	3000	0.9984
Riding (h)	3000	0.89	3000	0.9984
Break (h)	3000	0.89	3000	0.9984
Chasis (h)	60000	0.99	60000	0.99999
Cab (h)	4000	0.92	4000	0.9993
Electrical (h)	5000	0.93	5000	0.9997
Electrion(h)	5000	0.93	5000	0.9997
Hydraulic (h)	2000	0.84	2000	0.9947
Tractor (h)	350	0.37	1500[note]	0.9873

Note: this life data, not means MTBF, it is a life of the tractor when R=0.37.

$$MTBF = \frac{1}{\lambda_{tractor}} = \frac{1}{\sum \lambda \mathrm{i}} \tag{2}$$

A possible match of most assembly and the tractor result is listed in Tab.2 in column ‘example 3’, see also Fig.4. From the sheet, we might find that we could hardly control the failure and the maintenances. At the same time, we could not optimize the cost.

In this assumption, the shape parameter of all assembly is assumed to B=1.0, which is also called exponential distribution. From the table, we could found, the sketch might not be built to a economic project. Even we purchase many good parts, we even could not increase the MTBF to a high level. The circumstances would become more worst, if the quality of the parts is out of control, such as the situation of example 4 in Tab.1 .

How to change the dilemma circumstance is a worthy project of very high value. To actualize a perfect reliable design is not a simple task, which has puzzled many

engineers in past years. Now By the reliability engineering method, skill and tools, an earlier sketch and design, rapid development, or low cost machining would become more easily.

Now, we turn to the example 2 in Tab. 2. We should also assume that the distributions of all the assembly are as follow, the shape parameter is 3.0, and the architecture is a new one as the figure 2. Form the table, we can see, there is a high reliability tractor sketch on the paper. And from the further more matching, we could get a high reliability and live of the tractor plan, that the life reach 1500 hour's high when the reliability is 0.37, see Tab.2.

IMPROVE THE SKETCH BY RELIABILITY MATCHING

Although, above plans are all difficult when build a excellent product. We must consider the cost of manufacturing vs. the price market could accept. So we might find other sketch plans.

We should choose two plan with shape parameter of B=6.0 or B=3.0[6], example 5and example 6, the MTTF (MTBF) of some assembly might be matched to a normal level, such as 1000 to 2000 hours, and the architecture of the machine would adapt the new one as figure 2. So we get a sheet of data, as Tab.3 .

TABLE 3 THE NEWLY MATCHED TWO PLANS OF THE TRACTOR RELIABILITY

Example 5	MTBF	Reliability				
B = 3.0	hours	100	200	336	490	550
Engine (h)	1000	0.999	0.992	0.963	0.889	0.844
Transmissionn (h)	2000	0.9999	0.999	0.995	0.985	0.979
Electric (h)	2000	0.9999	0.999	0.995	0.985	0.979
Front Axle (h)	2000	0.9999	0.999	0.995	0.985	0.979
Operator Enviroment (h)	2000	0.9999	0.999	0.995	0.985	0.979
Hydraulic System (h)	1000	0.999	0.992	0.963	0.889	0.844
PTO (h)	1000	0.999	0.992	0.963	0.889	0.844
Trailer Hitchs(h)	1000	0.999	0.992	0.963	0.889	0.844
Break (h)	2000	0.9999	0.999	0.995	0.985	0.979
Rear AXle (h)	2000	0.9999	0.999	0.995	0.985	0.979
Steering(h)	2000	0.9999	0.999	0.995	0.985	0.979
Wheel ,Tyre (h)	1000	0.999	0.992	0.963	0.889	0.844
other(h)	1000	0.999	0.992	0.963	0.889	0.844
Tractor	550^{note}	0.9941	0.954	0.80	0.5	0.368
Example 6	MTBF	Reliability				
B = 6.0	hours	300	400	500	605	644
Engine (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Transmission (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Electric (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Front Axle (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Operator Enviroment (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Hydraulic System (h)	1000	0.9993	0.9959	0.985	0.952	0.931
PTO (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Trailer Hitchs(h)	1000	0.9993	0.9959	0.985	0.952	0.931
Break (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Rear AXle (h)	1000	0.9993	0.9959	0.985	0.952	0.931
Steering(h)	1000	0.9993	0.9959	0.985	0.952	0.931
Wheel ,Tyre (h)	1000	0.9993	0.9959	0.985	0.952	0.931
other(h)	1000	0.9993	0.9959	0.985	0.952	0.931
Tractor	644^{note}	0.9898	0.9443	0.80	0.5	0.368

The example 5 show an idea that, the distribution shape parameter of all assembly is 3.0, and the live of them varies from 1000 hours to 2000 hours. Then the sketch get a result of MTBF=550 hours. In example 6, the shape parameter is increased to 6.0, and all the assembly live is equal to 1000 hours. Fortunately, the result is MTBF = 664 hours. Both plans are enough for wining the market. And the assembly distribution plot of example 6 see Fig.5 , the distribution of tractor see Fig.6 .

How to realize the sketch, it is not a fairy tale, an enterprise has to give more attention to reliability during the whole life of the tractor, such as planning, designing ,building, testing, purchasing, assembling, selling, serving and maintenance, etc. Especially, when plan and design a new tractor, it is more important to give it a good reliability sketch.

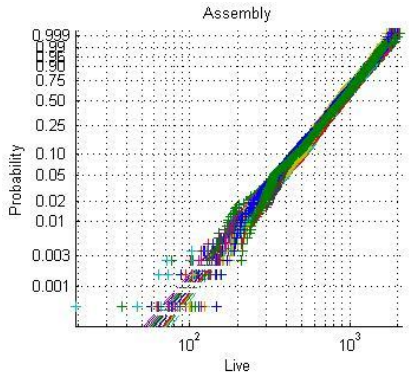

Figure 5 Assembly Live Distribution

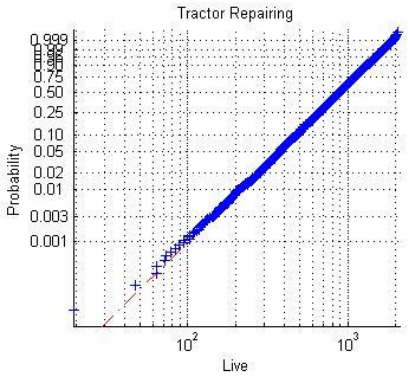

Figure 6 Farm Tractor Repairing Distribution

CONCLUSION

To built excellent farm tractor, it is important to match a high reliability plan first. To match a good plan, it is important to select a suitable architecture to reach high reliability and easy maintenance. The third, it is important to balance the quality and the budget .This means to get a excellent system, it is not the only way to purchase expensive parts, to optimum the reliability and live of tractor to a moderate level by matching the assembly or parts, is a good resolution. Otherwise, when adjust the parameter of a tractor or its assembly, such as matching the shape parameter from 1 to 6, it is important to respect the actual condition of the industry and control the influence in the whole product live cycle.

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Cover page

Title: *Preparation of Compound Copper Amino Acid Chelate from Concentrated Monosodium Glutamate Wastewater*

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ABSTRACT

Monosodium glutamate (MSG) wastewater is a kind of high concentrated and nontoxic organic effluent to be treated difficultly with low pH value and high-concentrated COD, NH_4^+-N and sulfate. Copper is the essential microelement for plant growth. This study focuses on the resource utilization of MSG-wastewater and exploring the structure of compound copper amino acid chelate. The compound copper amino acid chelate was prepared under specific synthetic conditions. And the rate of product preparation was measured by EDTA complexometric titration method, which was 89.2%. Infrared spectroscopy was used to analysis the structure of the chelates. It was found that glutamic acid preferred to reacting with copper. Amino and carboxyl were involved in coordination reaction, and water molecules found in samples were also involved in coordination reaction.

INTRODUCTION

MSG wastewater is a kind of recalcitrant and high concentration organic wastewater containing large amount of organic matter and nutrient [1]. With the development of MSG industry, MSG-wastewater emissions not only threaten the environment, but also can be a waster of resources. MSG-wastewater recycling use is the best strategy of the sustainable development in monosodium glutamate industry. The comprehensive utilization of monosodium glutamate waste water includes the following several ways: recycling glutamic acid; protein feed production; recycling ribonucleic acid (RNA); production of compound amino acid organic fertilizer; production of agricultural fungicides, etc[2-8]. Particularly, the compound amino acid organic fertilizer cause widespread concern in agriculture

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research and application[9-10]. And the main ingredient of organic fertilizer is microelement amino acid chelates with a unique ring structure[11-13]. Copper is the essential element for plant growth, which can increase production and has the ability to resist drought, cold and lodging. This paper is devoted to enrich the application range of reusing MSG- wastewater. Hence, the aim of this study is to compound Cu(Ⅱ)-compound amino acid chelates by the reaction of MSG-wastewater with copper sulfate. Main research contents include exploring the optimum parameters of preparation technology and applying infrared spectrum to analyze the structure of the chelates.

METHODS

Preparation of Compound Copper Amino Acid Chelate

50 times diluted WSG-wastewater was reacted with copper sulfate in beaker, and the pH value of the reaction liquid was adjusted to 10.5 by sodium hydroxide. The beaker was putted in thermostat water bath for 30 minutes. The temperature kept at 50℃. After reaction, the solution was centrifugated for 10 minutes at 7000r/min. And the supernatant was collected and standing it for 90 minutes. Finally, we could get the samples after evaporative concentration, purification and stoving, orderly.

Determination the Rate of Product Preparation [14]

Amount of compound copper amino acid chelate was accurately weighed and melted in 100ml volumetric flask with distilled water. The copper content was determined by EDTA complexometric titration method. 25ml solution under text was accurately moved in 250ml conical flask, added 50 ml distilled water and 2 -- 3 drops PAN indicator and titrated with 0.02 mol/LEDTA standard solution. The color of solution changed from purplish red to pale yellow when reached the titration end point. Besides, a same amount sample was melted in 50ml anhydrous ethanol. After fully mixing and centrifugal separation, the precipitation transferred into 100ml volumetric flask and constant volume. And also the copper content was determined by EDTA complexometric titration method.

$$\text{Rate of product preparation}(\%) = \frac{\text{Chelate copper content}}{\text{The amount of copper ions}} * 100 \quad (1)$$

Product Analysis

The kinds and content of amino acids in MSG-wastewater and chelate product were measured by automatic amino acid analyzer. And we made a comparative analysis. Infrared spectrum scanning was carried out on the product by KBr tabletting method to determine the structure of samples and analysis the changes of characteristic peak. And the measurement range was from 4000cm^{-1} to 400cm^{-1}.

RESULT AND DISCUSSION

The Rate of Product Preparation

Two samples were weighed of 0.2g, respectively. One was melted in distilled water, transferred to 100 ml volumetric flask and titrated with 0.02 mol/LEDTA standard solution. The EDTA solution was consumed 3.70ml. The other one was added in 50ml anhydrous ethanol. After fully mixing and centrifugal separation, the precipitation was dissolved in distilled water and transferred it to 100ml volumetric flask. And then the copper content was measured by EDTA complexometric titration method. The EDTA solution was consumed 3.30ml. The rate of product preparation was calculated at 89.2%.

The Content of Amino Acid Analysis

MSG production, extraction and separation process generates huge amount of wastewater. The wastewater contains protein, rich inorganic salt, organic acid, amino acids, residual sugar and nucleotide type of degradation products. The type and content of amino acid in concentrated MSG-wastewater were measured by automatic amino acid analyzer and listed in TABLE1. We could see that there were 12 kinds of amino acids in concentrated MSG-wastewater, in which glutamate content was the largest. The types and content of amino acids were very rich, especially glutamic acid residue was a lot during the production of monosodium glutamate. Carrying chelate reaction not only can make full use of amino acids, but also reduce the stress of monosodium glutamate wastewater to the environment.

TABLE2 listed the kinds and content of amino acid in chelate samples. It could be seen that there was a variety of amino acids in chelate samples, and the types were similar to those in MSG-wastewater. Glutamic acid content was the highest. Total content of amino acid reached 22.437%, which provided basis for the application of the compound copper amino acid chelate.

TABLE1. AMINO ACID CONTENT IN MSG-WASTEWATER

Amino acid	percentage composition (mg/100ml)	Amino acid	percentage composition (mg/100ml)
Aspartic acid	12.917	Tyrosine	69.438
Threonine	11.209	Phenylalanine	ND
Serine	11.161	Lysine	7.05
Glutamic acid	750.349	NH_3	140.348
Glycine	10.331	Histidine	2.499
Alanine	150	Arginine	ND
Cystine	ND	Proline	ND
Valine	71.848	Tryptophan	ND
Methionine	5.715		
Isoleucine	7.685		
Leucine	ND		
Total	1250.55		
ND: Not detectable			

TABLE2. AMINO ACID CONTENT IN CHELATE SAMPLE

Amino acid	percentage composition (g/100g)	Amino acid	percentage composition (g/100g)
Aspartic acid	0.282	Tyrosine	5.951
Threonine	0.139	Phenylalanine	ND
Serine	0.188	Lysine	0.049
Glutamic acid	12.264	NH_3	0.248
Glycine	0.259	Histidine	ND
Alanine	0.783	Arginine	ND
Cystine	ND	Proline	ND
Valine	2.274	Tryptophan	ND
Methionine	ND		
Isoleucine	ND		
Leucine	ND		
Total	22.437%		
ND: Not detectable			

Infrared Spectroscopic Analysis

Figure 1(a) showed the infrared spectrogram of sample, cupric glutamate and glutamic acid. The carboxyl symmetric stretching absorption peak of glutamate molecules was at 1421cm^{-1}. The amino asymmetric variable angle was at 1641cm^{-1}. And the amino stretching vibration ranged from 3200cm^{-1} to 2000cm^{-1}.

Whereas in the infrared spectra of compound copper amino acid chelate, carboxyl and amino absorption peak were displaced to 1409cm^{-1} and 1595cm^{-1}, respectively. It showed the production of new materials. Carboxyl oxygen and amino nitrogen of amino acid molecules were formed coordination bond with copper ions. Complexes had O-H stretching vibration peak at 3422cm^{-1}, which meant the presence of water molecules. Besides, complexes had O-H rocking vibration peak at 619 cm^{-1}, which showed the water molecules was involved in the coordination reaction.

In Figure 1(a), the peaks of cupric glutamate were similar to the peaks of compound copper amino acid chelate. It meant that glutamic acid mainly reacted with copper in chelating reaction, which proved the possibility that glutamic acid preferred to reacting with copper as glutamic acid had the highest content in concentrated MSG-wastewater.

In Figure 1(b), it showed the infrared spectrogram of two samples. The characteristic peaks of two samples were almost the same. They have same main functional groups and structural components, which means this chelating method is reliable, feasible and repeatable.

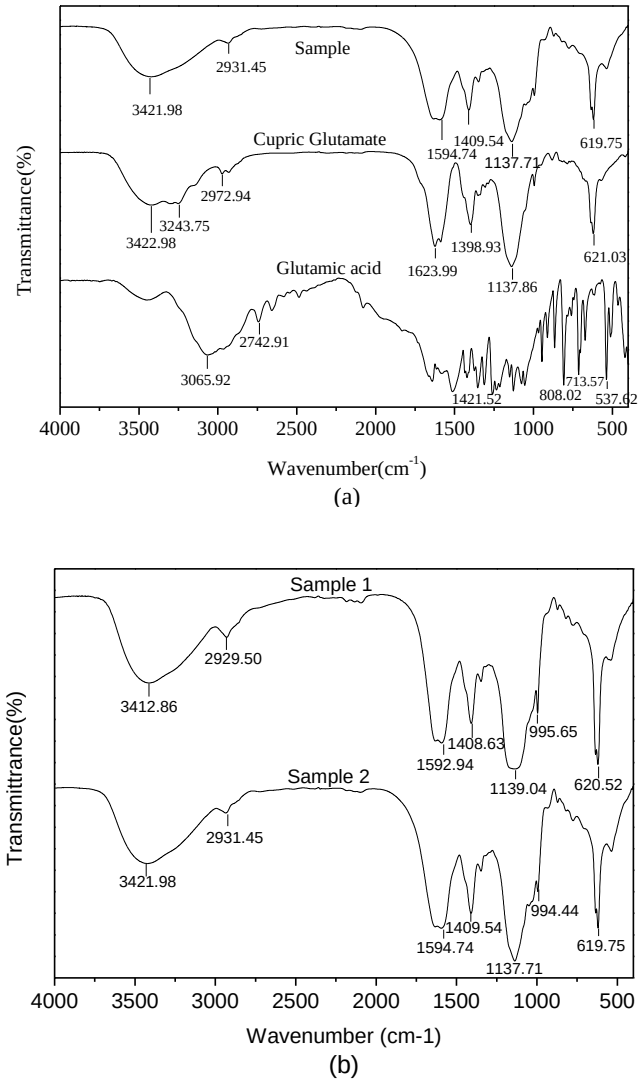

Figure1. a The infrared spectrogram of sample, cupric glutamate and glutamic acid
b The infrared spectrogram of sample 1 and sample 2

CONCLUSION

In this paper, we chose concentrated MSG-wastewater and copper sulfate as raw materials to prepare compound copper amino acid chelate. Automatic amino acid analyzer was used to analysis compound copper amino acid chelate. The content of chelate samples made in this experiment was 22.437%. And the rate of product preparation was 89.2%, which was measured by EDTA complexometric titration method. Infrared spectrum was used to analysis the samples and new characteristic peaks was found, which was respectively at 1595cm^{-1} and 1409cm^{-1}. Amino and carboxyl were involved in coordination reaction, and water molecules was found in samples and also involved in coordination reaction. The chelating method is feasible and repeatable, having extending significance in practical application.

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Cover page

Title: *Solar Optimisation Based on Different Tracking Techniques*

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ABSTRACT

The optimisation techniques employed during various solar tracking systems have improved the maximum efficiency of the generated output energy. This optimisation process constantly aim at aligning the surface of the solar photovoltaic (PV) panel to track the sunlight rays ensuring orthogonal orientation throughout the tracking period. The tracking process is achieved by the use of sensor devices (light dependent resistors (LDRs), photodiodes, phototransistors, webcam), computerised tracking program regardless of sensor devices such as artificial neural network (ANN) approach, fuzzy logic controller (FLC) approach, microprocessors, Global Positioning System (GPS) tracking system. This tracking process ensures effective uniform distribution and absorption of sunlight rays over the solar photovoltaic (PV) panel surface ensuring maximum power is absorbed for the conversion process to the load supply. The loss of energy associated with static installed solar photovoltaic (PV) panel gave rise to the need for optimisation of the solar photovoltaic (PV) system. This paper presents a review of the optimisation control in solar tracking techniques in enhancing maximum efficiency of the generated output energy, the attributes and briefly examines its technical limitations and concludes by providing future direction.

Keywords: Solar optimisation, Tracking techniques, Generated Output energy

1. INTRODUCTION

Recent technological advances in solar tracking optimisation have improved the means of harnessing the enormous daily released energy from the sun and reawakened the solar energy industry. The solar energy industry is classified as one of the cleanest green energy source since it is eco-friendly and pollution free. The transformation over the past two decades in the solar energy industry has brought about an improved efficiency in the generated output energy. The sensor tracking devices, integrated chips (ICs) are used for constant tracking of the sun to control the motor drive mechanism and positioning of the solar photovoltaic (PV) panel surface for maximum optimisation. The loss of continuous tracking in the static

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installed solar photovoltaic (PV) panel has led to reduction in the output generated energy and provided impetus for research in optimisation techniques. Various solar tracking algorithms and techniques have been proposed that are useful regardless of seasonal climatic change.

The solar tracking optimisations are categorised into these classification groups, the solar sensor circuit design group (involving the use of photo-resistors, photo-diodes, phototransistors, webcam, etc.), the hybrid group consists of the combination of solar circuit sensor, programmable logic controllers (PLCs) and integrated chips (ICs), maximum power point tracking (MPPT) group which has birth such tracking techniques such as particle swarm optimisation (PSO), perturbation and observation (P&O), artificial neural network (ANN) approach, fuzzy logic controller (FLC) approach, constant voltage tracking and many others.

Over the years, researchers in the solar energy optimisation have focused on the concept of software and hardware engineering aspect of solar tracking sensor devices, programmable logic controllers (PLCs) and integrated chips (ICs) devices for continual tracking in ensuring an improved efficiency in the solar technology industry. [1] proposed a one-axis sun tracking system technique for the optimisation of an improved efficiency solar tracking system. In this paper, the position of the sun are detected by three light-dependent resistors (LDRs). Each of these LDRs have specific designed function combining to produce a resultant signal fed into the drive mechanism unit of the solar tracking system causing a rotating effect of solar photovoltaic (PV) panel surface to attain orthogonal position to the sunlight rays continually. [2] proposed four electromechanical sun tracking systems design using a flat photovoltaic (PV) panel for the two axes, one-axis vertical, one-axis east-west and one axis north-south investigating on the best efficient means of the four tracking techniques deployed. This result showed an improved generated output energy of each of the proposed technique over the other. [3] proposed two axis sun tracking system with the use of a programmable logic controller (PLC) control coupled with the photosensors employing an open loop control system to control the motion of the sun tracking aligning to the surface of solar photovoltaic (PV) panel. The graphical results obtained from the research shows an evidence of a better improved solar tracking system. [4] proposed the design and construction of a two-axis tracking system for parabolic trough collector (PTC) efficiency improvement over the fixed tilted solar photovoltaic (PV) installed panel. The result obtained was about 46.46% efficient above the static installed solar photovoltaic (PV) system. [5] proposed a multi-axes sun-tracking system at different modes of operation, the results of the output was about 35- 40% efficient as compared to the fixed axis tracking system. [6] also proposed a multi-axes system with PLC control for photovoltaic (PV) panels, the result compared to the fixed system was 42.6% efficient. [5] proposed an efficiency improvement of solar photovoltaic (PV) panels using a sun tracking system combination of PLC, an electronic designed circuit and developed software to perform the technical tasks. [7] investigated the application of one-axis sun tracking system carried out with series combination of solar photovoltaic (PV) panels to establish a singular photovoltaic (PV) panel, the tracking process was carried out with the aid of the photo resistors and dc motor to control the movement of the solar photovoltaic (PV) panels to constantly be orthogonal with sunlight rays. The results obtained from the comparison with fixed solar photovoltaic (PV) system shows a better improved efficiency. [8] proposed an

algorithm to eliminate the trade-off existing between the tracking and the dynamic performance to enhance a high performance solar maximum power point tracking (MPPT). [9] investigated on the application of new control strategy for sun tracking, in this research an hybrid solar tracking system was built comprising of open and closed loop tracking systems eliminating the different sources of errors in the tracking system. The graphical comparison from the experimental results shows a credible efficiency gain in the generated output energy of the hybrid control system over the open loop tracking system. [10] proposed a design of a prototype micro-controller based multi-function solar tracking system, the maximum power point tracking (MPPT) data is transmitted in real time aligning the surface of connected solar systems constantly with the sunlight rays increasing the maximum efficiency generated.[11] proposed a feedforward maximum power point tracking of PV systems using fuzzy controller technique, reducing the burden of ripple effect and electromagnetic interference due to low ripple photovoltaic (PV) current thereby improving on the efficiency of the system.

The different reviewed tracking mechanism designs have shown reliability with certain degree of accuracy in the optimisation methods and improved efficiency in the generated output energy as compared with the fixed solar photovoltaic (PV) system. The objective of this paper is to present an overview analysis report on the various solar optimisation controlling units. This paper is organised as follows: Section 2 states the model of solar photovoltaic (PV) panel; Section 3 describes the Optimisation control techniques in solar tracking systems; Section 4 deals with Comparison of the output efficiency of the control tracking optimisation techniques and Section 5 concludes the results of our findings.

2. THE MODEL OF THE SOLAR PHOTOVOLTAIC (PV) PANEL

A solar photovoltaic (PV) panel is composed of a number of solar cells containing photovoltaic materials. The composition of the photovoltaic materials have advanced technologically in recent years because of the revolution in the solar renewable energy field as published by [12]. Some of the materials presently used include monocrystalline silicon, polycrystalline Fig. 1: The equivalent solar PV model silicon, armophous silicon, cadmium sulphide and copper gallium selenide/sulfide. The output energy generated from the solar photovoltaic (PV) modules depends on some factors, the watts rating of the solar cell, series or parallel arrangement of n-numbers of solar photovoltaic (PV) modules, solar irradiation level, temperature and solar optimisation control tracking techniques employed.

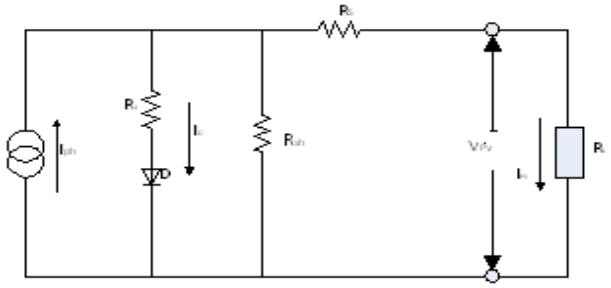

Fig.1 The equivalent solar PV model

The solar photovoltaic (PV) module is a non-linear device (power source) which converts the solar energy (sunlight rays) directly into electricity by photovoltaic effect. The equivalent solar photovoltaic (PV) model is descriptively represented in Figure 1. The model can be described using a current source having a diode across it ends and a shunt and series resistance connected across its load end. The mathematical model of an equivalent solar photovoltaic (PV) current module is given by the equation:

$$Iph = I_D + I_{sh} + I_{pv} \quad \text{(Kirchoff's Current Law)} \tag{1}$$

$$I_{pv} = I_{ph} - I_D - I_{sh} \tag{2}$$

The current flowing in the diode branch of the equivalent model is given by

$$I_D = I_{sat}\left[e^{\left(\frac{qV_{pv}}{Ak_bT_{cell}}\right)} - 1\right] \tag{3}$$

where I_{ph} (conversion current), is defined by the expression

$$I_{ph} = GI_{scr}\,(1 + K_o\,(T - T_r)) \tag{4}$$

also

$$I_{sat} = I_{rr}\left(\frac{T}{T_r}\right)^3 * e^{\left(\frac{qV_{pv}}{Ak_b}\right)\left(\frac{1}{T_r} - \frac{1}{T}\right)} \tag{5}$$

From eqn. (2) we have $I_{pv} = I_{ph} - I_D - I_{sh}$
and the solar cell voltage is expressed as

$$V = R_sI_{pv} + V_{pv} \tag{6}$$

The voltage measured across the diode is equal to drop across the shunt resistance R_{sh}

$$V = I_{sh}R_{sh} \tag{7}$$

Putting (7) in (6), we have

$$I_{sh}R_{sh} = R_sI_{pv} + V_{pv} \tag{8}$$

Fig. 2 Series/Parallel combination arrangement of solar PV modules

$$I_{sh} = \frac{R_s I_{pv} + V_{pv}}{R_{sh}} \quad (9)$$

therefore, from (2) the PV module current can be expressed as

$$I_{pv} = I_{ph} - I_D - I_{sh}$$

substituting the derived expression of I_D and I_{sh} respectively we have

$$I_{pv} = I_{ph} - I_{rr} \left(\frac{T}{T_r}\right)^3 * e^{\left(\frac{qV_{pv}}{Ak_b}\right)\left(\frac{1}{T_r} - \frac{1}{T}\right)} - \frac{R_s I_{pv} + V_{pv}}{R_{sh}} \quad (10)$$

$$I_{pv} = \frac{R_{sh}}{R_{sh} - R_s}\left[Iph - I_{rr}\left(\frac{T}{T_r}\right)^3 * e^{\left(\frac{qV_{pv}}{Ak_b}\right)\left(\frac{1}{T_r} - \frac{1}{T}\right)} - \frac{V_{pv}}{R_{sh}}\right] \quad (11)$$

The final photovoltaic (PV) module current is as expressed in (11) above. In order to increase the output generated energy of the solar photovoltaic (PV) module, parallel combination arrangement of solar photovoltaic (PV) are connected to get the desired output energy level. The pattern in the arrangement is as shown in Figure 2 such that the solar photovoltaic (PV) modules of X-parallel strings, each containing Y-identical solar cells in series can be expressed as in equation (12).

$$V_{array} = V_{cell} * X \qquad I_{array} = I_{cell} * Y \quad (12)$$

Therefore, the equivalent solar photovoltaic (PV) module current is defined by

$$I_{array} = \frac{R_{sh}}{R_{sh} - R_s}\left[YIph - YI_{rr}\left(\frac{T}{T_r}\right)^3 * e^{\left(\frac{qV_{pv}}{Ak_b}\right)\left(\frac{1}{T_r} - \frac{1}{T}\right)} - \frac{XV_{pv}}{YR_{sh}}\right] \quad (13)$$

TABLE I: Nomenclature

E_{gap}	Band-gap energy of the material
K_b	Boltzman's constant (1.38×10^{-23}J/K)
T_{cell}	Cell temperature (K)
q	Charge of an electron (1.6×10^{-19}c)
A	Ideality factor
I_{pv}	Output current (A)
V_{pv}	Output voltage (V)
I_D	PN junction diode current
I_{sat}	PV saturation current
I_{ph}	Light generated current
T_r	Reference temperature
I_{rr}	Saturation current at T_r
R_s	Series resistance
I_{scr}	Short circuit current at reference condition
K_0	Short circuit temperature coefficient
R_{sh}	Shunt resistance

Fig. 3: Block diagram of an open loop control system

3. OPTIMISATION CONTROL TECHNIQUES IN SOLAR TRACKING SYSTEMS

The optimisation control of the solar tracking system is very significant due to the goal of achieving correct orthogonal orientation aligning the surface of the solar photovoltaic (PV) module to constant reception of the sunlight rays. The control techniques employed in solar optimisation are namely the open loop control system, closed loop control system and the hybrid (combination of close and open) loop system. The improved efficiency in the optimisation control of solar tracking system depends on the choice of feedback sensors, the tracking mechanism device (dc motor, stepper motor, servo motor) and solar irradiation geometry models introduced into the system. The open-loop control system uses an actuating device to control the tracking process without a feedback device as shown in Figure 3. Most researchers prefer using a closed loop control system or an hybrid loop control system to perform the tracking task. The actuating device, programmable logic controller (PLC) is fed by a pre-programmed calculated input, i_{cal} stored in the computer system to control the tracking device, which controls the subject of orientation to give the desired output [13].

3.1 Open-loop Control Tracking System

In an open-loop control system the fed input signal cannot determine if the desired output is achieved, instead it relies on the control algorithms such as solar irradiation geometry model introduced in the system to compensate for disturbances or errors within the system [14]. The calculated inputs (i_{cal}) algorithms are pre-determined relative to the position and location of the installed solar tracking system parameters in terms of the geographical latitude (Φ), the declination (δ), the solar altitude (α), the sun azimuth angle (γ), the hour angle (ω)and the ecliptic orbit angle to the equator (ε).

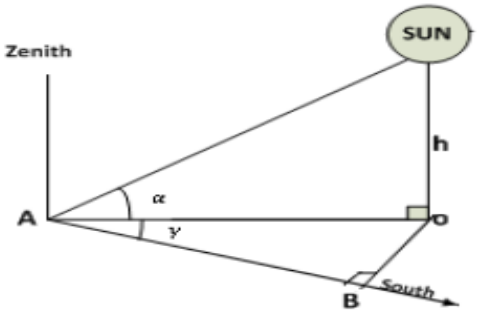

Fig. 4: The sun elevation angle and azimuth

These parameters form the vector equations describing the sun's precise position and the intensity of the rays of sunlight received by the tilted controlled solar panel plane. The sectional view of the celestial sphere (three dimensional co-ordinates x; y; z respectively) showing the trigonometry of the sun elevation, azimuth angle and the earth are as shown in Figure 4. Each of these calculated parameters are programmed into the computer system with standard mathematical expressions and thus briefly explained as follows:

(1) Ecliptic longitude of the sun: The ecliptic longitude, λ of the sun is the angle between vector S and the horizontal axis plane. The mathematical expression is used to determine the ecliptic longitude of the sun, n is the number of the day, such that n = 1st on the 1stof January, this implies the values of changes daily and computed for accuracy.

$$\sin\lambda = \sin\left[360^0\left(\frac{284+n}{365.25}\right)\right] (0^0 \leq \lambda < 360^0) \quad (14)$$

(2) Sun elevation angle: The sun's elevation angle, α is the vertical angle between the horizontal plane and the connecting plane to the sun. The mathematical expression is given as ;

$$sin\,\alpha = cos\omega cos\delta cos\phi + sin\phi sin\omega \quad (15)$$

(3) The hour angle: The hour angle, ω is defined as the angular distance that the earth has revolved in a particular day. This values for each hour of the day is constantly changing and at noon day the hour angle, $\omega = 0^0$. The mathematical expression is given as;

$$\omega = 15^0(hour - 12) \quad (16)$$

(4) Declination of the sun: The declination, δ is the angle that the vector S makes with the equatorial plane describing the latitude of the sun. The ecliptic plane and the equatorial plane intersect on the horizontal axis, the angle between these two planes is $\varepsilon \approx 23.45^0$. The mathematical expression is given as;

$$sin\delta = sin\lambda sin\varepsilon \quad (17)$$

(5) The Sun azimuth: The sun's azimuth angle, γ is the angle between the vector S and the sun projection in the south direction as shown in Figure 4. The value of the sun's azimuth changes every hour and daily. The mathematical expression is given as;

$$sin\gamma = (sin\omega cos\delta)(cos\alpha) \quad (18)$$

In eqn.18 above, if $sin\gamma > 1$ then eqn.19 is used to determine the azimuth angle.

$$sin\gamma = (sin\omega cos\delta)(cos\alpha cos\phi)^{-1} \quad (19)$$

3.2 Closed loop Control Tracking System

The closed loop control for the solar tacking system utilizes a measurement of the output signal which is feedback into the measuring device to achieve the desired orientation. A simple closed loop tracking system for a single solar-tracking module

is as shown in Figure 5. This provides a more efficient tracking system ensuring constant orthogonal reception of the solar photovoltaic (PV) module surface from the solar rays achieving maximum generated output energy during the day period. In the closed loop system, several components functions perform a particular function to achieve the desired orientation. These components functions are thus explained as follows:

(1) Measuring Device: The measuring device employed in solar tracking are referred to as solar sensor device such as photodiode, light dependent resistor, photo transistor, global positioning sensor, electronic compass and webcam etc.

(2) Desired Orientation: The desired orientation is to constantly achieve the solar photovoltaic (PV) module surface reception towards the sun at all times of the day.

(3) System Output: The system output is to ensure maximum efficiency of the desired tracking orientation is achieved.

(4) Tracking Device: The tracking device employed in solar tracking systems often are the dc motors and stepper motors. The resultant difference from the comparator controls the rotation of the solar photovoltaic panel (subject of orientation).

(5) Subject of Orientation: The subject of orientation are the flat solar photovoltaic (PV) modules, spherical photovoltaic (PV) modules either coupled with lenses or mirrors for higher absorption and conversion concentration.

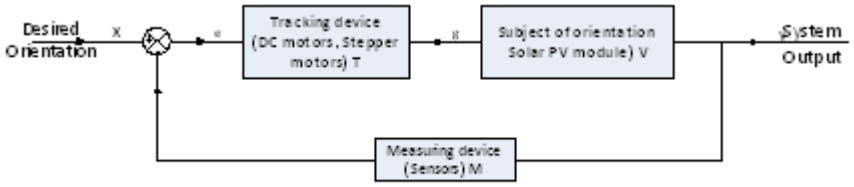

Fig. 5: A simple closed loop control solar tracking system

From Figure 5, the closed loop transfer function for the solar tracking system, H(s) is obtained if these assumptions are considered, tracking device T, the solar photovoltaic (PV) module V, and the measuring device M, are linear and time invariant, the system above therefore can be arranged using the Laplace transform on the variables. This gives the following relations:

$$Y(s) = V(s)G(s) \tag{20}$$

$$G(s) = T(s)E(s) \tag{21}$$

$$E(s) = X(s) - M(s)Y(s) \tag{22}$$

Putting eqn. (22) in eqn. (21) we have

$$G(s) = T(s)(X(s) - M(s)Y(s)) \tag{23}$$

Putting eqn. (23) in eqn. (20) we have

$$Y(s) = V(s)[T(s)(X(s) - M(s)Y(s))] \tag{24}$$

$$Y(s) = V(s)\,T(s)\,X(s) - V(s)\,T(s)\,M(s)\,Y(s) \quad (25)$$

$$Y(s)\,[1 + V(s)\,T(s)\,M(s)] = V(s)T(s)X(s) \quad (26)$$

$$H(s) = Y(s)\,X(s) = \frac{V(s)T(s)}{1 + V(s)T(s)M(s)} \quad (27)$$

The numerator component of the transfer function is called the forward (open-loop) gain and the denominator component of the transfer function is called the loop gain.

3.3 Hybrid Loop Control Tracking System

The hybrid loop control tracking system as shown in Figure 6, basically combines the open loop and closed loop control system in order to achieve a more efficient tracking orientation. The open loop system is based on the feedforward control while the closed loop system is based on the feedback controller method designed to correct the tracking errors made by the feedforward control. The actuating device is programmed with mathematical equations models of the sun's movement combining with the calculated solar radiation equations to produce the necessary signal controlling the movement of the tracking devices thereby subjecting the object of orientation to the sun reception. The measuring device sends the estimated positioning of the sun with the feedback signal from the system output in the closed loop to the actuating device in continuous closed loop. The hybrid orientation tracking system caters for the cloudy periods when the sensors are inefficient to provide tracking directions, the solar equations programmed into the actuating device serves as a backup to ensure constant orthogonal reception is achieved with the purpose of providing close approximation for minimising errors throughout the tracking period.

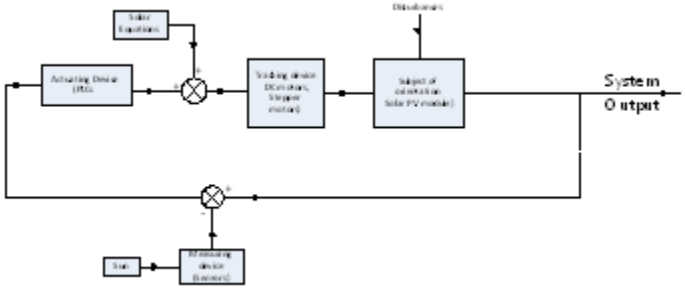

Fig. 6: Hybrid loop control solar tracking system

4. COMPARISON OF THE OUTPUT EFFICIENCY OF SOLAR CONTROL TRACKING OPTIMISATION TECHNIQUES

The introduction of different algorithms and techniques used in solar control tracking optimisation have proven to be more efficient compared to the mounted inclined solar photovoltaic (PV) tracking system relative to the generated output energy. The output efficiency fundamentally depends on the amount of solar irradiation and temperature captured by the solar photovoltaic (PV) surface. The

control tracking optimisation helps uniform distribution and absorption of the sunlight rays on the entire solar photovoltaic (PV) surface during the tracking period. Each of these three modes of tracking in solar control optimisation employs one or combines one of the classification groups of control techniques for effective energy absorption of the sunlight rays by solar photovoltaic (PV) surface. As discussed in the earlier section, each of this mode of control tracking techniques has its unique advantage over the other in terms of simplicity in design structure, robust efficiency in maximising the energy harvest by the solar photovoltaic (PV) surface. A comparative study of published papers employing one of these three classifications of control tracking techniques and the output energy efficiency performance are tabulated in Table II. Abdallah et al. (2004) used an open loop control system for the two axes sun tracking system with programmable logic controller (PLC) control. The results obtained compared with fixed surface showed a better performance. Hua et al. (1997) employed a closed loop control tracking system for maximum power tracking; the technique shows a higher efficiency in the MPPT control. Huynh et al. (1996) analyses a microprocessor-controlled peak power tracking system using a control-loop system stability criterion to establish the optimum dynamic performance and stability compared to the static installed solar photovoltaic (PV) tracking system. Khalifa et al. (1997) employs a closed loop tracking system to investigate the effect of two axis sun tracking on the performance of compound parabolic concentrators (CPC). The results compared to fixed collector proved a better performance with increase in the collected energy. F.R. Rubio et al. (2007) investigate an hybrid control loop for sun tracking system comparing its efficiency with an open loop tracking system. The results presented from the new control strategy were significant compared to the classical open loop tracking strategy. Kuo et al. (2001) presented a novel MPPT controller for photovoltaic energy conversion system employing an hybrid control loop tracking

TABLE II: Comparison of different control loop tracking efficiency performance

Algorithms	References	Efficiency above the static solar tracking
Open-loop control tracking system	Abdallah et al. (2004)	41.34%
	Abdallah et al. (2004)	43.87%,37.53%,34.43%,15.69%
	Sungur et al. (2009)	42.6%
Closed-loop control tracking system	Kalogirou et al. (1996)	-
	Enslin et al. (1997)	25%
	Khalifa et al. (1996)	75%
	Bahgat et al. (2004)	45.2%
	Taki et al. (2011)	33%
	Vorobiev et al. (2004)	30-40%,50-60%
	Sefa et al. (2009)	45%
	Stjepanovic et al. (2010)	24%
	Kaseem et al. (2011)	64%
	G.C.Bakos et al. (2006)	46.46%
	M.M. Abu-Khader et al. (2008)	30-45%
	A.Al-Mohamad et al. (2004)	40%
Hybrid-loop control tracking system	Kuo et al. (2001)	-
	Pandey et al. (2008)	94.6% MPP tracking system

The results obtained from the computer simulations and experimental demonstrates the superior performance of the proposed technique.

5. CONCLUSION

In this paper, the state of art of solar tracking optimisation has been reviewed. The three main control loop tracking systems employed in solar tracking optimisation to obtain maximum power point tracking (MPPT) have been discussed. This optimisation ensures uniform solar irradiation on the solar photovoltaic (PV) surface increasing the output generated energy efficiency compared with the fixed solar tracking system. The control tracking optimisation in some of the reviewed literatures are more effective as compared to that of the static solar photovoltaic (PV) system under varied solar irradiation levels, temperature and weather conditions capturing maximum solar photovoltaic (PV) power. The available evidence supports the need for solar tracking optimisation.

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Cover page

Title: *Bioaugmentation of Leachate Polluted Soil for Optimal Removal of Heavy Metals*

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ABSTRACT

Whereas many sources of heavy metal contamination of soil abound, leachate pollution is one of such which is gradual but persistent, and habours many environmental pollutants including heavy metals. Therefore technologies are been developed to remedy heavy metal contaminated soils in a more environmental friendly and sustainable form. Bioremediation has shown promising results in the direction of heavy metals removal from soil, yet how to manipulate the microbes to generate the combination that will yield optimal remediation is still limited. Hence, this study worked to optimize bioaugmentation as a tool for more effective removal of heavy metals from leachate contaminated soil. Leachate polluted site was characterized to obtain nine microbes which were considered persistent in the soil despite the presence of heavy metals. Therefore, the microbes were manipulated in both combinations and concentrations (1.3 ABS at 600nm), and reintroduced into 10% v/w induced leachate contamination on garden soil. Calculated percentage of removal for Pb and Cu showed that the use of three microbes (in combinations) out of the isolated nine yielded 71% and 86% removal, respectively, against fewer values obtained when the combination of the nine organisms was used. With the significant difference ($P < 0.05$) in the study, it concludes that a combination of *Bacillus* sp., *Lysinicbacillus* sp. and *Rodococcus* sp. alone posses a special interaction which give the inoculum a heavy metal removal edge over the use of a combination of all the isolated microbes, as well as, in situation of non-inoculation of the contaminated soil.

INTRODUCTION

Both terrestrial and aquatic ecosystems constitute the environment, with soil being the integral component that serves diverse purposes, including agricultural and industrial activities. However, the usefulness of soil may be hampered as a result of the introduction of substances that are either completely alien to soil environment or existing at higher/undesirable concentrations; hence the phenomenon known as contamination. But, considering the continued anthropogenic activities which initiate contamination either on terrestrial and aquatic ecosystems, technologies now

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emphasize on how to remedy polluted/contaminated sites in a green manner with the optimal interest of making them suitable for natural use.

It is well documented that human, industrial and other related activities induced negative impacts on biological systems and more in particular, the soil status [1]. Municipal solid waste (MSW) dump sites serve as a disposal option but pose detrimental impact to soil in most cases as a result of leachate generation. Leachate is liquid generated when rainwater percolates through waste dump to the ground and is one of the heterogeneous pollutants to the soil. The heterogeneous nature of leachate is in such a way that it contains monocyclic aromatic hydrocarbons, xenobiotic organics, polychlorinated biphenyls (PCB) and heavy metals, among other [2].

Among the mentioned components of leachate, heavy metals are of significant importance, because soil is a main sink for them. However, it is ironic because while most organic contaminants can be oxidized under microbial interactions to CO_2, metals tend not to be chemically and microbiologically degraded [3]. Hence, consequent upon the introduction of heavy metals in the soil, the total concentration persists for a long time [4]. Yet there are possibilities of having changes in their chemical forms and bioavailability. Environmentally, heavy metals pose a risk, and among the commonly implicated within contaminated sites are arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), zinc (Zn) [5] and even Fe.

Therefore, characterization and remediation are required for adequate protection and restoration of soil ecosystem that is contaminated with heavy metals from leachate pollution. In most cases, immobilization, soil washing and phytoremediation have been the adopted techniques for remediation of heavy metals contaminated soil [5]. However, such technologies are still commercially limited to developed nations considering the cost and operation principles. But with the increased awareness across governments, public and private sectors on the effects of contaminated soils on human and animal health, including agricultural production, scientists while considering scarcity of funds are saddled with the task of developing more green but cheaper technologies for the purpose of remedying heavy metal contaminated sites. Based on the foregoing, this study was designed to optimize bioaugmentation as a tool for removal of heavy metals from leachate contaminated soil. Therefore, the study intends to evaluate the effect of utilizing persistent microbes in leachate polluted soil in optimizing heavy metal removal.

MATERIALS AND METHODS

Samples Collection and Characterization

Raw leachate samples were collected from a non-sanitary landfill in Malaysia. These were analyzed to identify the physico-chemical properties [2]. Similarly, two classes of soil samples were collected; first from landfill environment polluted with raw leachate required for microbial isolations, and the other which was non-contaminated garden soil used for bioremediation experiment. Collection of soil samples were carried out according to 2004 ASTM E-1197 Standard guidelines for conducting terrestrial soil-core microcosm test [6].

Microbial Experiment

Bioaugmentation was the preferred method of bioremediation adopted in this study, and as such isolation of microbes from the leachate polluted soil was important. This is because the presence of microbes in the landfill environment may imply the persistent nature of the microbes, hence its potential involvement in some biological processes taking place within the contaminated soil. Therefore, 1 g of soil was mixed with 0.9% NaCl and the suspension vortexed for 2 hrs at 150 rpm using Lab-line 3521 orbit shaker. Serial dilutions were plated [7] on nutrient agar (NA) and subsequently incubated for 48 hrs at 33^0C. Single colonies were re-streaked separately on freshly prepared NA to obtain discrete pure cultures which were eventually identified using Biolog GEN III MicroPlate protocol [8, 9].

Microbial Formulation and Bioaugmentation Set-Up

The formula used in the bioaugmentation experiment contained nine strains of microbes isolated from the first class of soil (leachate contaminated site). Each strain was grown as a pure culture and discrete suspensions at the same physiological phase (1.3 ABS at 600nm) were then pooled in equal proportions to set-up inoculums for bioaugmentation. Soil microcosms of three treatments (A, B & C) were prepared from introducing 10% v/w of leachate concentration to the non-contaminated garden soil. Treatment A was inoculated with the nine strains of bacteria, whereas treatment B had only three of the strains, while C had no microbial addition. Portions of soil microcosms were sacrificed every 20 days (until 100 days) for onward metal analysis and assessment of microbial density. Each soil sample taken for sample analysis was acid-digested [10] using Multiwave 3000 microwave digester, while Optima 53000 DV was used to obtain the elemental concentrations of Pb and Cu.

Data obtained were further processed to calculate the percentage of Pb and Cu removal using;

$$\% \text{ of heavy metal removal} = \left(\frac{C_{0(x)} - C_{F(x)}}{C_{0(x)}}\right) X\ 100\% \quad (1)$$

where

$C_{0(x)}$ = initial concentration of metal "x" (Pb or Cu) in the soil at the start of experiment

$C_{F(x)}$ = final concentration of metal "x" (Pb or Cu) in the soil at the end of experiment

Similarly, first order kinetic model was used to compare the rate of heavy metals removal;

$$K = -\frac{1}{t}\left(\ln\frac{C}{C_0}\right) \quad (2)$$

where

K = first order rate constant for metal uptake per day

t = time in days

C = concentration of residual metal in the soil (mg/Kg)

C_0 = initial concentration of metal in the soil (mg/Kg).

RESULTS AND DISCUSSION

The soil which was originally contaminated by leachate that seeps from waste cells was characterized of microbes (bacteria) and Table I showed the list of nine isolated bacteria and the distribution in the bioaugmentation experiment.

TABLE I. ISOLATED BACTERIAL SPECIES AND DISTRIBUTION IN MICROCOSMS FOR BIOAUGMENTATION

Treatment A	**Treatment B**	**Treatment C (Control experiments)**
Bacillus sp.	*Bacillus sp.*	NU
Psuedomonas sp.	NU	NU
Stenotrophomonas sp.	NU	NU
Flavimonas sp.	NU	NU
Lysinibacillus sp.	*Lysinibacillus sp.*	NU
Acinetobacter sp.	NU	NU
Brevundimonas sp.	NU	NU
Microbacterium sp.	NU	NU
Rodococcus sp.	*Rodococcus sp.*	NU

"NU" means not used (meaning that such bacterial in treatment A was not used)

It showed diverse genera of bacteria that included both gram-positive and –negative *Bacillus*. Though many literature did not portray some of the identified microbes as enhancers of any sort of bioremediation (including heavy metals), yet their presence in the leachate polluted soil raises the interest on their existence and function in the contaminated environment. The spiked soil was characterized of heavy metals (Pb & Cu) as shown in Table II. It is worthy of note that the concentrations of the heavy metals were viewed for remediation based on the availability in the soil after being spiked with 10% v/w of raw leachate. The Table II also showed the residual/final concentrations of the heavy metals after bioaugmentation for the 100 days.

TABLE II INITIAL AND RESIDUAL CONCENTRATIONS OF HEAVY METALS FROM THE BIOREMEDIATION OF LEACHATE CONTAMINATED SOIL

Heavy metals	Initial Concentrations (mg/kg)	Mean Residual Concentrations (mg/kg)		
		Treatment A	Treatment B	Treatment C
Pb	2.068	0.84	0.60	1.21
Cu	0.241	0.09	0.03	0.11

Discrete concentrations of the metals across the various 20 day biomonitoring showed similar variations. One-way ANOVA for both metals took into account concentrations of the heavy metals at both initial and the intermittent monitoring for the 100 days and the result were significant with $P < 0.05$. The degree of significance were higher with the inoculated treatments (A&B) ($P = 0.02$). Reason may be the influence of the microbes as introduced into the soil microcosms and can be correlated with the fact that the bioremediation of polluted soils using microbes has been widely reported [11, 6].

However, a clearer understanding of the result was obtained when compared

across the treatments. Figure 1 showed the extents to which both metals were removed from the individual treatments. It reflects that while 71% of Pb was removed in treatment B in 100 days, only 59% and 42% were removed in treatments A and C. Basically, natural bioremediation cannot be ruled out in the control experiment since the soil was not autoclaved nor sterilized in any form, hence normally existing microbes may have some effect but in lesser extent when compared to other treatments. This implied that Pb concentration showed a significant reduction with the introduction of inoculums into the leachate polluted soil. Therefore, the calculated rate of Pb removal was 0.0124/day when inoculums B was utilized while 0.0087/day and 0.0053/day were removed in treatments A and C, respectively.

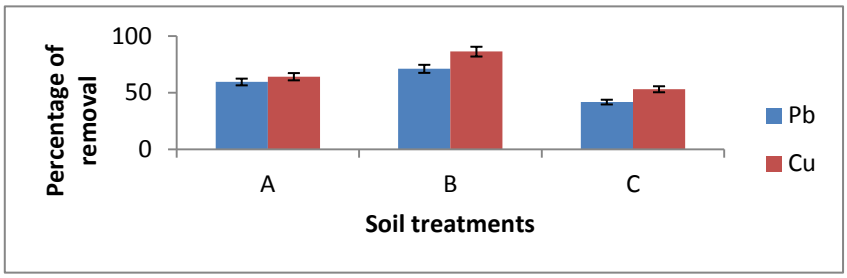

Figure 1. Perecentage of Pb and Cu removed during bioremediation

Similarly, treatment B recorded 86% of Cu removal as against 64% and 52% obtained in treatments A and C. The significant difference between treatment B and others were pronounced; B & A ($P = 0.03$); B & C ($P = 0.003$). Though Cu might be needed in minute concentrations as trace dietary mineral but its toxic effect in the environment led to the interest for its reduction via bioaugmentation. Cu removal rates across the treatments also showed that 0.0212/day, 0.0099/day and 0.0078/day was obtained for treatments B, A and C, respectively.

Therefore with highest percentage and rate of Pb and Cu removal pronounced most in soil amended with only 3 bacteria species, it might seem to be a surprise especially as treatment A contained all the 9 isolated bacteria species, since it may be easy to hypothesize that with increased diversity of microbes, bioremediation potential will increase. The mechanism behind the result may be found in interactions that exist among microbes when concentrations and diversity are manipulated. *Bacillus sp* which was found in the two inoculums (A&B) is known to have heavy metal removal capacity and has shown 77% removal of Pb and 8% Cu, from a mine extract [12]. Hence, this ability is enough to have enhanced Pb and Cu removal in treatments A and B, but seemed that the best interaction that yielded the optimal removal for the studied heavy metals existed among *Bacillus* sp, *Lysinibacillus* sp and *Rodococcus* sp, only. This argument can be buttressed further by the fact that it is expected that treatment A would have shown the optimal Cu removal considering the implication of *Pseudomonas* sp, *Brevundimonas* sp and *Stenotrophomonas* sp in previous studies of Cu removing potential [13,14]. Hence, the selective use of the 3 bacteria in treatment B might have special form of interaction especially as they were all gram-positive bacteria. Also considering that *Lysinibacillus* sp. possessed a hex-histidine tag (His_6--tag) at the C-terminus of its S-layer protein SbpA, it is possible that the metal binding property of His_6-tag was better expressed when in association with *Bacillus* sp. and *Rodococcus* sp., hence providing the bioremediation edge for treatment B.

CONCLUSION

In general, it is evident that the amended soils have bioremediation ability than the non-inoculated heavy metals contaminated soil. However, the study concludes that amendments differently prioritize heavy metals removal. Therefore leachate-site-persistent microbes can remedy heavy metals in leachate polluted soil. The use of *Bacillus* sp., *Lysinibacillus* sp. and *Rodococcus* sp. in combined state can avail optimal removal of Pb and Cu for heavy metals contaminated soil.

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Cover page

Title: *The Correlation of Fat Acidity, Antioxidant Activity and Phenolic Compounds of Brown Rice Following Storage*

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ABSTRACT

Although the changes in rice physical and chemical during rice ageing have been extensively studied previously, the understanding of ageing-induced changes in brown rice phenolic compounds (PC) and antioxidant activity (AOA) was very limited. This study showed that the content of Free-PC was lower or nearly equal to Bound-PC depending on rice cultivars. However, the corresponding AOA was otherwise. Storage at 37 ℃ led to a reduction of PC and AOA both in free and bound fractions, indicating they were all involved in rice ageing.

1. INTRODUCTION

Whole grains contain all the essential parts and naturally-occurring nutrients of the entire grain seed in their original proportions, which are usually made up of the endosperm, the germ, and the bran of the grain. It has been measured that whole grains contain higher level of phytochemicals than their refined grains. Epidemiological studies indicate that regular consumption of whole grains or related food products reduces the incidences of chronic disease and several forms of cancer[1]. The biologically plausible mechanisms may be due to the scavenging of free radicals by the bioactive phytochemicals present in whole grains[2]. The phytochemical compounds mainly present in whole grains are phenolic acids and flavonoids, existing as free and bound forms[3]. In recent years, the consumption of brown rice is becoming more and more popular because of its higher dietary fiber and antioxidants contents compared to white rice[4].The pattern of rice usage leads to the need to store rice over varying periods. The changes in rice chemical and physical properties have been have been extensively studied during storage (termed as rice ageing). However, the study on the influence of storage on the phenolic compounds and antioxidant activity of brown rice is very limited in the available literature review. Thus, the aim of the present researches to exam the changes of the content of phenolic compounds, flavonoid compounds, antioxidant activity and fat acidity when brown rice stored at 4 ℃ and 37 ℃, and the correlations among these parameters.

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2. MATERIALS AND METHODS

2.1 Rice Samples

Rice grains were cultivated in the 2009-2010 growing season in the North of China, named BLR and BRR. After the removal of hulls, the two brown rice cultivars were obtained in this study. BLR is a light brown rice, and BPR is a purple brown rice.Bulk samples (2 kg) of the two brown rice cultivars were stored at 4℃ and 37℃ in sealed glass bottles, and withdrawn at the different interval during the storage for up to 6 months. Prior to each analysis, the brown rice was ground using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO, USA) through a 0.5mm sieve screen. All the analysis was performed in triplicate.

2.2 Extraction of Free Phenolic Compounds (Free-PC)

Before the extraction of phenolic compounds, rice sample was de-fatted using 100% hexane for twice times (1:20 g/v for each treatment) to remove lipids of rice.1g of the rice residue was mixed with 20 mL of 80% methanol and homogenized for 2min. The mixture was magnetic stirred at room temperature for 1 h with the protection of light. The mixture was centrifuged at 2920 g for 10 min and the supernatant was collected. The residue was mixed with 10 mL of acetone/water/acetic acid (70/29.5/0.5, v/v/v) and repeated above procedure. 20 mL of 80% methanol (chilled, pH 2.0) was added to the collected residue for the third extraction. The three supernatants were combined together and used for the analysis of FPCs and antioxidant assay. The final residue was washed twice times using pure acetone, air-dried and then used for analysis of bound phenolic compound (Bound-PC).

2.3 Extraction of Bound Phenolic Compounds (Bound-PC)

After the extraction of Free-PC, the dried residue was used for the determination of Bound-PC according to an alkaline extraction protocol in Zhou et al[5].

2.4 Determination of Total Phenolics Concentration

The total phenolics in both Free-PC and Bound-PCwere analyzed using the Folin–Ciocalteu assay. Briefly, 0.1 mL of the extract (Free-PC and Bound-PC) was mixed with 0.25 mL of Folin-Ciocalteu reagent. After gentle shaking, 3 mL distilled, deionised water was added to the mixture. After incubation at room temperature for 2 min with the protection of light,1.0 mLof 15% sodium carbonate was added and left for 1 min, and then made the volume to 10.0 mL with distilled, deionised water. After incubation at room temperature under dark for 90min, the absorbance was determined at 760 nm against the reagent blank. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the total phenolic content was expressed as mg gallic acid equivalent per 100 g grains (mg GAE/100g).

2.5 Determination of Total Flavonoid Concentration

The total flavonoid concentration of rice samples both in Free-PC and Bound-PC was determined by using a colorimetric method[6]. 1.00 mL of the extract was mixed

with5 mL of distilled, deionized water in a 10 mL volumetric flask, then added 0.30 mL of 5% sodium nitrite. After 5 min, 0.30 mL of 10% AlCl3 was added to the mixture.After 6 min, 2 mL of 1 molL-1NaOH was added, followed by the addition of distilled, deionized water to the volume.The absorbance was measured immediately at 510 nm. The content of total flavonoids was calculated from the calibration curve of catechin standard. Measurements were calibrated to a standard curve of prepared catechin solution, and the total flavonoid content was expressed as mg(+)catechin equivalent (CE) per 100 g grains.

2.6 Measurement of Fat Acidity of Brown Rice Following Storage

Fat acidity was measured by the colorimetric method[7] in mg [KOH]100 g^{-1} dry weight (d.w.). Absorbance at 440nm was measured with a spectrophotometer. A calibration curve was previously made using a standard of linoleic acid.

2.7 Determination of Antioxidant Activity (AOA)

The antioxidant activity of the rice grain extracts (both Free-PC and Bound-PC) was assessed in terms of hydrogen donating or radical scavenging abilities. Extracts were tested at a standardized phenolic concentration of 0.1 mmol/L (gallic acid equivalents). There action for scavenging DPPH radical was conducted in 15 mL polypropylene tubes at room temperature (25 ℃). Extracts of rice tested at a phenolic concentration of 0.1 mmol/L (gallic acid equivalent) were added to 2.8 mL of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*, 98.9 mmol/L in methanol) and vortexed for 15 s. The decrease in absorbance of DPPH* was measured at 515 nm in a photodiode array spectrophotometer, starting at the time the solution was added and then every hour until no further change in absorbance was measured (ca. 6 h). Methanol served as blank solution (equivalent methanol was added to test samples); 0.020 mmol/L of Trolox was used as a positive antioxidant control; and 2.8 mL of DPPH* plus 100μ L methanol served as a control. The AOA was calculated as a Trolox equivalent (mmol TE) per g of grain by comparison to a standard curve.

2.8 Statistical Analysis

Experimental data were subjected to analysis of variance using Genstat 5 (release 4.1). Treatment means were tested separately for least significant difference (lsd) at a 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Cultivar Influence on Phenolic Compounds in Free-PC and Bound-PC Extracts

The content of phenolic compounds present in the two rice cultivars and the corresponding antioxidant activity (AOA) were listed in Table 1. It is clearly to see that cultivar BPR had a much higher content of phenolic compounds both in Free-PC and Bound-PC, respectively, than cultivar BLR, indicating the genetic influence on phenolic compounds of rice. It was also found the content of Bound-PC was higher

than that of Free-PC in cultivar BLR, whereas the contents of phenolic compounds between Free-PC and Bound PC for cultivar BPR were very close. This suggests that more proportion of total phenolic compounds distributes in the out layer (ie. the bran of rice) of the purple brown rice (BPR) compared to the normal brown rice.

TABLE 1. PHENOLIC COMPOUNDS (MG GAE PER100G RICE), FLAVONOID COMPOUNDS (MG CE PER 100G RICE), FAT ACIDITY (FA, MG KOH PER 100G RICE), AND ANTIOXIDANT ACTIVITY (AOA, MMOL TE PER G RICE).

Free phenolic compounds														
CUL	BLR							BRR						
ST		4℃			37℃				4℃			37℃		
SD	0	2	4	6	2	4	6	0	2	4	6	2	4	6
TP	60.5	62.3	58.7	58.5	37.3	34.4	30.3	317.2	324.8	312.0	297.4	302.4	290.2	279.9
TF	32.9	35.3	30.0	29.3	29.0	24.4	18.7	117.0	119.2	115.7	115.1	101.4	95.2	88.3
FA	25.7	27.9	29.7	33.5	39.3	47.8	66.7	27.9	29.4	31.2	33.5	36.8	48.1	60.2
AOA	4.5	4.0	3.9	3.6	4.0	3.6	3.0	19.4	18.8	18.3	18.0	17.8	15.2	12.8
Bound phenolic compounds														
TP	87.8	89.1	86.7	86.3	85.0	82.7	80.3	311.8	318.0	306.8	303.1	298.6	292.7	289.7
TF	44.7	46.8	42.2	40.1	42.4	40.0	37.2	121.3	123.9	118.5	112.2	117.6	102.1	97.7
AOA	3.7	3.6	3.4	3.3	3.3	3.0	2.6	11.3	11.0	10.7	10.3	10.5	9.2	8.3

CUL: cultivar; ST: storage temperature; SD: storage duration (month); TP: total phenolics; TF: total flavonoid; FA: fat acidity; AOA: antioxidant activity.

The antioxidant activity (AOA) of the two rice grains showed that AOA for Free-PC extract was significant higher for cultivar BPR than cultivar BLR(Table 1), suggesting that rice BPR may serve better as a reducing agent than rice BLR. The AOA difference in Free-PC extract between the two cultivars is highly consistent with their content of phenolic compounds. Although the content of phenolic and flavonoid compounds in Bound-PC extracts was either higher or nearly close to the content of phenolic and flavonoid compounds in Bound-PC extracts, their corresponding AOA showed otherwise level.This study might suggest that the reducing capacity/radical scavenging capacity of individual phenolic and flavonoid compound may vary depending on its molecular and configuration structure[8].

3.2 Effect of Storage on Phenolic Compounds in Free-PC Extract

The influence of storage temperature on the phenolic compounds was present in Table 1. Generally, the content of phenolics was slightly increased at the first 2-month storage, followed by a minor decrease but with no significant difference in the contents for the two rice cultivars stored at 4 ℃. However, a consistent decrease in the content of phenolics and flavonoids was found following the storage at 37 ℃ for both cultivars.Other studies also have suggested that phenolic compounds may suffer decomposition under high temperatures, and this impact may depend on the temperature, time of processing, type of compounds in the sample and other conditions [9,10].

Storage led to a great impact on AOA value (Table 1),which is a storage temperature related parameter. The change in the AOA value during storage was consistent with the change in the content of phenolic compounds.Besides the decomposition of phenolic compounds, other possible mechanism for the significant reduction in AOA for rice stored at 37 ℃ might be due to the interaction of the

phenolic compounds with other components in rice. One of the most outstanding chemical reactions occurred in rice during storage is lipid hydrolysis and oxidation. The fat acidity (FA) of the two cultivars significantly increased as a result of storage at 37℃ ($P < 0.0001$). Thus, it is commonly used as an index of quality deterioration during rice storage because lipid dissolution progresses more rapidly than other compositions in rice[7] . The release of more free fatty acids (FFA)makes rice becoming more susceptible to autoxidation than esterified fatty acids. Therefore, the control of the concentration of the FFA, particularly unsaturated FFA might be an effective way for retarding rice ageing progress.

3.3 Effect of Storage on Phenolic Compounds in Bound-PC Extract

Similar to the changes in Free-PC, the content of phenolic compounds in Bound-PC extract also showed a reduction during rice storage at 37° C Table 1. This indicates that the chemical reactions also occurred in Bound-PC during rice ageing. However, the magnitude of the reduction of Bound-PC after the storage was lower than that of Free-PC, suggesting that the phenolic compounds in Free-PC extract were more sensitive to ageing progress. This difference might be due to the different structures of phenolic compounds present in Free-PC and Bound-PC fractions, respectively, because structural difference is the determination of its individual reducing capacity of the phenolic compound. This further confirmed that phenolics existing in different fractions in rice demonstrated different roles on retarding rice ageing progress, which might be depending on the configuration structure of antioxidant compounds.

3.4 Correlation Coefficients between Phenolics and Antioxidant Activity

The correlations among the parameters measured in this study showed that the content of phenolics and flavonoid both in Free- and Bound-PC extracts were highly

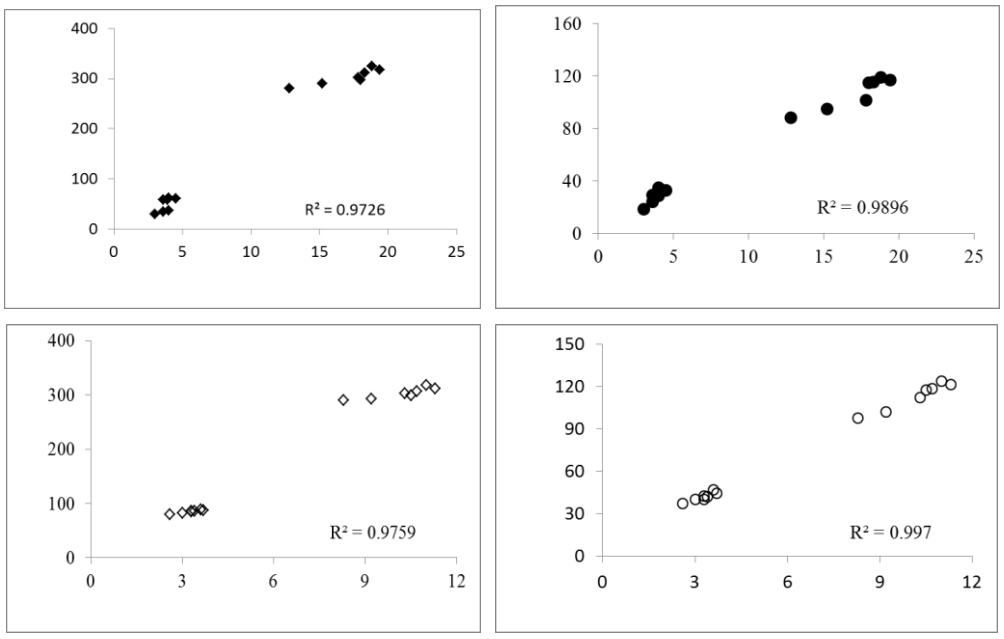

Figure1. Correlation coefficients between phenolics and antioxidant activity

correlated to the AOA value(Figure 1). This indicates that both free and bound phenolic compounds contribute to the antioxidant capacity of whole grain rice and were highly involved in rice ageing process.

4. CONCLUSION

Although the content of Free-PC was either lower or close to the content of Bound-PC in the two tested rice samples, their corresponding AOA is otherwise. This study might suggest that the structure and profile of phenolic compounds may differ between the two extracts. There was a significant reduction in the concentration of phenolic compounds and AOA both in Free-PC and Bound-PC fractions following the rice storage at 37 ℃, indicating both free and bound phenolic compounds are involved in the chemical reactions occurred during rice ageing progress.

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Cover page

Title: *Hedonic Property Value of Water Service in Tidal Lowland Agriculture*

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ABSTRACT

Despite its important role in agricultural water management in tidal lowlands, water service has not yet been considered as an input in crop production. The cost of water service is still born to the government budget such that budget cut will consequently results in abandoning of operation and maintenance of water infrastructures. This paper examines whether water service is a determinant of tidal farmland value. A survey involving 500 farmers was conducted in Telang, a rice production center in tidal lowland area of South Sumatra Province, Indonesia. Hedonic Property Value analysis was employed to provide answer to this examination. Analysis revealed that the value of tidal farmland with water service was significantly higher than that without water service. The value of tidal farmland was determined by soil fertility, distance to the local markets, the availability of water service, and farmland productivity. Being one of the significant determinants of tidal farmland value, imposing fee upon water service will expectedly be followed by farmers' willingness to pay for the fee.

Keywords: water service, tidal lowland, agriculture.

INTRODUCTION

Water charging as a policy issue has been adopted in Indonesia through the issuance of The Water Resource Law in 2004 (UU No. 7/2004). However, the term water charging is only implicitly stated in a phrase of "financial responsibility". Farmers (water users) financial responsibility according to the law includes construction, operation and maintenance of water infrastructures at tertiary level. These responsibilities in practice are shared among farmers within a tertiary block, which include routine gate operation (opening or closing of gates according to crop water needs) and tertiary canal and gates maintenance.

Water charging policy has not yet been implemented in tidal lowland irrigation eventhough it has been mandated in the Water Resource Law. Instead of charging, a payment is applied to water user for its membership in water user association (WUA), not for the water used for cultivation. Water charge remains unknown and un

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acceptable to water users. Therefore, water service fee is considered more appropriate term to emphasize that the charge is not for the water itself, but for the service of delivering water down to the tertiary canals to fulfill crop water needs,.

In tidal lowland area of Telang, one tertiary canal serves 16 ha farmland, 8 ha at each side. Two tertiary gates are installed, one at each end of the tertiary canal. Normally, these 16 ha farmland is owned by 8 farmers or 2 ha farmland in the average. Since water management actions directly affect the 16 ha farmland, the operation and maintenance of tertiary water infrastructures are collectively planned and implemented by the farmers, following the planned cropping patterns in the area.

The existence of water service and proper operation and maintenance of water infrastructures contribute to the farm output as well as the value of farmland. The value of farmland is also determined by farmland characteristics [1] such as fertility, relative distance to the market [2], and the existence of facilities to support cultivation [3]. Therefore, the objective of this study was to determine the value of water service and physical, spatial, and economic characteristics of tidal farmland. It was expected that the value of water service could be considered as basis for imposing water service fee for which water users (farmers) were financially responsible.

METHODS

Hedonic pricing (HP) and travel cost method (TCM) are two most commonly used of revealed preference methods. Both TCM and HP have advantages of observing actual behavior. While TCM has particularly been used in estimating non-market value of ecotourism and recreational sites [4], HP was frequently employed in valuing property with regard to the environmental characteristics and changes. It particularly extracts effect of environmental factors on price of goods that include those factors [5] [6] [7]. The hedonic property method has been used to value characteristics of goods that are not formally traded in the market [8]. This method has also been used to estimate the value of open space proximity [9], the improvement in air and water quality [10] and the evaluation of scenic views [11].

In water management research, hedonic method has been used in various studies with regard to water resource as a single resource or water in attachment to land resources. Hedonic method has been used to estimate the minimum payment an owner (of water right) would be willing to accept for the sale or lease of a water right in Douglas County, Oregon, USA [12]. Using the hedonic method, this study has successfully put a monetary value on irrigation water ($261 per acre-foot irrigation water) which is consistent with other studies and actual transaction in the study area. Similar to irrigation water, water service can be considered as an attribute of agricultural farmland which benefit can be valued similarly. Therefore, HP was considered appropriate in this study to value water service as an environmental characteristic that determine the price of tidal farmland as a property.

Hedonic property value of farmland is referred to as the market price of farmland. Its value has been studied based on several characteristics, including soil fertility [1], productivity and spatial aspects [2], and availability of water service [3]. In this study, the value of water service reflects the marginal willingness to pay for discrete change in water service, which is the change from without water service to with water service. Assuming two pieces of farmland that are identical except that one is with

water service and the other is without, the difference in market price between the farmlands is attributed to the value of water service for which the farmers are willing to pay.

In this research, the hedonic function for tidal farmland market price was stated as follow:

$$P_i = f\left(Q_S, Q_P, Q_{WS}\right) \tag{1}$$

where P_i = market price of tidal farmland
Q_S = the vector representing farmland quality
Q_P = the vector representing proximity of the farmland
Q_{WS} = the vector representing facility on the farmland

The quality of tidal farmland was measured in three indicator variables, namely farmland fertility, productivity, and income obtained from crop production. The proximity of the farmland was defined and measured as the distance of the farmland from the local market. The facility on the farmland was represented by the availability of water service on the farmland and the maintenance of gates and canal connected to the farmland.

Based on the above description, the following regression equation was proposed in order to estimate the function:

$$P_i = \beta_0 + \beta_1 FERT + \beta_2 PRO + \beta_3 INC + \beta_4 DIST + \beta_5 D_{WS} + \beta_6 MAINT \tag{2}$$

where P_i = the market price of tidal farmland per hectare
FERT = fertility level of farmland
PRO = farmland productivity
INC = income from crop production
DIST = distance of farmland to the local market
D_{WS} = dummy variables water service
MAINT = canal and gates maintenance

The above regression equation was predicted using ordinary least square (OLS) method to yield the predicted market price of farmland based on its affecting factors. Subsequently, some statistics were employed to examine the goodness-of-fit of the overall model and the significance of each of the affecting factors. In addition, interpretations on the significant factors were made in term of direction and magnitude of their effects on the market price of tidal farmland [13]. Supposed, the model contained only the intercept and the dummy variable water service, the interpretation was as the following [14]:

$$P_i = \beta_0 + \beta_1 D_{WS} + \varepsilon_i \tag{3}$$

where D_{ws} = 1 for farm with water service and 0 otherwise
The market price of farmland without water service was estimated as:

$$\mathrm{E}\left(P_i | D_{WS} = 0\right) = \beta_0 \tag{4}$$

The market price of farmland with water service was estimated as:

$$E(P_i | D_{WS} = 1) = \beta_0 + \beta_1 \quad (5)$$

Therefore, the intercept β_0 was the mean estimated market price of farmland and the slope coefficient (β_1) was the difference in mean estimated market price between farmland with water service and without.

This study was designed as a survey, conducted in the deltaic area of Telang, South Sumatra, Indonesia. Telang, a reclaimed tidal lowland area for agriculture, is located in the lower reaches of Musi River. Research sample of 500 farmers were drawn using random sampling from some 10,000 farmers, covering 12 secondary blocks (approximately 3,072 ha). Data were collected through field observation and structured interview with the sampled farmers.

RESULTS AND DISCUSSION

As discussed in the methods, tidal farmland value is determined by soil fertility, productivity, income obtained from farming, distance of the farmland from the local market, the availability of water service, and the maintenance of gates and canal connected to the farmland. The mean value of farmland as measured in farmland market price is US$5,261.07 (± 1,018.82). The farmland market price varies from as low as US$2,500.00 to as high as US$10,000.00 per ha. The mean market price of farmland with water service is US$5,612.36 per ha, whereas without water service is US$4,807.51 per ha. The mean market price of farmland with water service is significantly higher than that without water service (t=9.399, sig. t=0.000).

Table 1 presents the regression coefficients with the t statistics and significant level of each of the independent variables assumed to determine the farmland market price. Out of 6 independent variables assumed to have an effect on the farmland market price, only one variable that is significant at 90 percent confidence interval, which is farmland fertility. Others are proved to have statistically significant effect on the farmland market price at 99 percent confidence interval, which are: (1) productivity; (2) income; (3) distance of farmland from the local market, (4) the availability of water service, and (5) maintenance of canal and gate connected to the farmland. In Table 1 both direction and magnitude of the effect of these independent variables on the dependent variable are shown. The statistical tests of the regression indicate that the overall model is statistically significant. However, the variation in farmland market price explained by its determinants is approximately 26 percent. There is no collinearity detected in this analysis.

TABLE I. REGRESSION COEFFICIENTS AND THE VALUE OF STATISTICS

Variables	B	Std. Error	t	Sig.
(Constant)	33132758.632	3997802.536	8.288	.000
Fertility	2500777.738	1309442.781	1.910	.057*
Productivity (tons/ha)	1531738.264	497256.682	3.080	.002***
Income ($Rp10^6$)	178810.108	57019.518	3.136	.002***
Distance from the market	-1909067.120	358702.668	-5.322	.000***
Water Service (Dummy)	4058815.800	1256751.765	3.230	.001***
Canal & gate maintenance	3671982.726	1250081.538	2.937	.003***

*Significant at 10%; **Significant at 5%; ***Significant at 1%

Farmland fertility is a statistically significance determinant of farmland price (p=0.057). Its coefficient indicates that farmland fertility is positively related to its market price as expected. Fertility is an important attribute of farmland. Farmland fertility is a result of interaction between physical conditions of the farmland and its accessibility to water infrastructures. The higher the fertility the higher the farmland price would be. Measured in ordinal level, one level increase in fertility would increase its price by about US$250.08.

Productivity which is measured by the yield (tons of rice) per hectare farmland has a positive coefficient as hypothesized. Its effect on the farmland market price is significant. Its coefficient tells that every ton increase in productivity will be followed by an increase in the farmland market price of about US$153.17. Productivity remains an important target in tidal lowland agriculture, especially rice production. With current productivity level of about 5.35 tons per hectare, it can potentially be increased to 10 tons per hectare as has been achieved by few farmers in the study area. Higher productivity will consequently result in higher farmland market price of the farmland.

Income from farming contributes significantly to the price of the farmland. Its coefficient indicates that every million Rupiah increase in total income will be followed by an increase of farmland market price of US$17.88. While farmland productivity seems to directly affect the farmland market price, income affects the farmland market price indirectly through the perception regarding the value of property. Farmers with higher farm income tend to perceive higher value of their property than those with lower income.

The distance of farmland from the local market has a negative sign for its coefficient as expected. This indicates that the further the farmland from the local market, the lower the price of the farmland. For every km increase in the distance of the farmland from the local market, the price of the farmland decreases by US$190.00.

Maintenance of canals and gates as hypothesized has also a positive effect on the market price of the farmland. This variable was measured as a dummy variable which was coded 1 for maintained canals and gates, 0 otherwise. Farmland with maintained canals and gates connected to it has higher market price than that without canals and gates maintenance. Farmland which is connected to the maintained canals and gates has market price which is US$367.20 higher than that which is connected to un-maintained canals and gates. Maintained canals and gates enables farmers to properly irrigate, retain and drainage water from the field according to crop water needs. This condition would ensure the optimum crop growth and maximum yield obtained from the farmland.

The availability of water service significantly affects the market price of farmland. The positive value of its coefficient indicates that farmland with water service has higher price than that without water service. The difference in mean estimated market price between farmland with and without water service is indicated by the value of its coefficient (β_1). This means that the price of farmland with water service is US$405.88 higher than that without water service. This result is in accordance with the hypothesized relationship between the availability of water service with the market price of farmland, proving that water service is an important attribute of tidal farmland.

CONCLUSION

The result of hedonic property analysis indicates that, as a property, the mean price of farmland with water service is significantly higher than that without water service. The value of farmland is significantly affected by the availability of water service beside other characteristics of the farmland (farmland fertility, productivity, distance from the local market, and infrastructure maintenance) and farmer's income. The availability of water service accounts for approximately one half of the difference in the farmland price. Based on the findings of this study, the value of water service can be considered as basis for imposing water service fee for which farmers are financially responsible to contribute in the operation and maintenance of tidal water infrastructures at tertiary level.

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Cover page

Title: *Calcium-fortified Pineapple Juice for Prevention of Osteoporosis*

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ABSTRACT

Deficiency of calcium is associated with risk of bone fracture and osteoporosis. This malnutrition has been a focus of the governments and world organizations for decades and extensive efforts are made to address it. Being a popular and most common beverage of the population living in developing countries, fruit juice has gained an attention as a suitable carrier for calcium fortification. However, there is lack of the data on calcium-fortified fruit juice for the prevention of osteoporosis. Therefore, The main objective of this study was to determine that calcium-fortified pineapple juice (*Ananas comosus*(L) *Merr*) was potentially important vehicle for the prevention of osteoporosis. The results indicated that calcium citrate malate fortified pineapple juice CCM-PJ has good stability for 24 hours and well taste accepted by the sensory panelists. Calcium intake of young adult girls was 35.38% of the Recommended Dietary Intake for calcium. CCM-PJ had most marked effects on rates of bone density accretion of young adult girls after 6 months consumption of CCM-PJ. Moreover, CCM-PJ was potentially important carrier for increasing dietary calcium intake and prevention of osteoporosis.

Keywords: pineapple juice, calcium citrate malate, fortification, osteoporosis.

INTRODUCTION

Dietary intakes of calcium by Indonesian women, 254 mg/day, are far below that needed to build proper bone mass [1]. It is widely accepted in the literature that a low calcium intake over years contributes to the development of osteoporosis. Osteoporosis is a disease in which the density and quality of bone are reduced, leading to weakness of the skeleton and increased risk of fracture, particularly of the spine, wrist and hip [2]. Osteoporosis is a disease that is sadly overlooked, under-diagnosed and under-treated [3]. This silent epidemic causes human suffering and takes a heavy economic toll.

Osteoporosis has been recognized as a major problem since 2006. The prevalence of osteoporosis in Indonesian women age 50-59 and 60-70 years are 24% and 62%, respectively. To handle osteoporosis, Indonesian Government has allocated 162.000

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dollars in 2000 and 630.000 dollars in 2007 [4]. It was reported that 22-55% of elderly women are osteoporosis and 30-40% of elderly women are osteopenia (the early stage of osteoporosis) [5]. In addition, osteoporosis occurred in only 14% in the below 50 age range of Indonesians, while that figure rose to 28% in the 50 to 60 age range and 47% in the 60 to 70 age range.

The problems in getting enough calcium of the people in Indonesia are poor (low and inadequate) dietary habits, not many foods are naturally a good source of calcium (milk and dairy products), and another calcium sources (non-dairy foods) have to be consumed in large amounts to meet Recommended Dietary Intake (RDI) for calcium and low bioavailability (cereals, nuts, and green leafy vegetables). Another problem is consumers behavior such as low purchasing power, boring, preference, and special conditions (the teens years, the young adult years, the childbearing years, pregnant, breast-feeding, the later adult years, lactose intolerance, and vegetarian).

There are several solutions to increase calcium intake, one of these is calcium-fortified foods as a benefit product. However, less is known about the effect calcium fortification has on the sensory attributes of foods. Adding nutrients may increase the risk that a product "tastes less good". It is known that calcium fortification may create solubility problems as well as increasing acidity, chalkiness or bitterness of the food through calcium or associated ions [6].

Therefore, the study of Calcium Citrate Malate (CCM) fortification on Pineapple Juice (*Ananas comosus*(L) *Merr*) was conducted. Pineapple is a local fruit product utilized in this study. The main objective of this study was to determine whether CCM fortified pineapple juice (CCM-PJ) was potentially important vehicle for the prevention of osteoporosis. The specific objectives were: (i) to investigate the stability and likeness taste of CCM-PJ, (ii) to examine calcium intake of young adult girls as a sample, and (iii) to evaluate the effect of CCM-PJ on bone density of sample (*in vivo).*

MATERIALS AND METHODS

Processing, characterization, and fortification of CCM used the following materials and methods:

1) CCM used in the methods of this study has molar composition of calcium carbonate: citric acid: malic acid of about 5:1:1 and 6:2:3 (pharmaceutically-acceptable: safe and effective amount) in 3℃ and 55℃ (Stokiometry Principle).

2) CCM characterization: calcium content, pH, and solubility.

3) CCM fortification on pineapple juice based on standard of calcium fortified juice (450mg Ca^{++}/240mL) [7].

Calcium fortified juice was analyzed using the following methods:

1) Physical Analyses: physical stability.

2) Chemical Analyses: calcium content and pH.

3) Sensory Evaluation: taste (panelist 60 person).

The effect of the best CCM fortified juice on osteoporosis was analyzed using a randomized, double-blind, placebo-controlled trials of the effect of 6 months of CCM-PJ on bones density in 60 teenage girls/group, half of whom had a fortified juice intake of 450 mg Ca^{++}/day and half as control group received placebo juice.

Densitometry was used to measure bone density on wrist and forearm of sample at the beginning and the end of study.

Dietary Survey was conducted using recall 24-hour method (every 10 day during 6 months).

RESULTS AND DISCUSSION

Calcium Citrate Malate

The properties of CCM were white powder or granules with sandy, bland taste. When fortifying beverages, solubility dissolution characteristics and stability of ingredients in solution are major factors. Therefore, calcium salts with a good solubility and high.

The best processing in the method of this study was CCM has a molar composition of calcium carbonate : citric acid : malic acid of about 6:2:3 in 55℃ since it produced the highest calcium content (20.278%) and the highest solubility (0.5076%) among treatments. The solubility of CCM increased with increasing the temperature. Solubility of CCM is strongly influenced by the pH since the solubility of CCM typically increases with decreasing pH. Moreover, higher soluble CCM can give more free calcium ions.

CCM Fortified Pineapple Juice

Calcium content of pineapple was 76.06 µg/mL. Table 1 showed calcium content of CCM at every treatment and the amount of CCM fortification on pineapple juice. Based on calculation (Table 1), the amount of CCM fortification in juice is 2 gram.

TABLE 1. CALCIUM CONTENT (%) OF CCM AND THE AMOUNT OF CCM FORTIFICATION ON PINAPPLE JUICE

Treatment	Calcium Content (%)	Amount of CCM Fortification on Juice	
		300mg Ca^{++}/240mL	450 mg Ca^{++}/ 240mL
3˚C,5:1:1	17.785	1.687	2.530
3˚C,6:3:2	18.341	1.635	2.453
55˚C,5:1:1	18.744	1.600	2.400
55˚C,6:2:3	20.278	1.479	2.219
Average		1.600	2.400

Physical, Chemical, and Sensory Evaluation of CCM-PJ

The calcium content of pineapple juice was higher in low pH than that in high pH. Solubility is strongly influenced by the pH of the system since the solubility of CCM typically increases with decreasing pH of juice. Pineapple juice had a good stability on 24 hours but not in 72 hours (Table 2). The use of stabilizer (e.g. pectin, carageenan) can improve the stability of juice.

TABLE 2. PHYSICAL STABILITY OF CCM-PJ

Treatment	24 hours	72 hours
3˚C, 5:1:1	stable	not stable
3˚C, 6:2:3	stable	not stable
55˚C, 5:1:1	stable	not stable
55˚C, 6:2:3	stable	not stable

Note: stable = no separation, not stable = has separation

Since CCM had a sandy bland taste, therefore it was imparted a gritty mouth feel. Negative effects of CCM on taste of juice can be masked with chelating agents and the use of stabilizers as well as with the addition of flavorings. Based on the physical, chemical, and sensory analyses, the best treatment of juice were 55˚C, 6:2:3 CM-PJ. In vitro bioavailability (% Ca availability) of CCM-PJ (21 ±0.7).

The Effect of the Best Treatment of CCM-PJ on Bone Density

The calcium content of the best treatment pineapple was 427.368%. The subjects were required to consume one glass (240 mL) of the juice each day for a 6 months period. Juice was drunk in the evening, because evening calcium supplementation has been shown to suppress the nocturnal increase in bone resorption [8]. Calcium intake from dietary sources averaged 240.5 mg/day for the entire study group.

The baseline characteristics of the control and CCM-PJ groups are presented in Table 3. There were no significant differences between treatment groups in age, bone density, calcium intake, dietary energy, and protein. Means for each characteristics followed by the same letter within the same row are not significantly different at $p<0.05$.

TABLE 3. CHARACTERISTICS OF CONTROL AND CCM-PJ GROUPS

Characteristics	Control Group	CCM-PJ Group
Age (year)	11.41 ±0.54[a]	11.44 ±0.50[a]
Bone Density (g/cm^2)	0.22 ±0.09[a]	0.24 ±0.07[a]
Calcium Intake (mg/day)	244 ±10[a]	237 ±11[a]
Dietary Energy (Kcal)	1435 ±153[a]	1372 ±181[a]
Protein (g)	24.2 ±14.4[a]	25.9 ±13.8[a]

Daily calcium intake was 244 ±10 and 237 ±11 mg/day for control and CCM-PJ groups, respectively. After 6 months, the intake had increased to 569 ± 13 mg/day in the CCM-PJ group, and it remained unchanged at 252 ± 13 mg/day in the control group (Table 4). Compared with the control group, the CCM-PJ group showed significantly ($p<0.05$) higher calcium intake and greater gains in bone density. However, there was a non-significant dietary energy and protein in control and CCM-PJ groups. Means for each characteristics followed by the same letter within the same column are not significantly different at $p<0.05$.

TABLE 4. CHARACTERISTICS OF CONTROL & CCM-PJ GROUPS, 6 MONTHS

Characteristics	Control Group	CCM-PJ Group
Age (year)	11.91 ±0.30[a]	11.16 ±0.47[a]
Bone Density (g/cm^2)	0.25 ±0.06[a]	0.21 ±0.09[a]
Calcium Intake (mg/day)	241 ±11[a]	240 ±13[a]
Dietary Energy (Kcal)	1298 ±213[a]	1450 ±146[a]
Protein (g)	23.1 ±12.3[a]	29.2 ±11.7[a]

The results showed that an increase in calcium intake as CCM-PJ increased bone density in 11–12 years old girls with a low mean baseline calcium intake of 240.5 mg/day. Fortified juice as CCM-PJ had most marked effects on rates of bone density accretion during 6 months.

There is continuing uncertainty about the level of dietary calcium required to ensure adequate availability of calcium for optimal accrual of bone mass. Dietary calcium has a fundamental role in the development of growing bones. Calcium must be absorbed in sufficient quantities to adequately provide for period of rapid growth (such as in infancy and adolescence) and to offset daily excretory losses that would otherwise deplete previously obtain skeletal reverses [9].

There is a threshold requirement for dietary calcium, which is determined predominantly by skeletal response will occur when calcium intake is increased from deficiency levels to a threshold zone. Increasing calcium intake when the level of dietary calcium already exceeds the threshold will most likely not produce additional gains in bone mass [10]. Calcium threshold levels for optimal nourished children and adolescent were estimated to be approximately 1400-1600 mg/day [9]. In this study, CCM-PJ groups achieved significant gains in bone density with control groups with an overall calcium intake that exceeded the threshold.

This study was consistent with the conclusion of the meta-analysis that calcium supplementation is effective in enhancing increased the bone density of the total body (and arms) in children treated with calcium supplements fortification systems used in orange juice [11]. The mechanism was calcium supplementation results in a decrease in bone remodeling by suppressing PTH secretion, and the increase in BMD is a consequence of the filling in of the remodeling space [12]. Furthermore, it was reported stated that the greatest acquisition of skeletal mass occurs over several years in late children and adolescent (accounting for 40-50% of adult total body bone mineral) [13]. This period provides a window of opportunity when interventions may have their maximum effect to increase peak bone mass and reduce fracture later in life.

CONCLUSION

CCM has a molar composition of calcium:citrate:malate of about 6:2:3 with temperature 55˚C was the best instead of the highest calcium content and solubility. The best-fortified juice was pineapple juice fortified by CCM (6:2:3, 55˚C). The best-fortified juice had the highest calcium content and score of taste acceptance. Compared with control groups, CCM-PJ showed significantly greater gains in calcium intake and bone density ($p<0.05$). CCM-PJ is potentially important vehicles for the prevention of osteoporosis.

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Title: *Relationships between Aggregate Stability and Selected Soil Properties in Taleghan Watershed of Iran*

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ABSTRACT

Aggregate stability is a fundamental property influencing soil erodibility and main hydraulic and physical characteristics. Knowledge of soil components controlling aggregate stability is very important to soil structure conservation. Also, identification of the critical soil properties affecting aggregate stability is central to understanding where erosion might be located within a catchment as a result of natural patterns in aggregate stability. The objective of this study, which was carried out in surface soils from Taleghan, was to relate aggregate stability to selected soil properties. In this study, based on homogeneity in slope, aspect and litho logy, work units were prepared, and then by relying on observations and changes in the macroscopic scale of homogeneous work units in soils of Taleghan watershed, with 32.5 km2 in area, 84 points as the soil sampling points were selected. The index of stability used is the mean weight diameter (MWD). Correlation between mean weight diameter and measured properties were extracted using Pearson correlation coefficient and evaluated in terms of their statistical significance. The unexpected results have been obtained from correlation between soil properties and aggregate stability so that the correlation between aggregate stability and the percentage of Caco3, clay and soil saturation are negative while that with fine sand and very fine sand are positive. Thus the improvement of aggregate stability and soil erosion control in Taleghan watershed depends on management of these soil properties and soil conservation projects in order to adjust and optimize them.

Keywords: Aggregate stability, Soil properties, Work unit, Taleghan watershed, Iran.

INTRODUCTION

The aggregate stability is the ability of the bonds of the aggregates to resist

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when exposed to stresses causing their disintegration. Several studies have reported that aggregate stability can be used in erosion prediction equations instead of a soil erodibility, which often requires time-consuming and costly experimentation to determine (Valmis et al., 2005; Dimoyiannis et al., 2006). Due to these constraints, soil susceptibility to runoff and erosion, or soil erodibility, has also been evaluated through laboratory tests on small soil samples (< 100 g), which are easy to implement and far less expensive and time-consuming than field experiments. Among these laboratory tests, those relating to aggregate stability have received much attention.

Identification of the critical soil properties affecting aggregate stability is central to understanding where erosion might be located within a catchment as a result of natural patterns in aggregate stability. There are many literature references on which the relationship between soil properties and aggregate stability investigated (Dimoyiannis, 2011; Tayel et al., 2010; Cantón et al., 2009). But these references show that location and soil type play major roles in determining relationships between aggregate stability and soil properties. Relation of aggregate stability to soil properties thus, differs with climatic zones and soils. There is, therefore, a need to study these relationships in different regions in order to isolate which properties to manage for improving soil aggregate stability in a given location. While there is wealth of research on aggregate stability and its relation to soil properties, few studies have focused on Marly soils especially in IRAN. Marl or marlstone is a calcium carbonate or lime-rich mud which contains variable amounts of clays.

The objectives of the present study were (1) to assess the aggregate stability of calcareous Tertiary marl using Le Bissonnais's method; (2) to identify associations between soil properties and aggregate stability, (3) to compare the degree of similarity among different aggregate stability indices as well as identifying the main mechanism of instability.

MATERIALS AND METHODS

The Study Site and Soils

The study site, with 32.5 km^2 in area, is located in Taleghan watershed near Tehran Province in north of Iran (36 ° 10' – 36 ° 13' N, and 50 ° 45' – 50 ° 51' E). Annual average precipitation is 700 mm and means annual temperature is 7 ℃. The elevation ranges from 1900 to 2400 m. A set of 84 soil samples from two important lithotypes of the Upper Red formation were used for aggregate stability measurement. The soils are formed in Tertiary marl deposits. In this paper, the soils were referred to by their parent material, i.e. GY (Gypsiferous red and grey Mudstone and Siltstone) and NGM (Red Mudstone and Siltstone). Soil samples were collected from the surface layer (1–3 cm), which is affected by natural rainfall and is crucial to erosion processes. The soil samples were transferred to the laboratory; air dried, gently crushed and passed through a 2 and 5 mm sieves. 3 to 5 mm aggregates were separated for aggregate stability tests. Soil particles less than 2 mm were utilized for the chemical and physical analysis. Soil physical and chemical properties were determined using the routine method. The sand fraction was separated by wet-sieving, oven-dried, and then fractionated by dry sieving.

Aggregate Stability Tests

Aggregate stability was measured by Le Bissonais's method (1996). Aggregates of 3–5 mm in diameter were obtained by dry sieving and were oven dried at 40 ℃ for 24 h so that they were at a constant matric potential. The prepared samples were tested for the following three treatments: fast wetting (FW); slow wetting (SW); and mechanical breakdown by stirring after pre-wetting (WS). For the fast wetting treatment, 5 g of aggregates was immersed in deionized water for 10 min. After sucking off the water with a pipette, the slaking-resistant aggregates were transferred to a 0.05 mm sieve immersed in ethanol and gently moved up and down 20 times by hand to separate the <0.05 mm fractions from >0.05 mm fragments, dried in an oven at 40 ℃, and their size distribution was measured by dry sieving with sieves with 2.0, 1.0, 0.5, 0.25, 0.125, and 0.05 mm pore openings. In the slow-wetting treatment, the aggregates were placed on a filter-paper for 30 min and subjected to a tension of −0.3 kPa. The size distribution measurement procedure described above for the FW treatment was then performed. In the WS treatment, the aggregates were first immersed for 10 min in ethanol to preserve the soil structure, followed by then transferred in a flask filled with 200 cm3 of deionized water. Subsequently, the flask was corked and agitated end over end 20 times within 1 min. The drying and sieving procedure described above was then performed. Aggregate stability for each sample was expressed in terms of the mean weight diameter (MWD) for the seven size classes (Stones and litter >2 mm were removed):

$$MWD = \sum_{i=1}^{n+1} \frac{r_{i+1} + r_i}{2} \times m_i \tag{1}$$

Where ri is the aperture of the ith mesh (mm), $r_0=r_1$ and $r_n=r_{n+1}$, m_i is the weight fraction of the aggregates on the ith sieve, and n is the number of the sieves.

The relative slaking index (RSI) and the relative mechanical breakdown index (RMI) proposed by Zhang and Horn (2001), were used to determine the resistance to slaking and the mechanical breakdown of the study soils. These relationships are, respectively:

$$RSI = \frac{MWD_{SW} - MWD_{WS}}{MWD_{FW}} \tag{2}$$

$$RMI = \frac{MWD_{SW} - MWD_{WS}}{MWD_{SW}} \tag{3}$$

Where MWD_{FW}, MWD_{WS}, and MWD_{SW} are the mean weight diameters obtained by the fast-wetting, stirring, and slow-wetting treatments, respectively. In the present study, based on the hypothesis that the main mechanisms of aggregate breakdown during water erosion processes on Tertiary marl deposits are slaking by fast wetting and mechanical breakdown due to raindrop impact, we determined an aggregate stability index (K_a), which was proposed by Feng-Ling Yan et al. (2008). Actually this index, incorporate the main mechanisms of aggregate breakdown and can be determined according to the following formula:

$$K_a = RSI \times RMI \tag{4}$$

Physical and Chemical Properties of Studied Soils

All soil properties data obtained for the 84 surface soils are shown in Table1. From the Table1, it is evident that soils studied varied widely in their properties. According to the American soil texture classification, 53% of analyzed samples belong to clay texture.

As shown in Table 1, the study soils are calcareous, mostly clay textured and low in organic carbon. clay content and Caco3 are the major properties in these soils, representing 45.94 and 16.95 mean respectively. Thus, it can be said that the variation and range of soil properties are large enough to depict the degree and nature of the structural stability.

TABLE 1-STATISTICS OF SOIL PROPERTIES OF THE STUDY SOILS

properties					
	Mean	Max	Min	S.D.	C.V.
Clay (%)	45.94	80	22.00	14.5	0.32
Silt (%)	20.50	53.60	1.2	14.77	0.72
Sand (%)	33.56	66.8	2	20.24	0.6
CS	28.97	66.72	0.26	20.25	0.7
FS	1.4	11.7	0.06	1.99	1.42
VFS	3.19	16.44	0.3	3.26	1.02
SS(%)	46.87	65.7	32.55	6.46	0.14
SOC(%)	1.07	2.51	0.09	0.65	0.61
EC_{25}(ds/m)	0.73	3.47	0.08	0.7	0.96
pH	7.73	8.2	7.5	0.17	0.02
$CaCo_3$ (%)	16.95	25	2.9	5.59	0.33

CS = %Coarse Sand (0.2-2mm), FS = % Fine Sand (0.1-0.2), VFS = % Very Fine Sand (0.05-0.1), SS =% Soil Saturation, SOC = % Soil Organic Carbon

Effects of Lithotype on Aggregate Stability

Results showed that the distributions of the aggregate stability values for three aggregate breakdown mechanisms are normal. Analysis of variance (ANOVA) was made for all measured data by considering lithotype and aggregate breakdown mechanism as independent factors. The result showed that aggregate breakdown mechanism had a significant influence on aggregate stability but the influence of lithotype and the interaction of lithotype and aggregate breakdown mechanism not significant statistically.

Aggregate Stability under Different Breakdown Mechanisms

The aggregate stability values with different aggregate breakdown mechanism are presented in Table 2.The values of MWD always show the same trend across the three treatments for all off soil samples, so that it was greatest for the SW treatment, intermediate for the WS treatment and least for the FW treatment with 0.57, 1.04 and 1.39 mean respectively. Based on the result of compare means, there is a significant difference between each three treatments for MWD. The RSI values ranged from 0.03 to 0.83 and the RMI values from 0.005 to 0.55. An increase in RSI and RMI of aggregate implies a higher susceptibility of slaking and mechanical breakdown, respectively (Zhang and Horn, 2001). In the present study, 59 soil samples had high RSI values and low RMI values with 0.59 and 0.25 mean

respectively, indicating that slaking is the most important breakdown mechanism in wetting treatments in study soils.

Several methods for measuring the aggregate stability have been developed. Most of them consider only aggregate stability in relation to wetting processes or raindrop impact, but very few, consider both effects simultaneously. The LB-method reflected several mechanisms of aggregate breakdown in process of soil erosion by water. It could thus be argued that the index, Ka, reflects both the resistance of soil aggregates to slaking in sheet erosion as well as the detachment of soil material by raindrop impact, the latter one being the dominant process in interrill erosion. For ideal, perfectly stable aggregates, Ka has the lowest value, e.g. Ka = 0 and in the case of a completely unstable soil (RSI = 1 and RMI = 1), Ka = 1. In the present study, the Ka values ranged from 0.002 to 0.4, 30 soil samples had a Ka values less than 0.16, which was the mean of all of soil samples. According to the Ka values, it seems that the studied soils had a stable structure. It can be said that the Ka could not be used as a suitable index for describing aggregate stability in the present study.

TABLE 2- AGGREGATE STABILITY MEASURED BY LB-METHOD

Aggregate stability indices					
	Mean	Max	Min	S.D.	C.V.
FW	0.57	1.16	0.18	0.26	0.46
SW	1.39	1.93	0.51	0.37	0.27
WS	1.05	1.84	0.30	0.37	0.35
RSI	0.59	0.83	0.03	0.15	0.25
RMI	0.25	0.55	0.005	0.15	0.6
Ka	0.16	0.4	0.002	0.1	0.62

Correlation between Aggregate Stability Indices and Soil Properties

Correlation between aggregate stability indices and soil properties are presented in Table 3. Aggregate stability values (expressed as MWD for FW, SW and WS treatments) were positively correlated to organic carbon, fine sand, very fine sand and negatively correlated to soil saturation.

Many authors have demonstrated that SOC content is one of the most important factors determining aggregate stability in soil (Le Bissonnais et al., 2007). Our results agree, as SOC shows a positive correlation with the aggregate stability values. Taking into account that 1.15% of SOC content constitutes an important threshold of soil aggregate stability (Cerdá, 1998), the fact that 60% of the soil samples studied had less than 1.15% SOC content also explains the relatively poor aggregate stability of these soils. The role of clay content in aggregate stability is not as influential as the SOC content in these soils, which coincides with the findings of other researchers in different places (Tisdall and Oades, 1982). Tertiary marl soil has a very high clay content (ranged from 22% to 80% in all the samples analyzed), and as clay is the most important fraction controlling structural stability, this could explain the slight effect of clay on aggregate stability of marly soils. From Table 3, it can be said that sand fraction (fine and very fine sand) and silt has a significant effect than clay on aggregate stability in marly soils. Aggregate stability values for SW treatment negatively correlated to calcium carbonate. Clay content had a negative influence on aggregate stability values for WS treatment while the influence of silt was positive. There are a strongly correlation between

FW-SW, FW-WS and SW-WS with correlation coefficient 0.67, 0.7 and 0.84 respectively.

TABLE 3, CORRELATION BETWEEN AGGREGATE STABILITY INDICES AND SOIL PROPERTIES

	FW	SW	WS	SOC	$CaCO_3$	Clay	Silt	Sand	Coarse Sand	Fine Sand	Very Fine Sand	Soil Saturation
FW	1	0.68**	0.70**	0.30*	-0.22	-0.17	0.25	-0.06	-0.18	0.50**	0.49**	-0.40**
SW		1	0.84**	0.47**	-0.36**	-0.25	0.35**	-0.08	-0.22	0.50**	0.57**	-0.36**
WS			1	0.44**	-0.09	-0.29*	0.42**	-0.10	-0.23	0.43**	0.55**	-0.45**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

Under fast wetting situation, aggregate stability of clay soils in the Taleghan watershed is poor, so that, 80% of the samples studied had a mean weight diameter (MWD) less than 0.4. Therefore, we can conclude that the breakdown we observe corresponds to the breakdown caused by the compression of entrapped air during wetting. This mechanism is simulated by fast wetting treatment. No significant differences were found in aggregate stability between lithotypes (GY and NGM) in Upper red formation. As clay content in this soil is very high, the influence of this fraction on aggregate stability is very slight, even considering that the silt fraction and sand especially the finest, might contribute to aggregation.The SOC content has been found to be one of main factors controlling the aggregate stability in these soils.

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Cover page

Title: *The Extraction and Determination of Taurine in Scallop Viscera by Spectrophotometry*

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ABSTRACT

A rapid and simple method to determine taurine in scallop viscera by spectrophotometry was developed using formaldehyde and acetyl acetone. The optimum detecting conditions was determined by single factors experiment. The linear regression equation can be used for the quantitative analysis with R^2 being 0.999. Water boiling method was used as extraction method. Orthogonal design experiment, designed by the result of single factors experiment, showed the optimum technology was: solid-liquid ratio at 1:80, extracting temperature at 75 ℃， and extracting time at 2 h. Under the optimum condition, taurine extraction amount from scallop viscera was 7.636 mg/g.

1. INTRODUCTION

Taurine（$H_2N\text{-}CH_2\text{-}CH_2SO_3H$）, isolated from bovine bile in 1872 for the first time, is a free β-amino acid containing sulfur. It is widely distributed in biological fluids and tissues without being incorporated into protein. Taurine has many physiological functions such as being a neurotransmitter [1], an antioxidant [2], a modulator of intracellular calcium levels [3], a membrane stabilizer [4], and an osmolyte [5]. Some developed countries have added taurine to the milk to enhance its nutritional value. In addition, there is evidence that taurine is effective in alleviation of cardiacarrhythmia, hypertension, thrombus and atherosclerosis.

Taurine can be extracted from natural products or synthesized chemically. Chemical synthesis is applied commonly, which includes Ethanolamine, Dichloroethane Franc etc. Ethanolamine is employed to produce the largest percentage of taurine now [6, 7]. Chemical synthesis has a high yield, but some problems exist including toxic raw materials, complicated methods, high investment, and difficulty in recycling and environmental pollution [8]. Extraction of taurine from natural material is safe and effective. At the present time, the price of taurine extracted from natural product is much higher than those synthesized chemically, which benefits the development and application of nature-source taurine. The

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discovery of natural product containing a high amount of taurine and the improvement of extraction methods will shed a light on decreasing the cost of producing nature-source taurine.

It is previously reported that organisms living in ocean are rich in taurine than terrestrial animals especially shellfish. For example, the content of taurine in Dunker was up to 6.384 mg/g [9]. The content of taurine in bone skin was 4.265~6.654 mg/g [10], the content of taurine in *Pulse venosa* was 7.312 mg/g [11]. Scallop is a kind of valuable seafood. According to statistics in 2007, the farming area of scallop in China was 133,000 hm^2 in 2007. The total output was 1.2 million. The process of scallop will produce large amount of leftovers, in which about 17% of whole scallops is viscera, namely 20 million ton. It is a waste of resources and environmental pollution when discarding it [12]. It makes scallop viscera a kind of excellent raw materials to extract natural taurine that it is rich in taurine, fatty acids and many other inactive ingredients. There are few studies extracting taurine from visceral. Therefore, this paper used scallop viscera as raw material, studing the extraction and purification process of taurine and determining to establish a quick and easy way to detection. It provides a theoretical basis for a natural source of taurine and a high value of the use of scallop viscera.

2. MATERIALS AND METHODS

2.1 Materials

Patinopecten yessonensis; taurine, SIGMA; Sodium732cation exchange resin; Formaldehyde; Acetylacetone; Sodium acetate; all of the reagents were AR.

2.2 Instruments

High-speed organizational stamp mill; DHG-9030A-type electric heated blast drying oven; SHZ-III-type circulating water pump; freeze dryer; electronic balance; digital thermostat water bath; 722spectrophotometer; refrigerated high-speed centrifuge; DDS-307A-type conductivity meter; ion exchange column (46 mm × 50 cm).

2.3 Methods

2.3.1 COLORING MECHANISM

That taurine reacting with formaldehyde and acetyl acetone formed N-substituted-2,6 - dimethyl-3,5 - diethyl-1,4 - dihydro-pyridine. The product was yellow. This study choosed the visible region (400 nm) as the detection wavelength, because marine organisms of high concentrations generated background absorption below 390 nm [13].

2.3.2 DETECTION METHOD

1.0 ml chromogenic agent was added to taurine standard solution (0.6 mg/ml) in test tube, which was placed at 100 ℃ for 15min. Then cooled to room temperature. Absorbance was measured at 400 nm.

2.3.3 OPTIMIZATING DETECTION CONDITIONS

The main factors affecting the results were: the amount of color reagent, the amount of acetylacetone, heating time and cooling time. These four factors were selected to optimize detection conditions. We determined the optimum detecting condition of the four factors with single factors experiment.

2.3.4 SAMPLE HANDLING

Scallop viscera were homogenized by high-speed tissue machine[9],placed in oven keeping 70 ℃ to constant weight. The measured moisture content was 45.26%.

2.3.5 PREPARATION OF CATION EXCHANGE COLUMN

The resin was washed repeatedly, then soaked in 2.0 mol • L^{-1} NaOH solution for 24 h. Washed with distilled water to neutral. Soaked in 2.0 mol • L^{-1} HCl solution for 24 h. Washed with distilled water to neutral. Keep the sample at 4 ℃ before being filled in the ion exchange resin column (46 mm × 50 cm) 45 cm [11].

2.3.6 TAURINE CRUDE EXTRACTION FROM SCALLOP VISCERA

Distilled water and 0.5 g active carbon was added to erlenmeyer flask with scallops scraps, which was put into a water bath pot for water extraction. Supernatant was left after being filtered. pH was adjusted to 3 with 6 mol/L HCl solution to precipitate acidic proteins. Supernatant was left after centrifugation with 5000 r/min for 20min. Then pH was adjusted to 10 with 5 mol/L NaOH solution to precipitate basic proteins. Supernatant was left after centrifugation with 5000 r/min for 20 min. pH was adjusted to 4.5. The supernatant was concentrated using a rotary evaporator to 5 ml. The sample volume was 2 ml. Flow rate was 3 ml/min. Eluant was accessed when conductivity was from being down to being stable.

2.3.7 CRYSTALLIZATION

3 volumes of ethanol was added to the concentrated collection, which was put in refrigerator at 4 ℃ for 12 h to crystallization.

2.3.8 THE STANDARD CURVE OF TAURINE

The taurine reacting with formaldehyde and acetyl acetone forming N-substituted-2, 6-dimethyl-3,5-diethyl-1,4-dihydropyridine was used to determine the content of taurine by spectrophotometry. 1.0 mg.L^{-1}taurine standard solution 0, 1, 2, 4, 6, 8, 10 ml was added sequentially to 10.0 ml to volumetric flask. 1.0 ml reagent (acetyl acetone: formaldehyde = 2:3, V / V) was mixed with 2.0 ml of each solution in 10 ml test tube, which was put in boiling water bath for 15 min and cooled to room temperature. Absorbance was measured at 400 nm [12].

2.3.9 THE SINGLE FACTORS EXPERIMENT

According to the extraction process of taurine, the main factors affecting taurine extraction amount were solid-liquid ratio, extraction temperature and extraction time. Therefore, this test mainly selected these three factors at different levels for the single factors experiment.

2.3.10 OPTIMIZATION OF EXTRACTION PROCESS

Based on the single factor test, solid-liquid ratio, extraction temperature and extraction time as influencing factors, three levels were selected to orthogonal test.

3. RESULTS

3.1 Effect of the Amount of Color Reagent on Determination of Taurine

Different amount of color reagent (0 ml, 0.25 ml, 0.5 ml, 0.75 ml, 1.00 ml, 1.25 ml, 1.50 ml, 1.75 ml, 2.00 ml) was added to the tube, heating and cooling. Yellow and insoluble oily substance was produced on bottom of the tube when1.00 mL, 1.50 mL and 1.75 ml chromogenic agent was added, respectively. This result showed that 1.00 mL, 1.50 mL and 1.75 ml chromogenic agent was sufficient. However, there was no oily insoluble substance on bottom of the tube when 0.25 ml, 0.50 mL and 0.75 mL chromogenic agent was added. In order to react sufficiently, the amount of color reagent was 1.00 ml in the experiment (see Figure 1).

3.2 Effect of the Amount of Acetylacetone on Determination of Taurin

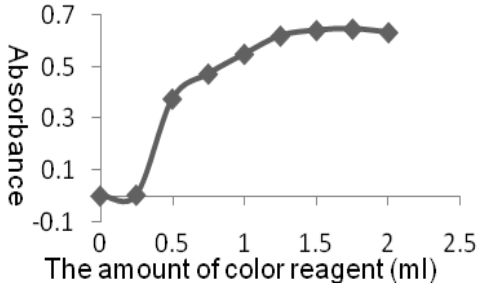

Figure 1. Effect of the amount of color reagent on absorbance

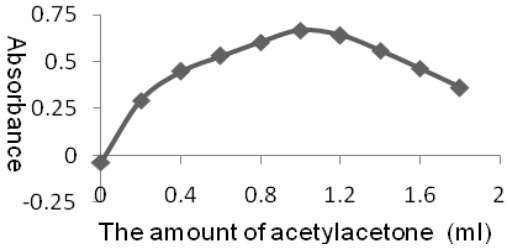

Figure 2. Effect of the amount of acetylacetone on absorbance

Taurine and formaldehyde reacted with different dosage of acetylacetone (0 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml) in the presence of sodium acetate. Figure 2 showed that absorbance was first increased and then decreased. When the amount of acetylacetone got to 1.0 ml, the absorbance reached the maximum. It may because excessive acetylacetone hampered the reaction. Therefore, the amount of acetylacetone was 1.0 ml in the experiment.

3.3 Effect of Heating Time on Determination of Taurine

Figure 3 showed that the absorbance increased with the heating time increasing. When heating 15min, the absorbance was up to maximum. Then the absorbance was stable. Therefore, the heating time was 15min in the experiment.

3.4 Effect of Cooling Time on Determination of Taurine

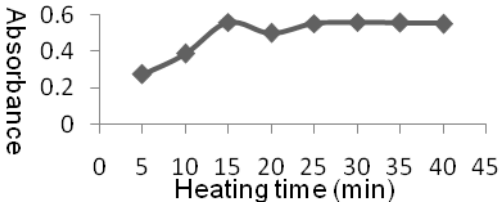

Figure 3. Effect of heating time on absorbance

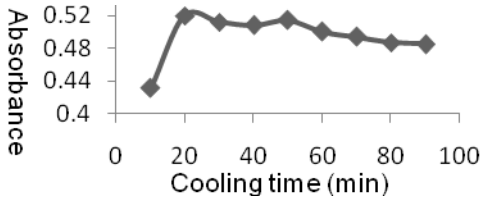

Figure 4. Effect of cooling time on absorbance

The effect of cooling time on determination of taurine was shown in Figure .4. As shown in Figure 4, the maximum absorbance was at 20 min after reaction. It may because long storage time would lead to unsaturated bond of the coordination complex being oxidized. Therefore, the cooling time was 20 min in the experiment.

3.5 The Standard Curve of Taurine

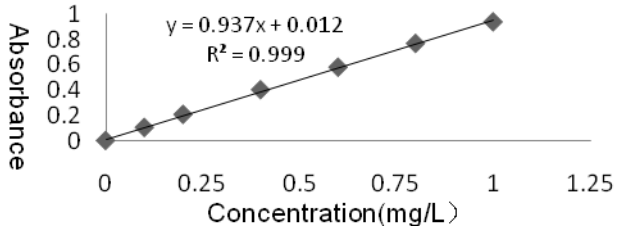

Figure 5. The standard curve of taurine

According to the optimum conditions, standard curve was drawed. The linear regression equation, y = 0.937x +0.012, R^2 = 0.999, can be used for the quantitative analysis.

3.6 Taurine Crude Extraction from Scallop Viscera

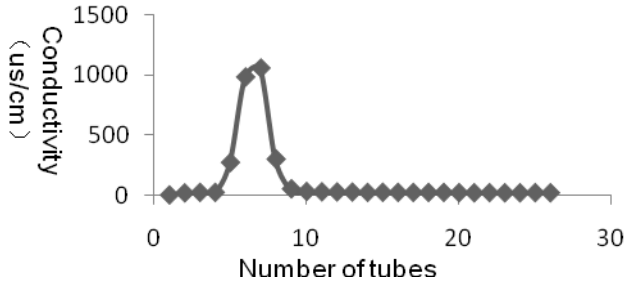

Figure 6. The out flow curve of conductivity

Conductivity can reflect the solution's capacity of conduction which can measure if the strong electrolyte in solution is eliminated after column chromatography. We can collect the eluant when the conductivity became stabilized. Figure 6 showed that the conductivity of 4-8 was higher, because the eluant contained large amounts of salts, sugars and other strong electrolytes. After 10 h, the conductivity became stabilized. The eluant with stable conductivity was collected.

3.7 The single factors experiment

3.7.1 EFFECT OF SOLID-LIQUID RATIO ON TAURINE EXTRACTION AMOUNT

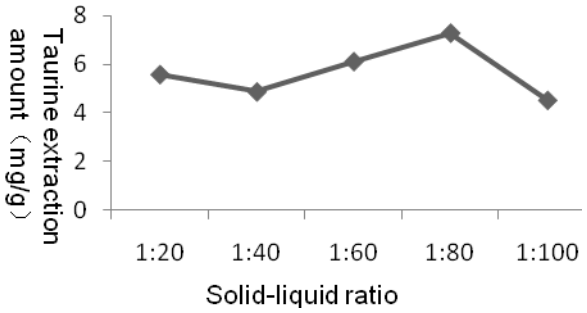

Figure 7. Effect of solid-liquid ratio on taurine extraction amount

As shown in Figure 7, with the solid-liquid ratio increasing, taurine extraction amount was first increased and then decreased. Taurine extraction amount reached maximum (7.2780 mg / g) when the solid-liquid ratio was 1:80. Therefore, the appropriate solid-liquid ratio was 1:80.

3.7.2 EFFECT OF EXTRACTING TEMPERATURE ON TAURINE EXTRACTION AMOUNT

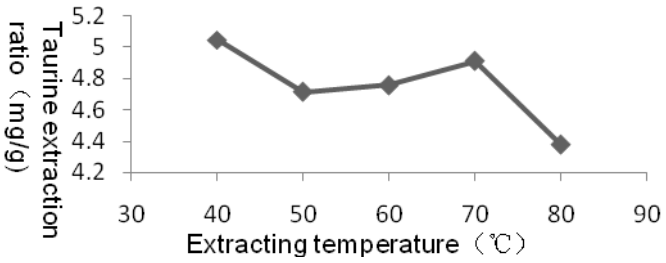

Figure 8. Effect of extracting temperature on taurine extraction amount

Figure 8 showed that with the extraction temperature increasing, taurine extraction amount was rising within a certain range of temperature, which was related to the solubility of taurine. Taurine extraction amount was up to 5.049 mg/g at 70 ℃, while reducing at 80 ℃. Therefore, the appropriate extraction temperature was 70 ℃.

3.7.3 EFFECT OF EXTRACTING TIME ON TAURINE EXTRACTION AMOUNT

The presence of certain enzymes in scallop viscera can decompose taurine under appropriate conditions, which can affect taurine extraction amount. Figure 9 showed that with extraction time increasing, taurine extraction amount was rising within 2 h. The maximum was 6.499 mg/g at 2 h, and then extraction began to decrease with the increasing time. Therefore, the appropriate extraction time was 2 h.

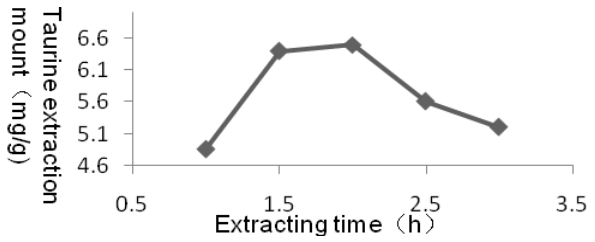

Figure 9. Effect of extracting time on taurine extraction amount

3.8 Orthogonal Design Experiment

Based on the single factor test, factors and levels orthogonal experimental design was established. The design was shown in Table I.

TABLE I. FACTORS AND LEVELS OF THE ORTHOGONAL TEST

Levels	Factors		
	Solid-liquid ratio A	Extraction temperature (℃) B	Extraction time (h) C
1	60	55	1
2	80	65	2
3	100	75	3

TABLE II. THE RESULTS OF ORTHOGONAL TEST

Test No.	Factors				Extraction amount (mg/g)
	A	B	C	Empty columns	
1	1	1	1	1	5.569
2	1	2	2	2	6.437
3	1	3	3	3	3.171
4	2	1	2	3	4.699
5	2	2	3	1	5.746
6	2	3	1	2	6.206
7	3	1	3	2	4.516
8	3	2	1	3	2.895
9	3	3	2	1	6.441
K_1	5.059	4.928	4.890	5.919	
K_2	5.550	5.026	5.859	5.720	
K_3	4.617	5.273	4.478	3.588	
R	0.933	0.345	1.381	2.331	

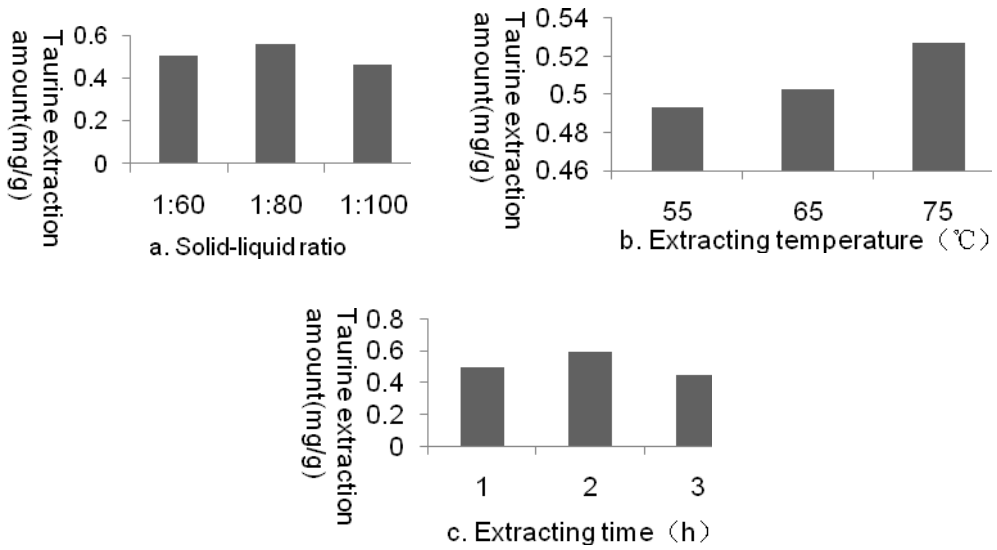

Figure 10. The trends of the three factors

The results of the orthogonal design in Figure 10 showed that the order of the three factors on the impact of the taurine extraction amount was: extraction time> solid-liquid ratio> extraction temperature. The optimum extraction condition was $A_2B_3C_2$, namely solid-liquid ratio at 1:80, extracting temperature at 75 ℃, and the extracting time at 2 h. Under this optimum condition, taurine extraction amount from scallop viscera was 7.636 mg/g.

4. CONCLUSION

In this paper, the extraction and determination methods of taurine were established. Firstly, through the single factor experiment, a simple and routine determination method of taurine was set up. Tested with standard taurine, this detecting method can be used for the quantitative analysis with R^2 being 0.999.

Secondly, the extraction technology of taurine was finished by orthogonal test, and the optimum conditions were solid-liquid ratio at 1:80, extraction temperature at 75 ℃, extraction time at 2 h. Taurine extraction amount was 7.636 mg/g under the optimum conditions, higher than the reported methods.

Scallop viscera was used as raw material to extract natural taurine, which had many advantages: simple process, low-cost equipment, eliminating the interference of other amino acids through the sample pre-treatment, recycling scallop waste and reducing environmental pollution. The established method can be used as an excellent way to extract taurine from aquatic products, and had important implications to promote the reuse of waste scallop with good practical significance and application prospects.

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Cover page

Title: *Effects of Polygonum Cuspidatum on Antioxidant and Selected Blood Indexes of Blood Parrot*

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ABSTRACT

A 2-week trial was conducted to study the effects of *Polygomum cuspidatum* of different mass fractions on antioxidant and part of blood indexes of blood parrot (). Experimental diet were prepared by supplementing the basal diet with *Polygomum cuspidatum* at 0.5%, 1%, 1.5%, 2%, 2.5% respectively, 3 parallel each group. Liver and blood were sampled in 7d and 14d. Results showed that superoxide dismutase (SOD) in 1.5% group, catalase (CAT) in 0.5% and 1.5% groups increased significantly for feeding *Polygomum cuspidatum* 7 days, while malondialdehyde (MDA) in 1.5%-2.5% groups, alanine transaminase (ALT) in 2.5% group, cholesterol (CHOL) and triglyceride (TG) in all groups decreased significantly. No significant difference on aspartate transaminase (AST) was observed for the same days. Feeding *Polygomum cuspidatum* for 14 days, SOD in 2% and 2.5% groups increased significantly, ALT in 1.5% and 2% groups, AST in all groups decreased significantly. These results suggested that *Polygomum cuspidatum* could enhance the antioxidant ability of blood parrot liver, alleviate hepatic injury, and improve the metabolism of lipid. Thus, a 7d continuous feeding with the dietary dosage of 1.5% was recommended.

INTRODUCTION

With the development of aquaculture industry, Chinese herbal medicine, used as feed addictive with no residue and pollution, has attracted a widespread attention [1] .Chinese herbal medicine is a kind of new feed addictive, the carbohydrates, alkaloids, glycosides in which can effectively enhance the animal's immunity. *Polygomum cuspidatum* Sieb. et Zucc. is the dried root and rhizome of *Polygomum cuspidatum* which has the effect of antivirus,antibiosis,anti-tumor and detoxication etc [2] . Recent years, it has been proven that *Polygomum cuspidatum* has a therapeutic effect on rat cerebral hemorrhage injury [3] , pulmonary microvascular endothelial cell injury [4] , nonalcoholic fatty liver and serum tumor necrosis factor [5] , etc. However, there are only few reports that focus on the research of aquatic organisms' physiological and biochemical indexes. Therefore, the present study choose blood

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parrot as research object and conducted a 2-week trial with *Polygomum cuspidatum* of 5 different addiction level, to provide a theoretical basis for the rational use of *Polygomum cuspidatum* as feed addictive.

1. MATERIALS AND METHODS

1.1 Fish and Feeding Trial

Blood parrot were obtained from Tianjin Kerry Freshwater Aquaculture Co. and were maintained indoors at Tianjin Key Laboratory of Aqua-ecology and Aquaculture. Fish were conditioned on a basal diet and acclimated to experimental conditions for 2 weeks prior to the feeding trial. Fish of similar sizes (initial mean body weight:68 g±1.5g) were randomly distributed as 6 groups into 18 plastic cases (70×50×45cm) , denoted as control group and A~E Group. Each diet was randomly assigned to three replicate cases. All groups of fish were fed their respective diets at the same fixed rate approaching satiation (3% of body weight per day) for 14 weeks. Fish were fed 2 times a day at 10:00 and 17:00. 1/3 of the water in cases was changed once a day, residual bait and feces were removed meanwhile. Water in the experiment was 24h aeration tap water. Water temperature remained at 27±1℃ throughout the trial.

1.2 Feed Used in the Experiment

Experimental diets A~E were derived from the basal diet (purchased from Tianjin Tianxiang Aquatic Products co.) by supplementing *Polygomum cuspidatum* at 0.5%, 1%, 1.5%, 2%, 2.5% respectively, The control diet did not add medicine. *Polygomum cuspidatum* powder was uniformly adhered with egg albumen, then naturally dried and stored in refrigerator at-20℃for next step.

1.3 Sample Collection

Fish from each plastic cases were anesthetized with MS-222, , and blood was collected from the caudal vasculature using a 1-mL heparinized syringe. Plasma was separated by centrifugation (5000r/min, 10min, 4℃). Fish were then dissected on iced tray directly. Samples of liver were washed with 4℃pre-cooling deionized water. More pre-cooling deionized water was added by 1:9 (W/V) to prepare 10% homogenate with glass homogenizer, after which the supernatant was separated by centrifugation (4℃, 3500 r/min, 10 min) as reserving liquid.

1.4 Determination of Indexes

Superoxide dismutase (SOD) , catalase (CAT) , malondialdehyde (MDA) , alanine transaminase (ALT) , aspartate transaminase (AST) , cholesterol (CHOL) , triglyceride (TG) were all determined using reagent kit produced by Nanking Jiancheng Biological Engineering Research Institute.

1.5 Statistical Analysis

Experimental data show as “average ± standard error (`X±SE) ”, the analysis

software is SPSS 13.0 . Perform the significance test on the test outcome, using the ANOVA one-way analysis of variance and Duncan multiple comparisons method. And the significance level displays as 0.05.

2. RESULTS

2.1 Effects of *Polygomum Cuspidatum* on Liver Antioxidant Indexes of Blood Parrot

TABLE 1 EFFECTS OF *POLYGOMUM CUSPIDATUM* OF DIFFERENT LEVELS ON LIVER ANTIOXIDANT INDEXES OF BLOOD PARROT

	SOD		CAT		MDA	
	U/mg prot		U/mg prot		nmol/mg prot	
Treatment	7 d	14 d	7 d	14 d	7 d	14 d
0%	219.5± 41.91^{ab}	204.8± 2.47^{a}	3.59± 0.42^{a}	5.83± 0.73^{b}	24.0 7± 5.23^{b}	17.85± 3.92
0.5%A	324.7± 88.50^{bc}	249.4± 62.13^{a}	11.54± 2.85^{b}	2.0 1± 0.27^{a}	30.30± 6.95^{b}	18.17± 0.63
1%B	205.2± 15.0 3^{a}	235.6± 46.34^{a}	2.64± 0.66^{a}	2.99± 0.54^{ab}	23.63± 3.80^{b}	19.24± 3.0 7
1.5%C	337.3± 33.32^{c}	156.3± 0.83^{a}	9.0 1± 1.60^{b}	4.30± 0.14^{bc}	13.44± 1.83^{a}	17.41± 4.14
2%D	224.6± 26.86^{abc}	471.6± 65.0 0^{b}	1.18± 0.45^{a}	4.14± 0.49^{b}	4.19± 0.95^{a}	15.51± 4.0 0
2.5%E	199.1± 18.75^{a}	379.4± 44.82^{b}	3.46± 1.32^{a}	3.0 9± 1.22^{ab}	10.69± 2.39^{a}	10.89± 1.55

Note: Numbers in the same line with different corner marks mean the difference between samples is remarkable (P<0.05)

From Table 1, Polygomum cuspidatum had different degrees of influences on blood parrots' SOD, CAT and MDA levels. During the first 7 days, treatment of 1.5% achieved the highest SOD level and it's significantly higher than control group (P<0.05) while other groups had no significant differences with it (P＞0.05). CAT level in treatments 0.5% and 1.5% were higher than others and they were conspicuously higher than control group (P<0.05), while treatments of 1%, 2% and 2.5% had no significant differences with it (P>0.05). The content of MDA could also be reduced in this period while it had no effects during the feeding trial of 14 days. Treatments of 1.5%, 2% and 2.5% was lower than other treatments.

During the feeding trial of 14 days, treatments of 2% and 2.5% achieved higher SOD level and had significant differences with control group (P<0.05) but others were without conspicuous difference compared with it(P>0.05). Besides, during the two feeding trials, there were significant differences among treatments of 1%, 2% and control group (P<0.05). What's more, the activity of SOD increased then decreased with the increasing of addition level. The effect on CAT was only obvious in treatments 0.5% and it had significant difference with control group (P<0.05). Comparing the two feeding trials, treatments 0.5% and 2% were conspicuously different while no differences are found among other groups (P>0.05).

2.2 Effects of Chinese Herbal Medicine Additive on Blood Antioxidant Indexes of Blood Parrot

TABLE 2 EFFECTS OF *POLYGOMUM CUSPIDATUM* OF DIFFERENT LEVELS ON BLOOD INDEXES OF BLOOD PARROT

	ALT		AST		CHOL		TG	
	U/gprot		U/gprot		mmol/L		mmol/L	
Treatment	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
0%	1.32±0.26^{ab}	1.45±0.16^{c}	14.84±3.87	14.01±1.80^{c}	11.02±1.48^{c}	14.02±1.08^{c}	5.47±0.86^{b}	9.53±1.20^{c}
0.5%A	1.57±0.28^{b}	0.37±0.05^{a}	14.06±1.16	10.76±1.33^{b}	7.08±1.36^{b}	8.44±0.38^{b}	1.94±0.27^{a}	4.55±0.95^{ab}
1%B	0.86±0.17^{a}	1.28±0.31^{c}	10.55±1.36	5.19±1.15^{a}	5.72±0.97^{b}	3.13±0.54^{a}	2.05±0.39^{a}	5.75±1.71^{b}
1.5%C	0.84±0.23^{a}	0.63±0.11^{ab}	10.38±0.04	6.28±0.57^{a}	1.63±0.27^{a}	2.72±0.52^{a}	2.26±0.17^{a}	2.37±0.56^{a}
2%D	1.68±0.0 4^{b}	0.46±0.04^{ab}	11.18±2.68	7.60±0.47^{a}	0.82±0.12^{a}	4.35±0.77^{a}	2.44±0.42^{a}	3.0 3±0.52^{a}
2.5%E	2.20±0.17^{c}	0.84±0.21^{b}	10.03±1.49	5.64±1.0 0^{a}	1.0 9±0.24^{a}	3.13±0.23^{a}	2.50±0.35^{a}	2.43±0.25^{a}

Note: Numbers in the same line with different corner marks mean the difference between samples is remarkable ($P<0.05$)

From Table2, it showed that ALT, AST, CHOL and TG levels decreased with the increasing of *Polygomum cuspidatum* levels. During the first feeding trial, treatments of 2.5% in ALT had significant difference with control group ($P<0.05$) while no differences were found among other groups ($P>0.05$). It also showed that the additive had no effect on AST in this period. Treatment of 2% achieved the lowest CHOL level and it's conspicuously lower than control group ($P<0.05$). During the feeding trial of 14 days, treatment of 0.5% achieved the lowest ALT and it's conspicuously higher than control group ($P<0.05$). Also treatments of 1.5%, 2% and 2.5% achieved conspicuous higher ALT levels than control group ($P<0.05$). Although *Polygomum cuspidatum* had no effect on AST in the first 7 days, it showd a definite effect on the five treatments in 14 days and treatment of 1% reached the lowest AST level. Obviously, treatments of 1.5% achieved the lowest CHOL and TG levels. The trend of the relationship among *Polygomum cuspidatum*, CHOL, and TG levels was displayed as negative correlation.

3. DISCUSSION

3.1 Effects of Chinese Herbal Medicine Addictive on Liver Indexes of Blood Parrot

MDA is known as a fatty acid peroxidation metabolites and is considered an indicator of liver damage [6]. There exists natural antioxidant system in fish, including CAT, SOD and other antioxidant enzymes, whose function is to remove these oxidant products to maintain normal physiological function [7]. Chinese herbal medicine contains a variety of ingredients, which can improve SOD and CAT level and decrease the level of MDA effectively at the same time. In a recent study, Hongbo Liu et al. reported that *Poria*, *Astragalus* and *Cyrtomium* Rhizome, as herbal additive, could increase SOD activity significantly [8]. What's more, Astragalus and Cyrtomium Rhizome can reduce the content of serum MDA effectively. Xun Liu et al. also indicated that the Chinese herbal compound could obviously increase the activitiy of

SOD in Acipenserschrenckii Brandt and it also decreased MDA effectively [9] . Ictalurus punctatus was taken as the study object to find the impacts of seven Chinese herbal medicines (*Polygomum cuspidatum*, *Belamcanda chinensis*, *Eucommia ulmoides* Oliver etc) on their physiological and biochemical indexes and the result showed that seven kinds of herbal medicines all had different degrees of influence on SOD and CAT activities in different organs [10] . In the present experiment, SOD and CAT activities can increase through feeding *Polygomum cuspidatum* for 7 days and the content of MDA reduced significantly. Furthermore, treatments of 1.5%, 0.5% and 1.5% achieved the highest SOD and CAT level respectively but treatments of 2% reached the lowest MDA level. Since the ratios between SOD and MDA, CAT and MDA can reflect different biological antioxidant levels, with the increase of the activities of SOD and CAT and decrease of MDA, ratios between SOD and MDA, CAT and MDA rises and the ratio of treatments of 1.5% was the highest. Therefore, feeding blood parrot with 1.5% *Polygomum cuspidatum* additive continuously for 7 days can improve liver antioxidant indexes effectively.

3.2 Effects of Chinese Herbal Medicine Addictive on Blood Indexes of Blood Parrot

Nowadays, there's a growing concern over the immunization of Chinese herbal medicine. ALT and AST, which are acid transaminases widely existed in animal mitochondria, play a vital role in protein metabolism. It is believed by most scholars that they are sensitive indicators of liver injury [11]. When liver cells get injured, acid transaminases tend to be released into blood, thus leading to an increase of blood transaminases. The content of CHOL and TG in Blood can indirectly reflect the lipid metabolism. It has been demonstrated that by supplementing compound Chinese herbal medicine (*Acanthopanax*, wolfberry fruit, honeysuckle and *astragalus*) in the basal feed of mirror carp, the activities of serum ALT and AST in compound group Ⅱ had significant differences ($P<0.05$) with the control group in 28 days, and a further serum ALT decrease in all groups was observed ($P<0.0$ 1) in 42 days [12]. Yingjiao Zhang et al.[13] studied the effects of compound Chinese herbal medicine on sturgeon serum CHOL, TG content and the result showed that Herbal Prescription two(*Astragalus*, *Codonopsisand* Angelica prescription) and Herbal Prescription three(*Capillaaris*, rhubarb, *CurcumaLonga*) could significantly reduce serum CHOL, TG content. Results from the present study suggests feeding the additive for 7 days, treatments of 1.5% in ALT was significantly lower than the control group ($P<0.05$）,while each group showed no significant difference in AST. Each treatment of CHOL and TG was significantly lower than the control group ($P<$ 0.05),by integrating traditional Chinese medicine for the effect of each group, feeding blood parrot with 1.5% *Polygomum cuspidatum* additive continuously for 7 days is the best choice.

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Cover page

Title: *Suspended sediment prediction by time Seri models and Artificial Neural Networks (Case study: Ghazaghly station in Gorganrood river of Iran)*

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ABATRACT

Accurate estimation of suspended sediment in rivers is very important from different aspects including agriculture, soil conservation, shipping, dam construction and aquatic research. There are different methods for suspended sediment estimation. In the present study to evaluate the ability of time-series models including Markov and ARIMA in predicting suspended sediment and to compare their results to Artificial Neural Networks it was tried to use daily suspended data from Ghazaghly station of Gorganroud River, as average monthly values in Minitab 16 software and Neurosolutions 5, and finally suspended sediment was predicted for 111 months. Calculation of the error measurement indices including MSE and NMSE based on the results of this study showed a good ability of Artificial Neural Network models in estimating and the average monthly suspended sediment. On the other hand between time series models, Markov model has better ability in estimating monthly suspended sediment.

Keywords: Suspended sediment, ARIMA, sediment prediction, Ghazaghly, Markov, Iran.

INTRODUCTION

Despite advances in the management of pollutant resources, water quality and sediment modeling remains as a complicated problem in hydrology [15]. The relationship between suspended sediment and river steam flow named as sediment rating curves has been seen with various correction coefficients in most studies related to this field from many years ago [2,3,4,5, 14] .

In recent years, there has been some study about suspended sediment modeling by time series models, are reported here: chen and Dyke [7] in 1996,used a time series model to estimate the suspended sediment, in their study, each value of suspended sediment were simulated based on its previous values, chen and et al [6] used the time series model to determine suspended sediment and associated water

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velocity in the Rifuji delta in Tanzania. Singer and Dunn [21], employed time series analysis to quantify the relationship between stream flow and suspended sediment concentration sediment records of (2-yr duration) were extended using time series models to calculate annual suspended sediment rate over 32- year period since dam struction. Melles and colleagues [12] used Arima, multiple linear regression, multiple non-linear regression and artificial neural networks (ANN) model to predict daily sediment data of three rivers and results indicated that ANN model is superior compared to other models. Nourani and Romyanfar [17] employed Arima model to rainfall-runoff modeling in Aharchay watershed.

With the advent of artificial intelligence and computer-based methods such as artificial neural networks in hydrology studies, sediment studies also impressed and researchers began to employ this new tool, in this field, Negy and et al [15], Jain [10], Kisi [11], Alp and Cigizoglu [1], Cobaner and et al [8], Nourani and et al[18], Rajaee and et al [19], Rezapour and et al [20] and Jafari Myanay and Keshavarz [9] have conducted some studies in this field, Application of neural networks in prediction suspended sediment were based on modeling the physical and climatic parameters affecting the suspended sediment load [22, 17] or by using hourly and sometimes the moment data resulting from intensive direct measurement of suspended sediment, since intensive suspende sediment data is not available in current situation of hydrometric stations of Iran, we can investigate suspended sediment by using monthly time steps. Therefore in this study, first neural network was used to predict the monthly sediment data and in other hand statistical time series models, due to their simplicity and ease of use, were compared with neural networks to predict monthly suspended sediment.

MATERIALS AND METHODS

In order to do this study, the daily water discharge (cms), and suspended sediments concentration (ppm) collected from 1970-2009, in Qzaqly station included 6777 data, since these data were not collected on regular basis, the average monthly data were calculated using Excel program in order to better fit the models. To run neural network modeling, monthly sediment data and its corresponding water discharge was used in Neurosolutions 5 software. Literature review showed that in most of the studies that used ANN for estimating suspended sediment, multi-layer perceptron neural network was applied, but Memarian and Balasundram in 2012 claimed that multilayer perceptron networks and radial basis network layer are inappropriate to predict suspended sediment of high stream flow discharge in semi-arid areas [13].There for in this study, 44 different structure from commonly used models including: Multilayer Percepteron, Generalized Feed Forward Modular Neural Network, Jordan / Elman Network, PCA, Recurrent Network survey were used, and finally, most suitable model was selected to predict suspended sediment. Then, the statistical time series models were used. Time series analysis has been presented originally by Box & Jen Kins. Linear function of this model is located based on three following linear components [16]:

Moving Average (MA), Integration (I), Auto regression (AR), The ARIMA time series can be written as equation (1)

$$\phi(B)(1-B)^d Z_t = \theta(B)\,\varepsilon_t \quad (1)$$

Where $\theta(B), \phi(B)$ are defined as relations 2 and 3

$$\phi(B) = 1 - \phi_1 B - \phi_2 B^2 - \ldots \phi_p B^p \quad (2)$$

$$\theta(B) = 1 - \theta_1 B - \theta_2 B^2 - \ldots \theta_q B q \quad (3)$$

In presented equations, Z_t is the original series, ε_t, is random series with zero mean and $\delta^2{}_t$ variance , ϕ , is parameter of correlated model (AR), θ ,is the parameter of the moving average (MA), P, is order of auto correlated model, q, is order of the moving average model, d, is the order of differencing and B, is the backward operator [17].

In order to apply the time series models, Minitab 16 statistical software was used. With regard to time series model requirement to normal databases, data were examined by normality test and then data were normalized by using logarithmic transformation and finally, Markov and ARIMA models were used on normalized data. The results of applying these techniques were compared using the sum of squares of deviations (SSR) (Equation 4) and Akaike information criterion (AIC) (Equation 5).

$$SSR = \sum_{i=1}^{n} (x_i - \hat{x}_i)^2 \quad (4)$$

$$AIC = n \ln(SSR) + 2(p+q) \quad (5)$$

In these equations, x_i is estimated sediment by model, $\hat{x}_i$ is measured sediment load, p and q are defined in above equations. It should be noted that the model with less SSD or less Akaike information criterion is more appropriate model.

RESULTS AND DISSCUSIONS

To apply times series models, first data trend should be removed, to do this, causing two lags in data sets seem appropriate. So Arima (1, 2) model can be used on the transformed data. However, for comparison, Markov or first-order autoregressive model and ARIMA (2, 1, 3) model was also used. SSD and AIC criteria were used to compare results of applied models (Table 1). As shown in this table, Markov model has lowest error. To evaluate the suitability of the selected time series model (Markov), the residues of the final model was examined (Fig. 1).

TABLE I- COMPARISON DIFFERENT MODEL BY AIC AND SSR

Model	SSR	AIC
ARIMA(1,1,2)	3003.99	3009.898*
ARIMA(2,1,3)	3003.89	3013.8987
Markov	3003.104*	3011.1048*

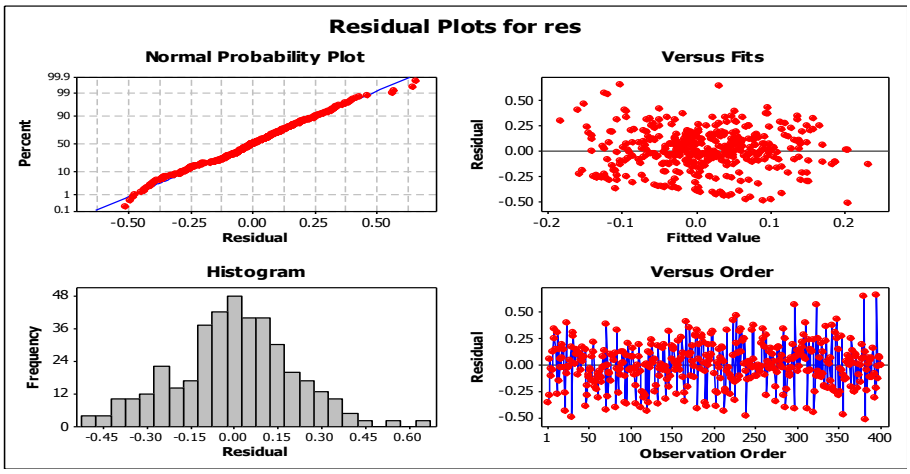

Figure1. Diagram of distribution of residuals from the Markov model

As shown in figure 1, in normal probability plot, points are located approximately along a straight line, and this shows that residuals from the Markov model are distributed in a normal form. This shows appropriateness of the selected model. The results of the predicted monthly precipitation for 111 months (25% data), using a Markov model and selected artificial neural network models are shown in Figures 2 to 4. As seen from Figures 2 to 4, artificial neural networks are able to better simulate monthly suspended sediment. To comparison selected artificial neural networks and statistical models (Arima and Markov model), Root Mean Square Error and Normalized Mean Square Error were used. The results are presented in Table 2.

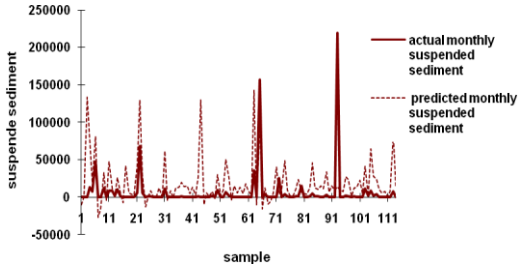

Figure 3 Comparison of the results of the tangent linear model Jordan / Elman Network with actual data

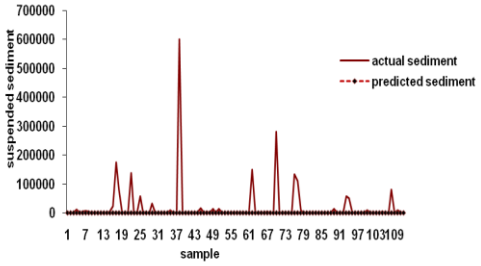

Figure 2 Comparison of Markov model results with actual data

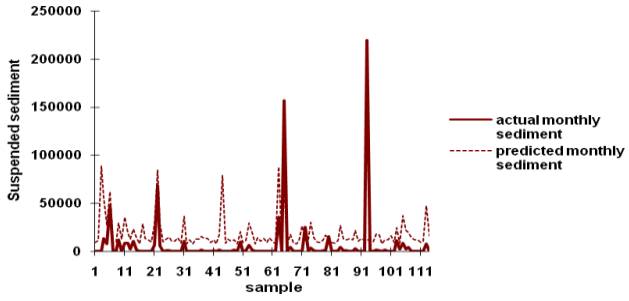

Figure 4 Comparison of model results with actual data Modular Neural Network tangent of function

TABLE Π - COMPARING MODEL RESULTS WITH THE NMSE AND RMSE INDEX

Model	Modular Neural Network	Jordan/Elman Network	ARMA(1,1,2)	Markov
RMSE	**28.99	*34.67	71.34	78.56
NMSE	**1.18	*1.78	2.48	3.2

Results suggest that the artificial neural network is a high priority. Statistical time series models in compare with artificial neural networks have less ability to estimate monthly suspended sediment, especially in flood condition, in fact they could predict average of monthly suspended sediment. Perhaps this is because of inappropriate sediment sampling system that almost always includes low and moderate steam flow discharge. Review of the literatures indicates that previous researchers, including Chen and Dyke [7], Singer and Dunn [21], and Melles and et al [12], in their studies, used time series models to evaluate daily suspended sediment and indicated that time series models are suitable, this is because of the quality of the data have been used in their studies, that is collected in compressed and momentary intervals. So it can be said that in current situation of suspended sediment sampling in Iran, the use of time series model is not recommended. However, if because of the advantages such as simplicity speed of performance, time series models are preferred, it is better that Markov and ARIMA (1, 1, 2) model be used. It should be noted that, usually in most studies of natural resources, a multilayer perceptron neural network is used but present study results showed that for monthly suspended sediment other models are accountable. Memarian and Balasundram [13] formerly noted that the MLP network is inefficient in estimating sediment concentrations in high steam flow discharge in semiarid area.

CONCLUSION

ANN models have good ability in estimation monthly suspended sediment. But MLP is not recommended always.

It can be said that in current situation of suspended sediment sampling in Iran, the use of time series models is not recommended for estimation monthly suspended sediment, but if time series models are preferred, it is better that Markov and ARIMA (1, 1, 2) model be used.

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Title: *The Set Pair Analysis and Its Application on the Correlation among Many Factors*

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ABSTRACT

Set pair analysis (SPA) explores micro-relational structure among factors with connection degree from microcosmic angle, the deterministic are divided into "the same" and "opposites", and the uncertainty is called "difference". SPA analyzes the objects and system from three aspects: the same, opposites and difference, the nature and proportionate relationships of the objects and system are analyzed; SPA is unique advantage to solve the determination-uncertain systems problems. The correlation among grain yield and agricultural irrigation water amount, grain planting area, the total power of agricultural machinery, and the rural electricity consumed, grain production-consumer price index, the government expenditure on agriculture, agriculture technological investment are studied with SPA, so their action characteristics and parallelism measures to grain yield are discussed. The results show: there is great uncertainty in the effect of agricultural irrigation water amount to grain yield, so the agricultural water-saving potential in Henan province is large; there are bigger positive implicative in the effect of the grain planting area, the total power of agricultural machinery, and the rural electricity consumed to grain yield, so Henan province should realize the grain production target by strengthening the protection of basic farmland, improving the level of agricultural mechanization, encouraging to improve rural electrification level as soon as possible; there are positive implicative in the effect of grain production price index, grain consumer price index and their difference, the government expenditure on agriculture to grain yield, That should improve food prices, and increasing agricultural subsidies, farmers to benefit, so as to realize the grain production target; there is great uncertainty in the effect of agriculture technological investment to grain yield, it relates the emphasis of the scientific and technological achievements.

Index Terms - *Set pair analysis, Correlation, factors*

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1. INTRODUCTION

In order to study the correlation of factors, correlation factors in common use are adopted to embody their correlation grade [1-2], correlation coefficient is a macroscopic quantitative index, it reflects relation between factors on the whole [3]. There are numbers of factors to influence grain yield, such as grain yield and agricultural irrigation water amount, grain planting area etc., and they indicate positive or negative correlation and the uncertain correlation in relevance. Correlation coefficient is used to reflect macroscopic feature, so it difficultly embody microscopic construction between grain yield and inflecting factors in micro-aspect, thus the detailed correlation can not be embodied. SPA [4] has its unique superiority to solve the question of certain-uncertain system, and proposed a theory basis for the question, so SPA is used in fields as Math. [5], Eco [6], Hydro. [7], rock and soil project [8] fields etc..

Henan province is one of the most important grain production area in China and a pivotal role to China's food security, the study on the correlation factors of grain yield in Henan province is very important to explore how to guarantee food security. SPA explores micro-relational structure among factors by connection degree from microcosmic angle, the nature and proportionate relation of the objects and system are analyzed; SPA is unique advantage to solve the determination-uncertain systems problems.

2. SPA CONCEPT

SPA (Zhao Keqing, China, in1989,) its core idea is to view the deterministic and uncertainty as the determination-uncertain system, and the uncertainty is called "difference", the deterministic are parted into "the same" and "opposites". And SPA analyzes the objects and system from three aspects: the same, opposites and difference, the nature and proportionate relationships of the objects and system are analyzed [9]. Set pair is the pair consisted of relevance set A and B, named as H(A,B). Correlation agree in SPA is introduced into study the same, opposites and difference among sets. The basis of SPA is set pair, and its key is connection agree[10].

Set A has factors of n to explore its feature, as $\mathrm{A}=(a_1,a_2,\cdots,a_n)$, so as for $\mathrm{B}=(b_1,b_2,\cdots,b_n)$, the connection agree to describe the relation of H(A,B) is determined as following:

$$\mu_{A\sim B}=\frac{S}{n}+\frac{F}{n}i+\frac{P}{n}j \tag{1}$$

Where S is the number of the same; F is the number of difference ; P is the number of opposite; S+F+P=n； i is uncertain coefficient of difference valued in interval [-1,1] according different condition, sometimes only acting as variation label; j is opposite coefficient with $j\equiv -1$, and sometimes acts as opposite label; $\mu_{A\sim B}$ is connection degree of H(A,B).

Opposing a=S/n, b=F/n, c=P/n, so for. (1)is following as:

$$\mu_{A\sim B} = a + bi + cj \tag{2}$$

Where *a*, *b* and *c* are component of connection degree and $a+b+c=1$, a, b and c are respectively the same degree, difference degree and opposite degree of H(A,B). The relation of *A* and *B* more tends to the same when *a* is more closed to1; The relation of A and B is more tended to the opposite when c is more closed to1; The relation of A and B is more tended to the difference when b is more closed to1. The uncertain correlation can be transformed into the same correlation or opposite correlation in particular condition. For example, difference degree can be transformed into the same degree when i=1, and the un-certain relation can be transformed into the same relation; difference degree can be transformed into opposite degree when i=-1, and the un-certain relation can be turned into opposite relation; difference degree includes in the same degree and opposite degree in specified ratio when $i \in (-1,1)$, and the un-certain relation includes in the same relation and opposite relation in specified ratio. Correlation degree μ and i is the foundation stone o f SPA theory, and the uncertain phenomenon such as random, fuzzy and grey etc. can be described by SPA theory [11]. For.(2)is named as 3-variant correlation degree, when b_i is expanded as $bi = b_1 i_1 + b_2 i_2 + \cdots + b_{k-2} i_{k-2}$, For.(2)is:

$$\mu_{A\sim B} = a + b_1 i_1 + b_2 i_2 + \cdots + b_{k-2} i_{k-2} + cj \tag{3}$$

Where b_1、b_2、$\cdots$、b_{k-2} is difference degree component, meaning as difference class or grade of difference; $a + b_1 + b_2 + \cdots + b_{k-2} + c = 1$, i_1、、i_2、$\cdots$、i_{k-2} is un-certain coefficient of difference. For.(3) is named as k-variant correlation degree.

3. APPLICATION FOR THE ANALYSIS OF CORRELATION FACTORS GRAIN YIELD IN HENAN PROVINCE

Henan province is a pivotal role to China's food security, and is the one of most important grain production area in China. so the research on the correlation factors of grain yield in Henan province is very important to explore how to guarantee food security. Grain yield is affected by many factors, and the main affecting factors are grain production-consumer price index, the government expenditure on agriculture, the rural electricity consumed, agriculture technological investment, agricultural irrigation water amount, grain planting area, the total power of agricultural machinery and so on. And these factors can be modulating controlled. So these factors are researched for the micro-correlation construction among factors and grain yield, and the feature of correlation construction and the ratio are also studied.

The selecting data is listed in table 1(detailed in water resources official report (1998-2009) in (Henan province, statistical yearbook in Henan province (2008), nation economy and society development official report in Henan province (2008)) from 1998 to 2009. Grain yield is taken as dependent variable Y, and $Y = (y_1, y_2, \cdots, y_{12})$,the agricultural irrigation water amount, the grain planting area, the total power of agricultural machinery, he rural electricity consumed, grain production price index , grain consumer price index, the government expenditure on

agriculture, agriculture technological investment and the difference between grain production price index and grain consumer price index are respectively X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9. Every effective factor $X_i = (x_{i,1}, x_{i,2}, \cdots, x_{i,12})\ (i = 1,2,\cdots,9)$ is the another sets, and Y and X_i constructed in set pair $H(X_i,Y)$, as $H(X_1,Y)$, $H(X_2,Y)$, $H(X_3,Y)$, $H(X_4,Y)$, $H(X_5,Y)$, $H(X_6,Y)$, $H(X_7,Y)$, $H(X_8,Y)$, $H(X_9,Y)$.

The set classification standard(Tab.1) is determined by set feature of 5, and the data in set are classified into 3 grade of smaller(Ⅰ), middle(Ⅱ), bigger(Ⅲ) according to $[0, \bar{x}-0.5s)$, $[\bar{x}-0.5s, \bar{x}+0.5s)$, $[\bar{x}+0.5s, +\infty)$, classification results are shown in Tab.2

TAB.1 THE CLASSIFICATION STANDARD

Class	X_1	X_2	X_3	X_4	Y
$\bar{x}$	123.26	9184.67	7263.67	167.42	4518.14
s	16.80	289.13	1630.90	48.44	609.39
Ⅰ	[0,114.86)	[0,9040.11)	[0,6448.22)	[0,143.20)	[0,4213.45)
Ⅱ	[114.86,131.66)	[9040.11,9329.23)	[6448.22,8079.12)	[143.20,191.64)	[4213.45,4822.83)
Ⅲ	[131.66,+∞)	[9329.23,+∞)	[8079.12,+∞)	[191.64,+∞)	[4822.83,+∞)
Class	X_5	X_6	X_7	X_8	X_9
$\bar{x}$	176.83	423.61	79.38	9437.83	246.78
s	30.90	76.51	76.47	10170.31	48.40
Ⅰ	[0,161.38)	[0,385.36)	[0,41.15)	[0,4352.68)	[0,222.58)
Ⅱ	[161.38,192.28)	[385.36,461.87)	[41.15,117.62)	[4352.68,14522.985)	[222.58,270.98)
Ⅲ	[192.28,+∞)	[461.87,+∞)	[117.62,+∞)	[14522.985,+∞)	[270.98,+∞)

TAB.2 THE CLASSIFICATION RESULTS

Time	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	Y
1998	Ⅰ	Ⅱ	Ⅰ	Ⅰ	Ⅲ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅰ
1999	Ⅲ	Ⅰ	Ⅰ	Ⅰ	Ⅱ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅱ
2000	Ⅱ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅱ	Ⅰ	Ⅰ
2001	Ⅲ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅱ	Ⅰ	Ⅰ
2002	Ⅲ	Ⅰ	Ⅱ	Ⅰ	Ⅰ	Ⅰ	Ⅰ	Ⅱ	Ⅰ	Ⅰ
2003	Ⅰ	Ⅰ	Ⅱ	Ⅱ	Ⅰ	Ⅰ	Ⅰ	Ⅱ	Ⅰ	Ⅰ
2004	Ⅰ	Ⅰ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ
2005	Ⅰ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ
2006	Ⅱ	Ⅲ	Ⅲ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅱ	Ⅲ
2007	Ⅰ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅱ	Ⅲ	Ⅲ
2008	Ⅱ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ
2009	Ⅱ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ	Ⅲ

The classification of Y and X_i is compared, the same classification is called the same, and its number added as S; difference one grade is called difference such as Ⅲ and Ⅱ, Ⅱ and Ⅰ, and its number added as F; difference two grades is called opposite such as Ⅲ and Ⅰ, and its number added as P. The correlation degree of set pair $H(Y,X_i)(i=1,2,3,4)$ can be calculated by For. (1):

$$\begin{cases} \mu_{Y\sim X_1} = \frac{2}{12} + \frac{7}{12} i + \frac{3}{12} j = 0.167 + 0.583i + 0.250j \\ \mu_{Y\sim X_2} = \frac{9}{12} + \frac{3}{12} i = 0.750 + 0.250i \\ \mu_{Y\sim X_3} = \frac{9}{12} + \frac{3}{12} i = 0.750 + 0.250i \\ \mu_{Y\sim X_4} = \frac{9}{12} + \frac{3}{12} i = 0.750 + 0.250i \\ \mu_{Y\sim X_5} = \frac{10}{12} + \frac{1}{12} i + \frac{1}{12} j = 0.833 + 0.083i + 0.083j \\ \mu_{Y\sim X_6} = \frac{10}{12} + \frac{2}{12} i = 0.833 + 0.167i \\ \mu_{Y\sim X_7} = \frac{10}{12} + \frac{2}{12} i = 0.833 + 0.167i \\ \mu_{Y\sim X_8} = \frac{5}{12} + \frac{7}{12} i = 0.417 + 0.583i \\ \mu_{Y\sim X_9} = \frac{10}{12} + \frac{2}{12} i = 0.833 + 0.167i \end{cases} \quad (4)$$

In correlation degree $\mu_{Y\sim X_i}$, a is positive correlation degree; b is un-certain correlation degree that is neither positive correlation degree nor negative correlation degree; c is negative correlation degree. There is the positive correlation degree among factors and grain yield if a>c; there is the negative correlation degree among factors and grain yield if a<c; the un-certain correlation is strong among factors and grain yield if b is bigger. The results is gained from For.(4):

1) In the correlation of grain yield and the agricultural irrigation water amount, positive correlation degree is close to negative correlation degree, but un-certain correlation degree is bigger with its value is 0.5833. Thus there is great un-certainty in the effect of agricultural irrigation water amount to grain yield. Which is resulted from the negligence mode of irrigation, and utilization efficiency of irrigation water is lower. So the agricultural water-saving potential in Henan province is large, the potential must be tapped by saving agricultural irrigation water amount.

2) In the correlation of the grain yield and the agricultural planting area, positive correlation degree is 0.75, and the correlation in the effect of the grain planting area on the grain yield is positive. So there is close relevance between increasing the grain yield and the grain planting area increase. Some measures must be done to strengthen the protection of capital farmland, so to further increase the agricultural planting area.

3) In the correlation of the grain yield and the total power of agricultural machinery, positive correlation degree is 0.75, and the correlation in the effect of the total power of agricultural machinery on the grain yield is positive. There is

hysterics in the effect of the total power of agricultural machinery to the grain yield, which is embodied in the difference degree with the value is 0.25. So there is close relevance between increasing the grain yield and the total power of agricultural machinery increase. Some measures must be done to strengthen the protection of capital farmland, so to further increase the agricultural planting area. So Henan province should enlarge policy to encourage peasants to buy agricultural machines and increase the level of agricultural machinery.

4) In the correlation of the grain yield and the rural electricity consumed, positive correlation degree is 0.75. So there is close relevance between increasing the grain yield and the rural electricity consumed increase. There is hysterics in the effect of the rural electricity consumed to the grain yield, which is embodied in the difference degree with the value is 0.25. Some measures must be done to strengthen the protection of capital farmland, so to further increase the agricultural planting area. So Henan province should speed up to reform the rural electric network velocity to encourage the level of agricultural electrification and accelerate the rural electricity to be consumed.

5) In the correlation of the grain yield and the grain consumer price index, positive correlation degree is 0.833, but negative correlation degree is in the correlation of the grain yield and the grain production price index grain consumer price index, so there is negative effect of the grain production price index to the grain yield. And there are un-certain correlation the indexed to the grain yield, and the uncertain correlation between the grain consumer price index and the grain yield is bigger, so there is more un-certainty in the effect of to the grain yield. Positive correlation degree is 0.833 in the in the effect of difference between grain production price index and grain consumer price index to the grain yield. So Henan province should increase the grain consumer price index or decrease the grain production price index to enhance the activity of planting grain.

6) Positive correlation degree is 0.833 in the correlation of the grain yield and the government expenditure on agriculture. So the activity of planting grain can be excited when the government increases agriculture expenditure and improves subsides. And there is more uncertainty correlation between the government expenditure on agriculture and the grain yield. The un-certain degree is bigger than positive degree shows that there is some uncertainty effect of the government expenditure on agriculture on the grain yield.

4. CONCLUSION

(1) SPA explores micro-relational structure among factors with connection degree from microcosmic angle, and analyze its feature and ration, so SPA is unique advantage to solve the determination-uncertain systems problem;

(2) In the researched main factors of 9, there is great un-certainty in the effect of agricultural irrigation water amount to the grain yield, so the agricultural water-saving potential in Henan province is large; there is much positive in the effect of the agricultural planting area to the grain yield, so some measures must be done to strengthen the protection of capital farmland, so to further increase the agricultural planting area. There is hysterics and much positive in the effect of the total power of agricultural machinery to the grain yield, So Henan province should

enlarge policy to encourage peasants to improve agricultural machines and increase agricultural machinery level; there is hysterics and much positive in the effect of the rural electricity consumed to the grain yield, thus So measures should be speeded up to reform the rural electric network velocity to encourage the level of agricultural electrification and accelerate the rural electricity to be consumed; positive correlation degree is in the grain consumer price index, the grain production price index and difference between grain production price index and grain consumer price index on the grain yield, so the grain consumer price index is increased or the grain production price index is decreases to enhance the activity of planting grain; Positive correlation degree is in the correlation of the grain yield and the government expenditure on agriculture, so there is more uncertainty correlation between the government expenditure on agriculture and the grain yield.

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Cover page

Title: *LCA Adapted Approach for Soybean Biodiesel Production from an Integrated Crop-Livestock System in Midwestern Brazil.*

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ABSTRACT

Brazil's Midwest region is one of the major soybean producing areas in the world. To achieve economic and environmental optimization in this region, extensive livestock systems have been replaced by integrated crop-livestock systems. This paper presents an adapted life cycle assessment approach for compa[1]ring these integrated systems regarding biodiesel production from soybeans.

INTRODUCTION

Brazil is one of the world's largest soybeans producers. Through systematic genetic improvement programs, today it is possible to cultivate high-yielding soybeans in all Brazilian regions. The Midwest region is the leading producer because of favorable factors such as climate, soils, relief, land availability and local infrastructure. The main activity in this region was traditionally extensive livestock breeding (mainly cattle) until the 1970s, when development of technologies started enabling better land use in the region, with cattle ranching yielding space for crops, including for biofuel production. This is a general trend in the "Cerrado" biome.

Two major technological factors have played major roles in soybean expansion in Brazil: development of specific biological nitrogen fixation organisms and no-till cultivation systems. In Midwest Brazil, another factor has been also crucial for its steady growth, the intermediate maize crop, which is cultivated between the soybean seasons, i.e. in the winter period between two soybeans crops, which are exclusively cultivated in the summer. This is technologically and logistically possible because of no-till cultivation systems. Another technology being adopted in the area is the intercropping of soybeans/maize with cattle ranching, with pastures serving as the fallow period for reestablishing soil conditions and fighting weeds and diseases.

Therefore, when carrying out an LCA for soybean biodiesel, it is important also to consider the expansion of these integrated systems, by including all relevant aspects of the production process that have bearing in the calculations, particularly the share of each necessary resource (natural or otherwise) to obtain soybeans and the byproducts.

As consequence, while contributing substantially for farmers' profitability, such

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integrated systems pose some challenges when carrying out life-cycle assessments for soybeans in the area, since inputs like machinery are used by both crops. Splitting their environmental costs can be done through different approaches, which can influence the final results. For this reason, this subject is addressed in this work.

For this case study, a farm in Mato Grosso do Sul was selected because of its location in a traditional soybeans production area, very good data availability, and a soybean production system that represents typical soybean farms in Brazil, but presenting the challenge of discriminating in the LCA the shares of inputs that should be attributed to each component of the whole integrated system.

CROP-LIVESTOCK INTEGRATION

In Midwest Brazil, farmers try to optimize production systems by planting maize in the soybean off-season (winter crop). In addition, some farms have been using a system that integrates crops and livestock. Farmers put the cattle to graze on fields in the period between maize and soybeans, for one or two months in the peak of the dry season (July and August) and/or only rotate between soybeans and livestock grazing.

The stages of crop-livestock integration, presented in Figure 1 and detailed below, are based on this case study farm, called Sapé Agro, a 4015-hectare farm located in the municipality of Maracaju, state of Mato Grosso do Sul. The cycle described covers one year.

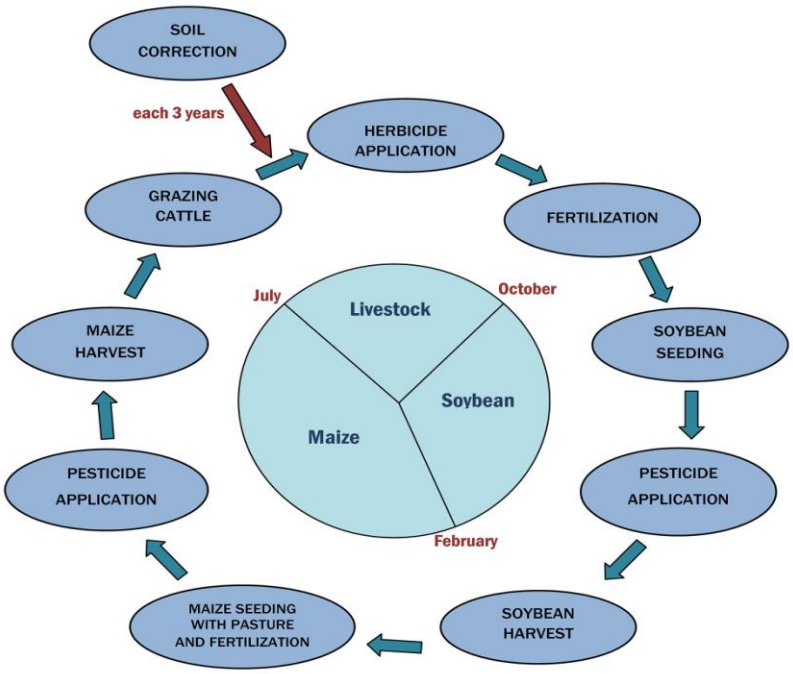

Figure 1: Flowchart of the integrated crop-livestock systems used at the farm.

To start any agricultural activity in the soils of the region, acidity correction is required. This operation is essential for large-scale agricultural production in the Brazilian Cerrado, and is usually repeated on average every three years. Lime and agricultural gypsum are incorporated uniformly in the layer up to 20 cm deep (plowed layer).

Lime serves to neutralize the acidity of the soil layers where it is incorporated. In turn, gypsum has the ability to correct the acidity of deeper layers by its characteristic of dissolution in rainwater and subsequent soil infiltration [1].

Soybean production takes place in the rainy season, between October and February. Soil preparation for sowing soybeans is composed primarily of killing weeds with broad-spectrum herbicides and fertilization.

Herbicides are responsible for eliminating pre-existing living matter, creating a mulch which allows the passage of light at the same time as stabilizing the soil temperature and moisture. Part of the KCl is spread in the fields before to seeding. The fertilizer applied at this stage is potassium chloride (KCl), the most used potassium salt in agriculture, containing 60-62% K_2O [2]. The presence of potassium is beneficial because it ensures greater vigor and disease resistance to the plants. For soybean seeding, some requirements must be followed, including:

- water absorption should be at least 50% of the dry weight of the seed, requiring adequate levels of moisture and soil aeration;
- seed should be placed on the ground when it has an average temperature between 20 and 30 ℃.

In general, the soybean sowing season runs from late October to early December, with crops planted in November producing the best yields and plant height.

Brazilian no-till seeding machines were developed specifically for the local environment. Their basic functions are to cut the straw and carve a deposition and distribution of seeds and MAP fertilizer in recommended amounts, then cover the seed and lightly pack the ground to remove air pockets. In this operation, deep root fertilization is carried out, taking care that it gets distributed beside and below the seeds, since direct contact would harm it or impair water absorption.

Monoammonium phosphate (MAP) is obtained by the reaction between phosphoric acid and anhydrous ammonia. It is a fertilizer rich in nitrogen and phosphorus, with 48% to 55% phosphorus pentoxide (P_2O_5) and 9% to 12% nitrogen. Its advantages are the high content of nutrients, high water solubility and greater P2O5 content, and it is fully compatible with all other components typically used in mixed fertilizer [3].

Soybean plants get most of the nitrogen they need through symbiotic fixation, which occurs with Bradyrhizobium bacteria [4]. From the two fertilizers used (MAP and KCl), soybeans require approximately 11 parts of nitrogen for each 52 parts of phosphorus and 60 parts of potassium.

The management of soybeans includes spraying of three types of pesticides (herbicides, insecticides and fungicides) uniformly on the crop. This practice is repeated around seven times per season, depending on infestation level.

Immediately after the soybean harvest, between February and March, maize is sown also without tillage, where through the use of specific machinery it is possible to seed maize on soybean straw, without plowing or other physical preparation of the soil, reducing the need for machinery, labor and fuel and generating various benefits for the soil.

In integrated systems, maize is sown with grasses, usually *brachiaria ruziziensis*, in a density of 2.5 kg per hectare. This system is used in approximately 80% of the area grown with soybeans, allowing extracting the benefits of the system without generating competition for maize plants. This pasture seeding has two main objectives: to give more cover to the soil, reducing erosion, and providing complementary forage

for cattle in the winter, if necessary.

For maize, NPK fertilizer is used, usually with the formulation 14:16:16, along with micronutrients, especially manganese, zinc, copper, boron and molybdenum.

The application of pesticides during cultivation of maize takes place in the same way as in soybean cultivation, differing only in the frequency of application. Maize usually needs about two applications. As the maize harvest progresses (early July), areas begin to be released for cattle grazing. In this winter period, rainfall declines and regular pastures are not able to support the existing stock, creating deficiency in cattle forage. In the integrated system, however, these cattle are taken to areas where maize was removed and they can feed on existing Brachiaria grass or maize straw.

In the remaining 20% (divided into 2 plots) of the area where no maize is planted, Brachiaria is planted alone, at a rate or 4.5 kg per hectare. This area supports the livestock in late fall/winter and cattle usually visit each plot three times. By mid-July, male and female calves are weaned and brought to the feed yard. Mother cows start grazing in areas where maize was harvested.

The complete rotation cycle on the farm takes five years, since it includes sugarcane, which is used until the fifth cutting, i.e., every five years, there is a movement in which:

- areas where sugarcane was planted turn into areas for planting soybeans/maize;
- areas used by livestock become areas for planting soybeans/maize or sugarcane;
- soybean/maize areas become areas for planting sugarcane or pasture.

However, sugarcane only has a partial participation in the life-cycle assessment, because it does not use the same area in the same season, only sporadically sharing a few resources such as machinery and infrastructure, which for this case study can be disregarded.

LIFE-CYCLE ASSESSMENT METHOD

To perform a soybean biodiesel LCA, it is necessary to analyze several subsystems, which range from the soybean production to transesterification process to obtain biodiesel. Figure 2 shows the complete cycle of biodiesel production from soybeans for a system with land use and other resources exclusively dedicated to soybean production. Each stage receives the product from the previous stage, the inputs and all the energy expended by the machinery needed for the current stage. The byproducts fall into the accounting as negative environmental impacts (credits), since they avoid atmospheric emissions and use of non-renewable energy that would be required to produce equivalent products by conventional agricultural and livestock production.

The environmental impact categories investigated in this work are non-renewable energy consumption and global warming factors.

Although maize cultivation and the land use for grazing are not in the traditional scheme of Figure 2, the impacts of these processes should also be considered in LCA for this area. In the case of maize, for example, straw left on the ground during harvesting provides a yield increase for the next soybean crop, by keeping the soil covered in the winter (no-tillage system). Also, the maize produced in soybean intercropping shares the same equipment and residues of soybean crop inputs.

Table 1 presents for each of the stages of the integrated crop-livestock (Figure 1) the equipment, inputs and byproducts of the case study farm.

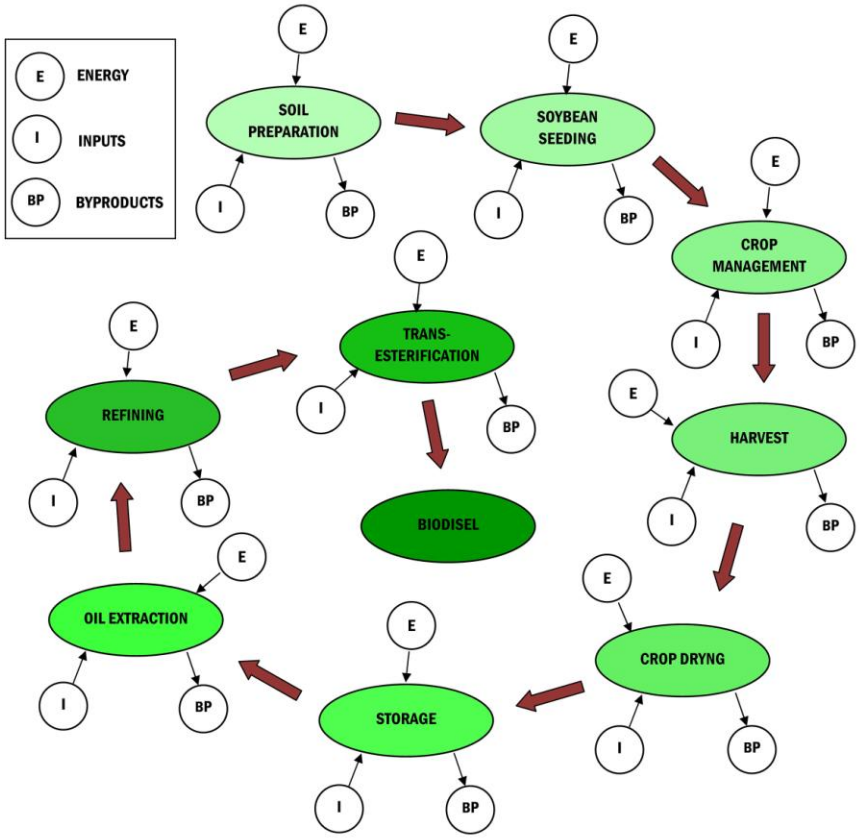

Figure 2: Flowchart of the soybean biodiesel Life Cycle Assessment.

TABLE 1: STAGES OF THE INTEGRATED CROP-LIVESTOCK.

	Stages	Equipment (energy)	Inputs	Byproducts
	Soil correction	Front loader, tractor and spreader	Lime	-
			Agricultural Gypsum	
Soybean	Herbicide application	Sprayer and water tank	Desiccator	-
	Fertilization	Seeder	KCl	-
	Seeding	Seeder, tractor, trailers and hauling wagons	MAP Soybean seeds	-
	Pesticide application	Sprayer and water tank	Herbicides	
			Insecticides	-
			Fungicides	
	Harvest	Combines, tractors, hauling wagons and trucks		Straw
Maize	Seeding for pasture and fertilization	Seeder, tractor, trailers and hauling wagons	NPK Maize and Brachiaria seeds	-
	Pesticide application	Sprayer and water tank	Herbicides	
			Insecticides	-
			Fungicides	
	Harvest	Combines, tractors, hauling wagons and trucks		Straw Maize
Livestock	Grazing cattle	Forage seeded together with maize, using the same machinery. Urea is spread four times a year over regular grazing areas, using a tractor, spreaders and wagons.	Brachiaria	Beef

CONCLUSION

From the specific data of each of the items of the stages of the integrated crop-livestock system (Table 1), an adapted approach for LCA, will allow the assessment of the impacts of each different subsystem. This will generate a comparative analysis of the SapéAgro integrated crop-livestock farm with the impacts of traditional systems for soybean production, to thereby determine whether there are actual environmental advantages of one in relation to another.

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Cover page

Title: *Ecotrophic Efficiency Comparison of Three Culture Modes of Grass Carp Based on Analyses of Ecopath with Ecosism*

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ABSTRACT

The grass carp is popularly and widely cultured in China as an important freshwater-fish species, however, traditional monoculture mode is limited due to its massive effluent discharge to the environment. Previous studies have shown that the polyculture of grass carp with silver carp and white-leg shrimp is an appropriate candidate, whereas more knowledge of ecotrophic efficiency of the polyculture mode is still poor. We analyzed and compared the ecotrophic efficiency characteristics of three grass carp culture modes using Ecopath with Ecosism model. The results showed that the ecotrophic efficiency of the culture system was significantly improved, which can provide the basic support for the ecological necessity of the polyculture.

INTRODUCTION

The grass carp *Ctenopharyngodon idellus* is one important species of the freshwater aquaculture animals in China and its yield per year occupies about 20% of the total freshwater aquaculture amount[1]. However, the impact of nutrient-rich effluent from fish farms using traditional monoculture mode on the environment has become a great concern with the rapid expansion and intensification of fish culture[2]. How to make fish culture sustainable together with improving the income of farmers is an urgent issue.

Our research group has focused on the fundamental study of polyculture modes of *C. idellus* with silver carp *Hypophythalmichthys molitrix*, white-leg shrimp *Litopenaeus vannamei* during the past five years under the support of National Basic Research Program of China (973 Program, No. 2009CB118706). These studies include the polyculture structure optimization[1, 3], composition of plankton and

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suspended particulate matter[4], energy budget and conversion efficiency[5], and bacterial abundance dynamics and community functional diversity[6]. In the present study, we used Ecopath with Ecosism model (EwE) to analyze and compare the ecotrophic efficiency characteristics of three grass carp culture modes based on the previous result that polyculture of *C. idellus*, *H. molitrix* and *L. vannamei* can reduce the effluent discharge and improve the income of farmers[3]. Furthermore, the bottleneck of the ecotrophic efficiency for the culture modes was analyzed and effective ways to improve the ecotrophic efficiency were proposed.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted in the fish ponds of Gaoqing County, Jinan City during April and September, 2012. Three of nine fish ponds were randomly set as one group and three total groups were set, which represented three culture modes, i.e., the monoculture of grass carp, polyculture of grass carp and silver carp, and polyculture of grass carp, silver carp and white-leg shrimp. The original densities of each species of animals were set according to our previous study[3]. The areas of the ponds were about 2000-3,300 m^2.

Definition and Determination of Experimental Parameters

Twelve to thirteen functional groups were set to meet the demand of EwE, i.e., three phytoplankton groups (small phytoplankton, microphytoplankton, picophytoplankton), two zooplankton groups (macrozooplankton, microzooplankton), two bacterial groups (water bacteria, sediment bacteria), two detritus groups (water detritus, sediment detritus), and one group of grass carp, silver carp, shrimp, feed and submerged plant. Details of the determination of these parameters were according to the methods of Zhang [7], whereas these of grass and silver carps were referred to the method of shrimp. Of the above parameters, phytoplankton, bacteria and detritus in the water and sediment, which play the crucial role in ecological systems were chosen and presented in the results below.

RESULTS

Variation of Phytoplankton Abundance

The abundance variation of the three sizes of phytoplankton during the sampling period are showed in the below three figures. The results showed that the densities of small phytoplankton in the polyculture mode of GS during the late culture period were significantly lower than the other two (Fig. 1). No different trends were found in the densities of microphtoplankton and picophytoplankton (data not shown). The densities of small phytoplankton and microphytoplankton in all the culture modes were $<10^7$ cells/ml during the culture period, whereas these of picophytoplankton were mostly $>10^7$ cells/ml. These indicated that the picophytoplankton (<10 μm) in the fish ponds was the most important primary producer.

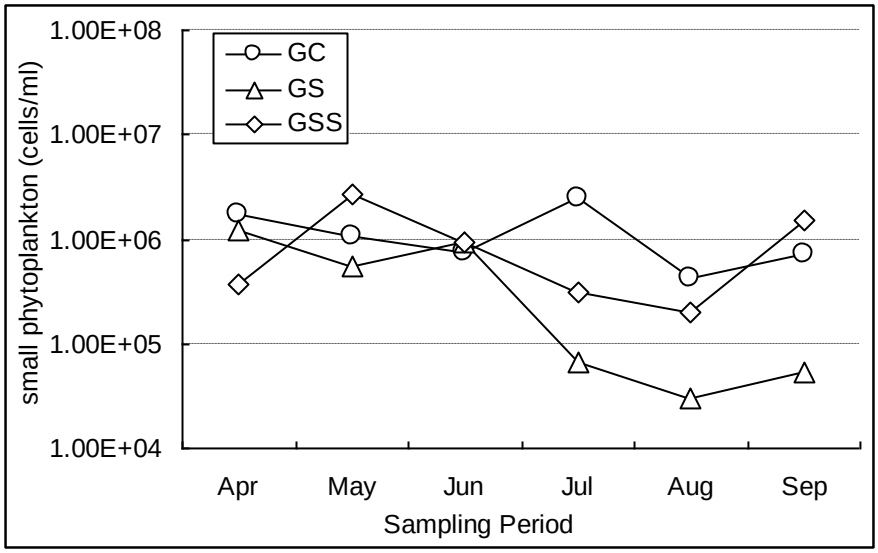

Fig. 1 The abundance variation of small phytoplankton during the sampling period. GC, GS and GSS indicated the three culture modes, i.e., grass carp, grass carp with silver carp, and grass carp with silver carp and shrimp.

Variation of Bacterial Abundance in the Water and Sediment

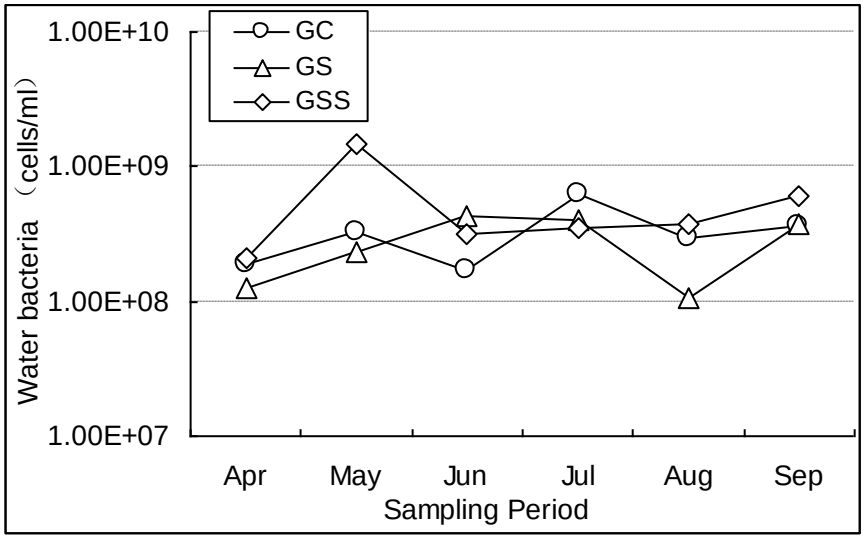

Fig. 2 Variation of bacterial abundance in the water during the sampling period. GC, GS and GSS indicated the three culture modes, i.e., grass carp, grass carp with silver carp, and grass carp with silver carp and shrimp.

The bacterial abundance in the water presented a relatively stable trend during the culture period and was in the range of 10^8 and 10^9 cells/ml on the whole (Fig. 2), whereas that in the sediment had an increasing trend and the cell densities were $>10^9$ (Fig. 3). Among the three culture modes, bacteria in the water did not show difference, however, these in GC in the sediment increased sharply and significantly higher than the cell number in GS and GSS. By the end of the culture, bacterial number in the

sediment of GS was between that of GS and GSS. The bacterial number differences in the sediments of the three culture modes might be due to the higher rate of feed sinking to the sediment in GC mode and the higher bioturbtion by shrimp in GSS mode.

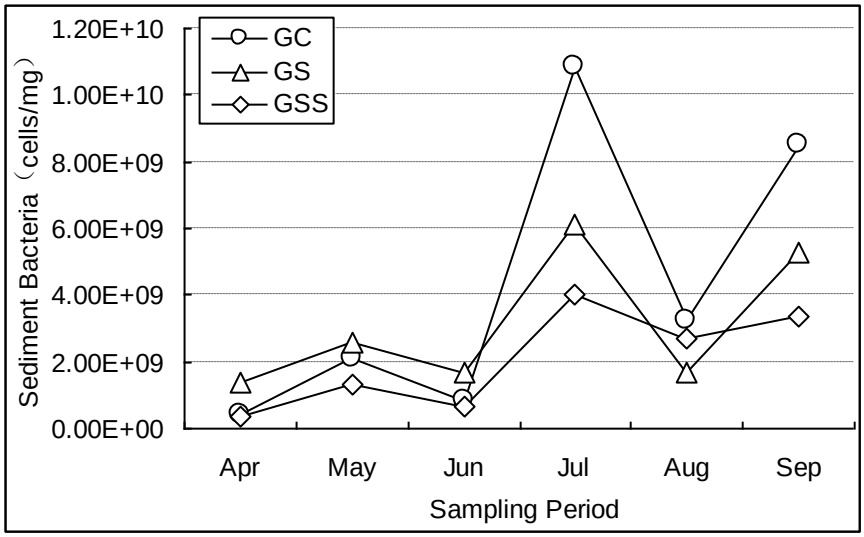

Fig. 3 Variation of bacterial abundance in the sediment during the sampling period. GC, GS and GSS indicated the three culture modes, i.e., grass carp, grass carp with silver carp, and grass carp with silver carp and shrimp.

Variation of Detritus in the Water and Sediment

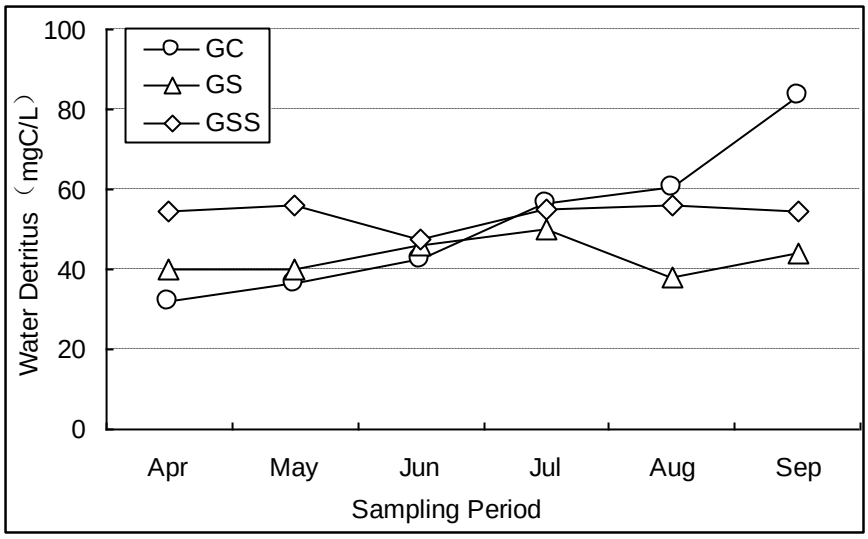

Fig. 4 Variation of detritus in the water during the sampling period. GC, GS and GSS indicated the three culture modes, i.e., grass carp, grass carp with silver carp, and grass carp with silver carp and shrimp.

The water detritus in GC increased slowly during the culture period and its content was significantly higher than those in GS and GSS (Fig. 4), which indicated that the filtering-feed of silver carp played an important ecological role. In the sediment, the detritus in GC was higher than those in GS and GSS on the whole and the detritus in GS and GSS kept the relatively stable condition (Fig. 5).

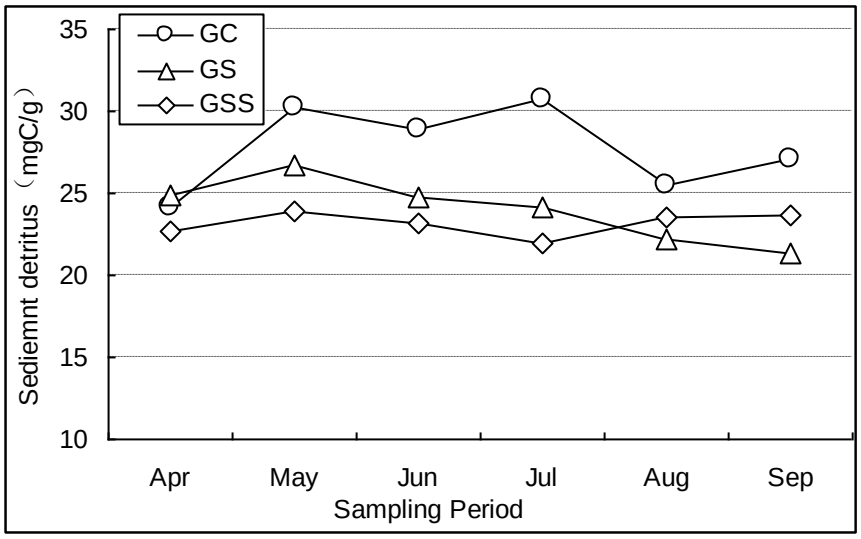

Fig. 5 Variation of detritus in the sediment during the sampling period. GC, GS and GSS indicated the culture modes, i.e., grass carp, grass carp with silver carp, and grass carp with silver carp and shrimp.

Comparison of ecotrophic efficiency of the three culture modes based on EwE

Tab 1. The comparison of ecotrophic efficiency of the three culture modes. GC, GS and GSS indicated grass carp, grass carp with silver carp, and grass carp with silver carp and shrimp.

	Functional Group	Ecotrophic efficiency		
		GC	GS	GSS
1	Small phytoplankton	0.52	0.75	0.79
2	Microphytoplankton	0.24	0.39	0.23
3	Picophytoplankton	0.19	0.38	0.29
4	Macrozooplankton	0.42	0.87	0.80
5	Microzooplankton	0.33	0.76	0.72
6	Water bacteria	0.49	0.66	0.64
7	Sediment bacteria	0.41	0.51	0.68
8	Grass carp	0.40	0.38	0.35
9	Silver carp		0.23	0.12
10	Shrimp			0.28
11	Feed	0.39	0.60	0.66
12	Water detritus	0.58	0.67	0.42
13	Sediment detritus	0.23	0.23	0.24

In the GC mode, the ecotrophic efficiency of only two groups, i.e., small phytoplankton and water detritus was >0.5, which meant that the material flow of these functional groups in the food web was good (Tab 1). In contrast, the functional groups of ecotrophic efficiency >0.5 in the polyculture modes of GS and GSS were nearly half of the total group number. These indicated that the polyculture of silver carp and shrimp significantly improved the material flow of the food web. The higher efficiency of feed in GSS (0.66) also showed that polyculure with shrimp was the most economical mode.

CONCLUSIONS

The intensive culture of the grass carp *C. idellus* is very popular in China, but the adverse impact of polluted effluents on the environment has drawn more and more attention. The previous studies have shown that the polyculure are environmentally friendly culture modes[1-6], whereas these need the basic support by the elucidation of the ecotrophic efficiency. In this study, we analyzed the ecotrophic efficiency of the three carp culture modes using the EwE modeling approach (http://www.ecopath.org), which is being widely used as a tool for analysis of exploited aquatic ecosystems, having reached 2400 registered users in 120 countries[8]. Our results indicate that silver carp and white-leg shrimp are appropriate polyculture species for grass carp, which can significantly improve the ecotrophic efficiency of the food web including artificial feed. Also, these can interpret the ecological necessity of the polyculure modes. This study was supported by the National Basic Research Program of China (973 Program, No. 2009CB118706).

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Cover page

Title: *Gasification of Torrefied Biomass in a Bubbling Fluidized Bed Gasifier*

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ABSTRACT

Torrefaction is a method to upgrade biomass properties for thermal application or transportation. It can be used in terms of increasing the energy density, grindability and decreasing moisture in biomass. In this study, *Crymtomeria japonica* used as feedstock was torrefied at 250 and 350 ℃ for 1 hour, with inert gas for gasification. The torrefied biomass enhances the gasification process due to a lower O/C and H/C ratio. Torrefied *Cryptomeria japonica* was gasified in a 30 kWth bubbling fluidized bed gasification system with a diameter of 7.6 cm in the bed region, 19.8 cm in the freeboard region and a total height of 1.9 m. The effect of equivalence ratio (ER) and torrefaction temperature on syngas composition and tar content have been investigated. Results indicated the H_2, CO, CH_4 and tar content decrease with increasing the equivalence ratio (ER) and torrefaction temperature, but CO_2 showed the opposed results**.**

1. INTRODUCTION

Gasification is a thermochemical conversion of carbonaceous feedstock such as waste, coal or biomass by partial oxidation at risen temperature into syngas (H_2, CO, CO_2, CH_4, etc.) for thermal application or power generation [1]. The gasification agents include oxygen, air, steam or mixing of these [2]. Air gasification produces syngas with lower heating value of 4-7 MJ/Nm^3, as oxygen and steam gasification may produce syngas with medium to high heating values of 10-18 MJ/Nm^3 [2]. Feedstock (biomass) properties may cause some problems in the gasification process. Low energy density, high moisture content, and poor grindability of biomass are the normal problems that have been faced. These problems may deteriorate the gasification efficiency in entrained flow gasificaion or co-firing with pulverized coal [3]. Many ways of pre-treatment have been developed to upgrade the biomass. Torrefaction is one of the effective ways which is conducted in the temperature range of about 200-350 ℃ near the atmospheric pressure with absence of oxygen (air) at a slow heating rate (< 50 ℃ /min) [4]. The advantages of torrefaction include increasing energy density [5,6], improving grindability, reducing the cost of transport and storage, generating dehydrated feedstock, and producing uniform properties [7-9]. The temperature of gasification of torrefied wood was higher than that of raw wood due to the low O/C, H/C ratio and low moisture content in the torrefied wood [3]. Chen et al

found that the cold gas efficiency of torrefied bamboo may reach 77% due to the higher concentrations of H_2 and CO in syngas [9]. Deng et al. reported that torrefaction of rice straw and rape stalk may increase the syngas production rate by 17% and 15%, respectively [10]. Couhert et al. found the syngas production was enhanced with the torrefaction temperature and the char from torrefied wood was less than raw wood in an entrained-flow gasifier [11].

In this work, the gasification of torrefied *Crymtomeria japonica* and raw *Crymtomeria japonica* were carried out to investigate the effects of equivalence ratio (ER) and torrefaction temperature on syngas composition and tar content.

2. EXPERIMENTAL

Crymtomeria japonica was used as feedstock and was pulverized and selected by sieving with screen to become uniform chips of about the size of 15-20 mm. Chips of *Crymtomeria japonica* were then torrefied in a 10 kW electric reactors for 1 hour at temperature of 250℃ or 350℃ before gasification. Compositions of torrefied *Crymtomeria japonica* were analyzed with Elementar vario EL for ultimate analysis and Perkinelmer, Simultaneous Thermal Analyzer, STA6000 for proximate analysis. Results of the analyses are shown in TABLE 1 on a dry ash free basis. The ratio of H/C and O/C of the torrefied biomass and raw biomass were shown in Figure 1. The torrefied biomass was reduced to the lower O/C and H/C ratio due to the removal of volatile matter and water.

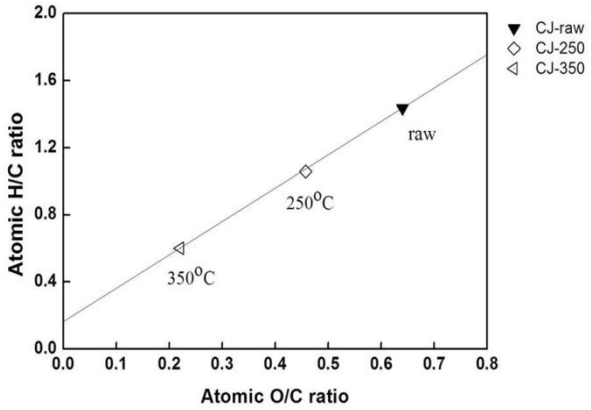
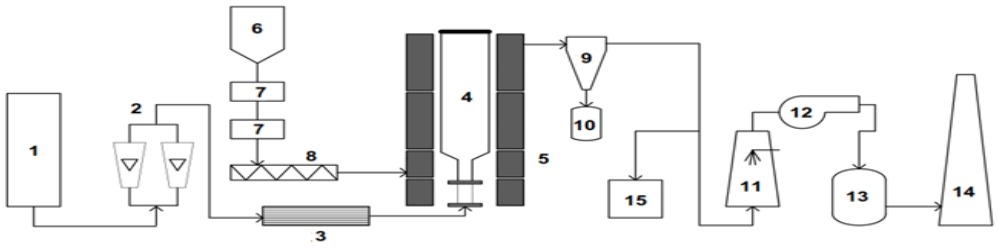


Figure 1. The H/C and O/C ratio of the raw and torrefied *Crymtomeria japonica*

The gasification experiment was performed in a 30 kWth bubbling fluidized bed gasification system as shown in Figure.2. The system consists of the gasification chamber with a windbox, the feeding unit, the air supply unit and the syngas cleaning unit, etc. The gasifier has the internal diameter of 7.6 cm in the bed region, 19.8 cm in the freeboard zone and a total height of 1.9 m with insulating of 6 mm of SUS310 stainless steel with ceramic fiber to shield the heat loss. The air supplied line was made by stainless steel of 6 mm, connected to a cylindrical windbox with a conical bottom, 30 cm in height and 7.6 cm in diameter. The air was preheated by electric heater worked as fluidization gas cross the air distributor of 2 mm stainless steel with an open area ratio 2%.



1. Air compressor 2. Rotameter 3. Pre-heater 4. Fluidized bed 5. Electric heater
6. Silo 7. Pneumatic knife gate valve 8. Screw feeder 9. Cyclone 10. Ash tank
11. Wet scrubber 12. ID Fan 13. Combustor 14. Stack 15. Gas and tar sampling

Figure 2. Schematic diagram of the gasification system

TABLE 1. ULTIMATE AND PROXIMATE ANALYSIS OF FUEL

	Proximate analysis (wt%, ar)				Ultimate analysis (wt%, daf)		
	CJ-raw	CJ-250	CJ-350		CJ-raw	CJ-250	CJ-350
Volatile matter	9.33	3.61	0.53	C	50.38	58.43	73.48
Fixed carbon	90.24	95.79	98.43	H	6.03	5.15	3.67
Ash	0.43	0.6	1.04	O	43.05	35.63	21.62
HHV (MJ/kg)	18.55	22.7	25.35	N	0.09	0.09	0.11
Empirical formala	$CH_{1.43}O_{0.64}$	$CH_{1.06}O_{0.46}$	$CH_{0.6}O_{0.22}$	S	0.02	0.33	0.07

[a] CJ-raw = raw *Crymtomeria japonica*, CJ-250 = torrefied *Crymtomeria japonica* at 250 ℃ and CJ-350 = torrefied *Crymtomeria japonica* at 350 ℃)
[b] Higher heating value

The gasifier was performed at steady state of operating temperature by electric heating unit around the bed region. The feedstock was input to the hopper above the bed region. The feeding system was composed of the hopper with two locks and screw feeder. Silica Sand was used as the bed material with mean size diameter of 0.437 mm. The syngas departed from the gasifier to a cyclone to remove ash and unburned char for the primary cleaning. Then the syngas was sampled in the tar sampling unit set up next to the cyclone. After this, the syngas came across a wet scrubber to burn in a combustor before sending to the stack.

3. RESULTS AND DISCUSSION

3.1 Gas Composition

The equivalence ratio (ER) is defined as the actual air-fuel ratio to stoichiometric air-fuel ratio. The effect of ER on syngas composition of raw and torrefied *Crymtomeria japonica* are presented as volumetric fractions (v/v %) in Figure 3. It is noted that H_2 and CO decreased as ER increased, while CO_2 increased. As ER increasing, more air is supplied to the gasifier that will enhance the burning of char to generate CO_2 with the compensation of product gas such as H_2, CO and CH_4. The reason of its is the lower in H/C and O/C ratio in the torrefied biomass, resulting the producing more syngas content (H_2 and CO).

3.2 Tar Content

A strong effect of the ER on tar content was observed in Figure 4. The tar content clearly decreases with increasing of ER. Increase in the ER enhances oxidation reaction, reduces the tar formation [12], and reduces the quality of syngas. The torrefied biomass produce the lower tar content compared with the raw biomass because of the lower in O/C and H/C ratio in the torrefied biomass consuming more oxidation reaction.

3.3 Gas Content

The syngas content is plotted in Figure 5. as a function of ER. The syngas content of the torrefied biomass generated more H_2 and CO compared with the raw, but CO_2 show the opposite trend. It means the lower O content of the torrefied biomass was less than the raw biomass.

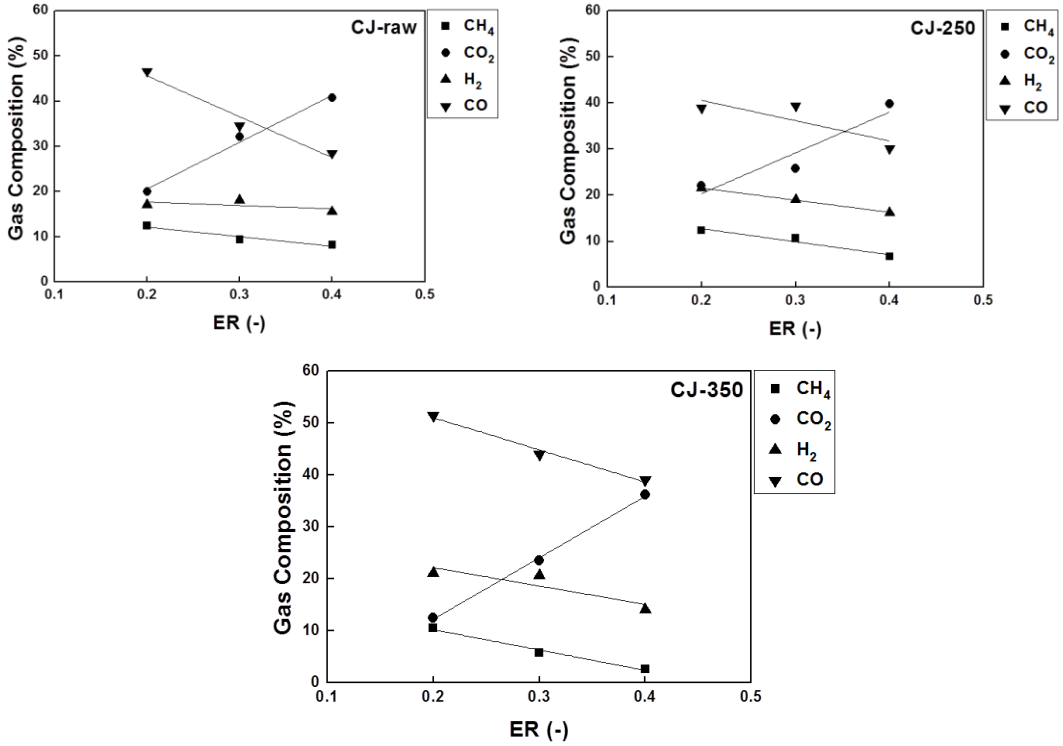


Figure 3. The effect of the ER on syngas composition (Gasification temperature =800 ℃)

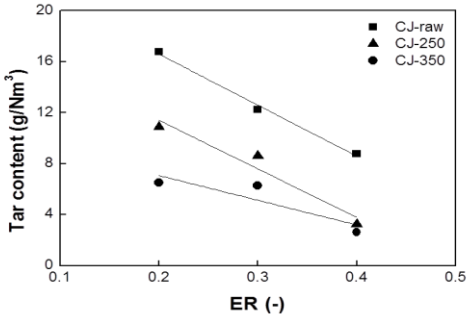


Figure 4. The effect of the ER on Tar content (Gasification temperature =800 ℃)

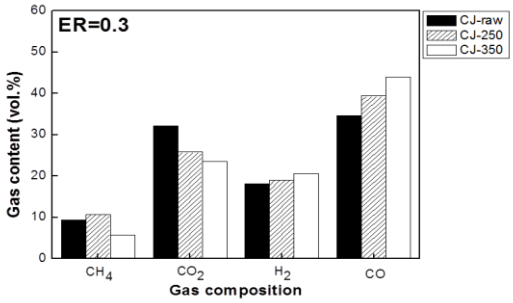


Figure 5.The effect of torrefaction temperature on gas content (Gasification temperature =800 ℃)

4. CONCLUSIONS

The gasification of torrefied *Crymtomeria japonica* and raw *Crymtomeria japonica* were performed in a 30 kWth bubbling fluidized bed gasification to observe the effect of the equivalence ratio and torrefaction temperature on the syngas. The H_2, CO_2 and tar content in syngas decrease with increasing the equivalence ratio and torrefaction temperature, but CO_2 present the opposing tendency. Due to the lower in O/C, H/C ratio and volatile matter of the torrefied *Crymtomeria japonica*. In addition, the torrefaction of *Crymtomeria japonica* improves the syngas quality in terms of increasing syngas content and reducing tar content.

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Cover page

Title: *The Importance and Involvement of Landscape Architecture in the Green Building Index (GBI) Practice*

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ABSTRACT

This paper fundamentally discusses on the importance of the Landscape Architecture scope of works and professionalism in the application of the Green Building Index (GBI). The aim of the paper is to investigate how the particular scope of works as well as the involvement of the Landscape Architecture professionals in the organization is being considered throughout the GBI practice. According to past researches, there are several professions that are mostly active in the green building and the GBI projects. The architects become the first place for the most involved and active profession during the design process of the green buildings then followed by the mechanical, electrical, structural and civil engineers; the interior designers and the quantity surveyors. Based on that research evidence, inactive involvement of the Landscape Architecture professionals and the GBI practice is seen as an issue whereby in consequence, the landscape architecture professionals are not put in place together with other allied professions in the green building team. The study basically used questionnaire survey and semi-structured interview, apart from secondary data collection obtained from the literature review and document analysis. From the evaluation of the survey and interview, the study found that the Landscape Architecture is a very important field in the green building and the GBI development. The finding indicates that the landscape design should be included from the very beginning of conceptual design stage in order to determine the right, sufficient and proper placement of the green areas. It is also supported that the role of the Landscape Architects are important to be imposed in the GBI practice. They also should be included in the green building design team to integrate the comprehensive knowledge of landscape design with other professionals in order to lead towards multidisciplinary approach.

INTRODUCTION

The green technology has become the most popular alternative that have been used by the developed countries such as United Kingdom, United States of America,

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Japan and many more. In regard to this technology, the idea of green building is introduced as a way of transforming the building market and transfiguring the common way of thinking about design, inhabit and operate buildings which is at the same time reducing the impact on environmental quality. A green building focuses on improving the efficiency of resource use – energy, water, and materials – while reducing building impact on human health and the environment during the building's lifecycle, through better sitting, design, construction, operation, maintenance, and removal. The GBI is aimed to develop high performance buildings without causing any harm to the living things and environment. Therefore, the global demand on the green building is increasing from time to time due to its advantage in producing lower development risks [1], [2].

In recent years, Malaysia has been introduced to green building rating system originating from the United Kingdom, the United States, Australia, Japan and Singapore. However, a green building rating system by its nature is very dependent on the local environment, including climate, resources, current state of development and not to be forgotten, culture [3]. Green building rating system is a very demanding system that provides variety of privileges and bonuses to the development and construction sector. It is essential because it is a great tool to exhibit environmental stewardship. It also enables the public to learn deeply about green buildings [4]. Green rating system also [5] by its nature and role is very dependent upon location and environment and thus climate. Hence, taking the advantages of having green building rating system, Malaysia has made a step further by adopting the Green Building Index (GBI) as the first local green rating building system. The GBI is a profession driven initiative to lead Malaysia towards a more responsible built-environment, for now and for the future [3]. As aforementioned, the GBI is a green rating system for buildings developed by Pertubuhan Arkitek Malaysia (PAM) and Association of Certified Engineers (ACEM). It was introduced in 2009 by the Honoured Senator Penny Wong, a Malaysian who is now Australia's Water and Climate Change Minister [6].

METHODOLOGY

Based on the determined research approach as well as the formulated research objectives, several methods were used to collect primary and secondary data for this research. For primary data collection, the methods used were questionnaire survey and semi-structured interview. The questionnaires were distributed and handed to the respondents face to face. The respondents were selected from the professional group who are working in Built Environment field such as architects, landscape architects, engineers, urban planners, academicians, quantity surveyors and others. These people were selected as the target respondents as they may probably acquire any knowledge and information related to the application of the Green Building Index (GBI) in Landscape Architecture scope of works. On the other hand, the interview sessions have been conducted with several selected key persons from Built Environment field who involved primarily in the GBI application as well as Landscape Architecture field. For secondary data collection, the methods used were literature review and document analysis. The questionnaire survey data was analyzed by using the Statistical Package for the Social Science (SPSS) version

17.0 software. The interview data was analyzed by using qualitative software, QSR NVivo Version 8. NVivo is a qualitative data analysis (QDA) software package that has been designed to simplify the process in managing, transcribing and analysing qualitative data

ANALYSIS AND FINDINGS

Level of Importance and Involvement in the GBI Practice

Table 1 below presents the results of the professionals' knowledge and expertise in the GBI practice.

TABLE 1: LEVEL OF PROFESSIONALS' KNOWLEDGE AND EXPERTISE IN THE GBI

Which professionals have the best knowledge, expertise and involvement in the GBI application and practice?	**M**	**SD**
Architects	4.31	0.873
Landscape architects	3.65	0.968
Engineers	4.06	1.023
Urban planners	3.44	1.018
Quantity surveyors	3.30	1.040
Total	3.75	0.984

Note. = M- Mean, SD- Standard Deviation

Based on the above table, it can be said that most of the respondents agree that the architects and the engineers have the best knowledge, expertise and involvement in the GBI practice. This is indicated by the high mean score = 4.31 for the architects and 4.06 for the engineers. Then, it is followed by the landscape architects with mean score = 3.65, the urban planners = 3.44 and the surveyors = 3.30. According to the results, approximately each of the professionals do have knowledge, expertise and involvement in the GBI but only the architects and engineers performed better in this particular item. Based on the total mean score = 3.75 and standard deviation = 0.984, it indicates that the professionals knowledge, expertise and involvement in the GBI practice can be rated as at medium level. Therefore, the GBI organisation should give an attention towards this matter in order to create balance knowledge widespread among the Built Environment professionals regarding the GBI application and practice. Not just that, the professional association like ILAM should also play its roles to be more active in dealing with the green initiative bodies, bond an attachment with them and educate its members about the importance of knowing and experience the green building projects.

Moreover, Table 2 presents the level of importance of the landscape architects' involvement in the GBI practice.

TABLE 2: LEVEL OF IMPORTANCE OF LANDSCAPE ARCHITECTS' INVOLVEMENT IN THE GBI

Do you think that the landscape architects are important to be involved in the GBI practice?	Frequency (F)	Percentage (%)
Yes	93	93.0
No	7	7.0
Total	100	100.0

Based on the above table, it can be seen that 93.0% of the respondents agree that the landscape architects are important to be participated in the GBI practice meanwhile only 7% are against it. This is most probably because the respondents think that landscape architecture may contribute to the development and execution of the green building works not only for outdoor aspect but also indoor aspect. This could be a valid reason on why they supported that the landscape architects to play a part in this new area of interest in the Built Environment field.

Besides the questionnaire survey, the information was also gathered through the interview session. The interviewees were asked about the current involvement of the Landscape Architecture professionals in the GBI. Most of them agree that the current involvement of the landscape architects is less in the green building and the GBI. According to an interviewee, at the moment, there was none of the GBI facilitators are coming from the Landscape Architecture background. They supported that the landscape architects should involve from the very beginning of the design stage; not only come in when the building has been completed. An interviewee mentioned that there is a new proposed act as abovementioned. This act may help in enforcing the landscape architects to come together at the preliminary stage of design and development. However, quite a few of the interviewees have listed down the problems of the landscape architects regarding this subject matter. Most of them supported that the landscape architects have not very contributing in the green building development and the application of the GBI because they did not understand the whole concept of the green building and also the GBI application.

Furthermore, the landscape architects do not have the ability to convince people especially the clients and they are not articulate to argue because they do not have sufficient knowledge, data and facts. Besides, another interviewee mentioned that the landscape architects did not have the effort to promote themselves in the green building field. The landscape architects in Malaysia are not innovative in selling their design ideas and they just want to make quick profit by selling the mediocre design. Furthermore, the landscape architects have not thought beyond their scope of works. However, another interviewee believed that the less involvement of the landscape architects in the green building and the GBI practice may occur due to several reasons. It is maybe due to the fact that the landscape architects themselves are not insist to involve; or maybe the situation hinders them to do so; or it may be occur due to the fact that the other professions are really confine into the idea that landscape design is only about plants. The landscape architects are rarely being called in the earlier stage of the development even during the initial phase of the GBI itself. Thus, the landscape architects might face some problems because at that particular stage, the landscape architects were already being left out.

DISCUSSION

In the aspect of the roles and future contribution of the landscape architecture professionals in the green building as well as the GBI application and practice, there were some suggestions that have been roped in. The building developers should be aware that they should hope in the landscape architects from the very beginning of the projects as they may help the architects in providing the strategies to incorporate the landscape design into the green buildings. This is because the Built Environment industry is leading towards multidisciplinary approach. Since there is quite less landscape architects in the green building development, the landscape architects' role should be taken into the project from the very first phase. Furthermore, the landscape architects themselves should see the green building as their responsibility; not a competition. They also must have a strong ability to convince the other professions that the green building is the area that maybe they can contribute. Regardless how big or small the contribution is, the landscape architects still have a role to contribute in the overall green building projects. The most important thing is they need to think and go beyond their comfort zone and try to bring a fresh and extraordinary idea to be incorporated in their design. They also may contribute a lot by having an attachment to the GBI organisation or becoming the GBI facilitators.

There were also some suggestions that the roles of the Landscape Architecture association or bodies such as Institute of Landscape Architects Malaysia (ILAM) are very important in encouraging the involvement of the landscape architects in the green building and the GBI practice. By having a liaison with PAM and ACEM, these bodies may encourage their members to attend the trainings, courses, workshops and info sessions that are related to the green building and the GBI itself. Another thing that can be done is through the regulation. If the government decided to make green building as the mandatory requirement, they need to suggest that the landscape architects should go into this green building area. This is very much related to the proposal of the new Landscape Architecture policies which are Landscape Policy, Landscape Development Act and also Landscape Architects Act. The Landscape Development Act is a guideline where certain areas should have the mandatory landscape architecture inputs. Thus, it is good in order to enable the landscape architects to play their roles. It spells out about the Landscape Architecture scope of works which indirectly securing their job for a long term span.

CONCLUSION

In conclusion, the Green Building Index (GBI) is conceived to be able to aid architects, designers, builders, government bodies, building owners, developers and end users to understand the impact of design towards the environment. It also helps to provide choice and solution in producing a better design with full consideration to the environment in the future. In relation to this research, the importance of landscape architecture in the GBI practice and application is very imperative to be looked upon. More than that, the roles of the Landscape Architecture professionals are also very essential to be explored in the green building and the GBI practice. Since it is proven that the involvement of the landscape architects is quite less,

proper strategies should be taken in order to promote and encourage the participation of this professional group. The strategies might also help to upgrade the image and the performance of the landscape architecture field in the eyes of the other professions as well as to secure the future of the landscape architecture professionals in the Built Environment field. They also may help to broaden the horizon of the landscape architecture as a profession as well as a niche area. Overall, it is hoped that this research has explored and opened up a new path and niche for the landscape architecture professionals to be as outstanding as other professions have performed.

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Cover page

Title: *Sludge Compost Applied to Agricultural Soil: Effects on Growth of Wheat and Environmental Risk of Heavy Metals*

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ABSTRACT

The effects of municipal sludge compost on the growth of wheat and the accumulation of heavy metals in soil were studied. Sludge compost application increased soil TN, TP, OM, available K concentrations. Clearly, the advanced growth of wheat can be achieved once the crop nutrient is enough in soil. On the other hand, there was also increase in the maximum content of heavy metals. It is very important to pay attention to the element of cadmium due to its content can increase significantly and migrate downwards. By using the methods of geo-accumulation index to evaluate the pollution of topsoil, it is clear that the others can not contaminate the soil during the test period except the element of cadmium.

1. INTRODUCTION

Sludge compost has been used as fertilizer to agricultural soil for many years in America and European Union [1]. There are some advantages for the land utilization of municipal sludge compost as follows: to improve the basic properties of soil, reduce volume weight of soil, promote crop growth and increase the quality of product[2], improve moisture holding capacity and aggregate stability of soil[3], moderate the soil harden and leanness conditions caused by the utilization of excessive fertilizer. However, it can also increase the risk of land utilization because there are salts, heavy metals and POPs in soil. The foreign researches showed that the application of municipal sludge compost on land can increase the content of heavy metals to a certain extent in soil and crops tissues [4-7]. The most of research results showed that the heavy metals can accumulated in the topsoil easily, but it is difficult to migrate down. Generally, the data of field trials are less than the ones of pot experiments for the land application of sludge compost. Due to the difference of sludge in China and foreign countries, it is not essentially useful to refer to these data reported from the foreign literature, and it is thus very necessary to carry out more experiments in field.

The purpose of this work is to investigate some issues about the application of sludge compost in Zhengzhou city, Henan province of China: the effects of sludge compost on the growth of wheat seedlings and environmental risk of heavy metals on soil; the accumulation and migration of heavy metals in different depth of soil.

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2. MATERIALS AND METHOD

2.1 Test Sites and Test Sludge Compost

The test field (34 °N, 114.4E, at an altitude of 108 meters) in Zhongmou country, of Henan province has a temperate and continental monsoon climate with average annual sunshine time of 3000 hours, rainfall of 600mm, frost-free period of 213 days and temperature of 14.5℃, this field soil has a bulk density of 1.32~1.53g cm^{-3} and a 25.2% (volume fraction) water-holding capacity in the topsoil. Municipal sludge compost is supplied by Bagang Sludge Disposal Plant.

2.2 Test Crops

The test crop is weak-gluten winter wheat, Zhongmai No.2, supplied by Henan Tianning seed industry, LTD.

2.3 Experimental Design

There were 21 experimental plots of 150cm×400cm were prepared for 7 experimental treatments in the test, the experimental treatments were CK(no urea, no sludge compost, group 1), 0.5N(group 2), 1N(group 3) 0.5N+0.5S(group 4), 0.5N+1S(group 5), 0.5N+2S(group 6), 0.5N+3S(group 7), respectively. The pure N content in urea is 112.5kg ha^{-1}(1N), as well as the pure N content in sludge compost (1S), all experimental treatments add equal amounts of P, K fertilizer as base fertilizer.

2.4 Analysis and Data Processing

These experiments were carried out in October 2012. Different depth of soil (0~20cm, 20~40cm, 40~60 cm, 60~80cm, 80~100cm) was collected prior to the experiment. Different depth of soil samples were also collected after 45 days. The samples were dried and ground with an Agate mortar and sifted out by the sample sieves with pore size of 0.149 mm and 1 mm. The final samples were kept in labeled polypropylene container at ambient temperature before analysis. The physicochemical parameters for the sludge compost and soil samples(pH, OM, TP, TN, available K) were measured by standard methods[8]. The digest method based on EPA3050B. A microwave digestion system (MARS, CEM Corporation) was used for digestion samples (soil, sludge compost). The determination of elements in digests of all understudy samples were carried out by means of ICP-MS ((ELAN 9000, PerkinElmer Corporation). National standard substance (GBW07448) was used to control the quality in testing and analyzing.

3. RESULTS AND DISCUSSION

With land application of municipal sludge compost, increased significantly the concentrations of TN, TP, available K and organic matter in topsoil, there was a significant difference in biomass of wheat. Contents of heavy metals in the topsoil used with sludge compost were higher than in CK, but still in safe. It wasn't an obvious tendency for migrate down.

3.1 Effects of Sludge Compost Application on Basic Property of Soil

The results showed that increase in the concentration of, 21.03%~88.02% (TN), 10.78%~90.06% (TP), and 6.99% ~ 145.53% (available K) and 4.27% ~ 43.56% (OM, organic matter) were obtained in the topsoil after applying the municipal sludge compost compared to the control experiments. It may be used as manure because it could significantly improve the soil fertility [9; 10]. The content of TN, TP, available K and OM in topsoil reached the maximum, increasing by 0.58 g kg^{-1}, 0.42 g kg^{-1}, 134.5 mg kg^{-1} and 5.93 g kg^{-1} than controls, respectively when the experimental treatments was 0.5N+3S (group 7), as shown in Figure 1-2.

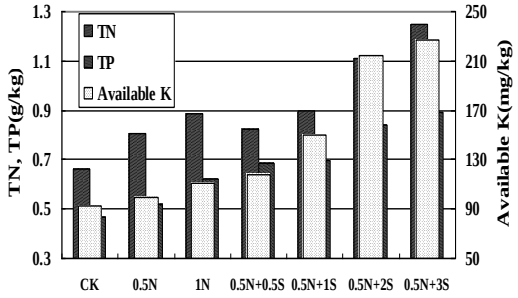

Fig.1 Effect of municipal sludge application on TN, TP, Available K value in the topsoil

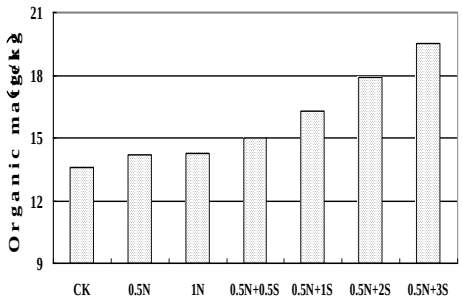

Fig.2 Effect of municipal sludge application on organic matter value in the topsoil

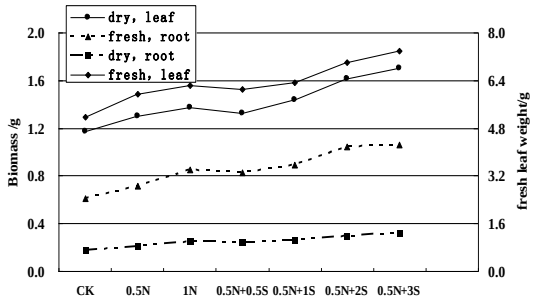

Fig.3 Effects of different treatments on biomass of wheat

3.2 Effects of Sludge Compost Application on Biomass of Wheat

Biomass is an important indicator for evaluating plant growing. The content of nutrients increased significantly after sludge compost application, so the nutrients affected the biomass of crops. Upperground and underground parts of a certain amount of wheat after sowing for 45 day were separated and dry weight and fresh weight were weighed, respectively. Clearly, the advanced growth of wheat can be achieved once the crop nutrient is enough in soil, agreeing with the result that the maximum biomass of wheat was obtained when the experimental treatments was 0.5N+3S, and there was increase in the weight of 42.8% (fresh, leaves), 45.1% (dry, leaves), 74.2% (fresh, roots) and 82.1% (dry, roots) compared to the control samples. The group 4(0.5N+0.5S) was lower than the group 3(1N), one possible reason was the sludge compost has slow-release effect[11]. Application of 0.5N+3S (group 7) could significantly enhance the biomass of wheat, which showed better effect than conventional chemical fertilizer treatments, as shown in Figure 3.

3.3 Effects of Compost Application on Heavy Metal Contamination

3.3.1 EFFECTS OF DIFFERENT TREATMENTS ON CONTENT OF HEAVY METALS IN SOIL

As shown Fig.4, the content of Zn, Pb and Cr in soil showed a decrease, increase and decrease again tendency. There are one peak and two valleys under 20cm. A maximum peak appearing at 60~80 cm and two minimum value appearing at 40~60cm, 80~100cm. Increase in the concentration of 15.7%~33.8% (Zn), 8.2%~28.9% (Pb) was obtained in the topsoil after applying the municipal sludge compost compared to the control experiments. However, increase in the concentration of 4.2%~19.1% for Cr was obtained in topsoil, it was not significant [12]. The results showed that the accumulation of Zn, Cr and Pb was mainly in topsoil, it was consistent with other studies[13; 14]. Zn content fluctuated present remarkably at 20cmto 100cm depth in soil different from Pb and Cr, it relevant to various factors of soil such as pH value, organic matter, texture and so on[15]. Besides, planting different crops and different carriers of Zn for adsorption and maintain may lead to discrepancy in case of different testing. Leaching phenomenon had not a great influence on deep soil for Cr, Pb and Zn. The content of Pb and Cr in different depths look quite different, the possible reasons were its own nature such as pH、OM、texture and biological activity had a combined action[16].

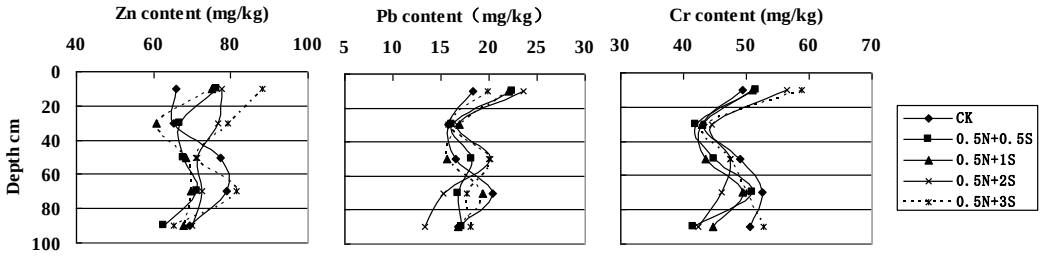

Fig.4 The variation trend of Zn, Pb and Cr content

As shown Fig.5, the results showed that increase in the concentration of 29.0%~42.4% (Cu), 3.9%~39.6% (Ni) and 7.3%~32.0% (Cd) were obtained in the topsoil after applying the municipal sludge compost compared to the control experiments. Cu, Cd and Ni content increased obviously in topsoil, this was consistent with other studies. [17; 18]. The content of Cu at 20~40 cm showed a sharply convergent tendency. The possible reason was the content of Cu and Ni increased rapidly in topsoil and the horizontal migration ability of Cu element was weak, so applying sludge compost had a lesser influence under topsoil. Ni content had an sharply decreasing underground. One possible reason was the migration ability of Ni was particularly weak. Cd content showed a certain related relation with the ratio of sludge compost in 20~40 and 40~60 depth soil different from the others. It is very important to pay attention to the element of cadmium due to its content can increase significantly and migrate downwards, yet the other elements of heavy metal can accumulate in the topsoil after the addition of sludge compost in soil.

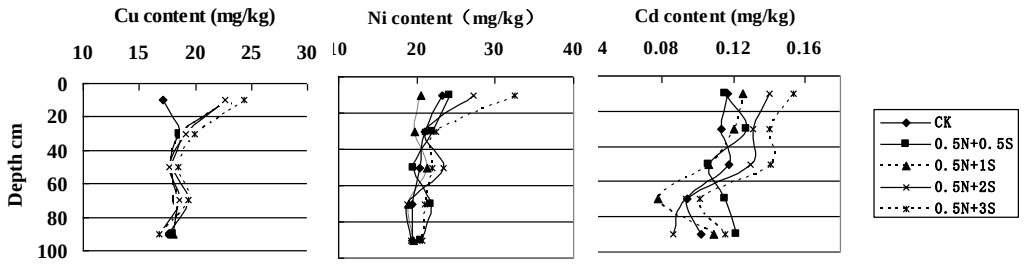

Fig.5 The variation trend of Cu, Ni and Cd content

3.3.2 EVALUATE THE SOIL HEAVY METALS POLLUTION LEVEL

The determination of metals in sludge compost is an important requirement for sludge application to farmland, because there is a risk of toxic elements accumulating in the soil [19]. The transfers of metals from sludge compost to soil and subsequently to plants pose potential health risks because they can enter the food chain and the environment[20]. The heavy metals content in topsoil had been evaluated by index of geoaccumulation. The index of geoaccumulation can be used to measure heavy metals pollution levels of sediments was proposed by Muller[21]. The formula of index of geoaccumulation: $I_{geo}=\log_2 (C_n/1.5B_n)$, where, C_n was measured mass fraction of particular element, mg/kg; B_n was background mass fraction of particular element, mg/kg.

TAB. 1 THE GEOACCUMULATION INDEXES OF HEAVY METAL OF TOPSOIL

	Pb	Cr	Ni	Cu	Zn	Cd
0.5N+0.5S	-0.35206	-0.86358	-0.69279	-0.33024	-0.19863	0.086964
0.5N+1S	-0.37506	-0.87781	-0.93671	-0.38388	-0.22224	0.198978
0.5N+2S	-0.2767	-0.73139	-0.52476	-0.34664	-0.1727	0.361609
0.5N+3S	-0.52915	-0.67111	-0.2671	-0.24057	0.011317	0.497164

As shown Tab.1, the pollution levels of Pb, Cr, Ni, Cu and Zn elements in topsoil (0~20 cm) was o level except the experiment treatment 0.5N+3S for Zn. But the content value of Zu was close to the standard value, needed to pay more attention. The pollution of levels of Cd element in topsoil was I level, belong to the range of mild-medium pollution. The results showed clearly that it doesn't cause pollution for Pb, Cr, Ni, and Cu and Zn elements. However, the content, for Cd, was belong to mild-medium pollution. When the dosage of sludge compost at minimum(0.5S+0.5N), it also caused mild-medium pollution Cd. It may be related the low background content of Cu in Henan. Compared to the Standard of State Environment Standard (GB15618-1995), the content of six elements were all in accordance with the primary level and in full compliance with the secondary standard for agricultural land.

4. CONCLUSIONS

Increase in the concentration of, 21.03%~88.02% (TN), 10.78%~90.06% (TP), and 6.99% ~ 145.53% (available K) and 4.27% ~ 43.56% (OM) were obtained in the

topsoil after applying the sludge compost compared to the control experiments. Clearly, the advanced growth of wheat can be achieved once the crop nutrient is enough in soil, agreeing with the result that the maximum biomass of wheat was obtained when the experimental treatments was 0.5N+3S (group 7), and there was increase in the weight of 42.8% (fresh, leaves), 45.1% (dry, leaves), 74.2% (fresh, roots) and 82.1% (dry, roots) compared to the control samples. On the other hand, there was also increase in the maximum content of 33.8% (Zn), 42.4% (Cu), 32.0% (Cd), 30.0% (Pb), 40.0% (Ni) and 19.1% (Cr) in the topsoil after using the sludge compost compared to the control samples. It is very important to pay attention to the element of cadmium due to its content can increase significantly and migrate downwards, yet the other elements of heavy metal can accumulate in the topsoil after the addition of sludge compost in soil. Applying sludge compost does not cause environmental pollution in a short time. For index of geoaccumulation, the pollution of levels of Cd element in topsoil was Ⅰ level; belong to the range of mild-medium pollution. It may be related the low background content of Cd in Henan. Compared to the Standard of State Environment Standard (GB15618-1995), the content of six elements were all in accordance with the primary level and in full compliance with the secondary standard for agricultural land.

From heavy metals accumulation rate and the current soil standard of view, the Cd element should be a priority for applying sludge compost. Heavy metals unlikely to migrate down underground, it should not affect the environmental quality of underground water. However, we suggest that more field tests should be performed in order to further investigate the potential risks of the land utilization of municipal sludge compost.

ACKNOWLEDGEMENTS

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Cover page

Title: *A Research on Performance Characteristics of Solar Drying System for Agricultural Products*

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ABSTRACT

The temperature and air velocity plays a critical part in drying quality and efficiency for subsidiary agricultural products. Different subsidiary agricultural products have different drying characteristics. This paper designed two types of solar drying system for different drying temperatures: medium and low temperature solar drying system with high heat-collecting efficiency. The average thermal efficiency is 66.5%, even in cloudy weather, the efficiency can reach 50%. This system adopted double wind in-let & out-let mode to cope with the nonuniformity of temperature and humidity which is caused by existing single in-let & out-let mode and heating up quicker with a better heat preservation effect. When the medium and low temperature solar drying system is used for notoginseng drying, the drying cycle will be shortened to half of the natural drying. The other type is solar groove-type concentrating heat-collection high and medium temperature drying system. The system adopted V-type metal heat absorption cavity of reliable performance, portable quality and low price with high drying air temperature above 200℃. It has been approved through the research on tobacco shred drying by using this kind of drying system that: the heating temperature of this kind of groove-type concentrating heat-collection high temperature drying system can fit properly with the required temperature for tobacco shred drying system and can be preferably applied in 80℃~210℃ high temperature drying.

1. INTRODUCTION

Drying is a necessary process for agricultural production which can directly affect the quality, appearance, color and luster, taste and production cost etc. of the product and has been applied extensively in various production and processing fields such as agriculture, forestry, food, medicine, chemicals, mineral products, paper manufacturing etc.

Solar drying is a process that the material directly or indirectly absorbs solar energy converts it to heat energy, vaporizing the water in the solid material and diffuse it to the air. After the material surface gets the heat, transfers the heat to the inside of the material, diffuses the water contained in the material as liquid or gaseous state

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gradually to the material surface and then to the air through the gas film on material surface, minimizes the water quantity of the material and finally reaches to dried status. Therefore, the drying medium shall remove the generated water vapor in a timely fashion to remain a certain level of water vapor driving force.

Solar drying has been mostly popularized and applied in tropical and subtropical countries and has conducted large quantity of research on it [1~8]. According to statistics, the energy used for drying in China occupies 12% [9] of the total energy consumption of national economy, in some fields, for example wood drying energy consumption even about 40%~70% [10] of the total energy consumption of an enterprise. Besides, the pollution caused by drying is an important source for environmental pollution. Thus the energy saving and environmental protection of drying technology is very important. For the traditional drying method such as drying in the sun or airing, the material can be easily affected by the weather, getting rotten or bad, with a long drying cycle and is easily polluted by insect flies and smoke dust, as a result of which it cannot meet the requirements of modernized agricultural production development.

The solar drying process utilizes the rich and accessible solar energy to dry. On one hand it can save energy, protect environment and achieve sustainable development, one the other hand the product will be good in appearance, full in shape, with high sugar content and excellent in taste, obviously shortened the drying time for subsidiary agricultural products, and enhanced the drying efficiency and quality. Since solar energy is a kind of intermittent energy, it has such disadvantages as low energy density, discontinuity and instability etc., which make it hard to realize continuous drying. Thus it is necessary to carry on research on the matching relation between the characteristics of solar drying, drying device and subsidiary agricultural products.

2. MATERIALS AND METHODS

Solar drying for agriculture products can be divided into two types according to different drying temperature: solar medium & low temperature drying and solar medium & high temperature drying. Researches on these two different methods are as below:

2.1 Solar Medium & Low Temperature Drying

2.1.1 EXPERIMENTAL SETUP FOR SOLAR NOTOGINSENG DRYING

The medium and low temperature solar drying system-test platform for notoginseng drying: fan, air flow meter, temperature sensor, pyranometer, ambient temperature sensor, anemometer, solar drying oven, air heating collector, bracket, etc... The laboratory system and picture of real product are illustrated in Figure 1.

According to the design, inlets and outlets are provided at both top and bottom of the drying oven. The operation mode can be shifted by adjusting the air valve-inlet at the top, outlet at the bottom or the opposite. Air deflectors are installed at the inlet and outlet to make the air equally distributed at the horizontal plane of the drying oven. The previous single in-let & out-let mode has been improved. Impacted by the material stacking density, the obvious nonuniformity between temperature and humidity is found in the vertical direction of the drying oven at the beginning period of the drying process.

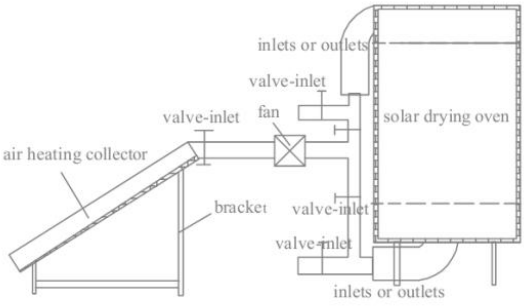

Fig.1 The medium and low temperature solar drying system

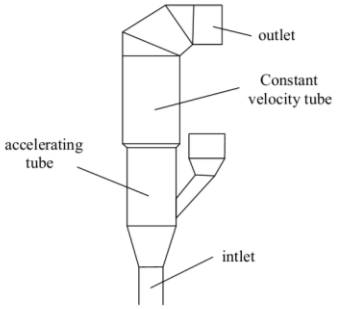

Fig.2 Solar drying tube

2.1.2 EXPERIMENTAL METHOD AND PROCESS

Two plate air heating collectors are connected in parallel. Both ends of the fan and the outlet of the heat collector are wrapped with heat insulation cotton. Three temperature probes are placed at the top, middle and bottom area of the drying oven in order to supervise the temperature distribution status within the oven.

In addition, three humidity sensors are placed separately at the inlet of the heat collector, inside the oven and the air outlet of the drying oven in order to measure the relative humidity at the related points. Anemometer is used to measure the hot air flow velocity within the air pipe. All the test data is recorded every 10 minutes.

Take 10kg of fresh notoginseng divide it into two parts with the same quantity. The first part is placed on the material grid structure in the drying oven of the solar drying device for drying. The second part is placed at a place with free air flow and abundant sunshine for natural air drying,

2.2 Solar Medium & High Temperature Drying Experimental Platform

The test device for tobacco shred drying includes groove type concentrator, line-focus V-type metal cavity, heat preservation tank, solar drying tube(As showed in Figure 2), heat insulated oil pipe and testing instrument. The test parameters of groove type light concentrating and heat collecting system includes temperature, direct radiation intensity, flow of heat conduction oil and wind velocity. The instruments needed are temperature sensor, direct radiation apparatus, flow meter, anemometer, data acquisition instrument, etc..

The drying system adopts liquid-gas heat exchanger to connect with the groove type light concentrating and heat collecting system. The heat exchanger will exchange heat between high temperature heat conduction oil within the light concentrating and heat collecting system and the air. The tobacco shred has been dried by the hot air after the exchange of heat. The temperature of the dried tobacco shred shall be measured at the exit with infrared thermometer. Finally, different brands of tobacco shred will be collected and then the moisture content will be measured.

3. RESULTS AND DISCUSSION

3.1 Solar Medium & Low Temperature Drying

Many groups of test data under various operation conditions were obtained after

conducting tests on solar heat-collecting and drying system for several days. The test results are showed in Fig.3 and Fig.4.

Fig.3 and Fig.4 shows that along with the change of solar irradiation values (cumulative radiation values) and environment temperature, the efficiency of the heat collector and the outlet temperature will undergo corresponding changes; the environment temperature has no obvious effects upon the efficiency of the heat collector and the outlet temperature which mainly depends on solar irradiation values. The variation tendency is showed in Figure 6. Despite the significant fluctuation of solar irradiation values (exceeding 20%/h), the hot air temperature at the outlet of the heat collector is relatively stable, almost above 45℃. The daily average temperature is 47.5℃, the average thermal efficiency is 53.9%. The average thermal efficiency of the solar drying system under sunny condition is above 60%, and no less than 50% under cloudy weather.

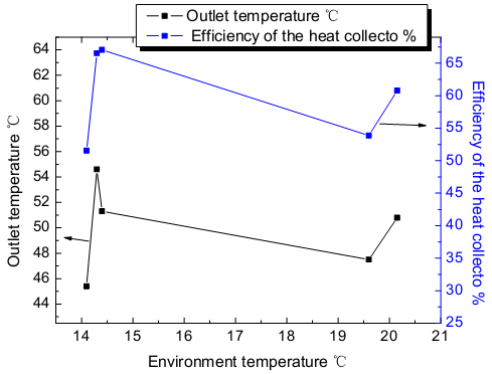

Fig.3 The influence of environment temperature on the dryer

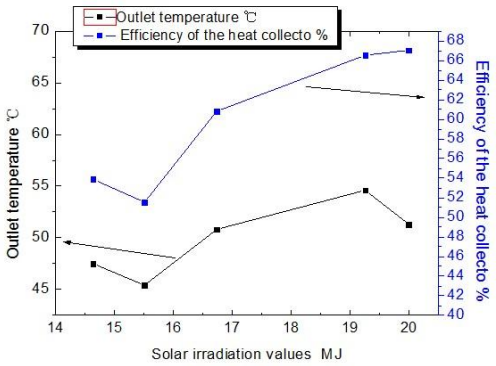

Fig.4 The influence of solar irradiation values on the dryer

3.1. 2 THERMAL PROPERTY OF THE SOLAR DRYING OVEN

As showed in Figure 1, two kinds of ventilation patterns are provided to supply heat for the drying oven by : The first one is that the hot air enters from the top of drying oven and discharges from the outlet at the bottom; the other one adopts the opposite flow method, namely bottom inlet and top outlet. Both of the two methods have the same inlet air flow rate. Test the temperature within the oven and the test results as showed in Figure 5.

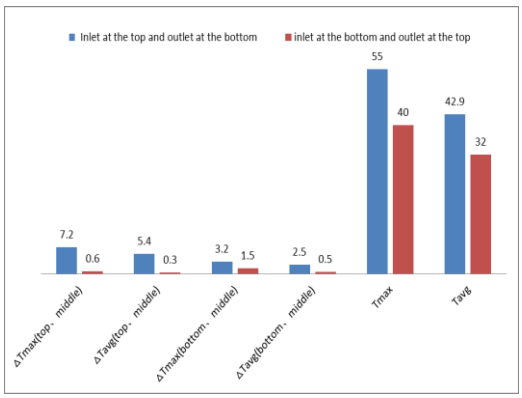

Fig.5. Two kinds of ventilation temperature contrast

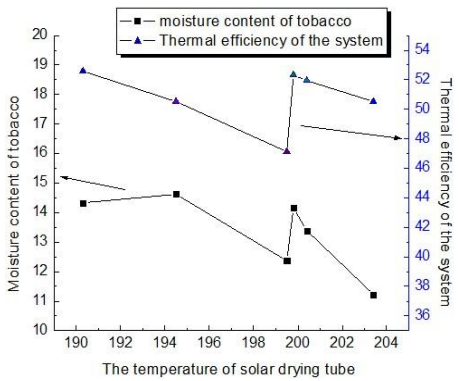

Fig.6. Characteristics of drying-tobacco shred

As showed in Figure 5, when adopting inlet at the top and outlet at the bottom pattern, it will have a larger temperature difference between top, middle and bottom layer. The highest temperature at the top and the lowest at the bottom. The exit discharging temperature and the bottom layer has bigger difference as a maximum diversity of 17℃, average temperature diversity is above 10℃. When adopting inlet at the bottom and outlet at the top pattern, it will have a closer temperature between top, middle and bottom layer. Although still has temperature diversity, but the difference range is small about an average of 1℃. The temperature of bottom is a little higher than the top. The exit discharging temperature and the bottom layer are also close to each other with a difference as a maximum diversity of 2.3℃.

3.2 Characteristics of Solar Medium & High Temperature Drying

When the airflow velocity is 15 m/s and the heat conduction oil flow is 0.4 kg/s, a drying test is conducted under the airflow temperature is 190℃.The characteristics of drying-tobacco shred are shown in Figure 6.

Heat efficiency of drying system can be used to evaluate heat utilization extent. Fig 6 shows that different temperature has different drying efficiency. The heat loss, air flow leakage of the actual tobacco shred drying device will reduce the thermal efficiency of the system. It is necessary to take actions for heat preservation and sealing for dryer. Heat efficiency of drying system can be improved by using discharged wet air excess heat to pre-heat fresh air or tobacco shred inlet or increase the temperature for the air at the inlet and tobacco shred.

TAB.1 HEAT EFFICIENCY OF DRYING SYSTEM

Type	Inlet temperature of drying tube/℃	Inlet temperature of heat-exchanging /℃	Outlet temperature of drying tube/℃	Efficiency of the drying system /%
A	199.5	122.9	163.4	47.12
B	199.8	117.8	156.9	52.31

From Table 1, we can get the conclusion. By controlling the air flow temperature at the inlet, speed and tobacco shred inlet moisture content, the moisture content of the tobacco shred can basically meet cigarette technology requirement. When the flow temperature and speed increase at the inlet, the moisture content of tobacco shred at the outlet of drying pipe will also decrease.

4. CONCLUSIONS

The article designed two sets of drying systems according to the different drying characteristic of subsidiary agricultural products. Such drying systems can use under different temperature. V-type heat absorber plate dual-channel air heating collector-solar drying oven medium & low temperature system and solar energy groove type condensation heat collecting-solar drying oven medium & low temperature system are used for drying materials under medium & low temperature (within 80℃) and medium & high temperature (within 80℃~240℃).

According to the design, inlet and outlet is installed at both top and bottom of the medium & low temperature drying oven. Through the performance testing for

notoginseng drying using medium and low temperature solar drying system, the test results turned out that the drying system drying temperature can reach 62.2℃, and the average thermal efficiency is 66.5% ; Even under cloudy weather, the thermal efficiency can be kept at over 50%. In addition, it is featured by heating rapidly and maintaining good heat preservation effect. Solar drying can shorten the drying cycle of notoginseng to half and avoid environment pollution and the effect to notoginseng quality and improve the quality of notoginseng.

It has proved through the research of solar energy groove medium & high temperature condensation heat collecting system to tobacco shred drying that: adopt V-type metal cavity as heat collector, the groove type condenser heat collector system can provide 230℃ heat for tobacco shred drying. Hot air of 201.7℃ can be obtained by heat transferring by heat exchanger and can fit the required temperature of tobacco shred drying system and realize clean production for cigarette production.

The use of solar energy conduct drying can not only meet drying requirements, but also save conventional energy, reduce production cost, and improve economy benefit; On the other hand, the solar drying process uses clean and renewable energy, avoids the large quantity of greenhouse gases and dust pollution from conventional energy and protects the natural environment.

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Cover page

Title: *Correlation between Activated Sludge and Methane Production of Eupatorium Adenophorum Spreng Anaerobic*

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ABSTRACT

With the application of modern biological techniques in ecological studies, it is possible for anaerobic environmental exploration the nature of microbial population structure and its interdependent environment. Using *Eupatorium adenophorum* Spreng as fermentation material, we extracted total DNA of activated sludge from different fermentation process, amplified PCR of 16S rRNA sequence of microflora in the fermented system, separated PCR amplification mixtures with DGGE method, compared with DGGE electrophoresis strips in different periods fermentation, then found the similarity of microbial population structure between different activated sludge.

SIGNIFICANCE OF ANAEROBIC ENVIRONMENTAL MICROBIAL POPULATION

Research Status of Anaerobic Environmental Microbial Population

With the application of modern biological techniques in ecological studies, we greatly promoted the development of microbial ecology, also made it possible for anaerobic environmental exploration the nature of microbial population structure and its interdependent environment [1].

In 1985, Prof. Qian Zeshu from Zhejiang Agriculture University determined the different kinds of hydrolytic bacterium, hydrogen-producing aectogenic bacteria, methanogens in cow dung biogas fermentation by using different culture medium and Hungate technology. It showed different microbial population structure change under the normal fermentation condition[2].

In 1997, Mr. Zhao Yizhang put forward that the nature habitat of anaerobic environmental microbial population was related with freshwater sediment, soil rice paddies, marine geological deep sediment, geothermal hot spring, rumen intestine, as well as a traditional Chinese wine fermentation digestor[3].

We mainly use nucleic acid probe technique, PCR amplification, rRNA sequence homology methods, gradient gel electrophoresis method in the study of molecular biology methods [4-9].

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At the beginning of this century, Prof. Qu Lianggu, from Gene Engineering Key Laboratory of Education Ministry, Zhongshan University, has obtained the information of methanogen structure and its diversity by 16SrDNA gene analysis of landfill leachate archaea community [10].

Based on 16S rDNA PCR-DGGE (Denaturing Gradient Gel Electrophoresis) spectrum analysis in combination with stripes tapping recovery of DNA sequence analysis, Mr. Zhao Liping from Shanghai Jiaotong University compared different microbial communities from two producing well, derived from the same water injection well. It showed that the similarity of microbial communities from water injection well and producing well was 20% and 30%, while the similarity of microbial communities from two producing wells was 50% [11].

Subsequently, Prof. Wu Xiaolei from the Department of Energy and Resources, Beijing University analyzed the microbial community structure of Daqing transition zone oil well by Terminal Restriction Fragment Length Polymorphism (T-RFLP) and Clone Library Analysis of 16S Rrna genes [12].

In 2005, dependent upon the review of total DNA extraction of soil microbial [13], Prof. Li Shunpeng from Key Laboratory of Environmental Micrology Engineering of Agriculture Ministry, Nanjing Agriculture University applied the method of DNA extraction and purification in biogas fermentation process, and overturned the wrong idea that was generally regarded as there being methanogens in the digested material without having control [14].

In 2006, Prof. Yu Jingquan from Agriculture and Biotechnology Institute, Zhejiang Universiry compared with soil community structure between bacteria and fungus of vegetable fields and paddy fields by means of molecular biology techniques, such as total DNA extraction, PCR amplification, DGGE analysis, and RFLP sequence [15].

In 2008, Mr. Wang Liuyang from the Graduate School of Chinese Academy of Agricultural Sciences attempted the biogas fermentation microflora change and methanogens community structure [16]. Meanwhile, Wang Jiangli from Huazhong University of Science and Technology studied cotton anaerobic digestion process and its molecular diversity of the microbial community at different periods [17].

In 2012, Zhu Xiuwen from Jiangnan University investigated the internal circulation (IC Reactor) anaerobic reactor by adding water volume load [18], exploring the relationship between microbial community structure and dehydrogenase activity [19] or coenzyme F_{420} content [20] in sludge.

Relationship between Anaerobic Degradation Efficiency and Microbial Community Structure

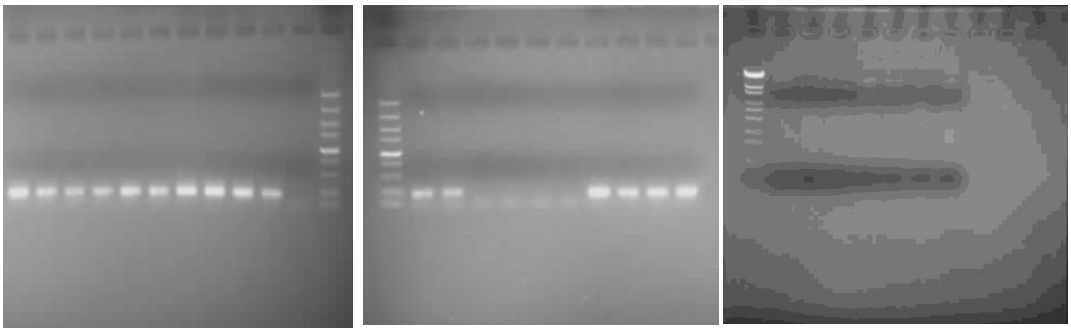
In the whole process of anaerobic fermentation, the anaerobic microbial diversity is influencing on anaerobic degradation efficiency [21]. In order to improve the material conversion rate and energy utilization efficiency, we conduct biogas production experiment using *Eupatorium adenophorum* Spreng as fermentation material, obtaining domesticated activated sludge enrichment method at various gas production stages, finding out coexisting relevance between optimum energy utilization efficiency and environmental microbial diversity of *Eupatorium adenophorum* Spreng.

Using methane-producing activated sludge as a sample, we extracted total DNA from different fermentation process, amplified PCR of 16S rRNA sequence of microflora in the fermented system, separated PCR amplification mixtures with DGGE method, compared with DGGE electrophoresis strips in different periods fermentation, then found the back-and-forth change rule between hydrolysis bacteria and methanogens.

CULTURE-INDEPENDENT ANALYSIS ON METHANE-PRODUCTION ACTIVATED SLUDGE

Genomic DNA Extraction of Samples

According to operating steps of PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA), we defrosted the bottom mud of biogas slurry, extracted DNA samples using 1% adarose gel electrophoresis, and measured DNA concentration with NanoDrop Micro-Ultraviolet Spectrophotometer.



Initial total DNA | Metaphase total DNA | Last phase total DNA

Figure 1. DNA experiment.

16Sr RNA Gene Amplification of Bacteria

We selected V3 hypervariable region of bacterial 16Sr RNA as DGGE detection section, which expanded product length is 250 bp.

Forward primer 341F+GC: 5'-CgC CCg CCg CgC CCC gCg CCC gTC CCg CCg CCC CCg CCC gCC TAC ggg Agg CAg CAg-3'

Reverse primer 534R: 5'-ATT ACC gCg gCT gCT gg-3' (Muyzer *et al.*, 1993)

After completion of amplification, take 4μL amplification products for 1% agarose gel electrophoresis banding detection. When we could see a bright stripe in 250 bp position, it was the successful amplification. Every sample should be amplified for 100μL products, which was concentrated to 15μL for DGGE electrophoresis sample.

DGGE Analysis

We selected V3 hypervariable region of bacterial 16Sr RNA as DGGE detection section, which expanded product length is 250 bp.

REAGENT PREPARATION

All reagents should be prepared according to Reagent Preparation in page 13 in The DCode™ Universal Mutation Detection System Technical Manual.

DGGE CASTING

Take 30% and 65% denaturation gel 17 mL respective, adding 340μL dye solution to 65% denaturation gel. Then add 170μL of 17% ammonium persulfate and TEMED respective. Uniform the denaturation gel for 30-40min under 28℃ rapidly.

ELECTROPHORESIS

Preheat 1×TAE to 60℃, blend the concentrated PCR products with loading buffer, then electrophoresis for 8 h under 60℃ and 150V. The electrophoresis apparatus was The DCode™ Universal Mutation Detection System (Bio-Rad) and Power PAC-3000 (Bio-Rad).

DYEING AND IMAGING

After electrophoresis, take out the gel, soak in 1×TAE buffer containing 50μg/mL ethidium bromide for staining 15min, rinse 15 min (shaking gently every 3 min), then image through ultraviolet imaging system (SYNGENE).

Analysis Result

INITIAL FERMENTATION

The sample serial number is (1) inoculum, (2) stem with HAC pretreated, (3) leaf with HAC pretreated.

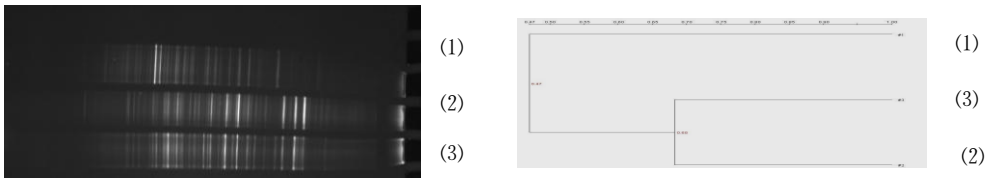


Figure 2. DGGE cluster analysis of fermentation starting.

We selected V3 hypervariable region of bacterial 16Sr RNA as

After adjusting pH value with HAC, the similarity of microbial population between stem and leaf fermentation is 68%, while it was only 47% between inoculum control and fermented sample.

METAPHASE

The sample serial number is (1) inoculum, (2) stem with HCl pretreated, (3) leaf with HCl pretreated, (4) stem 1 with HAC pretreated, (5) leaf 1 with HAC pretreated, (6) stem 2 with HAC pretreated, (7) leaf 2 with HAC pretreated.

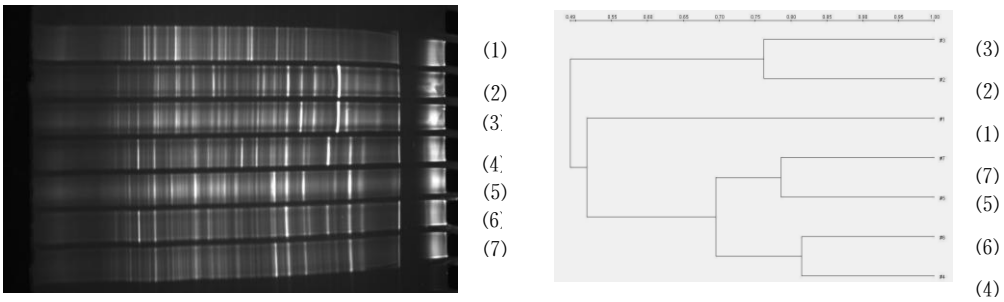


Figure 3. DGGE cluster analysis at the middle of fermentation stage.

We can see from the fig 3:

After adjusting pH value with HCl and HAC respectively, the similarity of microbial population is different. The similarity of microbial population of stem with HAC pretreated is 82%, leaf with HAC pretreated is 78%, between stem and leaf with HAC pretreated is 70%, between stem and leaf with HCl pretreated is 76%, between HAC and HCl pretreated is 76%, while inoculum control and with HAC pretreated is 51%.

LAST PHASE

The sample serial number is (1) inoculum, (2) stem with HCl pretreated, (3) leaf with HCl pretreated, (4) stem with HAC pretreated, (5) leaf with HAC pretreated.

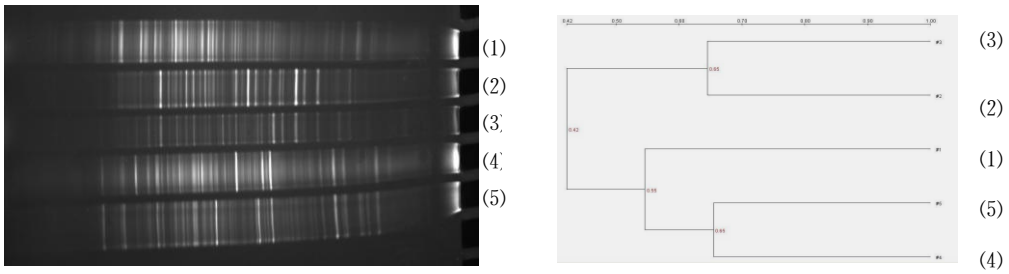


Figure 4. DGGE cluster analysis at the end of fermentation stage.

We can see from the fig 4: After adjusting pH value with HCl and HAC respectively, the similarity of microbial population between stem and leaf is 65%, between inoculum control and with HAC pretreated is 55%, while between HAC and HCl pretreated is only 42%.

DGGE Summary

INITIAL FERMENTATION

(1) The microbial community structure is different between inoculum control, HAC and HCl pretreated sample. The similarity of inoculum control and HAC pretreated is closer than HCl pretreated sample. We can propose that organic acid pretreated sample has much more similarity of microbial population structure with inoculum control.

(2) During gas production stability, the similarity of microbial community of stem and leaf is different. We can propose that raw material fermentation has influence on

microbial population structure.

(3) During gas production ending periods, microbial community distribution is population leveled off. It has the same similarity of microbial community regardless of HCl and HAC pretreated system.

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Cover page

Title: *Domestic and International Low-carbon Development Strategies and Responses to Global Climate Change*

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ABSTRACT

Scientific issue on climate change has become more and more influence to human activities. Carbon emissions are the main factors affecting global climate warming. Low carbon economy is the only pathway of coordination between climate change and development. Chinese low-carbon economic development is not only responses on global climate change, but also a new market power to energy conservation & emission reduction, transformation of development mode, and accelerating industrial restructuring.

SOME POLICIES RESPONDING TO GLOBAL CLIMATE CHANGE

Climate is not changeless. In the millions of years of earth history, the climate has changed for many times along with natural evolution, such as the energy increase caused by explosion of sun or volcano. Today when people talk about climate change, it is referring to the temperature shift which happened in the past one hundred year.

Human activity mainly through the following three important aspects to change the content of greenhouse gases in the atmosphere:

Deforestation

Trees absorb carbon dioxide. Trees reduction means more carbon dioxide accumulation in the atmosphere. Meanwhile, agriculture and industry production become the new emission source in the substitute for forest. Intergovernmental Panel on Climate Change (IPCC) estimated that about 5.9 billion tons of CO_2 was produced due to deforestation, which was equivalent to 18% of global CO_2 emission one year.

Fossil Fuel Combustion

Burning of coal, petroleum and natural gas will release greenhouse gas. In 2005, fossil fuel combustion discharged 2.7 billion tons of CO_2.

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Global Population Growth

This led to increase of food, domestic animal, and energy demand, as well as increase emissions.

In 1988, the United Nation set up the IPCC to analyze and report related scientific findings. The IPCC's first report found the earth has been increased by 0.5 degrees Celsius in the past century. It warned that only by taking strong measures to stop the greenhouse gas emission, could avoid strong global warming.

In 1992, the global summit was held in Rio de Janeiro. 154 U.N. Member countries have signed the United Nations Framework Convention on Climate Change (UNFCCC). The convention drafted the obligation of restrictions on exhaust emission to prevent the serious climate warming caused by greenhouse gas.

In 1997, the Kyoto Protocol was approved by the United Nation. UNFCCC agreed that it was voluntary goal. The Kyoto Protocol is the first international treaty of a legally binding about industrial countries to cut emissions, which was come into effect signed by 178 countries in 2005.

In 2007, the IPCC's fourth assessment report showed that the global average surface temperature rose by 0.74 ℃, and sea level by 0.17 m. The report forecasted the global surface temperature will rise by 1.1-6.4 ℃, sea level by 0.18-0.59 m, and perhaps frequent high temperature, hot wave and strong rainfall by the end of 21st century according to the current development situation.

On December 8, 2012, the two-week UN Climate Conference ended in Doha. The Doha meeting determined the second commitment period under the Kyoto Protocol, completed the negotiations of Bali Action Plan, mapped out preliminary the negotiations of Durban Platform, adhered to the principle of Common But Differentiated Responsibility, and maintained the basic institutional framework of the convention and the protocol.

As the UN IPCC's domestic leading organization, the Chinese Meteorological Administration is preparing for domestic governmental review work of the IPCC's fifth assessment report. Until October 2014, the IPCC' fifth assessment report will not be published.

SIGNIFICANCE OF LOW-CARBON ECONOMIC DEVELOPMENT

Now many countries have generally recognized that only choose low carbon economy can coordinate problems between climate change and development. Who progressed the low carbon economy first, who is likely potential for development advantages and spaces. Low carbon economy development is not only addressing climate change and reflecting major power responsibility, but also transformation of development mode.

The Chinese government always attaches great importance to the problems of climate change. Low carbon economy development has both opportunities and challenges. Low carbon economy development is both goals and means. Low carbon economy provides a powerful hand grab and a new market power to energy conservation and emission reduction, transformation of development way, and speeding up adjustment of industrial structure.

The essence of negotiations of climate change is competition of development right. Developing low carbon economy is an irresistible trend. How about low carbon economy development of various countries in the world currently?

In 2003, United Kingdom put forward the low carbon economy development firstly, which is used as a major developmental strategy. EU has staged a package of policies, as well as proposed three 20% Targets: firstly the greenhouse gas emission in 2020 will be decreased by 20% of 1990, secondly the energy efficiency risen by 20%, thirdly the proportion of renewable energy risen by 20%. EU carries out carbon trade system, blossoms carbon market, focuses low carbon economy on international cooperation. Japan comes up with the concept of low carbon society, advocates a well-off and sustainable society, and implements a serious of related policies and measures. Obama administration has presented an ambitious plan, which conduct new energy development as getting out of recession, bringing about employment opportunity, and grabbing commanding heights of the important strategic industry in the $780 billion economic stimulus plan. Obama proposed that renewable energy production should be increased by 1 times in the next three year, clean energy (such as solar, wind, biofuels) should be invested $150 billion. Some economic transition countries, such as Russia, India, Brazil, South Africa, South Korea, as well as developing countries also carry out low carbon economic policy research.

We put forward CO_2 emission per unit of gross domestic product (GDP) in 2020 down by 40-45% lower than that in 2005. It is certain difficulty to achieve the goal. How to think about the future development of low carbon economy facing challenge? For challenge, China's energy structure is given priority to coal, which is one of the largest CO_2 emissions. Coal consumption has accounted for 60-70% of proportion in the energy structure. It is difficult to change it. In addition, we need to perfect the market environment and technical foundation of low carbon economy. Our country is a big developing country, which should be considered imbalance of national economic development. Because the economic development base and CO_2 intensity reduction ability is different in eastern and western regions, low carbon economy policy system and management mechanism could not be one size fits all. It is a higher request to us for the next step of work. In addition, we are facing the great pressure from developed countries, which occupied certain advantages of international competition, dependent on their capital, the technical advantages, and domestic mature market environment of low carbon. On the other hand, in order that maintain their dominant position, the developed countries restrict the economic development of developing countries by setting green trading barriers such as the carbon tariffs. As during the Copenhagen Negotiations, Europe and America put forward respectively that they would take action of unilateral trade sanctions, establish carbon tariffs, impose border adjustment tax, or levy tax on imported products to America from developing countries. All of these will bring some pressure to our economic progress.

On November 25, 2009, the State Council established the control action target on greenhouse gas emission, including CO_2 emission per gross domestic product (GDP) unit in 2020 down by 40-45% lower than that in 2005, which was served as binding forces into the long-term plan for national economic and social development. Since 2009, the Chinese government has launched the first Green Book of climate change, which was named 《on climate change (2009) —to Copenhagen》. Then 2010, 《on climate change (2010) —the response to the challenges of Cancun and the action of

China》. In 2011,《on climate change (2011) —Durban's difficult position and China's strategic choice》. In 2012 focusing on low carbon finance,《on climate change (2012) —climate finance and low carbon development》.

MEASURES FOR LOW-CARBON ECONOMY IMPLEMENT

We should extensively propagandize the connotation of the concept of low-carbon economy and its developmental significance, to make the idea of low-carbon economy as mainstream consciousness, and further build the governmental leading, public participating, whole social force promoting to create the developmental atmosphere in Yunnan province as well as the western region.

It is necessary for government officials to firmly set up the social development view and low-carbon economic idea, transform the requirement of low-carbon economy to thinking of work plan, popularize it through science media, guide the public to form low carbon habits, find low-carbon lifestyle expression in dairy life, cherish every inch of land and each resource, stop driving a car for one day, use less a kilowatt hour electricity and a drop of water, and enjoy popular support of low carbon life, energy conversation, and emissions reduction.

Finally, it is urgent for enterprise to strengthen the consciousness of energy conversation and environmental protection, slow down the social shortage of energy resources, alleviate the pressure of the environmental damage, and promote social sustainable development.

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Cover page

Title: *The Economic Benefits of Water Absorbing Polymers: A Case Study of Drought Conqueror*

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ABSTRACT

As working by absorbing high quantities of water, in addition to beneficial nutrients, and then slowly releasing the water through osmosis, water absorbing polymers can be efficiently used in agriculture to reduce watering frequency, and save time and money spent on irrigation labor and water costs. This paper discusses the economic benefits of water absorbing polymers with a case study of the innovative product, Drought Conqueror, and investigates how new technologies and materials can be utilized to retain both soil moisture and nutrients in the root zone for the production of crops, thereby increasing productivity and net returns, and reducing input costs and environmental impacts.

INTRODUCTION

With climate change predicted to increase the severity and frequency of drought events in many parts of the world, water conservation is a growing concern. New water retention technologies using water absorbing polymers can help quench the thirst of parched crops while using less water, not only enabling crops to better deal with drought, but also improving crop yields in marginal areas [1].

Water absorbing polymers, also known as superabsorbent polymers (SAP), are soil additives designed to improve plant establishment and growth in arid environments. When irrigated, the dry polymer granules swell to form gel particles that hold several hundred times their original dry weight in stored water. The resulting gel fragments provide small, individual reservoirs of moisture and have been widely reported to influence water movement within soils and to increase plant available water storage in growing media.

Water absorbing polymers are especially useful in sandy soils, where the natural water retention at field capacity is low. These polymers do seem to be of some benefit when they are used in outdoor soil that is low in organic matter. Some researchers have found that it is possible to grow several types of plants outdoors with minimal irrigation in soil amended with water absorbing polymers. The best results are

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achieved when soil is amended with polymers, and some type of mulch is used to cover the soil [2].

Addition of small amounts of water absorbing polymers to sandy soils significantly increases the amount of water available at low moisture tensions. This, in turn, can improve plant survival rates, permitting a more efficient use of water resources, since water would otherwise percolate too quickly through the sand to support healthy plant growth. A further benefit of water absorbing polymers is that it can help decrease erosion by the formation of a light crust on the soil surface. The soil stability is further enhanced as the polymers can stimulate root growth, which will consolidate the protection against erosion, as they grow. Addition of water absorbing polymers can also facilitate the handling of fertilizer in dry climates where problems of fertilizer drying out and having to be re-wet can be largely overcome [3].

AN EXPERIMENTAL REPORT

The potential benefits of polymers to the production of cotton could be considerable, if water-use efficiency during production could be improved in circumstances where short periods of moisture stress are experienced, where rainfall is unpredictable or where irrigation costs are significant. The experiment described here examined the effects of Drought Conqueror, a new product of water absorbing polymers developed by Jiujiang Jirui New Material Industries Co. Ltd (www.jrhkx.com), on growth and output of cotton planted in coarse-textured and sandy soils, representing a low water-retaining, drought-prone substrate [4].

The experimental base, located in Hetian Cele County, Xinjiang, western China, has an area of 500 square meters. The base was divided into 10 units with 25 meters (length) and 2 meters (width) per unit. The water absorbing polymers mixed with fine soils were placed in plant holes prior to cotton seed planting. The dosage of water absorbing polymers was shown as follows (Table 1):

TABLE 1. DOSAGE OF WATER ABSORBING POLYMERS

Process / Unit Number	Usage Per Acre (kilogram)	Usage Per Unit (gram)
1	None	None
2	3	180
3	1	60
4	4	240
5	2	120
6	3	180
7	1	60
8	4	240
9	2	120
10	None	None

The experimental results of cotton output indicated that the application of water absorbing polymers significantly increased cotton output with the highest increasing rate of 35.55% (Table 2). By comparing the results, Unit 5 gained the highest seed

cotton output (197.59 kilograms) and ginned cotton output (75.08 kilograms) with 2 kilograms per acre of the polymer product. The results suggested that the usage of water absorbing polymers should be 2 kilograms per acre.

TABLE 2. RESULTS OF COTTON OUTPUT

Project / Process	Total Bolls	Seed Cotton Output (kilogram)	Ginned Cotton Output (kilogram)
1	74	130.93	49.75
2	72	170.42	64.76
3	73	181.68	69.04
4	82	184.39	70.07
5	84.5	197.59	75.08
6	75	182.86	69.49
7	71	160.24	60.89
8	71.5	167.75	63.75
9	86.5	184.92	70.27
10	61	127.35	48.39

Table 3 indicated that the application of water absorbing polymers resulted in differences in percent moisture. In the soil depth of 10-30cm, the differences in percent moisture were not significant with an average of 16% to 19%. However, in the soil depth of 30-40cm, the differences were significant with a large gap of 10.9% to 22.4%.

TABLE 3. RESULTS OF PERCENT MOISTURE

Depth / Process	0-10cm (%)	10-30cm(%)	30-40(%)
1	15.7	16.3	12.1
2	18.0	18.9	14.0
3	13.5	16.6	17.1
4	13.0	17.0	22.4
5	13.9	19.0	20.5
6	17.7	17.0	14.6
7	11.0	16.9	20.8
8	17.7	16.7	13.8
9	11.6	16.5	18.3
10	21.5	16.4	10.9

EVALUATING THE ECONOMIC BENEFITS

The positive experimental results of the application of Drought Conqueror in agriculture have been supported by many users since the product was on markets in 2011 [5]. According to users' feedback, there are four economic benefits of using Drought Conqueror:

Firstly, its capacity of slow release meets the watering needs for plants, and matches the on-going process of plant water absorption. This capacity overcomes the problem of evaporating and percolating water resources, and increases water use ratio nearly up to 100%. By increasing the amount of available moisture in the root zone, the length of the irrigation interval can be lengthened. Since evaporative losses are greatest during and immediately after irrigation, the capacity to reduce irrigation frequencies by lengthening the irrigation interval leads to significant improvements in irrigation efficiency and lower the need for supplemental irrigation [6].

Secondly, its capacity of aerification loosens the soil, and improves rooting conditions for plant germination and growth. Present injection techniques involve placing polymers as dry granules a few centimeters below the soil surface in seeding areas [7]. As the dry granule absorbs water and expands, it occupies additional space. As water is drawn from those hydrated granules by roots in close proximity or growing right through the polymer, the hydrated granules shrink. Depending upon the strength of expansion, the addition of water absorbing polymers adds an element of aerification from these shrink-swell cycles. An evaluation of soil cores indicated that the addition of polymers resulted in an average increase in root mass of 30-40 percent [8].

Thirdly, its capacity of fertilizer desorption strengthens the retention and supply of soil nutrients, and optimizes the utilization efficiency of the three key nutrient elements of Nitrogen, Phosphorus, and Kalium (Table 4). Drought Conqueror is specifically designed to improve the ability of the soil to retain water and nutrients and help protect the environment by preventing fertilizer and nutrient leaching.

TABLE 4. DATA ON CAPACITY OF FERTILIZER DESORPTION

Absorbing Capacity (g/kg)			Fixation Capacity (g/kg)			Desorption Capacity (g/kg)			Desorption Rate (%)		
N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
148	54	141	74	27	121	74	27	20	50	50	14

Table 4 indicated that Drought Conqueror possesses strong capacity to absorb and desorb Nitrogen, weaker capacity to absorb Phosphorus, and strong capacity to absorb Kalium. Incorporating fertilizers and water absorbing polymers in a single formulation makes Drought Conqueror more cost-effective, and practically beneficial to water and nutrient supply for plant growth [9].

CONCLUSION

This paper has demonstrated that there are economic benefits of water absorbing polymers to plant growth at all growth stages and to the water use efficiencies of a variety of soil-plant growing systems. In particular, the high permeability and poor moisture-storage properties of coarse-textured, sandy soils hinder plant establishment and growth. Water-storing, gel-forming synthetic polymers can assist in easing water shortage, and can be introduced as aids to plantation on such drought-prone substrates.

It is evident that because of its capability of absorbing large quantities of water and desorbing a range of nutrients, water absorbing polymers have been designed to

act as reservoirs in the soil from which developing plants can extract moisture and nutrients as required. The polymers have major potential applications as water conservation aids in arid and semi-arid regions to aid the establishment and growth of trees, shrubs and crops. Therefore, it is significantly important to study the water absorbing and desorbing capacity of polymer products when applied into soil as well as their effective time. Further research is needed to investigate the effect of different polymer types under the same moisture condition and the effect of the same polymer type under different moisture conditions on saturated soil water content, saturated soil hydraulic conductivity and soil water diffusivity.

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Title: *Recycling and fertilizer use reduction for clearing agricultural nitrogen of China*

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ABSTRACT

Agricultural chemical fertilizers caused worldwide degradation of aquatic and soil ecosystems. This letter challenges the cleaning up the rural environment particularly pollutants from agricultural sources in China. China's agricultural N-fertilizer application is currently 36 Mt N/yr with low N use efficiencies, accounting 35% of N fertilizers in the world. Moreover, agricultural N fertilizer consumption has been increasing significantly (R^2=0.95; p<0.01) for higher grain yield with considerably decreasing N use efficiency since 1980 (R^2=0.88; p<0.01). We posit agricultural effluent recycling *via* altered traditional irrigation system, and reducing fertilizers and teaching the local people for large reduction potential (i.e., around 70%) on currently anthropogenic-driven nitrogen additions.

Key words: rural environment, nitrogen, nitrogen fertilizer, agricultural pollution.

There have been rapidly increasing chemically N fertilizers to satisfy their crop demands. Currently, global N fertilizer applications (ca. 120 Mt/yr) are 8-fold global human N demands [1], resulting in worldwide eutrophication and water acidification. This excess loading of N and P will cause hypoxia, harmful algal blooming and losses of fishery production in aquatic ecosystem [2, Fig. 1]. Agricultural pollution sources have been the top priority as industrial effluents largely being curbed in China, and therefore become increasingly central for China's ambitious cleaning up the rural environment [3]. However, there is a long endeavor way because of promoting fertilizer uses and poor treatment facilities in the villages [4]. Prior research demonstrated strong linear relationships between N concentrations and chemical fertilizer uses in the Yangtze River especially in the recent two decades [cf. 5]. Similar nitrogen pollution is also popular in the Europe [6].

China's major reivers deliver about 5-10% of global freshwater input, 15-20% of global continent sediments, 9% of global riverine DIN exports and 1.5% of global DIP exports to the world ocean [cf. 7-9]. Thus, Chinese riverine loads of nutrients their changing stoichiometric ratios largely contribute to the composition and structure of phytoplankton community in the river plume. Here, we reported agricultural N and P fertilizers uses and their effects on riverine exports in China.

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Fig. 1. Fish-killed event in Sep. 2013 of Fuhe River in the Hubei Province due to substantial excess of nitrogen loads (http://www.china.com.cn/photochina/2013-09/03/content_29910417.htm)

There are substantial increases in riverine N and P loads at basin and national scales (Figs 2, 3, and 4) [5, 7-9]. In the Yangtze River, riverine fluxes of NO_3-N and DIP drastically increase as year ($p<0.01$), and they are respectively significantly related to N and P fertilizer uses in the drainage basin (Fig. 2). Fig. 2. illustrates much higher increases in nitrate loads than phosphate, which can be indicated by variation factor (maximum/minimum) of 22 for nitrate and 11 for phosphate, which consequently leading to pronounced increases in the ratios of NO_3-N:DIP ($R^2=0.45$, $p<0.05$). This suggests more severe phosphorus limitation for phytoplankton growth in China's river estuary and its adjacent coastal waters.

In the national scale, policy-driven increases in chemical fertilizer usage have been contributing to rising crop yields for feeding its huge populations particularly since 1978 (Figs. 3 and 4). China’s croplands accounting for 7% of the world total while consume 35% N fertilizers in the world for increasing grain production (Table 1). Grain production and agricultural N-fertilizer application thus currently reached 546 Mt/yr and more than 36 Mt N/yr nationally, respectively, increases of 68% and 230% as compared with 1981 (Fig. 4). Simultaneously, grain yield per Kg N fertilizer swiftly decreased (Fig. 4). Low N use efficiencies of 30-50% [10] or even below 10% [11] result in transformation of redundant nitrogen into powerful greenhouse gas nitrous oxide, dilution into freshwaters and ocean environments, and deposition in the soils. Guo et al. [10] reported that high levels of N fertilizer applications (ca. 500 kg N/ha/yr) have led to a significant soil acidification at the national scale, and the degraded soils will decrease grain production potential in return. Thus, optimal nitrogen-management strategies of reducing N surpluses without loss of crop yields are urgent for agriculture and environment.

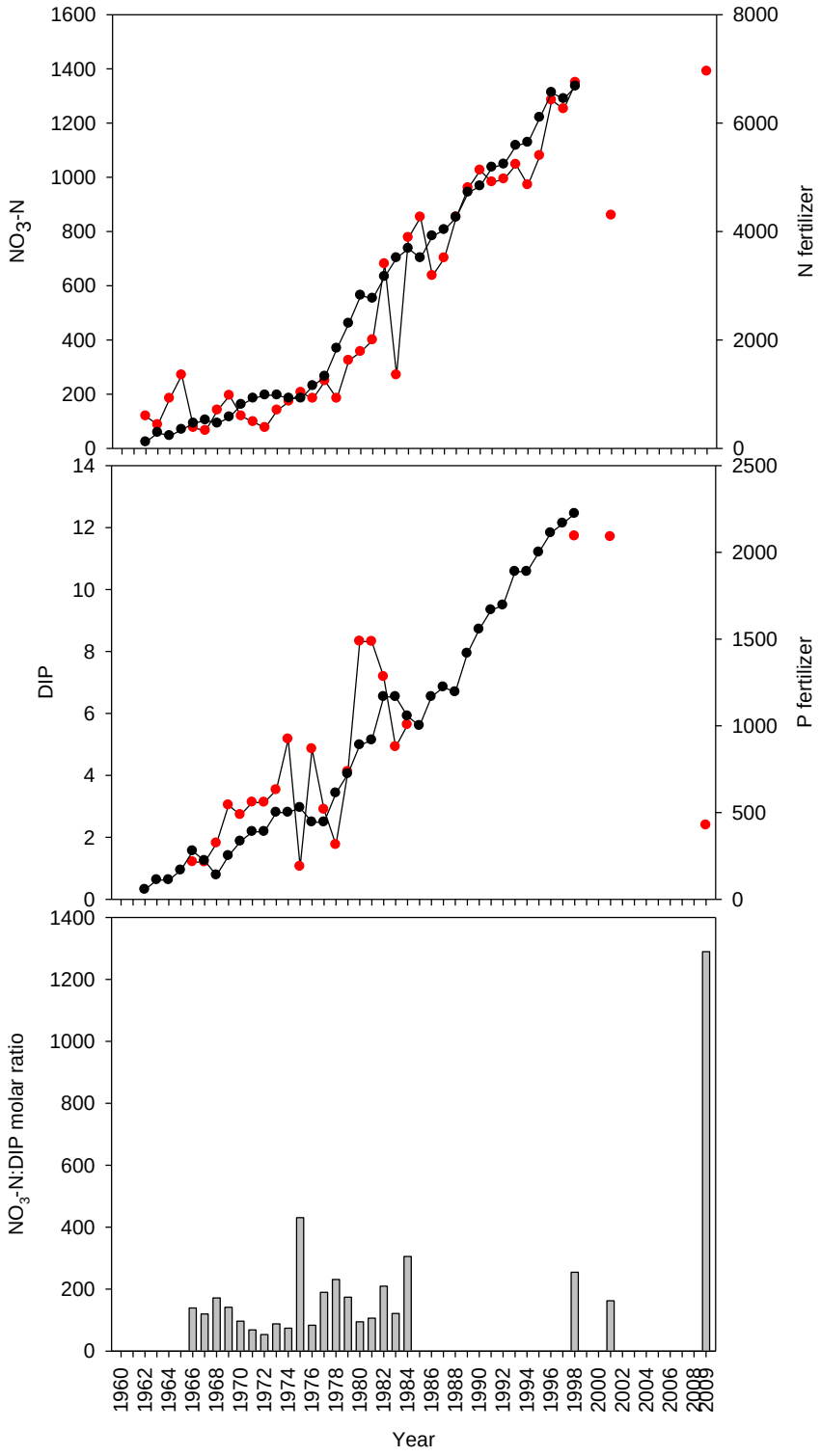

Fig. 2. Long-term changes in annual fluxes of NO_3-N and DIP from the Changjiang River, along with total chemical N and P fertilizer uses in the drainage basin. The NO_3-N and DIP data are from Duan et al [7], Liu et al. [8] and Muller et al. [9]. Data of chemical fertilizer uses are from Duan et al. [7] (unit in 10^6 kg/yr).

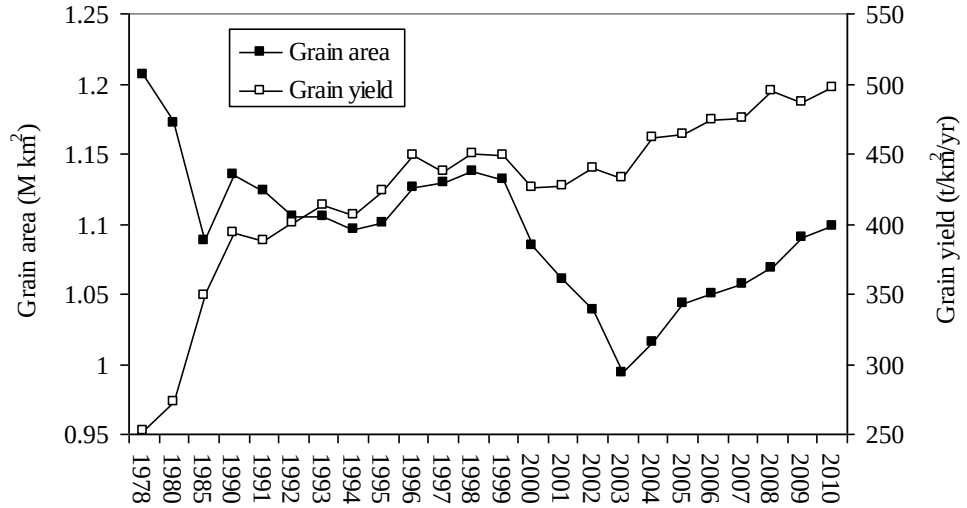

Fig. 3. Grain area and grain yield from 1978-2010 (grain yield increases significantly r^2=0.74, p<0.01). Data from National Bureau of Statistics of China (http://www.stats.gov.cn/).

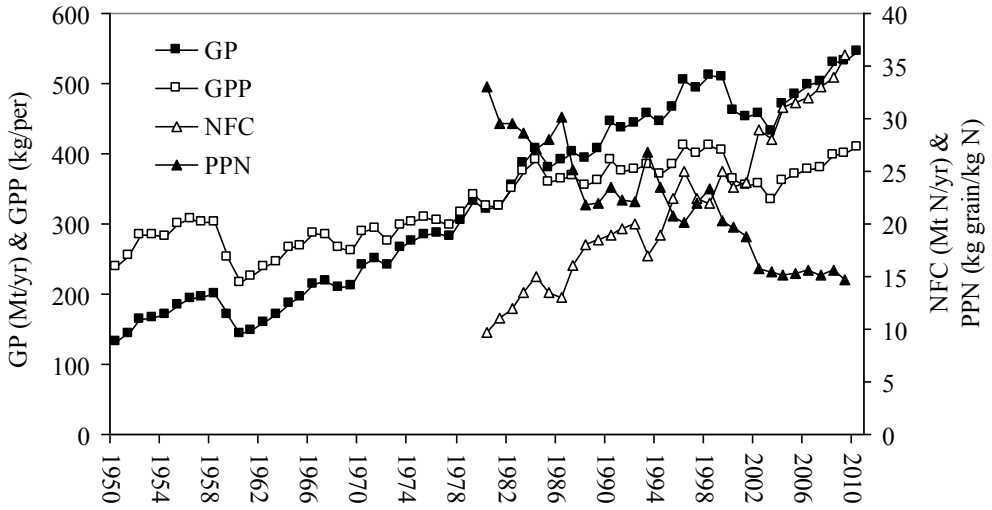

Fig. 4. Changes in annual grain production (GP), grain per people (GPP), agricultural N fertilizer consumption (NFC), and grain production and partial N fertilizer contribution (PPN) (the ratio of GP to NFC) in China during 1950 and 2010.

Here, PPN denotes N use efficiency. NFC sources from Guo et al. (2010), others from National Bureau of Statistics of China (http://www.stats.gov.cn/) and China Agriculture Yearbook.

Linear regression models:

GP=7.2Year+107.6; R^2=0.95, p<0.01; GPP=2.65Year+245.5; R^2=0.73, p<0.01; NFC=0.83Year+9; R^2=0.95, p<0.01; PPN=-0.57Year+31; R^2=0.88, p<0.01.

TABLE 1. COMPARISON OF AGRICULTURAL N FERTILIZER APPLICATION IN CHINA AND IN THE WORLD.

	Cropland 10^6 km^2	Agricultural N fertilizers Mt/yr	Total N fertilizers Mt/yr	Agricultural N fertilizer rate kg N/ha
China	1.1	36	41	330[a]
Global	15.7	103	120[b]	66
P use[c]	15.7	14	17.5	

Sufficient N use per hectare of cropland is 150 kg N/ha/yr (Deluca, 2011).
[a]500 kg N/ha/yr by Guo et al. (2010) and as high as 4000 kg N/ha/yr by Ju et al. (2007).
[b]Based on global N fertilizer use outpacing 7-fold human demand of 15 Mt/yr (Deluca, 2011).
[c]Global phosphorous usages (Elser and Bennett, 2011), P fertilizer consumption in China is around 8 Mt/yr.

Scientists have been seeking for sustainability of agriculture, i.e., survey of soil nutrients and cutting N fertilizers by 30-60% [10, 11], nevertheless, there are several challenges remained. Nutrient compositions in soils are clearly heterogeneous in space, i.e., N-rich in east China while N shortages in west and central China, and N compositions are remarkably distinguishable even in a county (http://www.hefei.gov.cn/n1070/n304634/n1813623/16099414.html). Moreover, soil quality, plant species, crop yields, fertilization technique, and hydro-climatology should be incorporated, rather than arbitrary decreasing N. Field survey and related mechanisms need huge costs and much manpower as well as long-time. Farmers in China are continuing the practice of fertilizers overuse for high crop yields (Fig. 3) [12]. Understandably, China's agriculture is responsible for more than 60% of nutrient discharges and chemical oxide demand of the national total and the proportions are further increasing. Thus, the first and also the important thing is to tell local farmers how, when and quantities for fertilizer applications.

Simultaneously, N use efficiency has been decreasing significantly since 1980 (r^2=0.88, $p<0.01$; Fig. 4), a variety of novel approaches to increase the nitrogen efficiency of cropland and livestock production are urgently available to farmers. On the other hand, this novel technology will be the key for China's food security due to China's shortages of some commercial fertilizers particularly potassium fertilizer, which largely relies on imports.

In addition to soil pH decline [10], more than half of the nitrogen in fertilizers and manures is lost to the aquatic environment [6,11], degrading water quality or leading to frequent algal blooming in the Chinese rivers and lakes, such as Taihu lake in the Yangtze River basin and Dianchi in the Yunnan Province (Southwest China), as well as water lettuce coverage in Minjiang of the Fujian Province in July 2011. This is corroborated by promptly increases of fertilizer usages of nitrogen and phosphorus (Figs 4 and 5). Promoting existing agricultural irrigational systems for effluent recycling from agricultural practices to crops should be incentivized for high nitrogen utilization efficiency, and this recycling is also urgently needed for phosphorus, i.e., 8 Mt/yr lost from global farms [13]. The measure also greatly decreases greenhouse gas fluxes of nitrous oxide. Such reduced N inputs are beneficial agronomically, economically and environmentally. However, this agricultural effluent recycling system represents a huge challenge to traditional irrigation systems.

China has planed huge investments on sustainable and equitable environment

development, i.e., US $600 billion on water infrastructures of reservoirs, wells, irrigation systems, and inter-basin water transfer projects over the next ten years (projected in the initial of 2011), US $5.5 billion (RMB 35 billion) from 2011-2020 for groundwater conservation (projected on 28 October 2011), and US $82 billion (RMB 600 billion) from 2011-2015 for improving cropland quality. The aforementioned costs are closely related to agriculture, which would greatly contribute to regional N fertilizers reduction and increasing N use efficiency, albeit these have been implemented in certain regions. Together with closing nutrient cycles by returning agriculturally derived nutrients to croplands particularly in the concentrated irrigated areas will cause greatly N loss abatements. Considering combined effects of cutting in half of N additions [11] and agricultural N recycling, additions of N fertilizer could be reduced dramatically by ~70% (assuming 50% of N lost to aquatic system recycled). However, it should be noted that policy-makers must recognize the substance of farmers in pollution reduction for clearly rural environment.

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Title: *The Comparison Research on the transformation efficiency of Regional Agriculture Science and Technology Achievements-- Based on the Model of DEA-TOPSIS*

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ABSTRACT

The transformation efficiency of agricultural science and technology achievements is an important index to evaluate the leading effect that the science and technology impact on agriculture, the paper build up a evaluation model for the transformation efficiency of regional agriculture science and technology achievements based on the input and output, combined with the relevant original data of the 30 provinces, cities and autonomous regions in China, used the *DEA* model to measure the transformation efficiency of each region, and meanwhile made a further evaluation for the effective decision making units by *TOPSIS* model, at last, put forward relevant suggestions in the agricultural science and technology application in different regions according to the results of the empirical analysis.

1. INTRODUCTIONS

As the basic industry and pillar industry, agriculture plays an important role, the develop status of agriculture also directly influence the development of social, economic and polity. Through the analysis of the data, the transformation efficiency of China's agricultural science and technology achievements is generally low, maintained at 30%~40%, the problem has become the bottleneck for agriculture to develop further in China.

Different from the industrial, commercial and other sectors, agriculture science and technology achievement has the characteristics of high cost, long period and high risk, meanwhile, the agricultural science and technology achievements has strong externalities, their interests are not effectively guarantee. In addition, a lot of China's agriculture science and technology achievement stranded in the laboratory and the experimental field, it is hard to popularize knowledge to farmers. Therefore, research on the transformation efficiency of regional agriculture science and technology achievements has a very high reference value.

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2. LITERATURE REVIEW

At present, scholars do lots of research on the existing problems of agricultural science and technology achievement transformation ,some scholars also tried to describe the calculation means of the transformation efficiency with the calculation method formula and model .Zhang Yu (2006) cites an calculation example about the agricultural science and technology achievements transformation rate, the measuring and calculating method for the transformation efficiency in farming, animal husbandry, aquaculture and other classification methods were introduced in the paper[1-2] ,further discussion was given on the operational mechanism of agricultural science and technology achievements transformation, Huang Wei Qiang (2008) defined the agricultural science and technology achievements transformation rate, he holds that the transformation rate includes two aspects, transformation cycle and results number, and gives the conversion rate of agricultural science and technology achievements transformation efficiency formula[3]. Zhao Qing Hui(2010) thinks that the primary cause of the low efficiency for agricultural science and technology achievements transformation is the distempered technology results transformation mechanism, unstable capital investment and the disjointed market demand[4]. Yue Fu Ju(2011) pointed out that a pressing matter of the moment to improve science and technology achievement transformation efficiency is to improve the quality level of agricultural science and technology[5]. Chen Xue Yun (2011) thinks that the basic reason for the low conversion rate of agricultural science and technology achievements in China is caused by the disjunction between the supply and demand [6]. Wang Jing Hua (2012) believes that the government's macro-control plays a key function in the transformation of scientific and technological achievements, government's duty is to "start" policy[7]. Lu Ling Xiao (2012) thinks that agricultural science and technology incubation center can well realize the commercialization of agricultural technology, reduce the cost and risk of agricultural science and technology research, create excellent environment [8].

3. THE EVALUATION MODEL OF TRANSFORMATION EFFCIENCY FOR REGIONAL AGRICULTURAL SCIENCE AND TECHNOLOGY ACHIEVEMENTS

3.1 The Model of *DEA*

DEA is convenient for the analysis of multi input and multi output relative efficiency of decision making units[9], so this paper evaluates the transformation efficiency of regional agricultural science and technology in *DEA* evaluation, and introduced *TOPSIS* method for sorting and analysis the effective decision making units.

CCR is a classic model which is used to evaluate the total efficiency of each decision making unit in *DEA,* according to the related literature, the *CCR*'s optimization model is constructed as follows[10]:

$$\max h_o = \frac{u^T Y_o}{v^T X_o} = V_{\bar{p}}$$
$$s.t. \begin{cases} h_j = \dfrac{u^T Y_j}{v^T X_j} \le 1 (j = 1, 2, ..., n) \\ u \ge 0, v \ge 0 \end{cases} \quad (1)$$

3.2 The Model of *TOPSIS*

TOPSIS method is an effective method used in multi criteria decision analysis, its basic principle is to sort the evaluation object by the distance from the evaluation object to the optimum or the worst solution, according to the related literature, the specific calculation steps of multi-objective *DMU* ranking as follows [11]:

The first step, constructs the virtual decision making unit, determining the ideal sample point.

The second step, distinguish and sort the efficient *DMU*.

4. EMPIRICAL ANALYSIS

4.1 Evaluation Index Design

The transformation of agricultural science and technology achievements is a complicated nonlinear activities, this paper intends to regard the process of agricultural science and technology achievements transformation process as a "black box", only consider the relationship between the input factors and output factors.

Based on the research results on the transformation efficiency of scientific and technological achievements [12-14] the paper constructs the input-output evaluation index, it was shown in table I:

TABLE I .REGIONAL EFFCIENCY EVALUATION INDEX OF THE TRANSFORMATION OF AGRICULTURAL SCIENCE AND TECHNOLOGY ACHIEVEMENTS.

Input of agricultural science and technology achievements	Output of agricultural science and technology achievements
Number of agricultural professional technicians. Number of agricultural research institutions The R&D funds expenditure	New agricultural plant varieties applications per unit yield of main agricultural products (grain) Net income of rural household management

4.2 The Evaluation Index Data Processing

Considering the hysteresis between input and output index, this paper choose the input index in 2011 and the output index in 2012, the data all stem from the China Statistical Yearbook and China Statistical Year book on science and technology, take 30 provinces, city, autonomous region of China as an example, using DEA-Solver-LV software, input the relevant data into the C^2R evaluation model, the results as shown in table II:

Table II. THE EFFICIENCY EVALUATION RESULTS OF REGIONAL AGRICULTURAL SCIENCE AND TECHNOLOGY ACHIEVEMENTS TRANSFORMATION.

DMU	total efficiency	*DMU*	total efficiency	*DMU*	total efficiency
Bei Jing	1.000	Zhe Jiang	0.278	Hai Nan	1.000
Tian Jin	1.000	An Hui	0.346	Chong Qing	0.552
He Bei	0.306	Fu Jian	0.539	Si Chuan	0.837
Shan Xi	0.171	Jiang Xi	0.354	Gui Zhou	0.211
Nei Meng Gu	0.496	Shan Dong	0.641	Yun Nan	0.601
Liao Ning	0.505	He Nan	0.636	Shan Xi	0.198
Ji Lin	0.563	Hu Bei	0.586	Gan Su	0.991
Hei Long Jiang	1.000	Hu Nan	0.226	Qing Hai	1.000
Shang Hai	1.000	Guang Dong	0.390	Ning Xia	1.000
Jiang Su	1.000	Guang Xi	1.000	Xin Jiang	0.361

4.3 A Sorting Based on the Model of *DEA-TOPSIS*

According to the results in Table II, the agricultural science and technology achievements transformation efficiency was 1 in Beijing, Tianjin, Heilongjiang and other nine provinces and cities, *DEA* model can't distinguish the effective *DMU*, it can be done by *TOPSIS* method, according to the calculation step and formula of *TOPSIS*, the sort of effective *DMU* efficiency based on DEA-TOPSIS model are shown in table III:

TABLE III. THE SORT OF EFFECTIVE DMU EFFICIENCY BASED ON *DEA-TOPSIS* MODEL.

DMU	Bei Jing	Tian Jin	Hei Long Jiang	Shang Hai	Jiang Su	Guang Xi	Hai Nan	Qing Hai	Ning Xia
d_j	0.48	0.55	0.42	0.66	0.27	0.67	0.61	0.84	0.71
Sorting of *TOPSIS-DEA*	7	6	8	4	9	3	5	1	2

4.4 Analysis of Evaluation Results

(1) The overall results evaluation. There has 9 effective decision unit overall, the regional agricultural science and technology achievements transformation efficiency is 0.626 on average, there are17 provinces' transformation efficiency is lower than the average, minimum value is only 0.171, that shows the transformation efficiency is low in China, and there is a big difference among different regions. The table II shows that the traditional agriculture province such as Heilongjiang, Henan, Shandong, Sichuan, Jiangsu, the agricultural science and technology achievements transformation is also effective, the agricultural science and technology can also enhance the core competitiveness of agriculture. The transformation efficiency of agricultural science and technology achievements China has "hourglass" distribution situation in the region.

(2) Analysis of the evaluation results of effective DMU. The 9 effective DMU were sorted by using the DEA-TOPSIS model, the sorting result is Qinghai, Ningxia, Guangxi, Shanghai, Hainan, Tianjin, Beijing, Heilongjiang and Jiangsu. In this

evaluation, the transformation efficiency in Qinghai, Ningxia, Guangxi and Hainan is better than the rest agricultural strong provinces, this is because the DEA is a measure method for the relative efficiency evaluation, the rank of the DMU will in front which can have more output with less input, so those backward provinces and cities in agricultural should increase the input of agricultural science and technology, make its capacity to fully exploit.

5. CONCLUSIONS AND SUGGESTIONS

We got some conclusions and suggestions according to the evaluation results:

(1) The average value of the transformation efficiency of agriculture science and technology in China is 0.626, the overall transform efficiency is low, and the transformation efficiency of agricultural science and technology achievements China has "hourglass" distribution situation in the region, the distribution shows that the transform efficiency in China should be integral improved, or the "bucket effect" will influence the application of agricultural science and technology.

(2) Qinghai, Ningxia and Guangxi were ranking at 1, 2, 3 in effective DMU, shows that those backward provinces and cities in agricultural are fully capable to improve the level of agricultural development with the popularity and promotion in the agricultural science and technology achievements, the centre government should encourage and support those areas with the input of agricultural science and technology infrastructures in order to invigorated the idle transformation ability.

(3) There has a waste phenomenon in the traditional agricultural provinces of agricultural science and technology resources waste phenomenon, which can't transformed the resources of agricultural science and technology fully into real productivity, input can't match the output relatively, such areas can be considered appropriate to streamline the related investment in agricultural science and technology, optimize the allocation of agricultural resources.

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Cover page

Title: *Effects of Neodymium on Extracellular Polymeric Substances Secreted by Aerobic Granular Sludge*

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ABSTRACT

Rare earth elements have a distinct impact on the metabolism and extracellular polymeric substances (EPS) secreted by aerobic granular sludge. The EPS secreted by aerobic granular sludge includes two forms: loosely bound EPS (LEPS) and tightly bound EPS (TEPS). We grew sludge in sequencing batch reactors in the presence and absence of Nd^{3+}. For both modified sludge (modified by neodymium nitrate) and control sludge, there is more TEPS than LEPS and the content of LEPS is about only one-fifth of the content of TEPS. Adding a low dose of Nd^{3+} stimulated the content of polysaccharides in LEPS, and the content of protein in TEPS, thus promoting the stability of the aerobic granular sludge.

INTRODUCTION

Studies indicate that low levels of rare earth elements positively affect the growth and metabolism of animals, plants and microorganisms, known as 'hormesis' [1]. Hormesis means a factor has a positive effect on the organism when the dosage is at a low level; although a higher dose may have a negative effect on the organism. The rare earths have a distinct impact on the metabolism and extracellular polymeric substances (EPS) secreted by aerobic granular sludge [2-4] In theory, moderate doses of neodymium will improve the COD degradation performance of aerobic granular sludge because neodymium will promote the activated sludge to produce EPS which promotes the formation of granular.

EPS is a kind of high molecular weight polymer which microorganisms secrete in a particular surrounding. EPS includes protein, polysaccharide, humic acid and other components. Among these, protein and polysaccharide are the main ingredients [5].In EPS, 40% can be biodegraded as energy and 60% cannot be degraded, but the latter makes up the basic skeleton for granular sludge. So EPS plays a great role in wastewater treatment and it can also indicate the growth state of the microorganism in the water and granular sludge[6]. Some experiments have shown that there are two kinds of EPS in the aerobic granular sludge system; one is called tightly bound EPS (TEPS), which mainly attaches to the cell wall of the microbes and is also the basis of bioflocculation; the other is loosely bound EPS (LEPS), which is free in the water. The

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former is the main component of sludge EPS, and the latter is the main component of supernatant fluid EPS [7].

METHOD

Description of the Reactors

The two column-type Sequencing Batch Reactors (SBR1 and SBR2) used in this study. The sequencing batch mode of operation of the SBR is beneficial to the growth and consolidation of activated sludge. The reactors were made of acrylic are 80 cm in height and with 9 cm internal diameter, giving them each a working volume of 5.1 L. Air was introduced at the bottom of the reactors through a porous stone. The flow of air was controlled by a rotameter to about 0.25-0.3 m^3/h. The influent and the effluent are controlled by a peristaltic pump and an electromagnetic valve, respectively. The SBRs were operated in successive cycles of 4h. In every cycle, the volumetric exchange ratio was 50%.

Influent Medium and Inoculated Sludge

The inflow used in experiment was synthetic wastewater with the following composition: CH_3COONa (as the sole carbon source); NH_4Cl (as the nitrogen source); $MgSO_4$, $CaCl_2$ and micronutrients ($FeCl_3$ $6H_2O$, $CuSO_4$ $5H_2O$, $MnCl_4$ H_2O, $ZnSO_4$ $7H_2O$, $CoCl_2$ $6H_2O$, KI). The specific content is shown in Table 2-2. The inoculated activated sludge was obtained from the Jinan sewage treatment plant. Before placing the sludge in the reactors with the synthetic wastewater, it was aerated for 3 days to allow it to consume the existing nutrients.

Running Conditions

The two SBRs were run under the same operating conditions. However, we added $Nd(NO_3)_3$ into SBR1 as the Test Group, and not to SBR2 which was the Control Group. As shown in Table 1, the experiment of culturing aerobic granular sludge was divided into three phases: start-up, modification and maturity.

TABLE I. THE SPECIFIC CONTENTS OF THE SYNTHETIC WASTEWATER

Process Parameter/ Stage	Start-up			Modification	Maturity
COD/mg Ł$^{-1}$	300	600	800	800	800
NH_3-N/mg Ł$^{-1}$	30	60	80	80	80
TP/mg Ł$^{-1}$	3	6	8	8	8
Micronutrient/mg Ł$^{-1}$	1	1	1	1	1
Nd^{3+}/mg Ł$^{-1}$	0	0	0	1-5-10-15	0
Ca^{2+}/mg Ł$^{-1}$	90	90	90	90	40

Start-up:

At this phase, the COD of the influent was from 300 mg/L to 800 mg/L and the C: N: P ratio was kept at 100:10:1. Temperature was controlled between 22 ℃—28 ℃. The SBRs were operated in successive cycles of 4 h. At first, every cycle included 6 minutes of influent filling, 219 minutes of aeration, and 10 minutes of settling and 7 minutes of effluent withdrawal. In order to screen out the sludge with better settling properties of sludge, we gradually shortened the settling time to 6 minutes. The times of each step were the same in the next two phases. All processes were controlled by an electromagnetic relay, and the gas surface velocity was kept at 0.005-0.009 m/s to maintain the DO and hydraulic shear force. The startup phase lasted 14 days.

Modification:

The sludge was stable and the removal rates of COD and NH_3-N were high. At this point, we added $Nd(NO_3)_3$ into SBR1. For fear of toxic shock of Nd^{3+} to sludge, the concentration of Nd^{3+} was increased step by step, from 1 mg/L to 5 mg/L to 10 mg/L and finally to 15 mg/L. By testing the MLSS(mixed liquid suspended solids). The optimum concentration of Nd^{3+} was determined to be 10 mg/L. (Higher dosage will lengthen the adaptation time of the sludge and the MLSS will dramatic decline.) At the 24^{th} day of this phase, the activated sludge in SBR1 had completely transformed to aerobic granular sludge. The average diameter was 2.5 mm. The granules in SBR2 formed 12 days later than in SBR1. The average diameter was 1.5 mm. Sludge which contained the rare earth neodymium grew better and had more compact granules.

Maturity:

The aerobic granular sludge had already been shaped. But the experiment continued for another 15 days to stabilize the granular sludge system. We call this phase maturity. In this phase, the average diameter of the granules in SBR1 and SBR2 increased to 2.8 mm and 1.7 mm, respectively. And the quality of the effluent water was clear and stable.

RESULTS AND DISSCUSION

The Contents of LEPS and TEPS

The sludge was grown through 3 phases: startup, modification, and maturity, to produce stable granular activated sludge. SBR-1 was modified with addition of $Nd(NO_3)_3$ and SBR-2 was the control.

The content of the two kinds of EPS in the phases of modification and maturity, are shown in Figure 1.

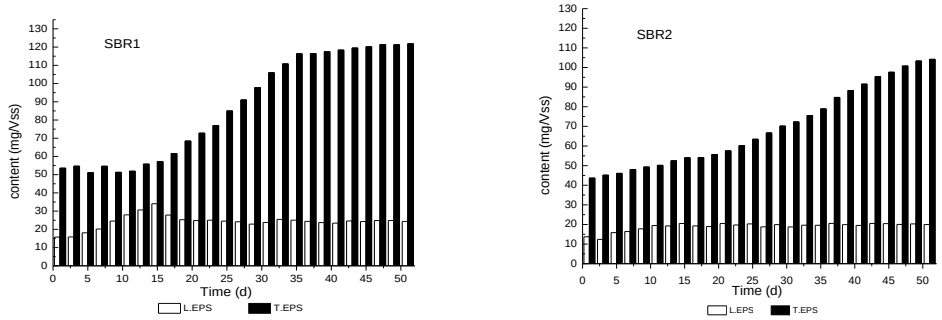

Figure 1 Comparison of LEPS and TEPS content in SBR1 and SBR2

In SBR1, the TEPS content did not change much in the first fifteen days of the modification process, remaining around the content of 53 mg/Vss. In contrast, the LEPS had a rapid growth from 15 mg/Vss to 35 mg/Vss. However, in SBR2, the content of LEPS was steady throughout the process of modification and maturity at about 15-20 mg/Vss. This result suggests that adding the rare earth neodymium could stimulate the microorganisms in the aerobic granular sludge to secrete more EPS to the liquid around them. The TEPS in SBR1 and SBR2 grew by the end of the whole process, but the content in SBR1 (121.8mg/Vss) was higher than in SBR2 (104.15mg/Vss). Thus, it can be concluded that Nd^{3+} could prompt the microorganisms to secrete more EPS, including LEPS and TEPS, but it had more influence on LEPS.

Composition of LEPS and TEPS

Proteins (PN) and polysaccharides (PS) are the main components of EPS. Their contents in LEPS and TEPS in both reactors are shown in Figure 2 and Fig 4 respectively and the ratio of protein/polysaccharide (PN/PS) is shown in Figure 3 and Figure 5, respectively.

In SBR1, during the first fifteen days of the modification process, the content of polysaccharides in LEPS grew rapidly from 12 mg/L to 23 mg/L. Then, it remained steady at around 17 mg/L. In SBR2, the polysaccharides did not change obviously, and the average value was about 14-15 mg/L, a little lower than in SBR1. The protein content in LEPS in the two reactors was similar. As the aerobic granular sludge developed, the content of protein increased from 3 mg/L to 9 mg/L, then remained steady at 7 mg/L. So, compared to protein, the polysaccharides in LEPS are more obviously affected by Nd^{3+}.

Figure 3 reflects the difference of the PN/PS ratio in SBR1 and SBR2. As a whole, the ratios in SBR1 are lower than in SBR2. From the above, the effect of Nd^{3+} is mainly focused on the polysaccharides in LEPS. Polysaccharide is the main hydrophilic material in EPS and it is the slime layer around sludge in LEPS. It protects the granular sludge. So, we can conclude that Nd^{3+}could improve the ability of the sludge to protect itself.

Comparing Figures 2 and 4, the main difference between TEPS and LEPS is that the protein content is lower than polysaccharide in LEPS. But in TEPS the contrary is the case. As is shown in Figure 4, the content of protein in TEPS is much higher than that of polysaccharides. From the general trend, the polysaccharides in TEPS remain at

a steady value. Comparing SBR1 and SBR2, we can see the total content of polysaccharides in SBR1 is higher; the average values of SBR1 and SBR2 are 17 mg/L and 14 mg/L respectively.

The protein contents in SBR1 and SBR2 are dissimilar. In SBR2, the content has no distinct change over the first fifteen days of the modification process. But during the next 20 days it rises quickly from 37 mg/L to 100 mg/L. In SBR2, the content rises steadily, from 31 mg/L to 85 mg/L. After a short period of adjustment, Nd^{3+} had a significant influence on the secretion of EPS by the microorganisms. However, the effect was mainly on protein secretion

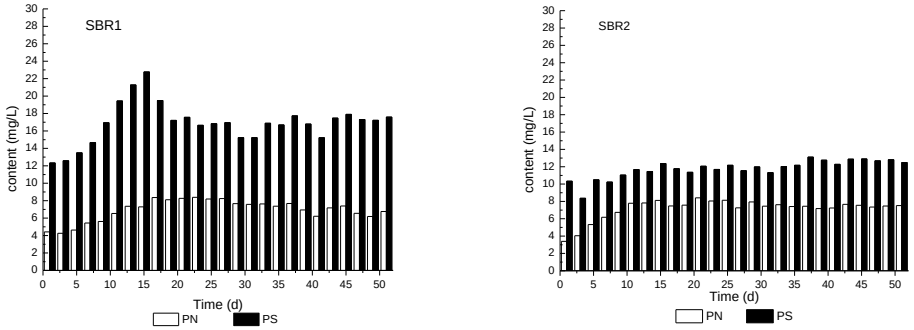

Figure 2 PN and PS of LEPS in SBR1 and SBR2

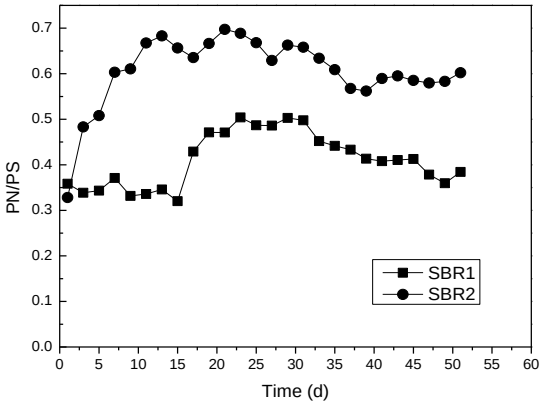

Figure 3 PN/PS of LEPS in SBR1 and SBR2

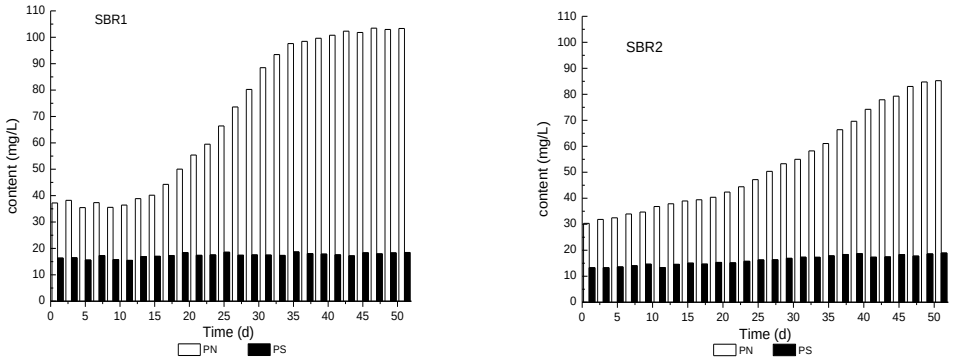

Figure 4 PN and PS of TEPS in SBR1 and SBR2

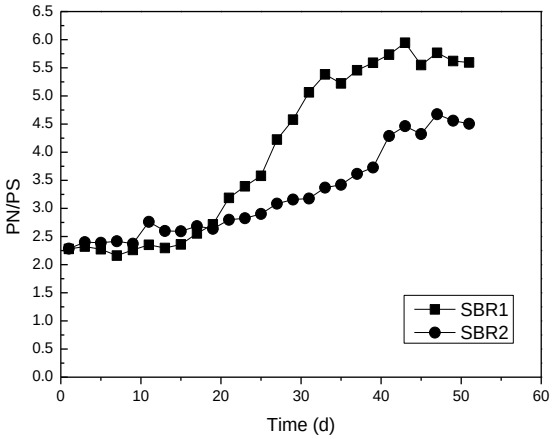

Figure 5 PN/PS of TEPS in SBR1 and SBR2

The ratio of PN/PS is obviously higher in SBR1 (Figure 5). Protein is the main ingredient in EPS, especially in TEPS. It is the main content of the basic skeleton in granular sludge. By stimulating protein secretion, Nd^{3+} strengthens the granular sludge. Also, as the protein is the main hydrophobic material in EPS, more protein in TEPS has greater bearing on the stability of aerobic granular sludge. So Nd^{3+} has a positive effect on the stability of aerobic granular sludge.

CONCLUSION

The extracellular polymeric substances (EPS) secreted by aerobic granular sludge include two forms: EPS in the supernatant (LEPS) and EPS in the sludge (TEPS). For both modified sludge and common sludge, there is more TEPS than LEPS and the content of LEPS is about only one-fifth of the content of TEPS.

In both the modified sludge and the common sludge, the main component in LEPS is polysaccharides. Adding a moderate amount of Nd^{3+} could stimulate the content of polysaccharides in LEPS, but had scarcely any influence on protein content. In this way, Nd^{3+} could improve the sludge self-protection. The main component in the TEPS is proteins. Nd^{3+} has notable influence on protein secretion into TEPS, but has little influence on polysaccharides. Therefore, Nd^{3+} has a positive, hormetic effect on the stability of aerobic granular sludge.

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Cover page

Title: *Relationship between Protease Activity and Biogas Rate during Anaerobic Digestion of Organic Fraction of Municipal Solid Waste*

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ABSTRACT

Hydrolytic enzymes, which are very key enzymes in hydrolytic step of anaerobic digestion, have an important effect on substrate hydrolysis and biogas production. To research those effect, this paper have investigated the relationship between protease activity and biogas rate with organic fraction of municipal solid waste (OFMSW) as substrate, at the mesophilic temperature and batch fermentation. The results showed that in the process of AD, the curve of protease activity was similar as that of biogas rate. With the increase and drop of enzyme activity, biogas rate increased and dropped. When biogas rate was 365 ml/d at its peak period, enzyme activity of protease was 7.52 μg tyrosine/ml.min at its peak period, too. It also indicated that there is an obvious co-relationship between protease activity and biogas rate in anaerobic digestion of OFMSW.

Key words: organic fraction of municipal solid waste (OFMSW); anaerobic digestion; protease; enzyme activity; biogas rate.

1. INTRODUCTION

Hydrolytic enzyme plays an important role in hydrolytic step of anaerobic digestion. Zhang Wudi etc investigated the relationship of various hydrolytic enzymes on biogas production rate of pig manure, and indicated that biogas production rate was related to hydrolytic enzyme activity [1]. Kim's research on the change of hydrolytic enzyme activity in digesting organic fraction of municipal solid waste (OFMSW) with a sequencing batch reactor at thermophilic temperature, showed that when protease activity was at very high level, the degradation rate of protein concerned was very fast[2]. Parawira etc also analyzed hydrolytic enzymes activity in two-phase anaerobic digestion of potato waste, and the result showed that the level of amylase activity is much higher than other hydrolytic enzymes, indicated a co-relationship between hydrolytic enzymes and substrate characteristics[3].

Based on the function of hydrolytic enzymes to catalyze substrate hydrolysis, some researchers added hydrolytic enzymes into anaerobic digestion, so that gas production was improved [1, 4, 5]. Other researchers also tried to investigate hydrolytic enzyme pretreatment, such as pretreatment with amylase in anaerobic

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digestoin of pig manure[6] and MSW[7], pretreatment with cellulase[8] in digesting MSW[8] and alcohol wastewater[9], and pretreatment with lipase in digesting high-fat wastewater[10-12]. These studies indicated that pretreatment group was better than control group.

Just as amylase, cellulase, and lipase mentioned above, protease could also promote biogas production, but there were few reports on protease pretreatment. This experiment will investigate the relationship between protease activity and biogas production rate using OFMSW as substrate, at mesophilic temperature and batch fermentation, developing a hydrolytic enzyme pretreatment techniques in anaerobic digesting OFMSW.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 RAW MATERIALS OF FERMENTAION

OFMSW was taken from a farmers market in Kunming City. Firstly, organic fraction was picked out from market wastes by manual sorting. Then, organic fraction was crushed and mixed by a garbage-crushing machine, with a total solids(TS) of 31.6%, a volatile solids(VS) of 24.39% (Based on total weight), and a pH value of 6.80. Finally, the crushed mixture was dried by thermostat oven at 105^{o}C and pulverized into powder by plant mill. The powder material was directly used as raw material of anaerobic digestion, some composition of this powder market waste as shown in table 1.

TABLE 1 PARTIAL COMPOSITION OF POWDER MARKET WASTE

Composition	TS	VS	mineral	Water- dissolved	Reducing sugar	Crude protein
%	98.28	75.87	12.80	31.77	3.96	16.00

2.1.2 INOCULUM

Inoculum was obtained from OFMSW activated sludge of anaerobic reactor in the National Engineering Center of Solid Waste Resources Recovery. TS of inoculum was 11.71%, VS was 7.95%, pH was 6.8.

2.2 Methods

2.2.1 EXPERIMENTAL DESIGN

This experiment was carry out by using batch fermentation at the temperature of 35℃.VS fermentative concentration was 6%, VS ratio of feedstock to inoculum was 2.5:1.

Before digestion was started up, loading quantity was calculated according to the following formula (1):

$$C_{VS} = \frac{W_1 \times VS_1 + W_2 \times VS_2}{W_0} \quad (1)$$

In formula, C_{VS} is VS fermentative concentration (%); W_0 is total amount of

fermentative mixture (g); W_1 is feedstock amount (g); W_2 is inoculum amount (g); VS_1 is the VS concentration of feedstock (%); VS_2 is VS concentration of inoculums.

In this experiment, 66 serum bottles, each bottle with a total volume of 250mL, were used as digesters and incubated under the same conditions. Three of them were used to determine biogas yield and methane content every day, and others were used to analyze enzyme activity.

With respect to activity analysis, operations were as follows. Firstly, pick up three serum bottles and put it into a freezer to terminate digestion when anaerobic digestion reaches the set time. Secondly, filter by using multilayered gauze, and centrifuge. Liquid centrifugated was transferred to a sample tube and stored in the freezer. Finally, accurately draw 1mL the centrifugated liquid every time to measure enzymatic activity according to analysis method introduced below.

Progress of the experiment was shown as table 2.

TABLE 2 EXPERIMENTAL PROCESS OF ANAEROBIC DIGESTION OF OFMSW

Digestion(d)	1-3	4-6	7-9	10-13	14-17	18-32
Sample interval(h)	8	16	24	48	96	124
Sample frequency(times)	9	4	3	2	1	3
Biogas yield and methane content	Using other 3 serums, recording and testing every day					

2.3 Analysis Methods

2.3.1 ANALYSIS OF RAW MATERIALS

(1) TS (Total Solid) and VS (Volatile Solid) were determined by the conventional analysis method of biogas fermentation [13].

(2) Soluble matter and reducing sugar. Weigh a certain amount of sample in beaker, add some distilled water into this beaker, and incubate at 50℃ thermostatic water bath for 30min, and then filter it. The filtrate was collected into a 100mL volumetric flask, adding distilled water up to the volume mark of flask. Take 2mL the liquid to test reducing sugar with DNS method.

The solid residue was dried in an oven at the temperature of 105℃. The difference between quality of raw materials and residues were the content of soluble substance.

(3) The protein content was determined by using a Kjeldahl method according to Liu Yu-lan etc. To improved and applied method for determination protein content of foods [14].

2.3.2 PROTEASE ACTIVITY

Definition of protease activity: 1g solid enzyme powder hydrolyzing casein to produce 1μg tyrosine in 1min was a unit of enzyme activity at the temperature of 40℃ and pH value of 7.5, indicated with U/g.

Principle of measurement: Folin reagent is extremely unstable in alkaline condition. It can be reduced by phenolic compound and appear blue. Due to proteins or its hydrolysate contains phenol-group amino acids, which can react with Folin reagent and appear blue, so this reaction can be used to determine the level of protease activity. In analyzing, casein used as substrate was hydrolyzed by protease at a certain pH and temperature, after hydrolyzing in a certain time, adding trichloroacetic acid to

terminate the reaction, and separating out hydrolysate by filtration method. Taking the filtrate (containning hydrolysate of protein), neutralizing with sodium carbonate, then reacting with Folin reagentr, using 721 spectrophotometer to determine the absorption rate.

Determination method: according to the standard of the People's Republic of china SB/T 10317-1999 (protease activity assay). Firstly, by using 721 spectrophotometer at the absorption wavelength of 680 nm, draw a standard curve by using L-tyrosine reacting with Folin reagent under the conditions of pH value 7.2, reaction time 10min. and then test sample at all the same conditions as that of drawing standard curve.

Protease activity of sampe was calculated according to the following formula (2).

$$\text{Protease activity} = VTK \quad (2)$$

In formula (2), V was the total volume of enzyme reaction, mL; T was the exact time of enzyme reaction, min; K was indicated with the tyrosine concentration of sample, gained from the standard curve at the absorbance value of sample, μg/mL.

2.3.3 BIOGAS RATE AND BIOGAS YIELD

Biogas production yield every day was tested by drainage gas-collecting method. The volume of biogas could be recorded from a cylinder.

3. RESULTS AND ANALYSIS

3.1 Change of Biogas Production

The curve of biogas production of OFMSW was shown in figure 1.

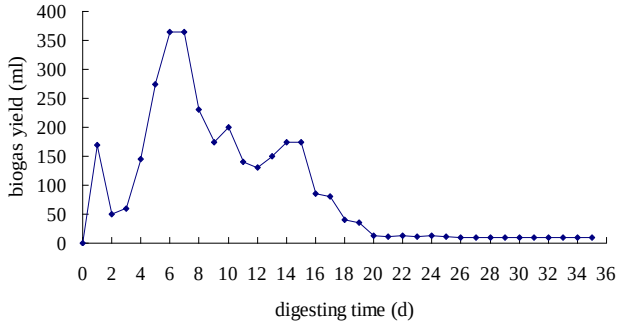

Fig.1 Change of biogas production in digesting OFMSW

According to the curve of biogas production as shown in figure 1, the process of anaerobic digesting OFMSW could be roughly divided into four periods: starting-up period (0-3 days), biogas rapid growth and peak period (4-9 days), sub-peak period (13 to 15 days) and biogas fade period (16 days later). In starting-up period, biogas production was very low. Though there was a peak of biogas in the first day, the gas was not real biogas, it was commonly known as a kind of "mixed gas" that contained air. After three days, anaerobic digestion went into biogas rapid growth and peak period. In this period, as shown in figure 1, daily gas production increased quickly from 60 ml/d at the 3rd day to 365 ml/d at the 6th and 7th day. After the 7th day, daily gas production began to decrease to 145 ml/d at the12th day. However, from the 13th

day to the 16th day, biogas production increased again, namely biogas sub-peak period. Beginning from the 16th, daily biogas production decreased gradually until biogas production termination. Such a change trend was similar with that reported in some literature, and indicated that this experiment was normal.

3.2 Relationship between Protease Activity and Biogas Rate

According to the set time in table 2, pick out the sample and analyze its protease activity, and then compare enzyme activity with biogas rate, as shown in figure 2.

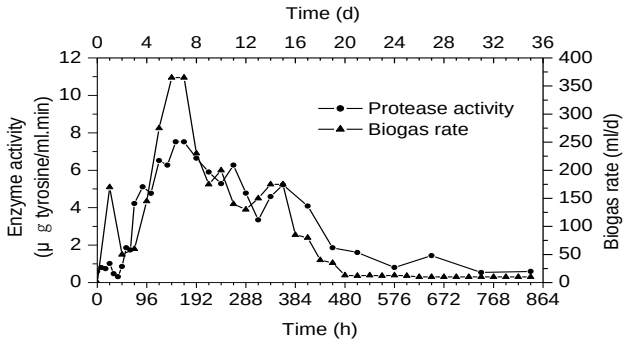

Fig.3 The relationship of protease activity and biogas rate

From the changing trend of two curves in figure 2, it could be seen that there was an obvious correlation between protease activity and biogas production. In starting-up period, biogas production and protease activity were relatively low. After starting-up periold, protease activity and biogas production increased rapidly. In biogas rapid growth and peak period, hydrolytic microorganisms began to grow and divide rapidly, secreting a lot of hydrolytic enzyme, so protease activity increased rapidly up to the highest level of 7.52μg tyrosine/ml.min at 152-168h (6-7 days), at the same time, biogas production rate also went up to the maximum value of 365 ml/d. After biogas peak period, protease activity and biogas production decreased to some extent. In biogas sub-peak period, protease activity and biogas production also increased again. After sub-peak period, with a large number of substrate consumed, enzyme activity and biogas production both decreased rapidly until biogas production termination.

The relationship between protease activity and biogas production as described above may be related to three stages of anaerobic digestion which are hydrolysis stage, hydrogen-producing and acetate-producing stage acid, methane-producing stage [15]. In three stages, the hydrolytic stage greatly affects anaerobic digestion. Hydrolysis was not only the beginning of the anaerobic digestion, but also the rate-determining step of methane production[16], so the faster rate of hydrolysis was, the faster speed of digestion and the greater of biogas production were. Furthermore, the rate of hydrolysis was often related to hydrolytic enzyme, therefore, the level of protease activity not only reflects the rate of protein hydrolysis, but also affects the rate of biogas production.

4. CONCLUSIONS

Experimental results above indicated that there was an obvious co-relationship

between protease activity and biogas rate in the process of anaerobic digesting OFMSW. With the protease activity increasing and decreasing in anaerobic digesting OFMSW, biogas production increased and decreased. When protease activity was 7.52 μg tyrosine/ml.min at peak level, biogas production rate was 365 ml/d at its peak period, too. So in order to promote substrate degradation and biogas production in digesting rich-in-protein OFMSW, pretreatment with protease in anaerobic digestion will be an effective method.

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Title: *A Research on Expanding Water Carrying Capacity of Karez in Extreme Arid Areas of Turpan Basin Based on Statistic Method*

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ABSTRACT

This paper analyzed the current situation and expanded carrying capacity of karez water located in Turpan Basin of Xinjiang Uyghur Autonomous Region of China, statistic data is from the two-year field investigation of this basin. Introducing the concept of saving lifestyle and traditional lifestyle, this paper proved the carrying capacity of limit-local water resource may be expanded to develop local industry and economy. The research converts consumed water of entire region into per capita value pertinent to saving lifestyle and traditional lifestyle, in which five aspects of industry, agriculture, living, health and courtyard as well consist of the basic indexes of carrying capacity. The difference of two lifestyles in effect of water reservation is analyzed and compared. The result from this research may be used for the other extreme arid areas in the southern part of Xinjiang Region where water scarcity restricted the development of local industry and economy.

INTRODUCTION

Turpan Basin is extreme arid area where water scarcity has been constraining the development of local industry and economy ever since a long time ago. As an extreme arid area in Xinjiang Region, annual evaporation and rainfall of Turpan Basin are respectively 3200mm and 16mm, water is very important for Turpan Basin and southern arid areas with same condition as Turpan. Karez structure consists of culverts and open channel and vertical shaft, which transmits the melt mountain water from Tian Shan into basin by avoiding evaporated under hot sunshine. Figure 1 shows the principle of karez structure in Turpan Basin. part of Xinjiang Region. Karez plays a very important role in local ecological protection and the local agricultural irrigation as well as the tertiary industry and tourism industry. Now, karez suffers from extreme, drought; the statistics data shows that there are 1091 karez in this basin in 2003, the length of karez culverts and channel are 3724.11km including 404 watery kazez with the total flow rate of 7.35 cubic meters per second, annual output water is 231 million cubic meters and total controlling irrigation area of 132300 acres, but the existing karez is only 200 in 2011. This study shows that the drought karez is 687 and 185 of them can be restored by repair and protection and 502 can no longer be recovered. Such rapid declination deeply affects the water demand in living and production, there are 360 million cubic meters of water is over exploited every year. Under this

condition, the method to save limited water resource is considered, this means to expand the carrying capacity of existing water.

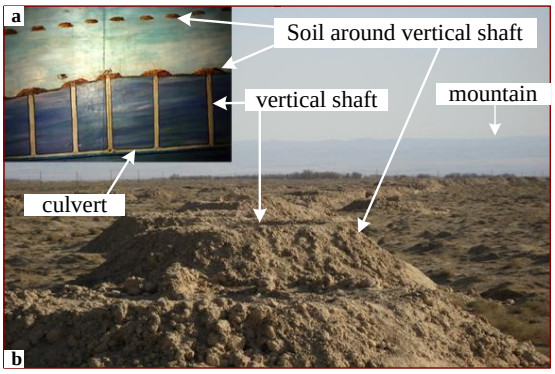

Figure.1 Karez structure in Turpan Basin
(Figure a is underground structure and b is ground surface structure)

STATISTICAND MATHEMATIC METHOD

General Procedure of Calculation

Fig.2 shows the related aspects of the carrying capacity of karez water resources in the calculation. This procedure considered the ecological environment and social economic system including population and livestock and other relates aspects in social economic system, the ecological system shows the related aspects in lands and complementary.

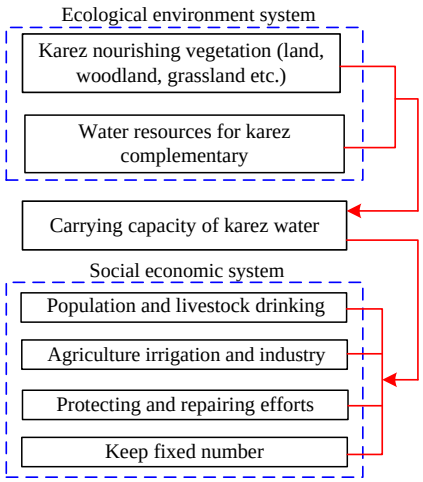

Figure.2. Related several aspects of carrying capacity of karez water resources for calculation

Statistic and Mathematic Basis

Weighted arithmetic average and weighted harmonic average value is used in this investigation as shown by equation (1):

$$\bar{x} = \frac{\sum xf}{\sum f} \quad \text{or} \quad \bar{x} = \frac{\sum m}{\sum \frac{m}{x}} = \frac{\sum xf}{\sum f} \tag{1}$$

Here, *x* and *f* and *m* are indicate the target elopement and frequency and number of samples related to investigation. Equation (2) indicates the standard deviation:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n\text{-}1}} \quad \text{or} \quad \sigma = \sqrt{\frac{\sum_{i=1}^{k} \sum \left(x - \bar{x}\right)^2 f}{\sum f}} \tag{2}$$

The meaning of alphabet is same as equation (1), *n* and *k* are respectively indicate the number of variation and group. The deviation coefficient is given by (3):

$$v_\sigma = \frac{\sigma}{\bar{x}} \tag{3}$$

In this research the standard score is also used for analysis on selecting the most efficient way to reserve energy, the index of standard score based on statistics method is shown in equation (4):

$$z_i = \frac{x_i - \bar{x}}{\sigma} \tag{4}$$

Developed type of (4) is equation (5) as shown below, *w* and *p* indicate energy consumed in transit and energy price of sample, and *s* is sample deviation.

$$z_{GEi} = \left| z_{Wi-j} + z_{Pi-j} \right| = \left| \frac{w_{i-j} - \bar{w}_i}{s_{Wi}} + \frac{p_{i-j} - \bar{p}_i}{s_{Pi}} \right| \tag{5}$$

The carrying capacity of water supply is shown as (6), here *Wk* is annual output water and *Wpu* is amount of water demand of traditional and saving lifestyle.

$$K_{CL} = \frac{W_K}{W_{PU}} \tag{6}$$

In this research, the times of population that can be supplied by karez water is considered to define carrying ability for local residents as shown in equation (7), here K_{CL} is water supply ability from karez and P_L is local population, hence, equation (7) can indicates the number of peoples that are nourished by the karez water.

$$K_{K-P} = \frac{K_{CL}}{P_L} \tag{7}$$

THE CONCEPT OF PHRASES APPLIED IN THIS PAPER

Saving Lifestyle

Based on statistic method mentioned above, the sample analysis is managed. To estimate the potential amount of conserving water, this research created per capita method namely saving lifestyle. Principle of saving lifestyle indicates the minimum water consumption in a person's life, but keeping suitable living demand. Consumed water in person's saving lifestyle integrates and represents all aspects related to people's life and converts the water to per capita value. The advantage of this method is easily applied for different arid areas with clear indexes, which may be used for comparing with traditional lifestyle formed in different areas and environment, and this method also shows entire consumed water in society system.

Traditional Lifestyle

Principle of traditional lifestyle indicates the normal water demand in a person's life, which usually formed in defined area and environment, this way lets people keep comfortable life with enough water demand. Consumed water in person's traditional lifestyle is managed by detail statistic investigation that reflects existing reality in water utilization. In this way, all aspects related to people's life are converted to the per capita value of water consumption. The advantage of this method is that it shows water utilization condition without wasteful behavior, hence this index has strong water saving character that provides the reasonable value in water consumption for local government.

Expanded Carrying Capacity of Water

The expanded carrying capacity of local water is an important index that shows the maximum potential in water supply and it equals to:

$$Q_{\exp} = Q_{tds} - Q_{svs} \tag{8}$$

Equation (8) indicates expanding carrying capacity equals to traditional lifestyle minus saving lifestyle. Because expanding carrying capacity is generated from entire society that relates to water utilization, the index reflects the full saving water.

APPLICATION RESULT

Resident's age is divided into four degree, less than 18 is the first degree (Deg.1) and 18 to 35 is the second (Deg.2), 35 to 60 is the third (Deg.3) and over 60 years old is the fourth degree (Deg.4) as well, other related indexes and data, such as amount of the water and feed demanded by livestock are omitted here. Table 1 indicates the annual water consumption converted from per capita's meat demand. Considering the page limit, the annual water consumption converted from other life's necessities, such

TABLE I. CONVERTED PER CAPITA WATER CONSUMPTION FROM MEAT DEMAND

lifestyle and index	Amount of meat consumed by per capita [kg/a]			
	Deg.1	Deg.2	Deg.3	Deg.4
traditinal lifestyle	50.40	46.80	46.80	54.00
saving lifestyle	30.96	28.80	28.80	33.12
Water equivalent of meat	Lamb: 48.75 [L/kg]		Beef: 120.27[L/kg]	
structure coefficient in consumption	Lamb: 0.800		Beef: 0.200	
Water equivalent of meat under structure coefficient	63.054 [L/kg]			
amount of water cacoused by meat consumptiom [L/a]	3178 Tra.lifestyle	2951 Tra.lifestyle	2951 Tra.lifestyle	3405 Tra.lifestyle
	1925 Sav.lifestyle	1816 Sav.lifestyle	1816 Sav.lifestyle	2088 Sav.lifestyle
average amount of water from meat consumptiom [L/a]	under traditinal lifestyle: 3121 [L/a] under saving lifestyle: 1918 [L/a]			

as fruit, vegetable, cereals and so on is omitted here. In Table1, the structure coefficient and water equivalent of meat is investigated and calculated based on the statistic samples. The research proved that the results deeply affected by local environment and life habit. The water equivalent of per capita is given by traditional and saving lifestyle. Due to page limit, the theoretical description of saving and traditional lifestyle is omitted; Table 2 and 3 are part of reference tables. Based on related data base like Table 2 and 3, the tables that represent annual water consumption concerted from the other aspects can be determined as Table 1.

TABLE2. IRRIGATION INDEX

lifestyle	daily consumption of cereal[g/d]			
	Deg. 1	Deg. 2	Deg. 3	Deg. 4
traditional lifestyle	500	650	650	450
saving lifestyle	475	618	618	428

TABLE 3: DAILY CONSUMED CEREAL

	Crops and irrigation water [m3/mu]			
	wheat	corn	melon	grapes
irrigation quota	345	366	600	800

As the results, the converted water consumption of per capita from six aspects is shown in Table 4, number 59476 and 82381[L/a · per capita] represent full water consumption of society. Since per capita is applied, the result in Table 4 may be used to estimate the potential water that can be used for developing industrial park in an arid areas, and this result also provides the information of how to approach progressively to the level of saving lifestyle in water demand. The investigation result proved that the efficient way to reach level of saving lifestyle should start from agriculture, this research also shows there are big saving effect in irrigation way.

TABLE 4. CONVERTED PER CAPITA WATER CONSUMPTION FROM SIX ASPECTS

lifestyle	related aspects to water consumption					
	people and livestock	courtyard and health	meat	cereals	vegetable	fruit
Tra. lifestyle [L/a • PC]	5823	37467	3121	26285	2700	6985
Sav. lifestyle [L/a • PC]	----	27045	1918	24970	1800	3742
in total	Tra. lifestyle: 82381[L/a • PC]			Sav. lifestyle: 59476[L/a • PC]		

Note: PC indicates per capita in Table 4.

Table 5 shows expanded water carrying capacity and multiple of population under traditional and saving lifestyle. It is clear there is big difference in carrying capacity of population under the saving and traditional lifestyle, which hints big saving effect of water. Carrying multiple indexes is derived from existing population and the carry-able population under two lifestyle. According to local ecology condition, the townships where carrying multiple over 2.5 may be regarded to develop industrial park considering type of industry. The expanded carrying capacity of water can be easily obtained from number of population presented in Table 4.

TABLE 5.VALUE OF EXPANDED CARRYING CAPACITY AND MULTIPLE OF POPULATION

Tur.Pre.	Township name	existing water supply [m3/a]	carrying index of population by Tra.lifstyle	carrying index of population by Sav.lifstyle	carrying multiple of population under Tra.lifstyle	carrying multiple of population under Sav.lifstyle
Turpan city	Sheng Jing	1985679	24104	33386	0.9527	1.3196
	Grape Valley	9878008	119906	166084	4.4477	6.1606
	Yar	16063847	194995	270090	3.4103	4.7237
	Chiat Kale	30037133	364612	505029	10.6906	14.8076
	Edin Tahu	9396518	114062	157988	5.3390	7.3951
	Yuan chongchang	3473073	42159	58395	17.7960	24.6494
shan shan county	Qiketai	7627323	92586	128242	5.6188	7.7826
	Tu Yugou	2118182	25712	35614	1.0356	1.4345
	Xue Zhan	3019576	36654	50770	1.3846	1.9179
	Dikan Er, Dalang Kan	2642596	32078	44431	4.2442	5.8787
	Lianmuqin	21303689	258600	358190	7.5760	10.4936
	Lukeqin	1261951	15318	21218	0.4763	0.6597
Tohsun county	Guo Le buyi	17454943	211881	293479	9.9163	13.7351
	Ira Tahu	1735043	21061	29172	1.2348	1.7104
	Bostan	616	7.4757	10.3548	0.0003	0.0004
	Xia	5934643	72039	99782	2.3072	3.1958

CONCLUSION

This research proved that the water consumption in extreme arid areas may be estimated and shown by traditional and saving lifestyle, which may be an important basis for establishing industrial park in an arid area. Saving lifestyle indicates the potential capacity of water that can be used for developing local industry and economy, and this index is essential in planning of future development in industry. Potential water also forms the optimal size of local population in urban and rural construction. It is expected to apply the research result to other arid areas in southern part of Xinjiang Region, where local industrial is constrained by water resource.

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Cover page

Title: *Emergy Analysis of Agricultural Eco-economic System Before and After Grain for Green in the Northwest of China*

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ABSTRACT

Comparative study is made by emergy analysis theory and method in terms of resource investment and yield on resource utilization, environmental stress, emergy index change and eco-economic system of Beizhuang Village before and after implementation of grain for green policy in the Northwest of China. The results indicate that, before grain for green, the total yield emergy of agriculture eco-economic system of the village was 7.53E+18 sej and, after grain for green, it reduced by 1.50E+17 sej, and the yield emergy of livestock products indicated a rise. The system stability index increased from the former 0.8091 to 1.0147. There was nearly no change in emergy investment before and after grain for green in this region. After grain for green, net emergy yield increased by 35.27%, and environmental load decreased by 8.31%, and system development sustainability increased by 47.38%. Pasture emergy yield increased by 148.12%, but stock raising yield only increased by 16.13%. The coupling coefficient of farming and animal husbandry has decreased by 41.74%. Development of stock raising was seriously delayed. In order to ensure more sustainable and stable development of agricultural ecosystem of this region, it is necessary to further adjust agricultural structure, take measures of high-efficiency stabling flocks and herds raising, continue develop stock raising, establish good farming and animal husbandry system emergy feedback mechanism, increase resource productive efficiency and finally realize coordinated and sustainable development of agriculture eco-economic system.

INTRODUCTION

Substance and energy are the most basic metabolic elements in the nature. All components and functions of natural system, human economic system and social system are involved with energy flow, conversion, reserve and dissipation. Energy is one of the indexes reflecting physics world, chemistry world and biology world. It an be used to represent the essence of nature and relationship between life and environment as well as human and nature[1-2].Since emergence of emergy theory, America pioneered to develop emergy research in 1980s[2-3]. Ulgiati and Brown, etc had made empirical study on eco-economic system of specific regions or drainage areas in Italy, Thailand and other countries. Japan also developed fast in 1990s. There were also many scholars in some developing countries of Asia, Africa and Latin

America starting emergy research [4-5]. In 1990s, some Chinese scholars applied emergy theory in eco-economic system analysis and made a series of case study [3][5-7]. In recent years, emergy theory and method have been applied in comparative analysis of agricultural mode [3][7-10], and the research method and index system have been continuously improved [11-12].

However, there have been few emergy studies on the agricultural ecosystem of loess plateau which is facing serious soil erosion. Especially fewer studies have been made on the emergy of influence on agricultural ecosystem by implementation of the policies of "Development of the West Regions" and "Grain for Green" raised in recent years. Accordingly, this article based on emergy analysis theory makes a comparative analysis on emergy structure and development level of agricultural ecosystem of typical villages in loess plateau before and after implementation of "Grain for green" policy; by means of quantitative research on system investment-yield emergy, gives an objective and accurate cognition and evaluation of the relationship between emergy investment and yield of agricultural eco-economic system as well as the real contribution of productive force level and natural resource to the social economy; provides theoretical reference to sustainable development of eco-economic system of ecologically fragile areas and enrich emergy theory. This is also quite significant to further implementation of "grain for green" policy, industry restructuring and ecological management, and provides theoretical reference to rational exploration of agricultural environment resource, sustainable agricultural development and assessment.

SURVEY REGION AND RESEARCH METHOD

Survey Region Summary

Beizhuang Village locates in southwest of Qingyang city in Gansu Province, which is in the ecologically fragile belt, agriculture-husbandry intermediate belt, semi-humid and semi-arid transition belt, attributed to loess plateau gully area with the geographical position at longitude 106°55' east and latitude 35°43' north. With a mean altitude of 1500 m, it belongs to continental climate with an annual average temperature 9.9℃, above 10℃ accumulated temperature of 2842℃, annual sunshine hours 2249～2437h, annual rainfall 403.8 mm, non-frost period about 150 d. It is a village mainly depending on crop farming. As a typical village of loess plateau, this village has both highland and sloped farmland with typical hill and gully landscape of loess plateau. The areas hit by soil erosion is 81% of the total land area, and the soil erosion modulus is 6211t/km^2 a. Serious soil erosion has resulted that the rich land on upper soil layer was washed away and fertility decreased, which led to low productivity, low and unstable grain yield, severely restricting sustainable economic development. The village has a population of 249 and arable land 38.87 hm^2, including 27.93 hm^2 highland and 10.94 hm^2 sloping field. This village is specified as the "grain for green" demonstrative base in the national strategy of western regions development, and 10.94 hm^2 sloping field has been gradually replanted with high-quality alfalfa during the period from 2004 to 2006.

Methodology and Data Source

In principle, emergy-based analysis can be conveniently used to measure and aggregate all heterogeneous natural resources and human-helped products. As the biosphere is usually thought driven by solar energy and most kinds of available energy are assumed derived from solar energy directly or indirectly, emergy as abbreviation of embodied solar energy is accepted as a common measure. Solar transformity, i.e. solar energy joules per unit energy joule in unit of sej/J, is used to measure the quality of resource and its position in the universal energy transformation hierarchy. The larger the transformity, the more solar energy required for the production and maintenance of the resource, product or service of interest, and the higher their position in the energy hierarchy of the universe [2,13].

Inputs to agriculture are generally categorized into four types: free local renewable resources (RR), such as sunlight, rain and wind; free local nonrenewable resources (NR), soil erosion, for instance; nonrenewable purchased inputs (NP), such as purchased fossil fuels and chemical fertilizers; and renewable purchased inputs (RP), such as water resources taken from outside. The system output (U), theoretically equals to the total inputs (RR+NR+NP+RP) in Odum's energy diagram, is different from the economic yield (Y) which includes the total products from cropping industry, forestry and livestock, respectively.

The detailed items associated with five kinds of fluxes (RR, NR, NP, RP, Y) are listed and calculated in Table 1 and Table 2. Most of the data are from inveatigated the official and farmars of before and after grain for green in 2003 and in 2007. On the basis of the fluxes mentioned above (RR, NR, RP, NP, U, Y), a series of system indicators are introduced and elucidated in detail [2,4,10,14,15].

Table 1 Emergy flow of agricultural eco-economic system of Beizhuang

Item		Expression	Before grain for green	After grain for green
Emergy investment	Renewable rerources from free environmental	RR	8.46E+16	8.50E+16
	Nonrenewable resource from free environmental	NR	2.41E+16	1.97E+16
	Nonrenewable purchases	NP	1.02E+17	9.56E+16
	Renewable purchases	RP	4.77E+16	4.43E+16
	Total environmental investment	RR+NR	1.09E+17	1.05E+17
	Total nonrenewable resources	NP+RP	1.49E+17	1.40E+17
	Total inputs	U=RR+NR+NP+RP	2.58E+17	2.45E+17
Emergy yield	Crop farming	Y_1	1.89E+17	2.21E+17
	Fruit tree planting	Y_2	1.19E+16	1.30E+16
	Stock raising	Y_3	1.85E+17	2.16E+17
	Government subsidy	Y_4	0.00E+00	3.80E+16
	Total emergy yield	$Y=Y_1+Y_2+Y_3+Y_4$	3.86E+17	4.88E+17

RESULT AND ANALYSIS

Emergy Investment Rate

Emergy investment rate is an index for measuring economic development degree and environment load. It is used to measure the competitiveness of economic activity under a certain circumstance and can detect the bearing capacity of environmental resource condition to economic activity. This index shows the utilization degree of the system to environmental resources. Besides, this index is also restricted by development policy, economic and technological level and other factors. There was nearly no change on emergy investment of Beizhuang before and after grain for green, respectively 1.37 and 1.34, lower than average of Gansu (2.08), Xinjiang (2.14), Inner Mongolia (4.56), Jiangsu (6.05), Zhejiang (2.56), China (2.33), world (2.00), Thailand (8.52), U.S.A.(7.00), and higher than that of Jiangxi (0.46). This indicates that utilization of each unit environmental resource is invested with little purchasing emergy, exerting less pressure to environmental resource. However, it also indicates that this village has good environmental resource condition for development and utilization. To be competitive enough, a system should have proper assortment of low-emergy renewable resource and high-emergy energy, i.e. adequate ratio of emergy to investment, to ensure high economic yield.

Table2 Comparison of emergy indexes of agricultural ecosystem before and after grain for green

NO.	Indices and indicators	Expression	Before grain for green	After grain for green
1	Emergy investment ratio (EIR)	(NP+RP)/(RR+NR)	1.37	1.34
2	Emergy yield ratio (EYR)	Y/(NP+RP)	2.58	3.49
3	Environmental load ratio (ELR)	(NP+NR)/(RR+RP)	0.95	0.89
4	System dominance degree	$\sum(Y_i/Y)^2$	0.4707	0.4078
5	System stability degree	$-\sum[(Y_i/Y)\ln(Y_i/Y)]$	0.8091	1.0147
6	Environmental sustainability index (ESI)	EYR/ELR	1.26	1.86
7	Agriculture-husbandry coupling degree	*	4.85	2.83
8	Emergy labor productivity	Y/t	6.84E+11	9.69E+11
9	Emergy density	Y/area	9.65E+11	1.22E+12
10	Emergy per capita	Y/population	1.54E+15	2.00E+15

*.Agriculture-husbandry coupling degree = $\frac{\text{Total emergy of animal product}}{\text{grain emergy} \times \text{utilization factor} + \text{emergy of pasture and stalk}} \times \frac{\text{high mass energy product emergy}}{\text{low mass energy product emergy}}$

New Emergy Yield Ratio (EYR)

New emergy yield ratio is an index for measuring system productive efficiency; the bigger ratio, the higher system productive efficiency; besides, net emergy productive efficiency reflects whether the system has advantage in economic input emergy and competitiveness in regional economic activity, representing to a certain

extent sustainable development of the system [7], and whether economic process is capable of providing basic resource to economic activity. After grain for green, the system net emergy yield ratio increased from 2.58 to 3.49, both of which are higher than the average emergy yield ratio of national agriculture (1.42). It indicates that the agricultural eco-economic system is of high emergy utilization rate, which has been increased further after grain for green. This is because that the purchasing emergy of agricultural eco-economic system of Beizhuang is of low investment proportion and production depletes high proportion of free natural environmental resource. After grain for green, the investment on purchasing emergy decreased by 6.27%, but the yield emergy increased by a large margin, forming a kind of agricultural eco-economic system of high return rate.

Environmental Loading Ratio (ELR)

ELR means nonrenewable emergy amount which unit renewable emergy amount bears. It is used to measure load degree of natural environment as an alert to economic system. High environmental loading ratio indicates that there are high-intensity emergy utilizations existing in economic system, keeping high pressure to environment system. If the system remains in high environmental loading ratio for a long term, irreversible function degradation or loss will happen to the system. In view of emergy analysis, external emergy input and over development of local nonrenewable resources are the main reasons for rising of environmental loading ratio. Table 4 indicates that the ELR of Beizhuang after grain for green decreased from 0.95 to 0.89, both of which are higher than Xinjiang (0.483) and Japan (0) but lower than China average level (9.89), Gansu (3.73), Inner Mongolia (3.13), Jiangsu (43.53), Zhejiang (11.25), U.S.A.(9.32), Italy (9.47) , Sweden (9.03) ,Thailang(1.04) and the world average level(1.5). This shows that grain for green has contributed in relieving environment system pressure of Beizhuang village. The pressure borne by environment is affordable to the ecological environment. It is still in large potential of resource development and utilization. It is necessary to enhance investment and develop artificial pasture stock raising of industrialized and intensified operation so as to increase the emergy yield.

Emergy Sustainability Index (ESI)

After long time span (1945-1994) analysis of Emergy Based Sustainability Index (ESI) of American system, Ulgiati et al stated that, as a relative assessment index, when ESI＜1, the system is a high consumer-driven economic system; when 1≤ESI＜10, the system is in a dynamic and potentially developing stage; when ESI≥10, it is a sign of underdeveloped economy, meaning that the system is in short of development and utilization of the resources [16].Gansu and Xinjiang are respectively 44.19 and 64.10, while Jiangsu and U.S.A. are 0.18 and 0.48. ESI of agricultural system of Beizhuang increased from 1.26 to 1.86 after grain for green. Comparing with that before grain for green, the agricultural system after grain for green had increased the productivity when environment load only increased a little. It is of great development potential, indicating that further implementation of grain for green policy will play an optimizing role for the system. However, comparing with other countries and regions, ESI of agriculture ecosystem of this region is relatively low. Therefore, it

is necessary to increase system yield capacity as much as possible on the condition of reduced load on environment in respect of gradual implementation of grain for green policy and adjustment of system structure so as to enhance the sustainability of agriculture eco-economic system of the drainage area.

Production Dominance and Stability

The dominance of agriculture ecosystem reflects the production unit equitability of integral structure[1]. Judging from Table 4, the agriculture ecosystem dominance decreased from 0.471 to 0.408 after grain for green, both of which are lower than the system dominance of Quzhou County of Hebei Province in the corresponding period (0.449) [5]. The main features are fruit tree planting subsystem is of low emergy yield and accounts for small proportion in the total emergy yield of system. Although the emergy yield of fruit tree planting system increased by 9.63% after grain for green, the emergy yield of fruit tree planting in 2007 only accounts for 2.67% in the total emergy yield, which is relatively quite small. Consequently, it is necessary to enhance fruit tree planting and increase production dominance of system. System stability index indicates the degree of stability of system production. The stability of agriculture eco-economic system increased from 0.809 to 1.015 after grain for green, indicating that the policy of grain for green has improved substance flow and energy flow connection network of agriculture ecosystem and enhanced system auto-regulation, adjustment and feedback function, leading to modulation of agricultural production structure.

Agriculture-Husbandry System Coupling Degree

Crop farming and stock raising are two interrelated systems. Activity of all kinds of flow (such as substance and energy flows) between the two systems has created a compound agriculture-husbandry eco-economic system. Coupling of agriculture and husbandry is considered as an important way for improvement of productivity. Agriculture-husbandry coupling degree can be used to measure the coupling degree of agriculture and animal husbandry and provide more theoretical reference for improving regional system productivity and development sustainability [17].

The ideal agriculture-husbandry conversion mode is to convert primary product of low mass energy into secondary product of high mass energy by means of grass-feeding livestock. Within the range of basic balance of pasture and livestock, the higher system coupling degree, the better coupling between agriculture and animal husbandry systems.

It can be seen from Table 4 that the coupling degrees of agriculture and husbandry systems of Beizhuang Village before and after grain for green are respectively 4.85 and 2.83, and the utilization rate of effective emergy of agriculture and husbandry systems are 2.08 and 1.64, both of which have decreased. On the whole, the agriculture-husbandry coupling degree of Beizhuang is higher than that of Wuchuan County of Inner Mongolia (2.68) [18]. Down the ages Beizhuang Village had the tradition of feeding cattle with crop stalks but not raising grain-consuming livestock such as pig. The internal breed structure of stock raising is relatively rational, which is the reason why agriculture-husbandry coupling degree is high. After grain for green however, the system coupling degree actually decreased by 41.74%, and the utilization

rate of effective emergy of system also decreased by 20.96%. It is mainly because that after grain for green the area of alfalfa growing increased a lot while the farmers kept the traditional extensive stock keeping mode instead of expanding raising scale, while resulted that a great deal of high-quality pasture was utilized in low efficiency and high dissipation. The pasture emergy yield increased by 148.12%, but stock raising yield only increased by 16.13%, which is far lower than increasing scale of pasture, leading to decrease of utilization rate of effective emergy of agriculture-husbandry system and consequently decrease of agriculture-husbandry system coupling degree. Therefore, stock raising should, on the premise of maintaining the original and rational internal breed structure, continue to develop raising of herbivore such as flocks and herds; besides, it is necessary to change traditional extensive raising mode, make use of fine breed, warm barn feeding and modern raising technology, and depending on pasture growing area, determine livestock amount and properly enlarge raising scale, and finally realize balanced development of crop farming and stock raising and change resource advantage into economic advantage.

Emergy-Labor Productivity

Emergy-labor productivity uses solar emergy to indicate labor product, equal to system emergy yield divided by labor time invested in system. Comparing with traditional labor productivity, this application can better represent the whole accumulated value including resource environment value. After gain for green, the emergy-labor productivity of Beizhuang Village increased by 41.7%, and it reached 9.69242E+11sej/h in 2007. Although grain for green decreased labor investment and emergy-labor productivity increased to a extent, the overall labor productivity is still relatively low and requires further improvement.

Emergy Density

Emergy density describes emergy flow in a unit area within a unit time, which can be used to measure emergy utilization and intensification in a country or region as well as flow consistence of emergy in the space. In highly developed countries or regions, because of frequent economic activity and large emergy conversion and utilization amount, the emergy utilization intensity is inevitably high. However, high emergy density indicates that the land will be a limiting factor against economic development in the future. This index of Beizhuang is close to average Chinese level before grain for green; after grain for green however, it is higher than many other countries and regions. This is due to increase of utilization efficiency of resources in agriculture eco-economic system after grain for green. Generally speaking, the emergy density of agricultural country or region is about $11.3\times10^{11}\sim41.0\times10^{11}$ sej/(m^2 a) [7]. Higher emergy density indicates more frequent economic activity in this region. The more advanced economic development, the higher position in the grade.

Emergy Percapita

Emergy per capita is an index which reflects people's living standard, living quality and economic development degree in respect of ecology. In this way all modes of substances and energy flow and economic flow are converted to the

emergy of unified standard for comparative analysis. The real wealth possessed by individuals includes economic emergy which can be embodied by currency as well as the emergy provided free of charge by natural environment which has not been marketized or monetized. So this index is more scientific and comprehensive than the traditional index of income per capita. Emergy application amount per capita can be usually used to reflect utilizability degree of real wealth such as resource, money and commodity but not to reflect the living quality and standard in sociology perspective (such as high educational level). In consideration of macroscopic ecological economic energetics, it is more scientific and comprehensive to measure people's living standard and quality by means of emergy per capita than by means of traditional economic income per capita. As restricted by total emergy and population, the emergy utilization amount per capita of Beizhuang before grain for green is 1.54×10^{15} sej, and it increased to 2.00×10^{15} sej after grain for green, both of which are lower than that of China and world. This is due to relative dense population and also indicates high developing potential of this drainage area.

DISCUSSION AND CONCLUSION

Initiation of emergy concept has provided theoretical and method basis for measuring and comparing energy movement of different types and levels, and has given solution to the problems of additivity and comparability of different energies which traditional energy analysis is confronted with[8]. This theory is a method for quantitative analysis combining ecosystem and economic system, which can be used to give a quantitative assessment on nature, economic system of human society, real value of resource and environment as well as their interrelationship, and perform objective measurement of resource, product and macroeconomy value. Emergy theory has provided a new idea for analysis of eco-economic system[7, 13].

After grain for green, the agriculture ecological and economic system structure of Beizhuang has changed a lot. Auto-regulation, adjustment and feedback functions of all internal subsystems have been enhanced, and the system productivity has also improved. However, comparing with other regions, the resource utilization efficiency is lower, and the structure is not adequately harmonious. In order to realize more harmonious development of agriculture eco-economic system, it is necessary to practically develop pasture growing, enhance pastureland construction, recover ecological balance and improve ecological bearing capacity of system; to prevent land erosion and natural disaster and protect agriculture production based on integrity of biological and engineering measures, traditional transactions and scientific technologies; to enhance opening degree and capital investment of agriculture ecological and economic system as well as agriculture-based facility construction, improve agriculture production condition, increase organic fertilizer investment, enhance agriculture production efficiency, stability and continuity; besides, to adjust agriculture production structure based on local circumstance, make full use of various biological resources, convert all kinds of organic matters in all levels of agricultural production into vegetal, zoogenic and deep-processed products, and establish ecological agriculture mode of sustainable development [9].

After implementation of grain for green policy, the total yield emergy of agriculture eco-economic system of Beizhuang Village was 4.8713E+17sej, increasing by 26.31% comparing with that before implementation. Before and after grain for green, the proportion of Alfalfa in yield emergy of crop farming was respectively 6.11% and 16.8%, and the farm product productivity in unit area increased from 5.00E+15sej/hm^2 to 5.83E+15sej/hm^2, indicating that implementation of grain for green has led to change of farm product growing structure and increase of productivity, and has supported basic grain ration of farmers and increased their income. After grain for green, the net emergy yield ratio of agriculture ecosystem increased by 35.14%, environmental loading ratio decreased by 8.31%, system development sustainability index increased by 47.38%, and system stability index increased from 0.809 to 1.015, indicating that grain for green policy has enhanced agriculture ecosystem productivity of Beizhuang and improved agriculture ecological environment. It is good for stable and sustainable development of agriculture ecosystem and should be reinforced continuously. After grain for green, the agriculture-husbandry coupling degree decreased by 41.74%, and pasture emergy yield increased by 148.12%; however, stock raising only increased by 16.13%, which is far lower than that of pasture, leading to decrease of effective emergy utilization rate of agriculture-husbandry system and consequently decrease of agriculture-husbandry system coupling degree. It is necessary to continue developing raising of herbivore such as flocks and herds; besides, it is necessary to change traditional extensive raising mode, make use of fine breed, warm barn feeding and modern raising technology, and depending on pasture growing area, determine livestock amount and properly enlarge raising scale, and finally realize balanced development of crop farming and stock raising and change resource advantage into economic advantage. The "Enterprise-artificial pasture-mutton" system of Chifeng City of Inner Mongolia is a paradigm of success [19].

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Cover page

Title: *A Control Strategy for Smooth Switching of Microgrid Operation Modes*

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ABSTRACT

To meet the requirement of islanded and grid-connected operation of microgrid, control strategies are proposed to realize the smooth switching between the two modes. PQ control is to ensure the balance of the power of microgrid in the grid-connected operation and a coordinate rotational transformation based virtual power V/f droop control method is to decouple the real and reactive power to provide the support of voltage and frequency in islanded mode. As considering the situation of reconnecting to the grid, a pre-synchronizing controller is designed. This control unit makes sure the system is stable and safety in the parallel connection. A simulation microgrid system is established with MATLAB/Simulink software and the simulation results show the effectiveness of the proposed smooth switching method and strategies.

1. INTRODUCTION

In the low-carbon context of the core of achieving smart grid, the microgrid is considered an important part of smart grid which reduces energy consumption and improves the reliability and flexibility of power system. The key to achieve technical and economic advantages is the ability of the two modes' operation – grid-connected mode and islanded mode. However, the most important part is the smooth switching between the control strategies used for the different modes of operation to ensure the stability of the switching process.

Research and implementation of the control of microgrid have increased in last few years in several ways. Literature [1] uses the improved droop control strategy, while control strategy unchanged when the mode switched to fit the plug-and-play feature. It did not take the restraining of impact current in consideration, and in the grid-connected mode, output power deviates from the nominal operating point of the

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droop control due to fluctuations in the grid voltage and frequency. Literature [2] suggests a dual-mode inverter where PQ control is used in parallel connection and droop control is adopted in the islanded mode. But the traditional droop control assume that the microgrid line was pure inductive or resistive, it lacks applicability that line inductance of the low voltage microgrid can't be ignored.

This article mainly introduced the structure of microgrid and control strategies of the different operation modes – grid-connected mode using PQ control, island mode using coordinate rotational transformation based virtual power V/f droop control strategy. A pre-synchronizing controller is added to keep voltage and frequency stable. The simulation results proved the effectiveness and excellent performance of the proposed control strategy.

2. THE STRUCTURE OF MICROGRID

Microgrid is a single controllable independent power supply system which combined distributed generation, load, energy storage device and a control device and can smoothly access power grid and independent autonomous operation. It is an effective way to express the efficiency of the distributed power. CERTS presented microgrid concept as: microgrid is a kind of the system which combines load and micro source; it can provide electricity and heat. Microgrid internal powers is mainly composed of power electronic devices that responsible for energy conversion, and provide necessary control. Microgrid act like a single controlled unit to the external power grid, while satisfying consumer's requirements of power quality reliability [3][4]. Based on distributed generation technology, microgrid combines energy storage, control and protection, and able to work in grid-connected and islanded modes. The structure of microgrid adopted in this paper is shown in figure 1.

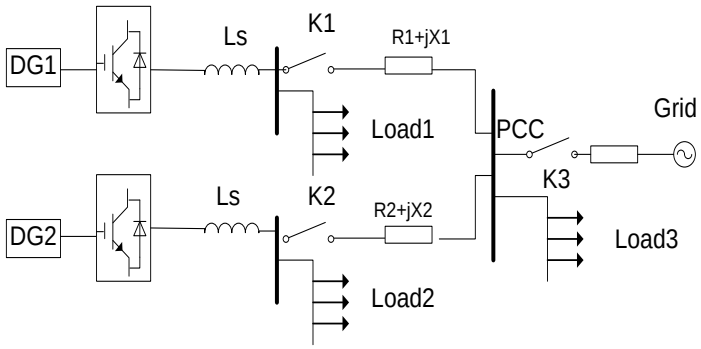

Figure 1. Structure of a Microgrid

Where DG1 and DG2 are distributed generations; Load1 and Load2 are two groups of important load; Load3 is interruptible load and connected to the point of common coupling (PCC). When power grid fault or power off or microgrid requests islanded operation from network operation on its own, K3 at PCC breaks and jilt some interruptible load though the isolation device to guarantee the important load or sensitive load of normal operate. Microgrid can operates in the mode of reconnecting to the grid when necessary.

3. CONTROL STRATEGIES OF INVERTER

PQ Control

PQ control is usually used in grid-connected operation. In this state, frequency and voltage are regulated by power grid, and DG doesn't need to consider frequency voltage regulation. As shown in figure 2, to a given reference value of active power and reactive power, microgrid controls inverter for constant power output[5][6].

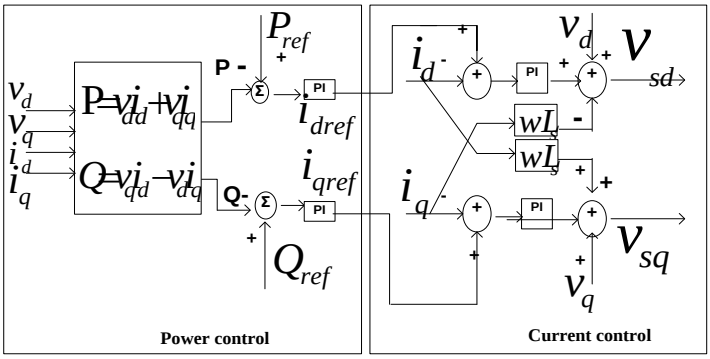

Figure 2. Structure of grid-connected PQ control

Coordinate Rotational Transformation Based Virtual Power V/f Droop Control

This control strategy is improved by droop control strategy[7]. The traditional droop control oversights line inductance, so it lacks of applicability to the microgrid. Considering the complexity of line impedance, a control strategy[8] is proposed, but the control strategy doesn't consider the problem of frequency recovery while the reconnecting grid. We put forward the pre-synchronizing control unit[9] to the virtual power V/f droop control, as show in figure 3.

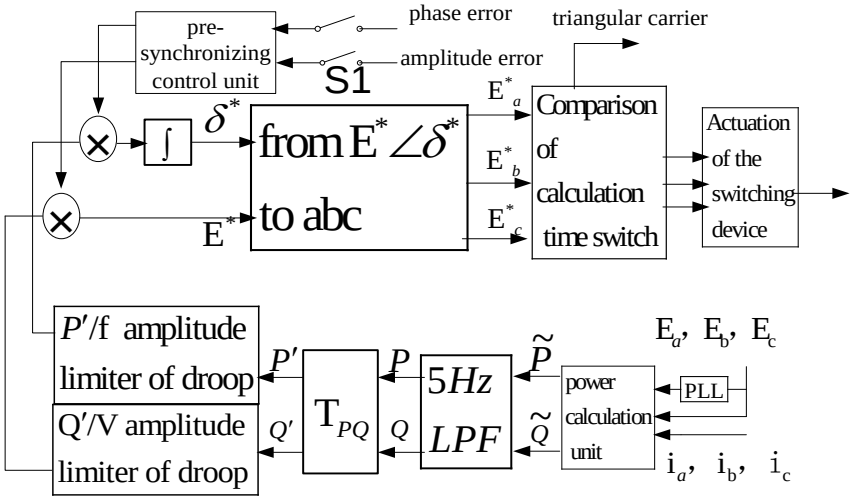

Figure 3. Block diagram of virtual power V/f control

Pre-synchronizing Control Unit

The pre-synchronizing control unit is used to control the inverter output voltage tracking external voltage and frequency. In order to reduce the impact current in the process of reconnecting to grid. As in (1) and (2), Δf and ΔU respectively represents the frequency and voltage compensation value[10].

$$\Delta f = \left(k_p + \frac{k_i}{s}\right)\left(\theta_{grid} - \theta_{inv}\right) \quad (1)$$

$$\Delta U = \left(K_p^{'} + \frac{k_i^{'}}{s}\right)\left(\theta_{grid} - \theta_{inv}\right) \quad (2)$$

From the formulation we can see, phase error and amplitude error need the PI control to get compensation value, ensure microgrid frequency and voltage amplitude and keep pace with power grid. When reconnect to grid, S1 was closed and the pre-synchronizing control unit starts to ensure the smoothness of the process of reconnecting; When disconnect S1 and exit pre-synchronizing control unit, the PI regulator will be reset.

4. SIMULATION RESULTS AND ANALYSIS

In order to verify the correctness and efficiency of the proposed method, we used Matlab/Simulink to set up microgrid simulation platform. Network structure as in figure 1. The initial parameters of the simulation are DG1: P_{ref1}=10kW, Q_{ref1}=0; DG2: P_{ref2}=20kW, Q_{ref2}=0. Voltage of power grid is 380V, and frequency is 50Hz. DG1 and DG2 comprehensive line impedance resistance R=0.64Ω/km, inductance L=0.1H/km.

Simulation Example 1: From Grid-Connected Mode to Islanded Mode

At t =0s, microgrid in grid-connected operation mode, DG1 and DG2 adopt PQ control; at t = 0.1s, PCC switch off, K1, K2 open, microgrid switch to the islanded state; DG1 receive a control mode switch instruction that switch from the PQ control into virtual power droop control. The simulation results as shown in figure 4-6. We can see that in the island state, after frequency mild concussion it keeps stable (in Figure 4), and after 0.15s active power and reactive power keep in stable state (in Figure 5) and the voltage at PCC keeps stable(in Figure 6).

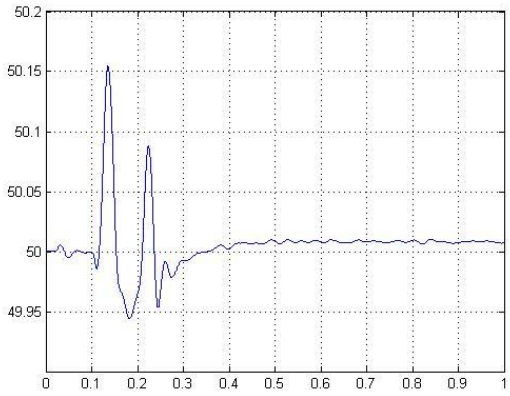

Figure 4. Frequency curve from grid-connect to island operation modes

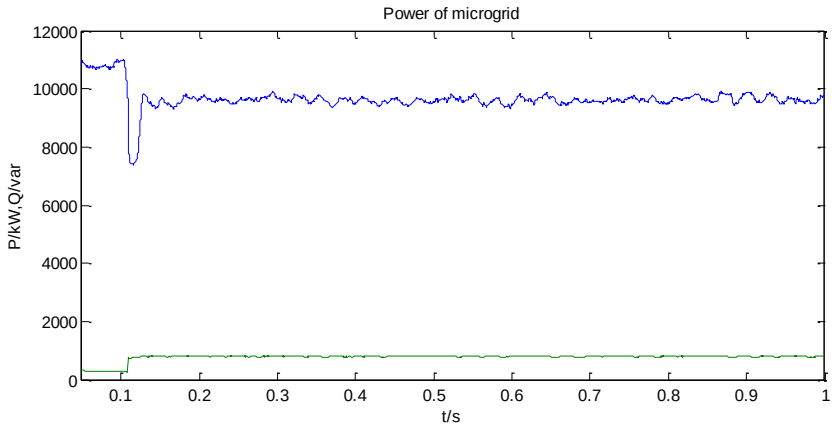

Figure 5. Power curve from grid-connect to island operation modes

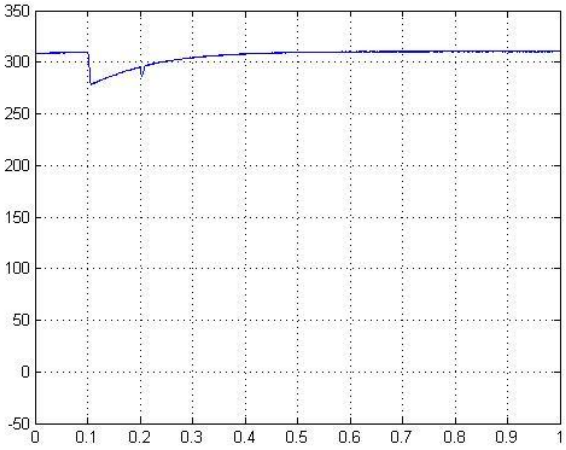

Figure 6. The voltage at PCC

Simulation Example 2: From Islanded Mode to Grid-Connected Mode

At 0.5s, pre-synchronizing control unit put into operation and from figure 7 we can see that microgrid frequency decreased, keep in previous parallel in state, and

confrontation remained stable. At 0.55s, K1 and K2 turned on to make microgrid reconnect to grid. Figure 8 shows that the system dynamic response quickly, active power and reactive power soon move into a steady state. Figure 9 shows the voltage at PCC can keep stable and smooth transition.

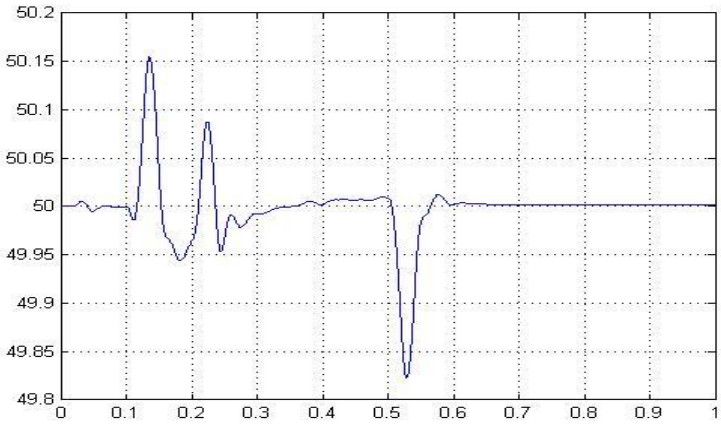

Figure 7. Frequency curve of reconnecting to grid

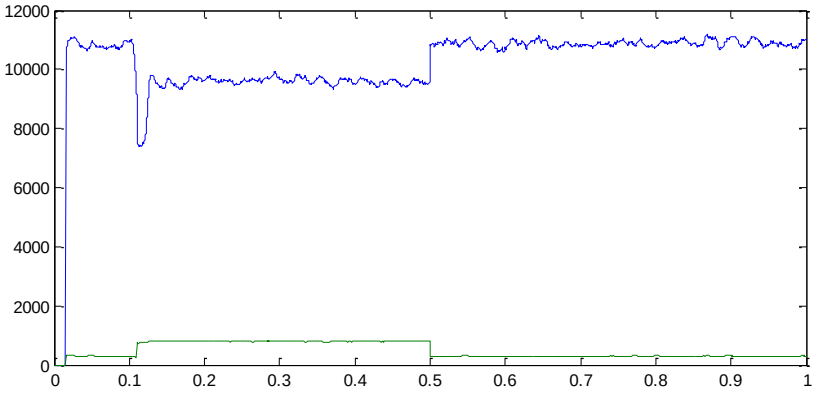

Figure 8. Power curve of reconnecting to grid

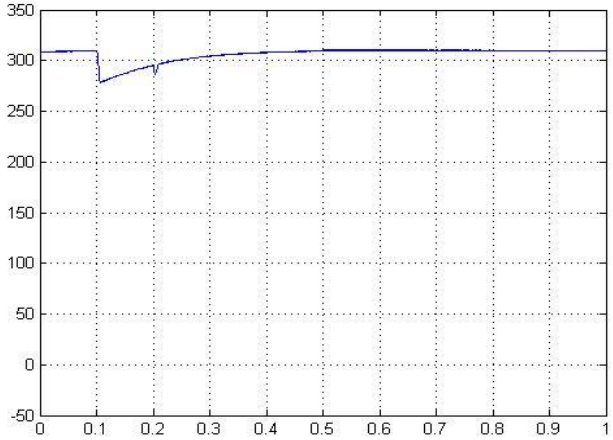

Figure 9. The Voltage curve at PCC of reconnecting to grid

5. CONCLUSION

Microgrid flexible and effective control strategy is the fundamental guarantee for its reliable operation. This paper analysis different control strategies of different operation modes, and proposed a control strategy and a pre-synchronizing control for smooth switching. Simulation results show that coordinate rotational transformation based virtual power V/f droop control strategy has good decoupling effect and can ensure the stability of the power output, and in the process of reconnecting, pre-synchronizing control unit effectively restrain the inverter process shock, improve the system security and stability. Microgrid realizing switch between grid-connect and islanded modes can play a great role in the future development of smart grid.

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Cover page

Title: *Research on Service Restoration for Distribution Network containing Distributed Generations*

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ABSTRACT

Service restoration in distribution network containing Distributed Generations is an important guarantee for realizing Distribution Management System and active distribution network. It has become more significant in improving power quality and meeting the demand of users. In this paper, the development and research status of the service restoration of distribution network are summarized based on existing research results. The concept, function, and model of active distribution network service restoration are expounded. The existing reconfiguration methods of distribution network are compared. Characteristics and deficiencies of the algorithms among the mathematical optimization method, the method of heuristic search and the artificial intelligence method are analyzed and a hybrid method is expounded.

1. INTRODUCTION

Distribution network acts as a direct link between the electric power producers and users, reflects the power supply service quality of electrical power system, and the security. Reliable and economic operation in power system, being taken in consideration more and more seriously[1].Client power failure is mainly caused by distribution network fault, while the fault reconfiguration of the distribution network is a optimization problem on the basis of the fault location and isolation to restore a power outage areas. The service restoration reconfiguration of distribution network is to satisfy certain constraint conditions, and comply with certain economic indicators to anew section switches and loop switches combination, improve the operation reliability and economy of distribution network.

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The fault reconfiguration of the traditional distribution network is to meet operational requirements, changing the status of the section switches and loop switches. Maintaining the open-loop running, transfer the non-fault outage area load to the normal feeder as much as possible, the optimal service restoration scheme [2] is a series combination of the switch operation. With the rapid development of renewable distribution energy, large-scale distributed generations are accessed to distribution network. The method of the traditional distribution network fault reconfiguration can not adapt the request of the large-scale distributed generation connection, and facing huge challenges. Active distribution network contains distributed generation, having the ability of control and operation[3], able to effectively linking the two sides of source and user's need , and deciding how to best run in real time. To meet this requirement, the level of control is much higher than the current level of the distribution network. Conventional distribution network is radial in nature, characterized by a single source feeding a network of down-stream feeders. The structure of distribution network is not radial after the distributed generation is connected. Meanwhile, the service restoration reconfiguration is changed. When the distribution network with distributed generation break down, the traditional recovery strategy is to remove all the distributed generation, although this can greatly protect network security, but it affects the reliability of power supply. By changing section switches and loop switches combination status in active distribution network, the position of distributed generation in the distribution network can be changed, and the range of distributed generation supply to fault area will also be changed[4]. Without removing the distributed generations, service restoration of active distribution network can improve operation reliability and economy of the distribution network.

2. MATHEMATICAL MODEL

The general objective of distribution network service restoration reconfiguration are: Maintaining connectivity and radial of distribution network after service restoration reconfiguration, the feeder capacity, the load transformer capacity, switches capacity and other demand. For achieving the minimum operation number of switches, shortest outage time, minimum network loss and restoring maximum load power, load balancing, the largest feeder capacity margin and other targets[5,6]. Objective functions of active distribution network service restoration are mainly included:

Objective Functions

1) Restore maximum important load

The reliability requirement of the electricity are different for the different loads which are cataloged into several priority levels. To maximum restore loads and ensuring that important loads are first restored, the objective function as follows:

$$\max f_1(x) = \sum_{i=1}^{n} A_i S_i P_i \tag{1}$$

Where n express the total number that participating in reconfiguration of all branch; x is the current state of the network, S_i is the status of the branch switch i where "0" express off and "1" express on; P_i and V_i respectively express the rated active power, reactive power, A_i express the priority weight coefficient of the load that connected to the branch switch i.

2) Minimum number of switching operation

$$\min N = \sum_{i=1}^{n} |K_i - S_i| \Rightarrow \max f_3(x) = \frac{1}{N} \tag{2}$$

Where K_i is the initial status of the branch switch i , N is the number of the switch operation.

3) Maximize the power supply of the distribution generation

$$\min f(x) = \sum_{i \in C} (P_{G\max}^{i} - P_G^i) \tag{3}$$

Where P^i_G and P^i_{Gmax} respectively express actual output and maximum output of the distribution generation i.

Constraint Condition

1) Power flow equality constraint.
2) Node voltage constraint where:

$$U_{i\min} \le U_i \le U_{i\max} \tag{4}$$

Where U_{imin} and U_{imax} respectively express the lower limit and upper limit of voltage at node i.

3) Line capacity constraint:

$$S_i \le S_{i\max} \tag{5}$$

Where S_i and S_{imax} respectively express the calculation value and maximum allowable value of the line power.

4) No closed loop after service restoration:

$$\sum_{k \in F_{ii}^{loop}} s_i + 1 \ge 0 \tag{6}$$

Where F_{ii}^{loop} is switch sets status of the closed loop.

5) Output constraint of the distribution generation:

$$P^i{}_{G\min} \le P_G^i \le P_{G\max}^i \tag{7}$$

Where P^i_{Gmin}, P^i_G and P^i_{Gmax} respectively express minimum output, actual output and maximum output of the distribution generation i.

3. APPROACH OF DISTRIBUTION NETWORK RESTORATION

Distribution network service restoration reconfiguration belongs to a complex discrete and continuous variables nonlinear optimizing problem with multi-constraints, multi-objective, multi-period and multi-combination [7]. These problem has been widely researched by many research scholars, literature [8], [9] respectively made a summary for the distribution network fault reconfiguration algorithm, which can be roughly divided into three types: mathematical optimization method, heuristic search method and artificial intelligence method.

Mathematical Optimization

Mathematical optimization methods consider the objective function and constraints to mathematical form and carry out mathematical model. Literature [10]-[12] solve distribution network fault reconfiguration problem with linear programming method, while literature [13] using mathematical programming method and literature [14] using branch and bound method. Traditional mathematical methods are mature, and able to get global optimum that not dependent on distribution network initial structure. However, with the distribution network structure becoming complex and the expansion of power outage regional, the computation time will greatly be extended. Since the complex large-scale systems can not be handled with these methods and only mathematical methods can not get perfect solution, there are now combination methods of artificial intelligence and numerical method.

Heuristic Search

Heuristic search limit the search direction based on certain rules , evaluate possible solutions at a certain criteria, reduce the search space and improve recovery time. Literature [15] proposed the concept of trees to restore, transformed service restoration of distribution network into restored tree cutting problem, greatly reduces the complexity of the problem. Literature [16] proposed minimum spanning tree theory basing on the graph theory to help search solution. Search results that bases on heuristic search are often depend on the network structure, resulting in unstable and huge number of feasible solutions. For large complex distribution network, the calculation efficiency is low, and may not find the global optimal solution. Literature[17] proposed best-first search strategy, as far as possible eliminate switch status combination that violate the constraint condition, to narrow the searching space.

Artificial Intelligence

Artificial intelligence algorithms general include: Particle swarm optimization, Fuzzy theory algorithm, Genetic algorithm, Artificial neural network, Expert system, Simulated annealing, and Multi-agent system.

1) Particle swarm optimization

Particle swarm optimization algorithm is an evolutionary computation technique based on intelligence, originate from the flock foraging behavior, simulating birds group behavior. Literature [18][19] combine the particle swarm optimization and

binary particle swarm optimization algorithm solve the problem of distribution network reconfiguration and power Optimization with DG injection. Particle swarm optimization has the strong ability of global convergence and robustness, the algorithm is simple and easy to implement, but this algorithm local search ability is poor and prone to premature convergence.

2) Fuzzy theory algorithm

Fuzzy theory algorithm simulates human daily reasoning model, and uses membership function to establish relationship between fuzzy sets. Distribution network fault reconfiguration using fuzzy theory algorithm, usually consider switching times, load transfer, load degree of spare feeder as membership degree, and use fuzzy membership degree on a weighted sum of the membership function to evaluate optimal solution[20]. This algorithm does not depend on the initial state of the system, and can get the global optimal solution, but generally limited by search solutions, and online processing ability is poor.

3) Genetic algorithm

Genetic algorithm is an optimization method based on the mechanism of natural selection and population genetics. Literature [21] described a power recovery genetic algorithm based on minimum spanning tree coding, eliminated the generation of infeasible solution and greatly improved the computational efficiency. Literature [22] represented the switch state of the network with the binary string corresponds to a fitness function. Literature [23] described an improved non-dominant genetic algorithm which introduced depth of the node coding technology into multi-objective optimization algorithm. Genetic method has strong robustness and good optimization ability, but poor local search ability, poor real-time performance and longer calculation time and easy to premature.

4) Artificial Neural Network

Artificial neural network uses neurons and their directed weights to deal with problems. Literature [24] applied artificial neural network in power distribution network fault recovery problem. This method does not need power flow calculation, and has strong parallel computing and anti-jamming abilities, strong real-time performance, which is suitable for mapping of complex non-linear function relation.

5) Expert system

Expert system [25] storages expert knowledge in a knowledge base, and use the knowledge base and experience accumulations to get the optimal solutions. Literature [26] implemented a integrated intelligent expert system with strong real-time performance and no dependence of power distribution network structure. Literature [27] adopted heuristic, topology identification rules to reason reasonable switch operating sequence. Expert system can satisfy the real-time requirements used for large-scale network and service restoration. But some knowledge is not easy to express by the form of rules, and it’s hard to cover all the failure mode and operation mode.

6) Simulated annealing

Simulated annealing [28], derived from solid annealing principle, is based on the natural evolution process simulation and abstract for a class of random adaptive optimization algorithm. Literature [29] used simulated annealing to solve

combinatorial optimization problems, use stochastic search iterative process to seek the optimal solution. The algorithm uses a kind of random optimization methods. Probability is used to guide the search in the algorithm other than conventional methods using deterministic rules. The algorithm has the parallel processing ability, and is easy to parallel implementation and the final solution has nothing to do with the initial solution. For large-scale systems, it needs to do a lot of random iteration to find the global optimal solution or approximate the global optimal solution.

7) Multi-agent system

The multi-agent system, by planning, reasoning and decision making, can accomplish a specific task and achieve the intended target, has coordinated control and strong information communication ability, fast and efficient recovery failure characteristics. Literature [30] established a three layer multi-agent fault restore model, and the recovery process was divided into four stages of substation, distribution network, whole network and remote recovery.

According to the shortcomings of single algorithm, the effect of distribution network service restoration is not very good. Many hybrid optimization algorithms [31]-[34] have been proposed. Literature [31] combines the expert system method and mathematical programming method, in which the expert system decomposes the problem first, and then mathematical programming calculates the sub problems. Literature [32] based on chaos and immune hybrid algorithm, combines the global search algorithm and local search of artificial immune algorithm, effectively improve the speed and accuracy of service restoration. Literature [33] combines simulated annealing and genetic algorithm, with two layers of parallel search structure and improve efficiency. Literature [34] combines the clone genetic algorithm and tabu-search algorithm to reduce the search range and improve the search efficiency.

4. CONCLUSION

Each algorithm in service restoration for distribution network has its own advantages and disadvantages, while different algorithm suit for different situation. Using the hybrid algorithm can improve the optimization performance of algorithm, and avoid falling into local optimization, enhance the exploration ability and efficiency of the algorithm in the solution space. Search capabilities of various algorithms can be mutually complementary, hybrid algorithm can overcome shortcomings of single algorithm and improve reliability and efficiency.

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Cover page

Title: *Prediction of the New Rural Construction Demands for the Agricultural College Services Based on BP Artificial Neural Network.*

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ABSTRACT

BP artificial neural network model is used to predict the new rural construction demands for the agricultural college services. Starting from the brief introduction of the usages of BP neural network, we analyze the demand factors of the agricultural college services and the affective elements of the demands, use the BP neural network model to predict, and then run the BP neural network model on the MATLAB platform, and finally carry out the case studies of Heilongjiang Province.

The demand prediction of agricultural college services is the scientific analysis, estimation and inference of the future service needs of agricultural college services using the existing historical information and data, and the appropriate methods and techniques. The approach of artificial neural network modeling is an artificial and functional neural network based on human's knowledge and understanding of the brain neural networks. With the highly non-linear function, it's a system that is able to perform the complex logic operations and to achieve the non-linear relationships. Moreover, it can better reveal the relativity of the nonlinear time series in the time-delay space so as to achieve the purpose of prediction. We predict the demands for agricultural college services based on the multi-layer artificial neural network learning algorithms - BP algorithm.

1. BP NEURAL NETWORK

Among many different types of artificial neural networks, the error back propagation algorithm - BP algorithm is the most widely used and popular model. BP algorithm, by definition, is calculated from backward to forward. Usually, it has a multi-layer neural network, and the BP network information flows from the input layer to the output layer. Therefore, it is a multi-layer forward neural network. The difference between BP neurons and other neurons is that the transfer function of BP neuron is non-linear, and the most commonly used function is logsig function and tansig function. Some of the output layer is linear function—purelin with the output A = logsig (W • P + b). After determining the BP network structure, the network can be

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trained with the input and output sample sets, that is to learn and correct the network thresholds and weights, so that the network can achieve a given output mapping.

The basic idea of BP algorithm is to assign the initial network weights and threshold, and calculate forward. And then, based on the error between the achieving results and the expected results, network weights and thresholds are modified repeatedly and reversely until the minimum error is achieved.

In a three-layer BP neural network, the input vector is X = (x_1, x.. x_n) and the desired output vector is d = (d_1, d_2, d_3 ... ... d_m).The actual outputs of nodes on each level can be calculated with the forward propaganda among networks, and the input u_i of the neurons on each hidden layer is calculated as

$$u_i = \sum_{j=1}^{n} w_{ji} x_j - \theta_i, \text{ i = 1,2, ..., p,} \tag{1}$$

where w_{ji} is the connection weight between the j-th neuron on the input layer and the i-th neuron on the hidden layer; θ_i is the threshold of the i-th neuron on the hidden layer. P is the neuron number on the hidden layer. The output of each neuron on the hidden layer is

$$y_i = f(u_i) = \frac{1}{1+e^{-u_i}}, \text{ i=1,2,…,p.} \tag{2}$$

The input u_i and the output y_i of each neuron on the output layer are

$$u_t = \sum_{i=1}^{n} w_{it} x_i - \theta_t, \text{ t=1,2,…,m, and} \tag{3}$$

$$y_t = f(u_t) = \frac{1}{1+e^{-u_t}}, \text{ t=1,2,…,m.} \tag{4}$$

w_{it} in Formula (3) and (4) is the connection weight between the i-th neuron on the hidden layer and the t-th neuron on the output layer; θ_t is the threshold of the t-th neuron on the output layer; and m is the neuron number of the output layer.

With the comparison of the expected output d_t with the actual output of the neural network y_t, the learning error which can be expressed as the mean square error e_t can be achieved as follows

$$e_t = \frac{1}{2}\sum_{t=1}^{m}(d_t - y_t)^2 \quad . \tag{5}$$

2. THE MAIN DEMAND ELEMENTS OF THE AGRICULTURAL COLLEGE SERVICES

In the new rural construction, the main demanding subjects for the agricultural college services are mainly college students, farmers, farmers' cooperative organizations, agribusiness, and relevant government departments. The new rural construction demands for the agricultural college services mainly include education, research and social services. There are 34 indicators in the three categories in total

which include the educational needs, the research needs, and the social service needs. The educational needs include formal education, adult education, basic education and training; the research needs include the promotion of scientific research, technological innovation, etc.; and the social service needs are covered by the community, life, information services and consulting and so on.

Based on principal component analysis of the 34 indicators, and according to the principle of the over 85% accumulated variance contribution rate, the cumulative contribution rate of the three selected common factors, such as formal education, training and the promotion of the scientific and technological achievements is 89.38%, which can give a full explanation and generalization of the most data. Therefore, these three main components are selected as the indicators of the service demands because they can be a good overview of this set of data.

The calculation of the demands for the agricultural college services on the new rural construction is mainly expressed by the sum of the formal education, training and the promotion of scientific and technological achievements. Among them, the "formal education" is represented by the product of the agriculture-related employment with a full-time agricultural college education or higher diploma and the average wages of college graduates that year; "Training" in this article specifically refers to the total revenue of the paid training offered by the agriculture colleges in the agriculture-related field; "the promotion of the scientific and technological achievements " is represented by the total technological achievement(patent) transfer of the agricultural universities.

3. FACTORS OF THE PREDICTION ON THE NEW RURAL CONSTRUCTION DEMANDS FOR THE AGRICULTURAL COLLEGE SERVICES

There are many factors of the prediction on the new rural construction demands for the agricultural college services. Because of the complex relationships among them, we should consider the demands for the agricultural college services not only by the rural economic development, but also by the rural social development, and take into account the agricultural college input and many impact factors. The demands for the agricultural college services by the rural economic development is mainly reflected in the GDP of primary industry, fixed asset investment, labor productivity and other factors. The demands for the agricultural college services by the rural social development exist in education, culture, family, life, and other aspects. The life indicators like the rural disposable income per capita and the consumption expenditure per capita indirectly reflect the changes in living standards and people's ability to pay for educational services.

The core of the agricultural college services to the new rural construction is technology. Therefore, the technological development has a close affect on demands for the agricultural college services. The technology development provides the prerequisite for fulfilling the functions of agricultural colleges, and opportunity to grow for the development of agricultural colleges as well. Major scientific and technological development activities are in three areas of science and technology, the scientific research expenditure, and the technical market conditions.

In the factor correlation analysis of the demands for the agricultural college services, the five indicators with most correlative coefficients in order are the GDP of

primary industry, the fixed asset investment, the rural consumption expenditure per capita, the R & D expenditure, and the agricultural university research inputs. The results of the further regression analysis are shown in Table 1.

TABLE 1 INDICATOR REGRESSION DATA

	Multiple R	R Square	Adjusted R Square
GDP of primary industry	0.93678	0.87639	0.83436
Fixed asset investment	0.92713	0.85386	0.81231
Rural consumption expenditure per capita	0.91955	0.83127	0.78502
R & D expenditure	0.91109	0.82304	0.77298
Agricultural university research inputs	0.91001	0.82297	0.77036

Table1 shows the correlation coefficient (Multiple R) and the coefficient of determination (R Square) of the primary industry's GDP, the fixed asset investment, the rural consumption expenditure per capita, the R & D expenditure, and the agricultural university research inputs. The adjusted R square of the Primary industry's GDP is 0.83436, indicating that this variable can explain the 83.43% of the variation for the demands for agricultural college services, which indicates that this variable has a good explanatory power. The fixed asset investment（0.81231）, the rural consumption expenditure per capita（0.78502）, the R & D expenditure（0.77298）, and the agricultural university research inputs（0.77036） can respectively explain 81.23%, 78.50%, 77.30%, and 77.04% of the variation for the demands for agricultural college services, which indicates that these variable have a fine explanatory power. Based on the correlation analysis, we choose the indicators of the GDP of primary industry, the fixed asset investment, the rural consumption expenditure per capita, the R & D expenditure, and the agricultural university research inputs to predict the new rural construction demands for the agricultural college services.

4. THE PREDICTION OF THE NEW RURAL CONSTRUCTION DEMANDS FOR THE AGRICULTURAL COLLEGE SERVICES IN HEILONGJIANG PROVINCE

For the sake of the prediction analysis of the demands for the agricultural college services in Heilongjiang Province, we select the input variable of BP neural network model as the demands for services for the agricultural college services. When the significance level is 5%, the following relative indicators are significant: the GDP of primary industry, the fixed asset investment, the rural consumption expenditure per capita, the R & D expenditure, and the agricultural university research inputs. And the output variable is the amount of agricultural college services with the input vector X = (x1, x.. Xn) and the desired output vector d =(d_1,d_2,d_3……d_m) . The termination of the learning conditions is that the global network error E= 10^{-4}, or that it has learned 100 times.

The prediction of the demands for the agricultural college services by BP neural network is shown in Table 2:

TABLE 2 THE PREDICTION RESULTS OF THE DEMANDS FOR THE AGRICULTURAL COLLEGE SERVICES IN HEILONGJIANG PROVINCE

Sample	1	2	3	4	5
Year	2007	2008	2009	2010	2011
Demand amount for agricultural college services(100 million)	1713.65	2266.41	2282.87	2481.32	2813.98
GDP of primary industry (100 million)	737.6	915.4	1089.1	1154.3	1302.3
Fixed assets investment in rural areas(100 million)	295.6	242.2	316.2	333	519.9
Consumption expenditure per capita in rural areas(yuan)	2618.2	3117.4	3844.7	4241.3	4391.2
R & D expenditure (100 million)	133.7	199.7	256.5	266.6	299.1
Agricultural labor productivity (yuan / person)	9160	11408	13593	14692	15918
Predictive demand amount for agricultural college services (100 million)	1714.28	2267.15	2281.32	2481.97	2813.71

Source: the data from Statistical Yearbook of Heilongjiang Province (2008-2012) and the agricultural colleges in Heilongjiang Province

5. THE ANALYSIS OF THE PREDICTION RESULTS

To make sure of the good predictive accuracy of the BP neural network model, we use the residual test to test the results of the model. The residual calculation is shown in Table 3.

TABLE 3 THE RESIDUAL CALCULATION

Year	K	Actual value	Predictive value	Error	Relative error
2007	1	1713.65	1714.28	0.63	0.0368%
2008	2	2266.41	2267.15	0.74	0.0327%
2009	3	2282.87	2281.32	-1.55	-0.0679%
2010	4	2481.32	2481.9 7	0.65	0.0262%
2011	5	2813.98	2813.71	-0.27	-0.0096%

As can be seen in Table 3, this model, with a goodness of fit, has a relatively small absolute error, indicating that the BP neural network prediction model has a very excellent accuracy; we can use this model for the scientific prediction of the total demands of the new rural construction for the agricultural college services. The prediction results can be calculated by the above BP neural network prediction model of the demands for agricultural college services as shown in Table 4.

TABLE 4 THE TOTAL PREDICTIVE VALUE OF AGRICULTURAL COLLEGE SERVICES IN HEILONGJIANG PROVINCE, 2012-2014

Year	2012	2013	2014
Total agricultural college services	3119.62	3406.17	3920.44

CONCLUSION

To judge from the predicted results, the total new rural construction demands for agricultural college services in Heilongjiang Province from 2012 to 2014 is respectively 3119.62,3406.17, and 3920.44. The total agricultural college services in Heilongjiang Province grow steadily with a demand increase each year. The residual test and a posteriori error test show that the BP neural network prediction has a good accuracy.

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Cover page

Title: *Biological Purification Efficiency in Long-Distance Raw Water Distribution System*

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ABSTRACT

Making full use of biological purification in raw water distribution pipelines was beneficial to improve water quality in long distance water distribution pipelines. For this purpose, a laboratory pilot plant was devised and connected to the considered water network. Water and biofilm samples were taken and examined during the experiment. The biofilm had been exposed to the bulk water at approximately 18C, for at least 45 days to allow the formation of a mature quasi-stationary biofilm. In the present study, biological purification was reflected by investigated water quality variation with time of raw water in long distance water distribution pipelines. We determined that the initial concentration of ammonia nitrogen (NH_4^+-N) was approximately 1mg/L. The effluent of NH_4^+-N fell to 0.2mg/L, and nitrite nitrogen (NO_2^--N) remained stable at 0.05mg/L. The removal rate of orthophosphate (PO_4^{3-}) ranged from 18.58% to 28.94%. The removal rate of UV_{254} held steady at 22%, and biofilm biomass stabilized at 68 nmol/cm^2. Nitrifying bacteria and heterotrophic bacteria counts (HPC) in the biofilm had higher level than in the bulk water. Biological purification of biofilm could significantly improve the water quality in long distance water distribution pipelines.

INTRODUCTION

The method of using inter-basin water transfer and re-allocation of water resources can ease and solve the urgent needs of the water shortage region. Many countries such as the United States, the former Soviet Union, Canada, France, Australia, Pakistan, India and so on, have tried, and some are made great benefits [1].China has designed and implemented a number of long distance water supply works also, such as the project of “Luan River to Tianjin”, the project of dahuofang water supply in Shenyang, the project of ”drawing Pitt into even” in Dalian.[1]

Long-distance water pipelines will inevitably attach growth biofilm in the long running process. Nitrification and microbial degradation of organic matter are two main biological actions. Numerous studies have shown that nitrification in drinking

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water distribution is undesirable because it has a negative impact on water quality. With nitration reaction cause chloramine attenuation, pH and alkalinity reduction, the number of heterotrophic bacteria, and the concentration of nitrite nitrogen and nitrate nitrogen increased [2-3]. But the nitrification in the water distribution system is different. Nitrifying bacteria oxidizes NH_4^+-N to NO_2^--N and nitrate nitrogen (NO_3^--N), thus effectively reduce the concentration of NH_4^+-N in raw water [4].Ammonia nitrogen, nitrite nitrogen, pH, COD_{Mn} and TOC are declined, at the same time, nitrate nitrogen is rising along the long-distance water supply project [5]. In low turbidity, high algae and more serious micro-polluted water distribution pipelines, the color, turbidity, ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N) and organic matter are significantly reduced [6]. Zhu and Qu are both reported on the variation of water quality parameters in water distribution pipelines, but no mention of water process of change with time. However, few studies have reported on the water quality variation with time in long distance water distribution pipelines.

Therefore, the overall purpose of this project is to evaluate the raw water quality changes in the process of transportation, and make full use of biological purification in long-distance water distribution pipelines to improve the water quality and reduce water pollution load of water purification plant, as well as provide theoretical and technical support for regulation and optimization of biological purification in long-distance raw water distribution pipelines.

MATERIALS AND METHODS

Test System and Experiment Methods

The experiments were performed in biofilm annular reactor (BAR) to simulate the effect of pipeline hydraulics on biofilm. Each BAR contained a reaction tank, a central rotor, coupon tanks, a motor, inlet pipe, outlet pipe, dosing tubes and coupon sampling ports. There were 20 rectangular coupon slots in the rotor surface, and the coupons mounted on the rotor in the reactor. The rotor connected to the motor, and the motor driven rotation to simulate the effect of shear on biofilm in water distribution pipelines. BAR could be installed coupons of different materials to simulate a variety of pipes in water distribution system, and the test used polyethylene (PE) coupon. The effective volume of BAR was 1L, and used a peristaltic pump to control influent water. The hydraulic retention time was controlled in 2h.The simulated system was run for 60 days to allow the microorganisms to colonise the surfaces in and achieve a mature biofilm. Biofilm samples and effluent water samples were taken regularly during this period to determine colonization kinetics. Biofilm samples were collected by swabbing the entire surface of the coupon with a sterile cotton bud three times to collect the attached bacteria. The bud was then transferred to 10mL sterile buffer solution, and ultrasound for 20 minutes to release the bacteria. The effluent water samples were sampled from the reactor at the same time as the biofilm.

Analytical Methods

Turbidity was analysed with an HACH 2010N turbidity meter. NH_4^+-N, NO_2^--N, NO_3^--N, UV_{254} and orthophosphate were analysed with an spectrophotometer [7]. Dissolved oxygen (DO) was measured by a online DO meter. HPC analysis was done

with the spread plate method using R_2A agar and incubation time of 7 days at 22℃ temperature. Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were cultured using the most probable number (MPN) technique and Soriano and Walker medium, and incubation time of 28 days at 28℃ temperature. Lipid phosphorus was measured by the same method as orthophosphate. The result expressed in nmol P/cm^2, and 1 nmol P equivalent size of E.coil cells 10^8.

Sampling

Water samples were prepared by adding natural colloids and filtered domestic wastewater to local tap water (Beijing, China) at a volumetric ratio of 4:15:1000, and then standed for one day. Quality characteristics of the prepared water were as follows: turbidity 1.71～2.13 NTU, NH_4^+-N 0.916～1.120 mg/L, NO_2^--N 0.034～0.05 mg/L, DO 5.31～6.25 mg/L, UV_{254} 0.018～0.022 cm^{-1}, PO_4^{3-} 0.215～0.287 mg/L.

RESULTS AND DISCUSSIONS

Removal of Nitrogen and Phosphorus

Water samples were taken regularly and analysed by NH_4^+-N (Fig.1). The effluent of NH_4^+-N decreased after running three days. The removal rate of NH_4^+-N was increasing, stabilized at 0.2 mg/L after running 43 days, and the removal rate of NH_4^+-N reached in 82%. The removal rate of NH_4^+-N was increased by leaps and bounds during the experiment. Indicating a logarithmic growth of nitrifying bacteria, and including adaptation period, logarithmic growth phase and stationary phase three stages. The variation of NO_2^--N was shown in Fig.2. The effluent of NO_2^--N was increased first, and then reduced and gradually stabilized. The accumulation of NO_2^--N reached the maximum value 1.281mg/L after running 19 days. The effluent of NO_2^--N decreased gradually and stabled at 0.05 mg/L after 41 days. Analysis of the reason was that different growth rates of AOB and NOB at initial stage of biofilm formation resulting in the accumulation of NO_2^--N.

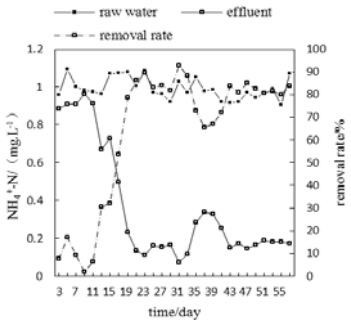

Fig 1. Variation of NH_4^+-N

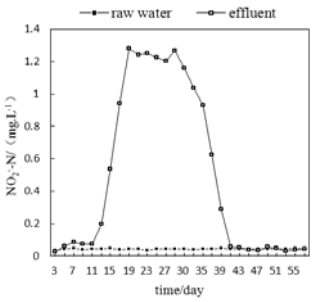

Fig 2. Variation of NO_2^--N

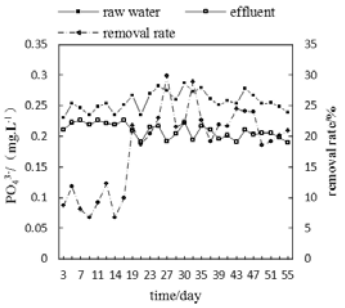

Fig 3. Variation of PO_4^{3-}

Total phosphorus was the sum of exist in various forms of phosphorus in water, and orthophosphate (PO_4^{3-}) was likely to be directly absorbed by the bacteria source of phosphorus [8]. The experiment used PO_4^{3-} to reflect phosphorus removal. The variation of PO_4^{3-} was shown in Fig.3. Effluent PO_4^{3-} had a certain degree of reduction,

illustrating that the simulated reactor could remove some phosphorus. After running 27 days, the removal rate of PO_4^{3-} reached the maximum value 29.93%. With the extension of running time, the removal rate of PO_4^{3-} ranged from 18.58% to 28.94%.Phosphorus was an essential element for microbial growth. Nitrifying bacteria and HPC needed consume phosphorus to growth. Biological phosphorus removal was mainly done by phosphorus accumulating bacteria (PAOs). To remove biological phosphorus, you must provide PAOs anaerobic/aerobic or anaerobic /anoxic environment. PAOs released phosphorus in the state of anaerobic, and absorbed of phosphorus in the state of aerobic [9]. In this experiment, the simulated biofilm reactor running under high concentration of DO conditions, there was no anaerobic conditions, so the removal of PO_4^{3-} for little effect.

Variation of Microbial

The results of AOB and NOB in the bulk water and biofilm were shown in table 1. The bulk water and biofilm were both detected nitrifying bacteria, indicating that the nitrification was the outcome of combined action of nitrifying bacteria in the bulk water and biofilm. After running a certain time, the quantity of nitrifying bacteria in the biofilm significantly higher than the bulk water, indicating that nitrifying bacteria in the biofilm was dominated. The results were consistent with the findings in drinking water distribution system [10].The quantity of AOB and NOB were first increased and then decreased last stabilized. To the biofilm mature, the quantity of AOB was greater than NOB, indicating that AOB was the dominant species compared to NOB. The results were consistent with the findings of Liao [11]. Wang [12] using the Monod equation established nitrification kinetics model, and the result showed that the quantity of AOB was dominated than NOB. AOB was widespread in drinking water distribution system and had a high level of its existence, while NOB was only existed in a small amount of biofilm samples [13]. Through the above experimental results, we could conclude that NOB was also existed in the bulk water and its quantity lower than in the biofilm, which was different from the drinking water distribution system.

TABLE 1. THE QUANTITY OF AOB AND NOB IN THE BULK WATER AND BIOFILM

time/day	bulk water/(MPN.mL^{-1})		biofilm/(MPN.cm^{-2})	
	AOB	NOB	AOB	NOB
8	4.5	0.7	1.85	0.25
15	15	9	15.43	2.47
25	45	25	277.78	18.52
35	30	45	185.19	98.77
45	30	20	154.32	86.42
57	25	25	154.32	86.42

The results of HPC in the bulk water and biofilm were shown in table 2. High activity, rapid propagation speed and the weak growth performance of adherent made the quantity of HPC in the biofilm less than the bulk water in the early running. As time went on and the adhesion state of microorganisms increased, the quantity of HPC gradually increased, and the quantity of HPC in the biofilm reached the maximum after running 35 days. However, the total amount of microorganisms in the biofilm could not be further increased, and also gradually reduced. The quantity of HPC in the

biofilm decreased, because the major component of the biofilm adapted to a large number of growth cycle longer and attached growth of microorganisms. As the further change of microbial population, microorganisms in the biofilm eventually reached a steady state that the most suitable for the attached growth. The number of HPC in the biofilm stabled at 2.8×10^5 CFU/cm^2 after running 45 days. And to the biofilm mature, the quantity of HPC in the biofilm was significantly higher than the bulk water. Therefore, the degradation of organic matter was the outcome of the HPC both in the bulk water and biofilm. The quantity of HPC in the biofilm was significantly higher than the bulk water, so its dominated in the long-distance water distribution pipelines.

TABLE 2. THE QUANTITY OF HPC IN THE BULK WATER AND BIOFILM

time/day	The bulk water HPC/(CFU.mL^{-1})	The biofilm HPC/(CFU.cm^{-2})
8	1.1×10^5	3.3×10^4
15	1.1×10^6	4.1×10^4
25	6.8×10^4	1.7×10^5
35	1.1×10^5	7.4×10^5
45	6.1×10^4	2.8×10^5
57	7.5×10^4	2.7×10^5

Organic Matter Degradation

The UV_{254}, TOC, DOC and COD had a certain correlation, could indirectly reflect the degree of organic pollution in water, and had been widely recognized the UV_{254} as indicator reflected of organic matter content in water quality monitoring [14]. The variation of UV_{254} was shown in Fig.3. The effluent of UV_{254} was decreased, indicating that microorganisms could degrade organic matter and purify water. To the biofilm mature, the removal rate of UV_{254} held steady at 22%.

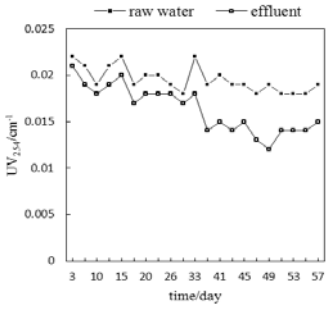

Fig 3. Variation of UV_{254} in the BAR

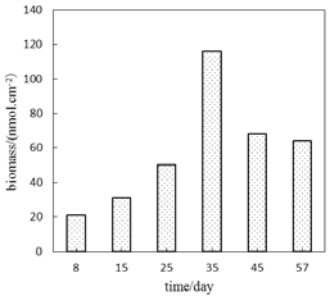

Fig 4. Variation of biomass in the biofilm

The activity of the biofilm only related with the active biomass, and lipid phosphorus could be used as the indicator of active biomass in the biofilm [15]. The experiment used lipid phosphorus to reflect the variation of biomass in the biofilm, and the result of biomass in the biofilm during the experiment was shown in Fig.4. The variation tendency in biomass was consistent with the change of HPC in the biofilm that was first increased and then reduced last stabilized. The maximum and stable time of biomass was as same as HPC in the biofilm, and the biomass stabled at 68 nmol/cm^2 after running 45 days.

CONCLUSION

The main conclusions are as follows: (1) To biofilm mature, the effluent of NH_4^+-N fell to 0.2mg/L, and the effluent of NO_2^--N remained stable at 0.05mg/L. The removal rate of PO_4^{3-} ranged from 18.58% to 28.94%. (2) The removal rate of UV_{254} held steady at 22% after biofilm matured, and biomass in the biofilm stabilized at 68 nmol/cm^2. (3) Nitrifying bacteria and HPC in the biofilm had higher level than in the bulk water. The nitrifying bacteria and HPC in the biofilm dominated the nitrification and the process of organic matter degradation respectively in long-distance water distribution pipelines.

ACKNOWLEDGMENTS

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Cover page

Title: *Property of Electroplating Ni-Fe Alloy for Mems*

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ABSTRACT

The microstructure and electromagnetic properties of electroplating Ni-Fe alloys were studied in this paper. The results indicate that the deposit is bright and compact, the crystal-planes of the plating were (111), (220) and (200). Electrodeposited Ni-20%Fe has a strong paramagnetism effect with the smallest remanence of 0.5 emu, The coercivity show a monotonic decrease with increasing Fe content in deposit, and the saturation magnetization was only 10% of that of the IJ85 permalloy, which proved that the electroformed Ni-20%Fe alloy has good electromagnetic property and could be used in MEMS actuator manufacturing.

INTRODUCTION

Ni-Fe plating has a copmpact structure, bright and smooth surface and was widely used in the production of new materials that require specific mechanical, chemical and/or physical properties [1,2]. Due to the possibility of substitution of up to 35% of nickel by iron, Ni-Fe alloys may be fabricated which are considerably less expensive than bright nickel. These alloys also exhibit properties of levelling, ductility, corrosion resistance, and brightness. Due to their magnetic properties they have many applications in the area of memory devices for computers, as perpendicular magnetic recording media for rotary encoders, as stampers for disk pressing and the cores of on-chip inductors. Recently micro electromechanical systems (MEMS) technology has been successfully applied to the fabrication of many tips such as microsensor and micro-actuators [3,6].Various compositions of Ni-Fe alloys such as Permalloy (80%Ni-20%Fe), Invar alloy (36%Ni-64%Fe) and high strength Ni–Fe alloy can be used to produce microsensor, microactuators and other MEMS device.

In the present work, we investigated several important factors on the nickel-iron plating electromagnetic property. The work will beneficial to the fabrication of many magnetic microactuators with Ni-Fe alloys.

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EXPERIMENTAL

Experimental Condition

There are many kinds of solution for Ni-Fe solution. An acid sulphate solution [4], containing metal sulphates and some brightener, was chosen for this study. The main content of solution and devices using in the experiment was presented as follows:

Main Salts: Nickel Sulphate, Ferrous Sulfate. Buffer Solution and Stabilizer: Boric acid, Citric acid. Anode activators and conductive agent: Sodium Chloride, Sodium Sulphate and some additive.

Main instrument:JSM-6300 type Scanning Electron Microscope/Energy Spectrometer, D/max-rC type X-ray Diffractometer, Lakeshore 7307-9309 type Vibrating Sample Magnetometer, The deposited alloys were to determine the nickel and iron contents by the Energy Dispersive X-ray Spectrometer.

The cathode was stainless slice with 1mm thick (1Cr18Ni9Ti). Before plating, the cathode was polished followed by surface activation in 20% Sulphuric Acid solution. Electrical pure iron and electrolysis nickel board with the area ratio Ni/Fe = 10 was used as the anode. The anode was protected by the terylene pocket.

Experimental Method

Since the magnetic properties of Ni-Fe alloys are seriously affected by their compositions and structures, a reliable control of the composition and textural properties (e.g. crystalline structures, morphologies, roughness, etc.) through plating is an important issue in designing the magnetic functionality of these materials. The key of the experiment was to control the process parameters such as the content of iron ion, PH value, temperature, current density in the solution.

When preparing metal salt solutions of different concentrations, the amount of Nickel Sulphate was been kept 300 g/l, the amount of iron ion was adjusted by the Ferrous Sulfate additives. The pH was adjusted to a value of 2.5 with sulphuric acid. The main electroforming parameters: pH value of the solution: 2.0～3.5, current density was set from 2.5 to 4A/dm^2, stiring velocity: 500～700 rpm/min, the solution temperature was ranged from 50 to 70℃, $FeSO_4 \cdot 7H_2O$ 3～7 g/l, several different process parameters were proceeded in the experiment.

DISCUSSION

Composition of the Deposit

According to Brenner [2,5], the electrodeposition of Ni–Fe alloy is classified as anomalous, meaning that iron which is the less-noble metal is deposited preferentially. In fact, it has been observed that the nickel deposition rate is inhibited in the presence of iron species. Nevertheless, the chemical composition, thickness and morphology of the films can be precisely controlled by electrochemical parameters such as the deposition potential (or current), bath composition, stirring, etc..The optimal process parameters to obtain Ni-20%Fe deposition were as following: $FeSO_4 \cdot 7H_20$

concentration 6 g/L; PH value 2.5; current density 3.5 A/dm^2; electrolyte temperature 55℃.

Property of the Plating

The properties of Ni-Fe deposit are affected by several factors such as the texture, compositions, etc. The main properties of the plating were presented as follows [3,4]:

SURFACE MORPHOLOGY

The deposit surface morphology was sensitive to the impurity in the solution. Some organic additives were adopted in the experiment to obtain the bright and smooth plating surface. Ni-Fe deposit has a more compact structure than that of Ni. Using conventional scanning electron microscopy, the grain size of the electrodeposits cannot be resolved. Fig.1 was the SEM photograph of the plating (Ni-22.27%Fe) after 2 days and 7 days eroding in 2 M HCl. The deposition rate is about 5μm-10μm per hour. The electrodeposits showed no curling or buckling after removing from the substrate. Which indicated that internal stresses are relatively low.

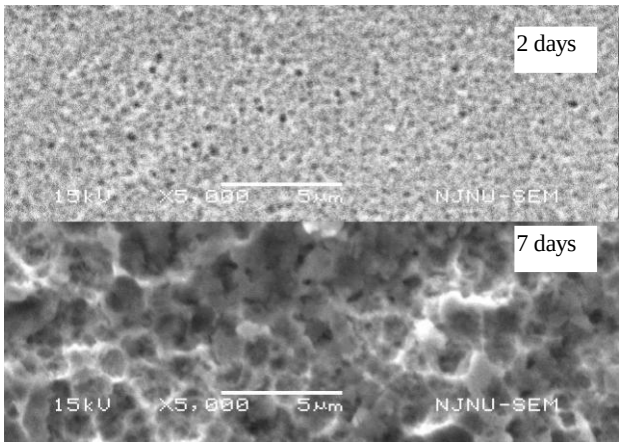

Figure 1. SEM photograph of electroforming Ni-20%Fe alloy.

Figure 2 shows the X -ray diffraction of electroforming alloy. The plating has a well crystal structure. The X -ray diffraction peak were (111),(220) and (200). When the iron content reached to 41%, the highest diffraction peak fall from (111) to (220). Pure Ni has a diffraction peak of (111).The change of the crystalline orientation may be attributed to the cathodic overvoltage and the change of the ion concentration. There are some inhibition material such as H_2, $Ni(OH)_2$ on the cathode-solution interface. They not only affect the plating morphology, the selective adsorption alter the crystal growth mode also.

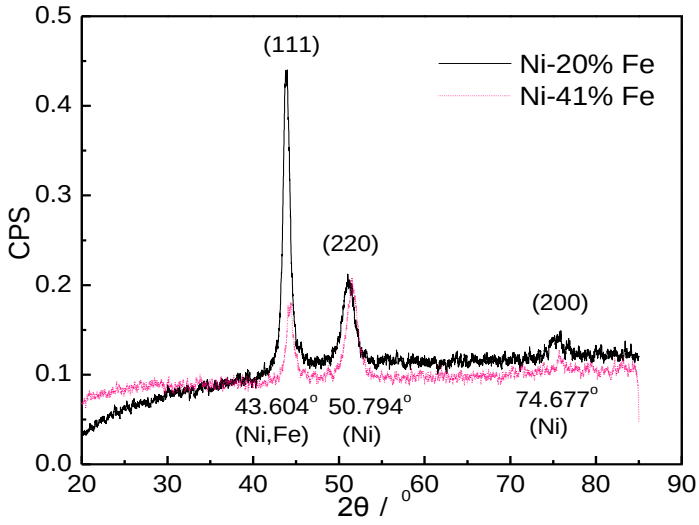

Figure 2. X-ray diffraction of electroforming alloy.

MAGNETISM PROPERTY

To compare the magnetism property with permalloy, the hysteresis loop of Ni-20%Fe deposit and permalloy (IJ85) were tested at the same condition. As showed in Figure 3, Ni-20%Fe deposit has super paramagnetic characters with magnetic remnant was 0.5 emu, coercivity was 2.819 Oe. The remanence and coercivity were less than that of IJ85 permalloy. Saturated Magnetization Value of deposit was only 10% of the IJ85.The remanence and coercivity comparison of the Ni-Fe deposit was showed in Figure 4. Ni-20%Fe deposit has a lowest remanence and the coercivity show a monotonic decrease with increasing the Fe content in deposit.The test data indicate that the Ni-Fe deposit has a satisfactory magnetism property.

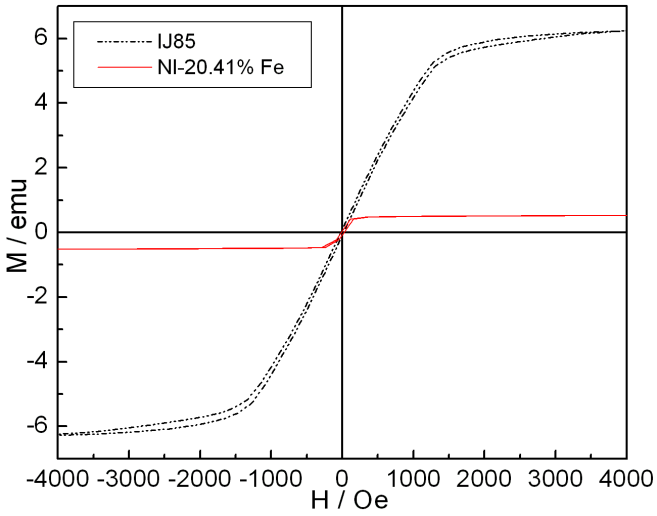

Figure 3. The hysteresis loop of electroforming alloy(Ni-20%Fe) and IJ85 permalloy.

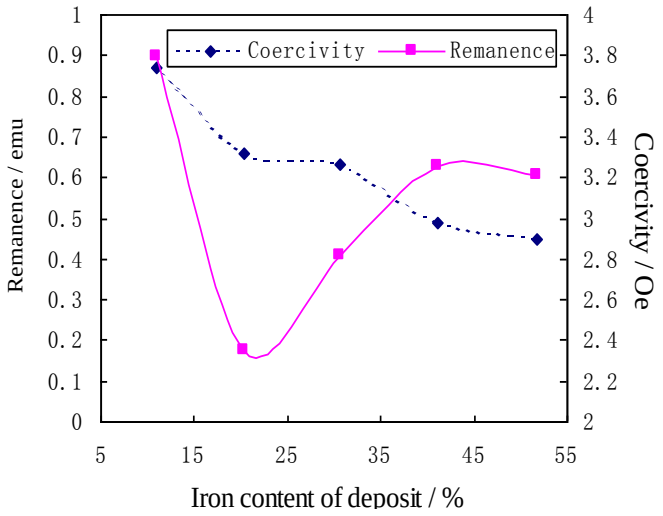

Figure 4. The Fe content of /Ni-Fe deposits against the coercivity and remanence.

CONCLUSION

Ni-Fe electrodeposit technology and physical property was discussed in this paper, and given the detail trend of the iron content in the plating. From the experiment it can concluded that the Ni-Fe deposit has fine electromagnetic property, bright and smooth surface and compact structure, and could be used in MEMS actuator manufacture.

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Cover page

Title: *Detecting Water, Light and Temperature Responses in Leaf Activity Using Dynamic Laser Speckle Analysis*

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ABSTRACT

This paper reports our recent result on the detection of water, light and temperature response in cabbage leaf activities using laser speckling technology with a normal vector based dynamic laser speckle analysis. There are two folds. Firstly, we show that the use of normal vector based statistical methods which we developed recently enables the dynamic laser speckle analysis to serve as a sensor measuring plant behaviour via its leaves. It demonstrates the capability of the underlying technology to build a non-contact and non-destructive sensor which is a key component in a feedback control system of agriculture. Secondly, we present experimental results showing that the sensor works. We implement three growth chamber experiments to investigate the response of leaf activity to different leaf water status, light and temperature separately for healthy cabbages. Our experimental results show leaf activity being responsive to the variation of water and light status but not so to temperature.

INTRODUCTION

Dynamic laser speckle analysis is widely recognised as an effective tool for the detection of sample surface activity. The distribution of laser speckles on a sample surface and the degree of its temporal evolution are strongly correlated with the activity of sample surface, which enables a real-time, non-contact and high resolution measurement method, known as dynamic laser speckle analysis, to measure micro-activities of the scale as smaller as laser wavelength on a sample surface. The technique has been widely applied for measuring activity of a variety of materials [1], such as plant seed activity analysis [2], blood flow detection [3-5], visualisation of tissue perfusion [6], burn scar perfusion [7], and works of art [8].

The migration of dynamic laser speckle analysis for plant leaf activity measuring is not straightforward. Conventional dynamic laser speckle analysis methods implicitly assume a uniform lighting distribution over the sample imaging area and the dynamics of speckles is thus characterised by the variation of image intensity over time. In general, the larger the temporal variation of image intensity is, the higher the speckle

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dynamics and sample activity are. However, for plant leaves under natural environment, the intensity based dynamic speckle analysis can cause severe biased results as a consequence of harsh measurement conditions, such as non-uniform sample reflectivity, non-uniform laser intensity, or uncertain ambient lighting etc. In our recent work in [9], a normal vector based statistical method was proposed to address the above issues and we demonstrated the capability of the normal vector based methods for measuring leaf surface activity. By using the normal vector based method, we successfully demonstrated in a well defined environment that the activity of a plant leaf can be detected using the technique of dynamic speckle analysis, and leaf activities are correlated with leaf water status [10-11]. The motivation of this paper is to further show the concept of plant leaf sensing via the technology of dynamic laser speckle analysis by presenting preliminary experimental results.

METHODS AND EXPERIMENTAL ARRANGEMENTS

In this section, the principle of normal vector based dynamic laser speckle analysis and the experiment set up are briefly given below.

Normal Vector Based Dynamic Laser Speckle Analysis

A fundamental difference of the normal vector based method over the conventional intensity based methods is the use of relative image intensity via the rotation of local normals to quantify the dynamics of speckles. This effectively filters out the influences of abnormal measurement condition, such as non-uniform sample reflectivity and laser illumination, time varying ambient light and diversity of sample colour etc.

With the collection of a sequence of sample speckle images, a pixel-wise statistics over time is performed. First, the images are smoothed using the method in [12] which increases the accuracy of estimation of local normals. Next, local normals are estimated with the normal vector voting algorithm proposed by Page *et. al* [13]. Once the normals are obtained, their rotations are evaluated using one of the three algorithms proposed in [9]. In this paper, the chosen statistics is the normal vector based sample entropy (NVBSaEn).

Experiment Set Up

The laser speckle imaging system used in this experiment is shown in Fig. 1. The laser diode, operating at 650nm, was placed in front of the abaxial leaf surface to illuminate it, resulting in the production of laser speckle patterns, registered by a CCD digital camera (CCE-B013-U) with a 12mm-focal-length lens (M1214-MP). The computer sends commands to the control module to control the entire speckle measurement process. Consecutive speckle images were taken and stored in the computer where they were processed using NVBSaEn algorithm.

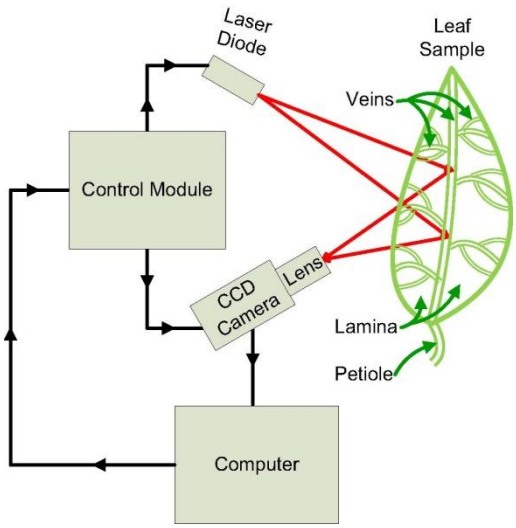

Figure 1: A schematic of the laser speckle imaging system consisting of a 650nm laser diode, a CCD camera and a control module, illustrating the region of laser illumination and camera imaging on a leaf.

Figure 2: A view of the experimental plant and the leaf clamping system on the laser speckle imaging apparatus placed insidethe chamber.

Fig. 2 illustrates how the leaf sample was clamped on the portable laser speckle imaging device. The leaf clamp was built using the sealing lips on the gas chamber of LiCor-6400, which have been proven to be able to maintain the health of the clamped leaf. The leaf clamp was held about 1cm above the case to make the boundary layer condition of the clamped leaf similar to that of other free leaves on the plant.

A total of 100 speckle pattern frames of 450×200 pixels each were recorded every measurement at 30 frames per second (fps). In parallel, the optical image of the leaf was also taken using the same camera just before taking the speckle images. The camera exposure time was 15ms and the lens aperture was adjusted so that the speckle diameter is about 6 pixels. The pixel size of the CCD sensor is $4.65 \times 4.65 um^2$ which resulted in a speckle size in the image plane of $611um^2$.

It is well known that leaves are responsive to ambient climatic variables such as light, temperature, humidity, CO_2 concentration, and wind speed [14]. In order to investigate the impacts on leaf activity individually, the experiments were performed in Conviron Controlled Environmental Cabinets, CMP5000, BioLab, Industrial Technologies, Australia located at Dookie College, University of Melbourne.

Experimental Scenario

Water response: The plants were deprived of water until leaf wilt was observed, after which laser speckle measurements were taken periodically. At 12:10pm, 3 hours after the commencement of measurements, the plant was watered sufficiently for it to recover from water stress. The temperature was controlled to be $28C^o$, and the lamps were always on during this experiment. Two leaves were measured every 5 minutes under this treatment.

Light response: In order to decouple the impacts of light and temperature, white LED lamps of cool operation are used as the light source. In the first 30 minutes, the

lamps were off. Then they were turned on and off periodically for 3 circles at a duration of 1 hour per circle. Plants were water hydrated and temperature was controlled to be 23C^o during this experiment. 3 leaves from 3 plants were measured every 3 minutes under this treatment.

Temperature response: Air temperature was controlled to be 23C^o in the first 30 minutes. Then temperature was increased to 26C^o which takes about 1 hour, and maintained at 26C^o for about 2.5 hours. Plants were water hydrated and the lamps were always on during this experiment. 2 leaves from 2 plants were measured every 3 minutes under this treatment.

EXPERIMENTAL RESULTS

The dynamic speckle image sequences collected from the experiments are processed using NVBSaEn algorithm and thus the distributions of leaf activity are obtained. Leaf veins are extracted from optical images via the Hessian based tubular object extraction algorithm [15]. The evolutions of averaged vein activity under different treatments indicate the response of water, light and temperature of the underlying plant leaves.

Water Response Curve

The time evolution of averaged vein activity in the water response experiment is illustrated in Fig. 3. In the two replicates (leaves 1 and 2), the vein activity increases gradually with the rise of leaf water content in the first 3 hours after being watered and reaches a stable level as the leaf becoming water hydrated. Such an observation agrees with the underpinning physiological principle of a leaf. Plant water transportation occurs in xylem vessels which is a significant composite of veins. Hence the micro-motion of veins is correlated with plant water status, which results in higher vein activity in a water hydrated leaf than in a water deficit leaf.

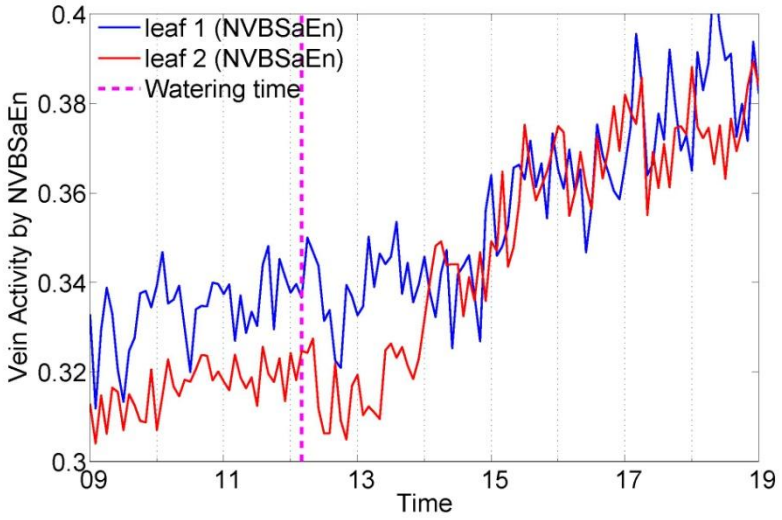

Figure 3: Water response curve of vein activity, where the time of watering water stressed plants is indicated by the magenta dashed line.

Light Response Curve

The influence of dark and bright lighting to vein activity is illustrated in Fig. 4, where a strong correlation between vein activity and photosynthetic active radiation (PAR) can be observed. The sizes of veins measured in the three replicates are different. A thick vein has higher activity than a thin vein does, leading to different activity levels from these three replicates. However, their activity trends versus time are consistent.

We should mention that the vein activity response to light is not an artefact of the light because the activities measured right after the lamp state toggle points (indicated in red dots in Fig.4) are in the same level with that measured before the lamp state toggles. The activity jumps up/down rapidly at the second measurement (sampling point) after the lamp state toggles and fluctuates at that level until the next lamp state toggle. The measurement (sampling) rate is 1 per 3 minutes hence the response time is faster than 3 minutes. As one of our future works, we will increase measurement sampling rate in the experiment to investigate the time response of vein activity to instant light variation.

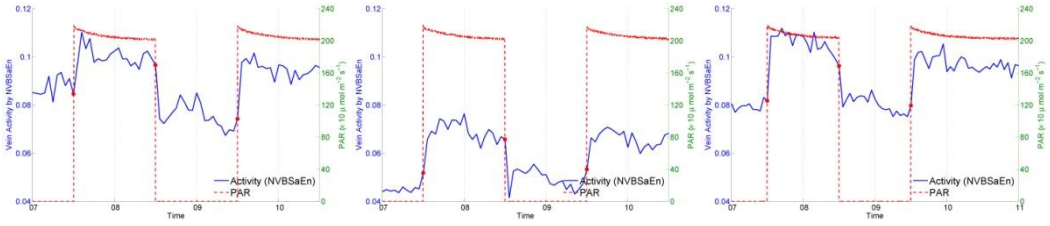

Figure 4: Light response curve of vein activity. The blue line is averaged vein activity and red deshed line is PAR.

Temperature Response Curve

The temperature response curve of leaf activity is presented in Fig 5. In contrast to the water and light response curves in Fig. 4, the vein activity seems not responsive to temperature change. In the two independent experiment replicates, the vein activities measured by NVBSaEn algorithm are fluctuating at a stable level when there is an increase of air temperature.

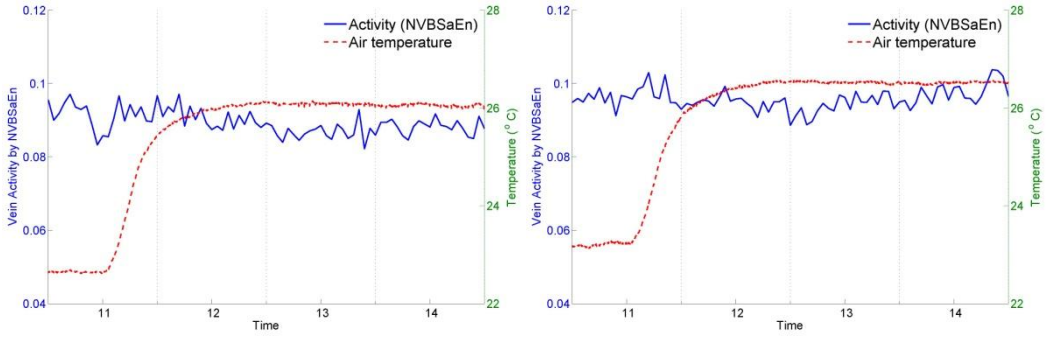

Figure 5: Temperature response curve of vein activity. The blue line is averagedvein activity and red dashed line is air temperature.

CONCLUSION

The response of cabbage leaf activity to water status, light and temperature is measured using a normal vector based technique of dynamic laser speckle analysis in several carefully designed growth chamber experiments. Experimental results confirm two points: 1) the vein activity has a positive relationship with leaf water status and light, but does not change with temperature; 2) in proof of concept, the technology of dynamic speckle analysis is capable to address the problem of non-contact and non-destructive plant sensing in a natural environment. Further experiment, result analysis and discussion in this topic will be presented in a separate paper elsewhere.

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Title: *Effect of Roasting on Anti-Inflammatory Activity of Oriental Melon (Cucumis melo l. Var. Makuwa) Seeds*

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ABSTRACT

The aim of this study was to investigate the triacylglycerol (TAG) profiles of hexane extracts from oriental melon (Cucumismelo L. var. makuwa) seeds subjected to roasting. For the first time, understanding relationships between the inhibitory effect on nitric oxide (NO) production in macrophages and different fatty acid contributed TAG. When the temperature fixed at 250 ℃, linoleic distributed individual TAG (LLL) increased by 1.1% comparing with that roasted at 150 ℃. In addition, in vitro inhibitory effect on NO production was positively correlated with the LLL content. At a concentration of 200 μg/ml, seed roasted at 250 ℃ showed powerful anti-inflammatory effect with a NO inhibitory activity of 54.3% in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The change in cytotoxic activity is considered to be correlated to the chemical alteration of constituents by the heat-processing.

INTRODUCTION

The biological functions of fatty acids, which are usually derived from glycerides, have been studied extensively. n-3 and n-6 polyunsaturated fatty acids are the two major classes encountered in the diet, and both classes of fatty acids are required for normal human health. Oriental melons (Cucumismelo L. var. makuwa) are widely grown in Korea and favored by consumers, largely due to their high qualities and special flavors. Oriental melon seeds have been reported to possess various pharmacological activities, such as anti-inflammatory [1], anti-ulcer [2], anti-cancer [3]. The extracts of oriental melon seed, which may have better health-promoting effects, are of interest to us and other researchers focused on the utility of this by-product in development of functional foods or food additives. Our previous study has shown that oriental melon seed could be used as a potent alternative for controlling type 2 diabetes [4]. It is well known that natural nutrients could be significantly lost during the thermal processing due to the fact that most of the bioactive compounds are relatively unstable to heat. However, other current studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities during heat treatment [5, 6]. Therefore, this study examined the effects of roasting on the composition and positional distribution of fatty acids in oriental melon (Cucumis melo L. varmakuwa Makino) seeds. In addition, the potential of the extract from the

seeds roastedat different temperatures to inhibit NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells was also evaluated.

MATERIALS AND METHODS

Plant Materials

Raw oriental melon seed were supplied from an industry located in Seongju-gun, South Korea. The seeds were deserted as agricultural waste. Nevertheless, care was taken that all of the samples analyzed were representative of the same initial batch, which was homogeneous. Several roasting experiments were performed on a laboratory scale using an electric muffle furnace (Model DMF-802; Daeil Engineering, Masan, Korea). After roasting at 250 ℃, seeds were stored at 4 ℃ before extraction.

Preparation of Oriental Melon Seed Extract

Ten g of melon seeds were extracted with 250 ml of hexane solvent at 150 ℃ for three hours and repeated twice. The extracts were filtered through Whatman No. 42 paper and evaporated in vacuum (JP/N 1000S-W; Eyela, Japan) and water bath (Digital Water Bath SB-651; Rikakikai, Tokyo, Japan) at 45 ℃ to remove extract solvents. For GC-MS analysis, the sample was directly converted to trimethylsilyl (TMS) derivatives.

Determine TAG content by HPLC

According to the method of Yanty et al [8] with minor modification, TAG profile was determined on a HPLC (Waters, Milford, MA, USA) associated with UV-Visible detector (Jasco, Japan).TAG was separated using a ZORBAX Eclipse XDB-C18 (4.6 cm × 250 mm, 5 µm, Agilent, USA) column. The detector was UV at 210 nm. The mobile phase consisted acetone/acetonitrile (63.5/36.5, v/v) at 1 ml/min flow rate, and injection volume was 10 µL.

Anti-inflammatory activity in RAW 264.7 cells

CELL VIABILITY

The RAW 264.7 cell line was obtained from Korea Cell Line Bank (Korea). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 100 units of penicillin, and 100 streptomycin sulfates at 37 ℃ in an atmosphere of 5% CO2. Cell viability was based on 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay in 96-well plates at a density of 5 × 105 cells/well. After overnight incubation, the medium was removed and replaced with fresh medium containing phosphate-buffered saline (pH 7.4) and dimethylsulphoxide DMSO (vehicle control) or indicated amount of seed extracts. 25 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml in phosphate-buffered saline, pH 7.4) was added to each well and incubated further for 4 h at 37 ℃. Upon termination, the supernatant was aspirated and the MTT formazan, formed by metabolically viable cells, was dissolved in DMSO

(150 μl) by mixing for 30 min on a gyratory shaker, and the plates were scanned at 540 nm with spectrophotometer.

Nitric Oxide (NO) Inhibitory Effect

Nitric oxide (NO) concentration in the medium was assayedas an indicator of NO production according to the Griess reaction method, as previously described [9]. In brief, RAW264.7 cells (5 × 105 cells/well) were seeded in 96-well plates incubated for 24h at 37℃. Cells were treated with LPS (1 μg/ml) or only with different concentrations of extract for 18 h. Then, 100 μL of culture medium from each sample was mixed with the same volume of Griess reagent and incubated at 37℃ for 10 min. The absorbance was then read at 550 nm. NO concentration was determined by comparison to dilutions of sodium nitrite as a standard.

Statistics Analysis

Statistical analysis was performed by Statistical Analysis System (SAS, Cary, NC). Data were subjected to the analysis of variance (ANOVA), followed by mean comparisons by Ducan's multiple range test at $p < 0.05$. Results of three replicate readings were pooled and expressed as mean ±standard deviation.

RESULT AND DISCUSSION

Major TAG Profiles of Oriental Melon Seed

The seeds extract differ in the degree of unsaturation, of the fatty acids constituting their triacylglycerols, as well as in the quantity and quality of the compounds [6] in their non-saponifiable constituents. Differences in composition are translated into differences in the stability, sensory and technical qualities of the oils. The types and the relative concentrations of various TAG species were the most important index in evaluating the functional properties of oriental melon seeds. TAG peaks were identified based on the retention time of HPLC of standard [8]. Hexane extract of melon seed contained four major TAG acid rich in linoleic acid: LLL, OLL, PLL, where L, O, and P were linoleic, oleic, and palmitic acids, respectively (Figure 1).

TABLE 1. TRIACYLGLYCEROL PROFILES OF HEXANE EXTRACT FROM ORIENTAL MELON SEEDS ROASTED AT DIFFERENT TEMERATURES.

Roasting Temp(℃)	Roasting Time (min)	Triacylglycerol (%)		
		LLL	OLL	PLL
RAW	0	33.1 ±1.1	30.6 ±0.3	14.9 ±1.2
250	60	31.7 ±1.1	29.4 ±0.7	14.6 ±0.6

LLL: trilinolein, OLL: dilinoleoyloleoylglycerol, PLL: dilinoleoylpalmitoylglycerol.

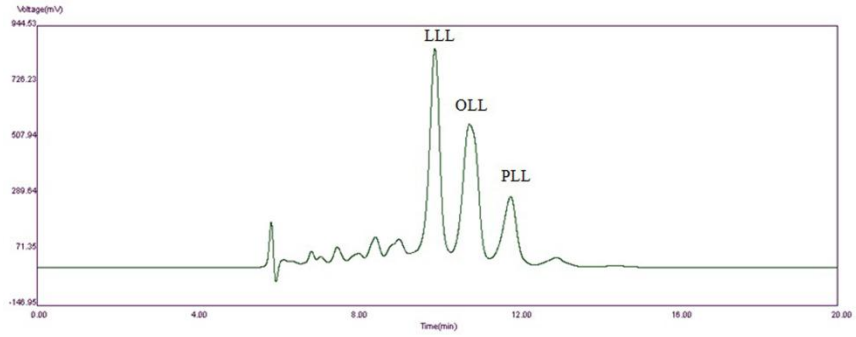

Figure 1 HPLC profiles (210 nm) of hexane extracts from oriental melon seed.

The representative changing profiles of composition and positional distribution of fatty acid of the TAG were compared before and after roasting (Table 1). With processing of roasting, differences were observed in the patterns of the fatty acid composition and positional distribution in the residual TAG. Linoleic distributed individual TAG contrarily increased, when the temperature fixed at 250 ℃ and the roasting time increased to 90 min. Previous study reported that linoleic primarily occupied the sn-2-position of the TAG molecules, while palmitic and stearic, were mostly located in the sn-1, 3-position, oleic was almost evenly located in the sn-1, 2, 3-position [12]. Fatty acids linked at the sn-2-position of glycerol are more stable towards thermal oxidation than those with the same acid at the sn-1-, or sn-3-position. Assuming that TAG oxidized continually as the roasting time's increasing, the facts that oxidation product diacylglycerol (DAG) will be accumulated simultaneously. Nakatsugawa and Coworkers [13] reported that DAG was stable and could be proposed as an antioxidant, similar to a sugar alcohol, or as a donor for the chelation of trace metals.

Anti-inflammatory Activity

NO has been well established as an intracellular messenger [14]. Moreover, its role in the regulation of cellular functions such as vasorelaxation and inflammation and in the elimination of pathogens and tumor cells is well known. Overproduction of NO in RAW 264.7 macrophage cells contributes to numerous pathological processes including inflammation. Therefore, the inhibition of NO production might be an important target in the treatment of inflammatory disorders. To determine the effect of oriental melon seed extract on cell toxicity, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used in RAW264.7 cells. As shown in Figure 2A and Figure 3A, no significant toxic effects were found in the RAW 264.7 cells when treating the cellswith oriental melon extracts at a concentration of 200 μg/ml. The effects of oriental melon seed extracts on LPS-stimulated NO production in RAW 264.7 cells were investigated by measuring the amount of nitrite release into the culture medium using the Griess reaction. Nitrite accumulation in culture medium after stimulation with 1 μg/ml of LPS was 21.45 μM (the values were obtained by using a sodium nitrite standard curve). It is seen in Fig. 2B, after incubation for 24 h, that 1.81 μM of NO was produced in the un-stimulated RAW 264.7 cells (DMSO). Extracts from different times and temperatures roasted seed significantly inhibited NO production in LPS-stimulated RAW264.7

macrophages and nitrite accumulation was in a concentration-dependent manner. All the extracts of the seeds roasted at different temperatures, RAW, Seed (150 ℃), Seed (200 ℃), Seed (250 ℃) and Seed (300 ℃), showed 14.6, 9.6, 19.1, 53.1, and 16.4% inhibition of NO at 200 μg/ml, respectively, indicating that seed (250 ℃) has more significant inhibitory activity ($p < 0.05$). In addition, all the samples roasted at 250 ℃ had a strong time-dependent inhibitory effect on NO production (Figure 3B). As the roasting time was increased, the inhibitory activity increased slightly to a maximum value of 87.7% (90 min). On the other hand, when the roasting time was increased continuously to 180 min, the inhibitory activity decreased to a significantly lower level.

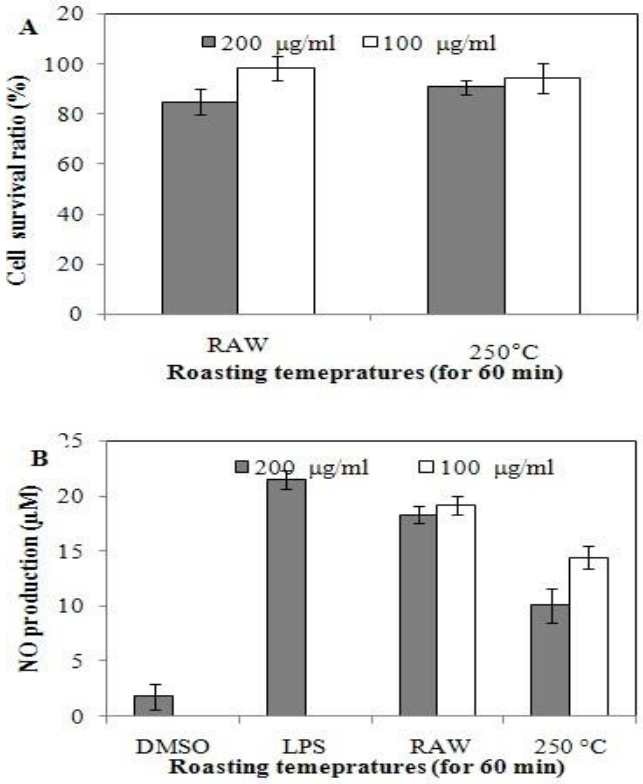

Figure 2 Cell viability and dose-dependent inhibitory effects of oriental melon seed roasted by different temperatures on the LPS-stimulated NO production. A: Effects of extract on viability of RAW 264.7 cells. Cells were treated with 200 μg/ml concentrations of samples for 24 h. DMSO: No extracted sample. B: RAW264.7 Cells were treated with two concentrations of samples and LPS (1 μg/ml) for 24 h. After incubation, the NO accumulation in the media was determined by the Griess reaction, LPS: only LPS (1 μg/ml) treatment. Bars represent standard error.

CONCLUSION

Although little attention has been paid to oriental melon seed which causes environmental problems as an agricultural waste product in the countryside near farms, we demonstrated that the extract of stalks is an effective inhibitor of NO in RAW 264.7 cells. The data are useful and informative for producers to develop a new class of anti-inflammatory agents from agricultural waste products.

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Title: *Study on Biological Control of the Probiotic Bacillus Coagulans CGMCC 6681*

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ABSTRACT

[Objective] The paper was to study in biological control about *Bacillus coagulans* CGMCC 6681 against crop diseases. [Method] By screening for strains, which had obvious antimicrobial action against rifampicin that is a broad-spectrum antibiotic were colonized on tomato leaf surface and roots, and thus to study the colonization. [Conclusion] The paper provides the result about the inhibitory effect of *Bacillus coagulans* CGMCC 6681 against *B. cinerea* was best, it has laid a good foundation for biological control.

1. THE DESCRIPTION OF AGRICULTURE

Nearly 20 years, the disease problems of the crop all the country have been suddenly intensified because of cropping systems change, climate variability, industrial structure adjustment and other factors [1]. Crop fungal disease control plays an important role in protecting capacity and quality of crops production [2]. People use the existing resources to regulate plant microbial environment, it is conducive to planting growth and development, so as to achieve disease prevention and control, reduce pollution, improve quality and maintain the ecological balance. The yields of the world's crop on the field have now increased by 30% to 40%. This is to accelerate the development of modern agriculture has laid a certain foundation [3].

2. *BACILLUS COAGULANS* AND APPLICATION PROGRESS

2.1 Main Features of *Bacillus Coagulans*

Bacillus coagulans is a gram-positive, and showing as dull circle, but straight (Fig.1).It has oval spores. Some sporangium is swelling, and can exercise. The colonies of *Bacillus coagulans* are flat round with irregular edges. They can grow under aerobic and anaerobic conditions, the optimum growth temperature is 35 ~ 45 ℃, and the optimum pH is 6.6 to 7.0[4].

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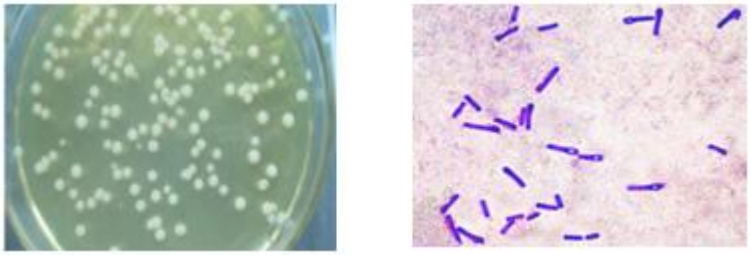

Fig. 1 The colony morphology and optical microscope *of Bacillus coagulans*

2.2 Applications *of Bacillus Coagulans* in the Field of Agriculture

Currently screened beneficial strains of *Bacillus coagulans* can be used for antagonizing fungal diseases of economic crops strongly, such as *Verticillium dahliae Kleb*, *Alternaria brassicicola*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*, rice sheath blight and so on [5]. Jorjani Marjan, who applied *Bacillus coagulans* B2 to sugar beet seeds in greenhouse tests, seedling mortality has been effectively controlled [6].

3. *BACILLUS COAGULANS* IN BIOLOGICAL CONTROL RESEARCH

3.1 Materials

Biocontrol bacterium: *Bacillus coagulans* CGMCC 6681, by Biotechnology and Pharmaceutical Engineering College, Nanjing University of Technology.

Plant pathogenic bacteria: *Verticillium dahliae Kleb*, *Alternaria brassicicola*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*, rice sheath blight and so on, isolated and preserved institute of plant protection, Tomato: Japanese Meiyu, presented by the Jiangsu academy of agricultural sciences.

3.2 Methods

3.2.1 MEDIUM CONFIGURATION AND CULTIVATION METHOD

(1) solid medium of *Bacillus coagulans* (g/L): peptone 10, yeast extract 5, NaCl 5, agar powder 20, pH 7.0～7.2
(2) seed medium of *Bacillus coagulans* (g/L): glu 60, peptone 10, yeast extract 5, pH 7.5
(3) fermentation medium of *Bacillus coagulans* (g/L): glu 22.00, peptone12.00, yeast extract15.00, $MnSO_4 \cdot H_2O$ 0.0002, $CaCO_3$ 0.02, pH 7.5
(4) Biocontrol fermentation: Transferred kind of age 18h, 4% of seed solution to 500 mL triangular flask which contains 10% fermentation medium, 180 r / min in fermentation medium for 40 h under vibration condition.
(5) Preparation of carbendazim tablet: dissolve 0.02mol/L dilute hydrochloric acid solution to carbendazim to prepare the mother liquor.

3.2.2 DETERMINATION OF INHIBITION ZONE

By plate dual-culture method, we used a punch (d = 6mm) to take the fungus cake

from colony, then picked it into 1/3 tablet midline to culture for 24 h, drilled holes in the 1/3 same line to 15 μL of fermentation broth, inverted culture on 28 ℃ [7], each treatment was repeated for three times. The control group is with using carbendazim.

$$\text{Inhibition rate} = \frac{\text{Control colony diameter} - \text{Processing colony diameter}}{\text{Control colony diameter} - \text{fungus cake diameter}} \times 100\% \quad (1)$$

3.2.3 POT EFFICACY TEST

The tomato seeds were cultured in the greenhouse. When four true leaves were grown up, the seedlings were transplanted separately. The conidia of *Bacillus coagulans* was washed by sterile water, and made into spore suspension with concentration of 4×10^6 spore/mL; 10 mL of suspension was sprayed on tomato leaves, and 10 mL of suspension was used to irrigate the root. The biocontrol strain was diluted into 1×10^6 CFU/mL, and 10 mL dilution was sprayed on leaf [8]. The treatment only inoculated with spore suspension and sterile water was set as control; treatment 1 was simultaneously inoculated with spore suspension and fermentation liquid of biocontrol strain; treatment 2 was first inoculated with spore suspension for 2 d, and then inoculated with fermentation liquid of biocontrol strain; treatment 3 was inoculated with fermentation liquid of biocontrol strain for successive 2 d, and then inoculated with spore suspension; treatment 4 was first inoculated with spore suspension, and then sprayed with 10 mL of 50 mg/L pyrimethanil. Each treatment contained 60 pots. The temperature in greenhouse was between 20℃ and 25℃.The disease classification was based on the percentage of lesion area in total leaf area; 0 level, no lesion; 1 level, ≤ 5%; 3 level, 5% -10%; 5 level, 10% -20%; 7 level, 20%-50%; 9 level, ≥ 50%.

3.2.4 *BACILLUS COAGULANS* ON THE COLONIZATION STUDIES OF TOMATO

3.2.4.1 The colonization on the leaf surface and root

Many plant pathogens are present in the leaf as a residence, the period of residence on these favorable sites is for occupation and breeding can produce infection after a certain number of virulence. Colonization is the key to occupy favorable sites and achieve the desired control effect for *Bacillus coagulans* on plants.

To exclude naturally occurring colonization results for the determination of the interference, the paper selected broad-spectrum antibiotic which was named rifampicin in order to screening strains. Put rifampicin solution into the solid medium with biocontrol to prepare different concentrations (100,150,200,250,300,350,400 μg / mL) of rifampicin selective medium [9]. The last is to select the maximum growth concentration to screen rifampicin antagonistic strains to subculture.

When tomato seedlings achieved to be 20cm, sprayed the microbial of Rifampicin marked which were cultured for 40 h (108 cfu / mL) on tomato leaf surface with 0.1% Tween 80, 20 seedlings per treatment. We can keep the temperature from 18 to 25 ℃ and the light was 12 h / d. For sampling after 1 h, measuring the initial amount of bacteria and we should taken 20 cm2 leaves from plants, cut into pieces, use 50 mL sterile water washing 30 min, then got 0.1 mL of different dilutions of the washing liquid coating on the solid medium, Each dilution gradient was deal with 3 dishes, then cultured 24 h on 37 ℃, counted the number of colonies to convert into the number of colonization, taken samples after 1,3,5,7,11,15,21,27,35 d

respectively. The amount of bacteria expressed with cfu/cm2. Each treatment was repeated three times. Tomato seedlings during the trial period do not apply to any other chemical reagents [10]. The treatment use the same way of irrigating root then take root soil sampling in 5 grams, with sterile water washing. The control group was set without Bio-control bacteria.

3.3 Results and Analysis

3.3.1 INHIBITION RATE OF *BACILLUS COAGULANS* CGMCC 6681

Table 1 shows the best inhibition rate on againsting *B. cinerea* was 72.64%. The *B. cinerea* which is one of the most common diseases has expanded each year. According inhibitory results, the paper would choose the *B. cinerea* to further study.

TABLE 1 THE INHIBITION RATE ON PATHOGENIC FUNGUS OF DIFFERENT MEDICAMENTS

inhibition rate (%)	pathogenic fungus				
	B. cinerea	*Verticillium dahliae Kleb*	*Rhizoctonia solani*	*Alternaria brassicicola*	Sclerotinia sclerotiorum
Fermented liquid	72.64	48.25	36.25	25.19	17.46
Carbendazim	100	100	100	100	100

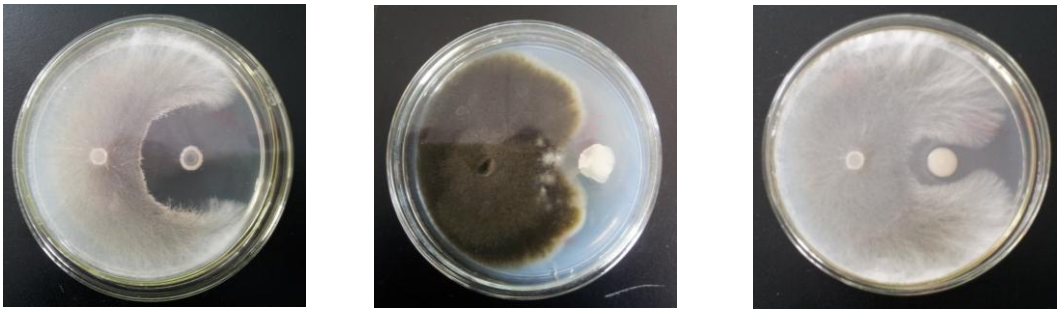

Fig. 2 The antibacterial effect of *Bacillus coagulans* CGMCC 6681 to *Verticillium dahliae Kleb*, *Alternaria brassicicola* and *Sclerotinia sclerotiorum*

3.3.2 CONTROL EFFECT OF *BACILLUS COAGULANS* AGAINST *B. CINEREA* IN POT TEST

Table 2 showed that treatment 3 (first inoculated with fermentation liquid of biocontrol strain for 2 d, and then inoculated with *B. cinerea* spore suspension) had the best control effect, and the control efficiency was 78.5%, equal to the control group which was treated by 50 mg/L pyrimethanil. Pyrimethanil was the pesticide commonly used to control *B. cinerea*, but the pathogen would gradually produce resistance. Test results showed that the effect was not as good as *B. cereus*. The control effect of treatment 3 was better than that of treatment 2, because advanced inoculation of fermentation liquid of *Bacillus coagulans* tomato seedlings increased the resistance of plant, and its incidence rate was lower than the treatments with equal amount of pathogen.

TABLE2 CONTROLLING EFFECTS OF *BACILLUS COAGULANS* CGMCC 6681 ON *B. CINEREA*

Treatment	Disease classification	Average disease index (%)	Control effect (%)
Control	7	38.35	-
1	5	17.60	57.61b
2	7	20.62	48.23c
3	5	9.26	78.50b
4	5	9.54	74.36a

3.3.3 EFFECT OF *BACILLUS COAGULANS* COLONIZATION ON TOMATO

3.3.3.1 Results on the colonization of tomato leaf surface and root

We can get 200 μg / mL rifampicin resistant strains of *Bacillus coagulans*. Its resistance remains stable when cultured three more generations, and the antagonism to *Botrytis cinerea* was not lost.

The ability for *Bacillus coagulans* CGMCC 6681 on the colonization of tomato leaf surface is very strong, It could be seen from the figure 3, the test for the first 5 days, the number of bacteria decreased slightly, until to the first 7 days it produced peak amount of bacteria, the number of bacteria had already reached 4.42×10^6 cfu/cm^2. The reason was probably *Bacillus coagulans* had formed dominant species in the short term. During the later period of the test, the quantity of bacteria was gradually stabilized, after 45 d, its colonization remains relatively stable, it might be the original microbial restore competitiveness.

The trend on the root was basically similar to the leaf surface (figure 4), but the all amount of bacteria was more than on the leaf, and this cause was bio-control bacteria occur huge complex interactions with microbial populations in the soil.

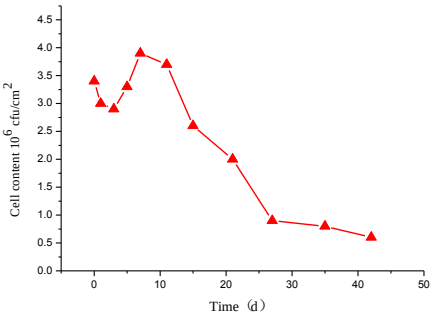

Fig. 3 deal with on the leaf surface

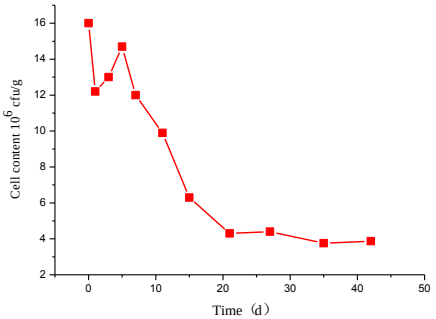

Fig. 4 deal with on the root

Figure 5 The colonization on the leaf surface (left) and root (right)

4. CONCLUSIONS AND DISCUSSION

In the case of having the same initial concentration about *Bacillus coagulans*, the colonization on tomato root was significantly better on the leaf surface of its colonization .We guessed that *Bacillus coagulans* were screened from the soil, so the growth environment in the soil such as temperature, moisture, nutrients and other were more suitable for growth. The study has laid a good foundation for biological control.

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Cover page

Title: *Effect of Water Stress on Physiological Ecological Characteristics, Photosynthetic Rate and Yield of Cotton*

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ABSTRACT

In order to improve the cotton production, we studied the physiological, ecological indexes and the reflectivity of cotton which is under different drought duration and different soil water stress. From blooming and bolling period, the variations of cotton (MeiMian 99B) leaf chlorophyll, plant height, photosynthetic rate, yield and reflectivity were studied by controlling dry time and varying degrees of soil moisture treatments. We establish the model between the production rate and the canopy reflectance. The results showed that all the indexes measured from plant height, chlorophyll content, photosynthetic capacity and cotton production are decreased significantly when ender water stress, and it became more serious with the strengthening of stress decrease. We have established the relationship between normalized difference vegetation index (NDVI), enhanced vegetation index (EVI) and cotton production rate. It can provide the basis for monitoring crop yields and drought risk prediction.

INTRODUCTION

Drought has become the important restriction element to cotton production in China. Cotton cultivation is mainly distributed in Xinjiang, the Middle-Lower Yangtze Plain and Huanghuaihai plain, and the cotton growth is limited by moisture in Xinjiang and Huanghuaihai plain. Drought has caused the cotton production deceased sharply in past years. With the rapid development of economy and human society, increasing the use of cotton and exploring the drought and other natural disasters which lead to cotton production decease has become a hot topic nowadays.

Water stress will affect plant growth and development and lead to severe crop failure. Cotton leaf is an important index of cotton growth. When the cotton is under water stress, the leaf water content and chlorophyll content will decrease[1-3]. Drought also affects cotton photosynthetic rate, . Studies showed that droughts destroy the chloroplast activity and affected the stomata conductance which; lead to the photosynthetic rate reduced[4-6]. Moisture impact on cotton growth majorly, and the water requirement is different under in different times, For instance, appropriate water deficit will impact on yield less before flowering and boll and improve the droughtresistance of cotton, but water deficit in flowing will cause huge losses [7-9]. Cotton in blooming and bolling period needs a lot of water, and it is sensitive to water

moisture disorders, metabolic processes is blocked, and a large number of abscission of cotton buds and bolls shed which cause premature aging and affect the cotton growing process seriously [10-11]. So studying on the ecological effects of cotton under water stress is meaningful.

1. MATERIALS AND METHODS

1.1 Experimental Design

The experiment was performed in Liangxiang testing ground of Beijing normal university from august to October 2012. It was included 21 plots which divided to the experimental group and control group. The lenth and width is 3 m*3 m. There is 1m interval between each plot to prevent soil moisture loss. The local soil is cinnamon soil and the basal soil fertility is pH7.72, total nitrogen 0.27 g kg-1, total phosphorus 0.53 g•kg-1, total potassium 16.9 g•kg-1. The test cotton is MeiMian 99B.

Frome the blooming and bolling period of cotton, the experimental group used the awning to eliminate rain and control the soil moisture. Experiments were equipped with seven treatment respectively: A1-short time of drought (August 24 - September 5, a total of 12 d), A2-moderate drought time,(August 24 - September 23, a total of 30 d), A3-long time of drought (August 24 - on October 10, a total of 47 d); B1-high moisture treatment (more than 60% of field capacity), B2-medium water processing (40% - 50% of field capacity), B3-low water treatment (less than 30% of field capacity); Control group S -normal water supply.

1.2 Test Item and Measuring Method

Cotton test item include photosynthesis, plant height, chlorophyll, yield and optical properties, etc.

Photosynthesis: useing the Li - 6400 portable photosynthesis meter (USA) to measure the plant leaves and every plant measured 3 times. The instruments equipped with red and blue LED light source and the photon flux density (PFD) is 1000μmol•m-2•s-1.

Plant height: useing ruler to measure the plant height which were selected in every plot.

Chlorophyll: useing SPAD - 502 chlorophyll meter to measure the plant leaves which were selected in every community.

Optical properties: useing the FieldSpace ® 3 portable spectrometer (American ASD company). Choose sunny windless weather, from 10:00 to test time "(the Angle of the sun is more than 45 °).

2. RESULT AND ANALYSIS

2.1 The Influence of Photosynthesis under Water Stress

The photosynthetic rate and transpiration will be suppressed when cotton under water stress, so they will affect the cotton normal growth. Table 1 shows the photosynthetic rate of each disposal is lower than the control group when the cotton

under water stress for 12 days, reduced respectively: 37.4%, 38.2%, 41.9%, 8.4%, 19.6% and 17.5%. All of them passed the test of significance, and A1, A2, A3, B2, B3 are extremely significant. After 30 days, Pn, Gs, Tr have a downward trend, among of them, the Pn value of A3 is minimum, dropped 36.8%, the second is the A2,dropped 36.5%. And the Pn value of B1 is maximum, dropped 10% compared with the control group. After 47 days, the cotton is close to maturement, the photosynthetic characteristics of cotton are down significantly. Especially its biggest falls is under drought intensity processing.

Overall, the decrease of A1, A2 and A3 treatment were larger than B1, B2, B3. The reason is that there was no moisture to join in the process of class A, and the moisture of class B was always keep in the specified range, it can provide the moisture that the normal growth of cotton need. Water decreasing made the photosynthetic characteristics to decrease significantly.

TABLE 1 THE PHOTO, COND AND TRMMOL IN DIFFERENT MANAGEMENT

dispose	September 5, 2012 (12 day)			September 23, 2012 (30 day)			October 10, 2012 (47 day)		
	Pn	Tr	Gs	Pn	Tr	Gs	Pn	Tr	Gs
A1	12.44**	3.59	0.18	17.65**	3.97	0.19	8.02**	1.55	0.05
A2	12.27**	3.67	0.17	14.85**	3.95	0.19	7.19**	1.23	0.07
A3	11.54**	3.87	0.17	14.78**	4.1	0.12	6.81**	1.35	0.06
B1	18.2*	5.88	0.32	21.07*	6.88	0.33	12.85*	3.09	0.13
B2	16.17**	4.87	0.26	19.1**	5.91	0.28	10.88*	2.5	0.10
B3	16.40**	5.11	0.27	19.41**	5.50	0.29	8.32**	1.69	0.07
S	19.87	6.54	0.29	23.39	6.31	0.32	14.03	4.66	0.14

Note: * significance level of 0.05, ** significance level of 0.01.

Cotton was subjected to water stress, it will affect the plant cell activity, resulting to the growth rate slow, and reduce chlorophyll content and the ability of net photosynthesis. Meanwhile, the plants's ability to protect themselves leads to Tr decreased and Gs reduced. which will reduce crop photosynthesis further.

2.2 The Influence of Plant Height under Water Stress

Fig.1 and Fig.2 show that the cotton plant height is on the rise with the growth. But the growth rates are different under different water stress. Fig.1 shows the plant height of A3 is the lowest, and it was dropped 12.4% compared with control group before removal apical dominance. A1 is the least affected by water stress, and it was dropped 4.5%. There is a massive gap between A1 and others (A2 and A3), because at the end of treatment of A1, rewater the cotton lead the plant height of A1 increased significantly.

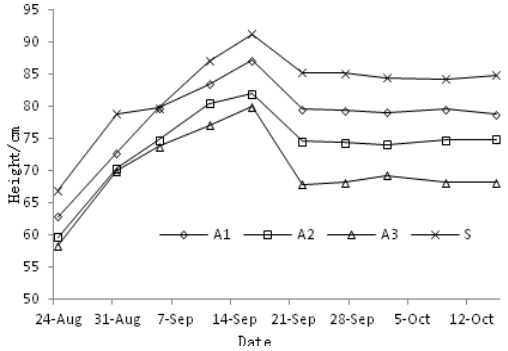

Fig1 Effect of drought intensity on plant height of cotton

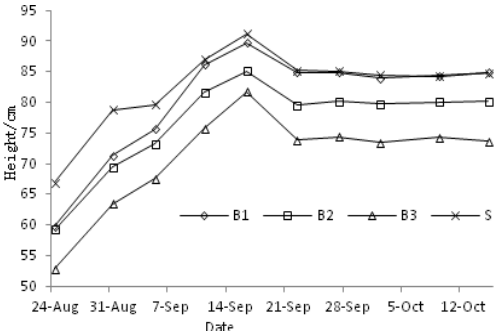

Fig2 Effect of water deficit on plant height of cotton

In fig.2, the trends of plant height which are gradually increasing are similar. B1, B2 and B3 were lower than control group, and the plant height of B3 is much lower, it was dropped 10.4% compared with control group before removal apical dominance. Among them, B1 has the most moisture, and the plant height is the highest, opposite, B3 was the least in moisture content, and the height is always in the worst level.

In conclusion, moisture impact on cotton plant growth greatly. That is the less moisture conditions, the worse the level of plant growth, on the contrary, the growth level is well. Cotton's matter distribution will change if it lack of water in the growth process. The cotton root grow lushly, and the canopy material significantly reduced.

2.3 The Influence of Chlorophyll under Water Stress

The value of chlorophyll (SPAD) can directly reflect the pace of cotton growth. Fig.3 shows the chlorophyll's trend increases first and then decreases with the growth of cotton. At beginning, the chlorophyll of A1, A2 and A3 had declined, but there were little difference between each other. The chlorophyll has increased dramatically compared to the others when A1 rewater, The chlorophyll of A2 also rose rapidly similarly. It is said that the soil moisture content will impact chlorophyll of cotton signally.

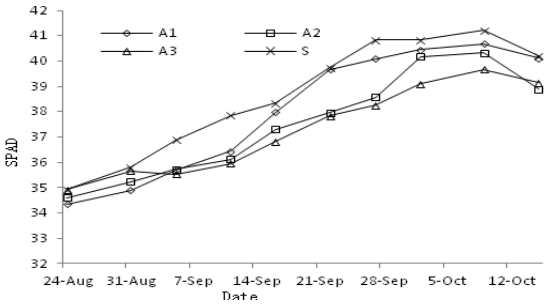

Fig3 Effect of drought intensity on content of chlorophyll

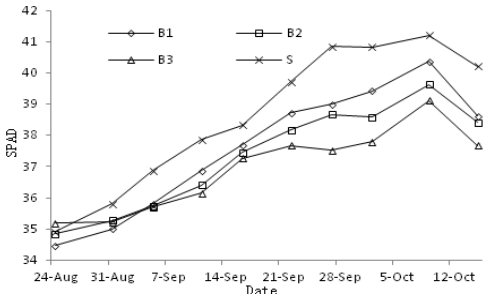

Fig4 Effect of water deficit on content of chlorophyll

Fig.4 shows that chlorophyll content were lower than the control group under different water treatments. The chlorophyll content of B3 is the lowest, and it was dropped 8.4% compared with control group, and B1 was the highest.

In summary, A1 and B1 had the largest water conditions, the chlorophyll content was always at the highest level; A2 and B2'smoisture conditions and chlorophyll content were keep in intermediate level; A3 and B3 water conditions were the worst, and the chlorophyll content were minimum and always lower than the other treatments. Thus, there is a positive relationship between the chlorophyll content and soil moisture.

2.4 The Influence of Biomass under Water Stress

Table2 shows that the cotton in different levels of water stress during the growing season would reduce the boll. And it descends with the increase of water stress. The number of boll dealing with A3 is only account for 66.3% of control group which is not significant, and followed by A2 and A3 which falls 16.4% and 27.9% respectively. On the water stress, the number of boll dealing with B1 has minimum decrease which is 10.6%. B2 and B3 fell 26.9% and 36.5% respectively, and exceed 95% confidence level.

TABLE2 THE NUMBER OF COTTON BOLLS AND STRAW WEIGHT UNDER DIFFERENT PROCESSING (ONE PLANT)

dispose	A1	A2	A3	B1	B2	B3	S
Cotton boll number	9.67	8.33*	7.78*	10.33	8.44*	7.33*	11.56
Straw weight	102.15	76.74*	67.93*	131.79	101.95	91.73*	134.68

The weight of crop straw has been influenced by water stress. Weights of crop straw of six processing are lighter than the control group. Among them, A2, A3 and B3 were significant. When water is the least, the straw weight reaches minimum, and vice versa. When cotton subjected to different levels of water stress, net photosynthesis rate were significantly reduced, resulting to the decrease of the amount of photosynthetic products. And during its distribution process, the plant's self-protection ability made large amounts of water assign to the root system to enhance its water capacity, thereby reducing the canopy biomass. Sufficient moisture is the important condition for the growth of cotton. When moisture is sufficient, number of bolls will increase. It will affect the production increase of cotton.

2.5 The Relationship between Yield Reduction and NDVI, EVI

The yield and the spectral reflectance (NDVI) has a close relationship[12]. We put the NDVI and EVI as independent variable, and put the yield reduction rate as dependent variable, construct the relational model to provide the basis for cotton yield monitoring.

NDVI and EVI calculation is as follows:

$$\mathrm{NDVI} = (\rho_{NIR} - \rho_{\mathrm{Red}})/(\rho_{NIR} + \rho_{\mathrm{Red}}) \tag{1}$$

$$\mathrm{EVI} = (\rho_{NIR} - \rho_{\mathrm{Red}})(1 + L)/\ (\rho_{NIR} + C_1\rho_{\mathrm{Red}} - C_2\rho_{Blue} + L) \tag{2}$$

Where ρ_{NIR}, ρ_{Red} and ρ_{Blue} is the canopy reflectivity correspond to near infrared wave band 2 (841~876 nm), red light band 1 (620~670nm), blue light band 3 (459~479 nm). L=1. C_1 and C_2 coefficient and C_1=6, C_2=7.5 [13].

TABLE3 RELATIONSHIP MODELS OF YIELD REDUCTION RATE OF COTTONT WITH NDVI AND EVI UNDER DIFFERENT DROUGHT DURATION

dependent variable	independent variable	fitting model	Model type	R^2	RMSE
yield reduction rate %	NDVI	y=--950.7 x^2 +1314x-422.3	quadratic	0.9667	2.654
yield reduction rate %	EVI	y=-126.8 x^2 -225 x -94.17	quadratic	0.9943	1.095

TABLE 4 RELATIONSHIP MODELS OF YIELD REDUCTION RATE OF COTTON WITH NDVI AND EVI UNDER DIFFERENT WATER DEFICIT

dependent variable	independent variable	fitting model	Model type	R^2	RMSE
yield reduction rate %	NDVI	y=--14.77 x+18.51	line	0.7243	8.582
yield reduction rate %	EVI	y=2523 x^2 -3491 x+ 1207	quadratic	0.8689	5.919

Table 3 and table 4 show that the relational model between yield reduction rate and EVI is significant under long drought. But under different soil moisture, the relational model between yield reduction rate and NDVI is great, which can provide forecast for cotton reduction by according to the spectral characteristics of cotton under different water stress modes.

3. CONCLUSIONS

The mechanism of crop drought tolerance is very complicated, and it is not enough to research the mechanism of injury from one level. Plant height, leaf area, chlorophyll and net photosynthetic rate contain a lot of information. Therefore, study on cotton physiological and ecological changes in drought conditions can help us to understand the impact of water stress on cotton.

Water stress will lead to stomata closure and affect the supply of CO2 to the chloroplast, while it will cause the chloroplast activity and net photosynthetic rate decreased. The tests showed that cotton net photosynthetic rate increased with timing and intensity of water stress reduce. In the growing period, chloroplast activity will strengthen and stomata resistance will decrease when remove the water stress, and the net photosynthetic rate will have a rising trend.

Water stress can affect the growth conditions of cotton. It impact the plant height, chlorophyll content and photosynthetic products. The study demonstrates that cotton plant height, chlorophyll content, photosynthetic rate and yield increased with the degree of water stress decreased.

Cotton normalized difference vegetation index (NDVI), Enhanced Vegetation Index (EVI) and production rate can establish a good relational model. Through

analyzing agronomic parameters of early growth stage cotton, it can forecast production condition and provide crop yield monitor and drought risk prediction.

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Cover page

Title: *Optimization of Irrigation Scheduling using Genetic Algorithms and AquaCrop: A Case study for Cotton in Northern Greece*

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ABSTRACT

This paper presents a two-stage procedure for optimizing irrigation scheduling in cotton farming. In the first stage the amounts of water for each irrigation are calculated assuming that the irrigation days are known. In the second stage the irrigation days are determined assuming that the irrigation amounts are known. Genetic algorithms are used to perform the two optimizations and the whole procedure is repeated three times to ensure convergence. This approach is tested for a typical cotton farm in Northern Greece using six years of meteorological data and the model AquaCrop. The simulation results shows that this approach leads to significant irrigation reduction compared to fixed irrigation based on the amounts of water allowed by Greek legislation or irrigation triggered according to fixed thresholds for soil water depletion.

INTRODUCTION

Increased water demand for agriculture, caused by global population growth coupled with global warming, has resulted in dwindling of fresh water resources. The misuse of the available fresh water resources threatens sustainable agricultural development and economic growth. Maximizing the effectiveness and efficiency of fresh water use in crop irrigation is therefore crucial.

Various approaches can be implemented to ensure that agriculture uses water more efficiently. These include changing the timing of irrigation so that it follows closely the crop water requirements, adopting more efficient techniques such as using micro-irrigation systems, implementing the practice of deficit irrigation, and large-scale use of reclaimed wastewater. The present work focuses on the first issue, namely on optimization of the irrigation schedule. Cotton cultivation in Northern Greece is used as a case study.

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OPTIMIZATION PROCEDURE

Assuming that the number of irrigation events for the whole season (denoted by *N*) has been set *a priori*, the basic questions are when to irrigate and how much to irrigate each time so that yield is maximized while the amount of irrigation water is minimized. This complex "Min-Max" optimization problem can be replaced by a simpler minimization problem if we consider that the desired yield is given *a priori*. In this case the basic questions are when and how much to irrigate so that the amount of irrigation water is minimized while still reaching the desired yield. In other words we need to determine *2N* unknowns, the irrigation days D_1, D_2, ... D_N and the corresponding irrigation amounts (in mm): I_1, I_2... I_N, , so that the target yield is reached.

Mathematically:

Find $\{D_1, D_2, \ldots D_N, I_1, I_2, \ldots I_N\}$ such that $\sum_{k=1}^{N} I_k \rightarrow minimum$

subject to $yield \geq target$

We propose to solve this problem in two stages: (1) optimization of the irrigation amounts (assuming that the irrigation days are given) and (2) optimization of the irrigation days based on the irrigation amounts obtained at Stage 1. The whole procedure is repeated three times to ensure convergence. Initially, the irrigation days are set at regular intervals throughout the season. For the second and third iterations the results of the previous iterations are used as starting point.

Each optimization was performed using genetic algorithms (GA) [1, 2]. Genetic algorithms mimic natural selection to efficiently search very large solution spaces. Compared to traditional optimization methods based on the gradient of a function, GA are more appropriate when the function includes some complexities and/or discontinuities. The major advantages of the GA are that the derivatives of the objective function are not required and nonlinearities can be handled.

In the first optimization stage the chromosomes corresponded to the irrigation amounts and in the second stage they corresponded to the dates of irrigation. In both cases the population was described by a vector of *N* integers. The size of the population was set to 50 and the optimization was performed for 20 generations.

CASE STUDY

The performance of the procedure described above was investigated using as case study a farm cultivating cotton in Northern Greece. Six years of meteorological data were available (2004-2009). The crop growth model Aquacrop developed by FAO [3] (http://www.fao.org/nr/water/aquacrop.html) was used to simulate the crop development. This model was selected since it has been developed specifically to predict crop response to various irrigation regimes. The following input files were used in addition to the measured weather:

- Crop File – Cotton.CRO (Default Cotton, Calendar, Cordoba – 15Apr86) with a modification in the active roots depth (1 m instead of 2 m)
- Soil Profile – Aquacrop default file (SandyClay.SOL) – Permanent wilting point 27% vol., Field capacity 39% vol., Saturation 50% vol.

The initial soil moisture was assumed to be at wilting point. For each year the optimization procedure was repeated seven times with increasing target yields (4.68,

4.95, 5.00, 5.20, 5.40 and 5.46 t/ha), assuming 8, 10 and 12 irrigation events (*N*=8, 10 and 12) (altogether 21 combinations per year). For the sake of comparison, the following scenarios were also simulated using the same AquaCrop model:

- Utilization of the maximum amount of monthly irrigation water according to current Greek legislation (Table 1), with irrigation performed every ten days
- Utilization of 80% of the maximum amount of monthly irrigation water according to current Greek legislation, with irrigation performed every ten days
- Utilization of 60% of the maximum amount of monthly irrigation water according to current Greek, with irrigation performed every ten days
- Irrigation schedule generated by AquaCrop based on allowed depletion of soil water corresponding to 20% of total available water (TAW) (i.e. irrigation is triggered every time the soil water reaches 80% of TAW). Each time the irrigation replenishes the soil to field capacity
- Irrigation schedule generated by AquaCrop based on allowed depletion of soil water corresponding to 50% of total available water (TAW).

RESULTS

Figure 1 shows the results (yield vs. irrigation) associated with all the irrigation schedules obtained at the end of the GA optimizations for year 2009 assuming 10 irrigation events. From these results the Pareto front corresponding to the optimal yield/irrigation combinations can easily by determined (solid line in Figure 1). Figure 2 shows these Pareto fronts for all six years. It can be seen that overall the number of irrigation events (*N*) has only a limited impact on the results.

TABLE I. WATER QUOTAS FOR COTTON IRRIGATION IN GREECE.

	April	May	June	July	August	September
Quota, mm	75	104	120	136	130	94

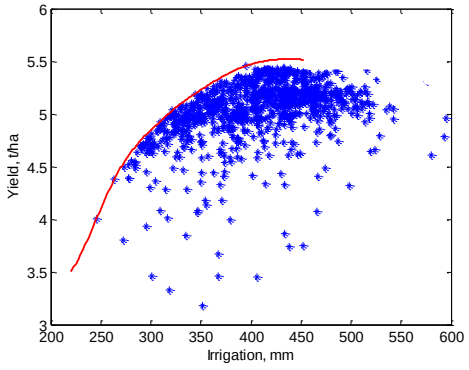

Figure 1. Relationship between irrigation and yield for irrigation combinations investigated during the optimization procedure. The solid line corresponds to the Pareto front (highest achievable yield with given amount of water). The results are for year 2009 assuming 10 irrigation events.

Results such as those presented in Figure 2 could be used by a farmer to estimate the amount of irrigation water required to achieve a desired yield (assuming that the

weather is known until the end of the season). For the sake of discussion, let us assume that the desired yield is 5.3 t/ha. For each year the corresponding optimal irrigation is indicated by '•' in Figure 2, and the top rows of Table 2 show the monthly amount of irrigation required. Since the monthly water quotas were not taken into account in the optimization procedure these quotas are exceeded in some months: April, July and/or August of 2007, 2008 and 2009 (emphasized by bold in Table 2). Introducing these constraints in the optimization procedure slowed down the computations significantly. Therefore, rather than implementing these constraints during the optimization we suggest two alternatives.

TABLE II. MONTHLY IRRIGATIONS. BOLD INDICATES THAT THE MONTHLY QUOTA IS EXCEEDED.

	Year	April	May	June	July	Aug.	Sept.	Yield (t/ha)	Yield reduction, %	Irrigation reduction, %
Optimal solution without quota constraints	2004	30	56	45	65	81	79	5.3	-	-
	2005	92	76	73	88	15	111	5.3	-	-
	2006	**134**	0	9	118	**159**	62	5.3	-	-
	2007	42	44	101	**183**	**138**	92	5.3	-	-
	2008	70	70	93	78	**207**	48	5.3	-	-
	2009	25	74	29	41	**147**	86	5.3	-	-
Best solution that satisfies quota constraints	2006	72	63	51	104	116	45	5.2	2	8
	2007	74	10	98	136	105	81	4.8	10	20
	2008	68	49	91	134	130	56	5.0	6	6
	2009	56	60	0	99	120	81	5.3	0	-3
Optimal solution modified to satisfy quota constraints	2006	75	59	9	136	130	73	5.2	2	0
	2007	42	44	120	136	130	94	4.9	8	35
	2008	70	70	93	136	130	67	4.9	8	0
	2009	25	74	29	58	130	86	5.3	0	0

The first approach is to take as best irrigation the irrigation that gives the highest yield while not exceeding the water quota (one of the combinations investigated during the optimization process – Figure 1). The results of this approach are summarized in the middle section of Table 2. This approach leads to yield reductions ranging from 2 to 10%. These are partially offset by 6-20% reductions of the corresponding amounts of irrigation, so that the overall results remain close to optimum.

The second approach consists of transferring the excess of water to the previous or following month (to the last and first planned irrigation event respectively, so that no additional irrigation event is required). The corresponding results are summarized in the lower section of Table 2, and shading is used to emphasize the events which were adjusted. For instance, in 2005 the very large irrigation originally planned for April was divided between April and May. Note that in 2008 it was not possible to maintain the same total irrigation since the quotas were originally exceeded in consecutive months and not enough spare water was available. This approach leads to yield reduction 2 and 8% without reduction of the total irrigation in 2006 and 2008, and to 35% reduction of irrigation with only 8% yield reduction in 2008.

Figure 2 also shows the results obtained with the five non-optimal scenarios described above (irrigation every 10 days based on water allocation to cotton cultivation or irrigation triggered by soil water depletion). It can be seen that all these strategies lead to results that are far from optimal. In many cases such strategies even result in no yield at all (missing symbols on graphs) due to severe soil dryness during the initial crop development.

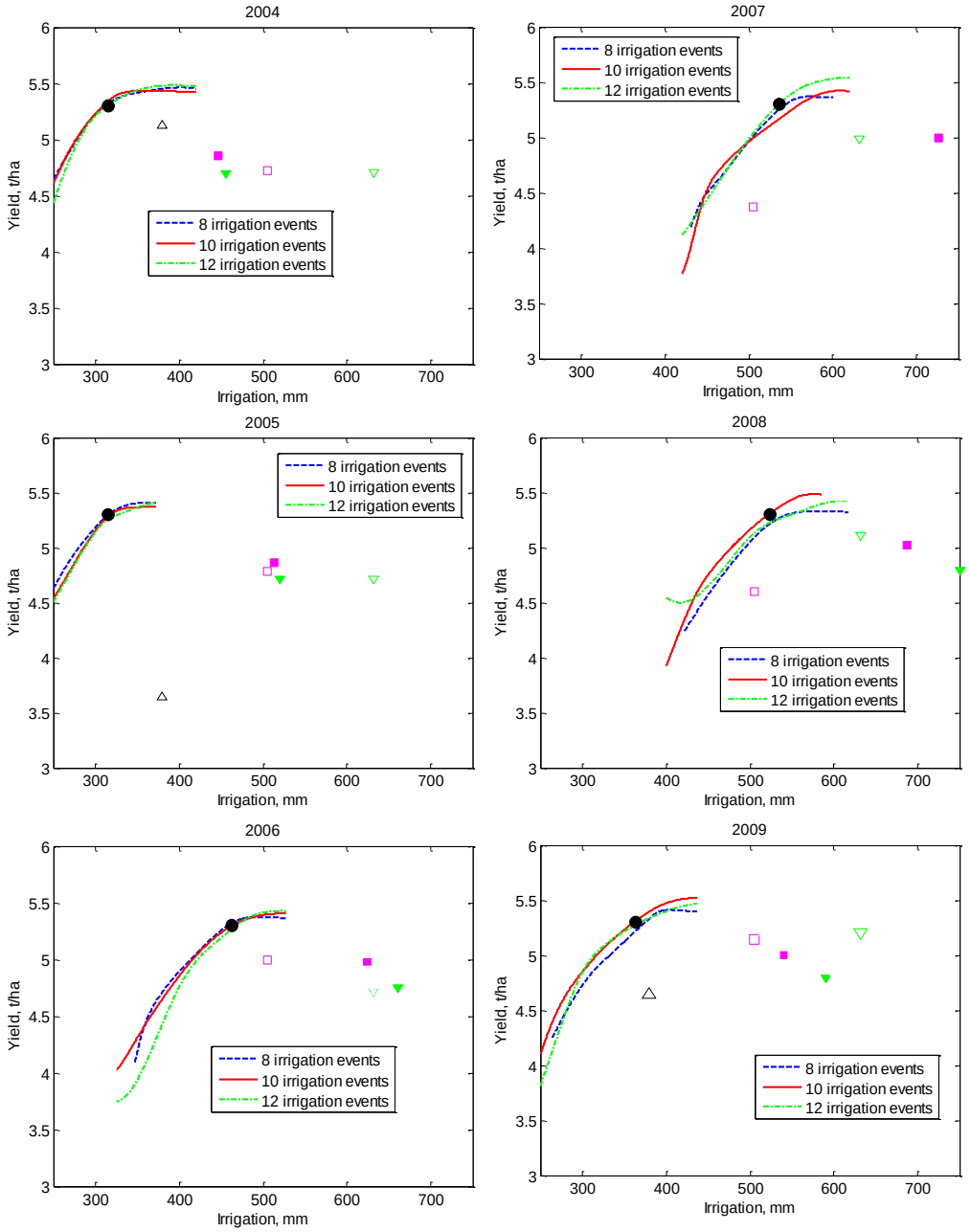

Figure 2. Relationship between highest achievable yield and irrigation (Pareto front) assuming 8, 10 and 12 events (lines). The open symbols show the results when using 100% (▽), 80% (□) and 60% (△) of water available according to Greek legislation. The closed symbols show the results when irrigating according to 50% TAW (▼) and 80% TAW (■). Amount of rain during season: 2004:291mm, 2005: 367mm, 2006, 185mm, 2007: 154mm, 2008: 112mm, 2009: 274mm.

CONCLUSIONS

The simulation results show that the two-stage optimization procedure developed in this work can effectively be used for optimizing irrigation scheduling. Furthermore, these results illustrate that more common strategies such as fixed irrigation amounts at regular intervals or irrigation triggered by soil water depletion lead to very significant waste of water.

ACKOWLEGMENTS

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Title: *Investigation and Analysis on Rural Residential Energy-Saving Transformation in Northern China ——Taking Balin Zuoqi for Example*

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ABSTRACT

To explore the residential energy-saving transformation in the north, this paper makes a field research into the energy-saving transformation of farmer houses in Balin Zuoqi, Inner Mongolia. Through a contrastive analysis on the methods of residential energy-saving transformation, the effect of residential energy-saving transformation, the correlation between various transformation measures and indoor temperature, and residents' sense of happiness before and after transformation, it is found that farmers are more active to the aesthetic transformation and not much enthusiastic about heat preservation transformation and the transformation of doors and windows has not much influence upon indoor heat preservation.

Keywords: Rural residence, energy-saving, transformation and contingency analysis

FOREWORD

Energy is the motive power for social economic development, and as a developing country, China's demand for energy is increasing constantly. For a long time, the buildings in the rural area of northern China have many drawbacks, such as residences occupying too large areas, weak building technical level, lacking uniform scientific planning, low requirement in the thermal performance and comfort of buildings and blindly pursuing the floor area. During the period of the 11th Five-Year Plan, based on the "Provisions on the Administration of Energy Conservation for Civil Buildings" passed and issued on October 28, 2005, the government again issued and implemented the "Provisions on Quality Acceptance of Building Energy Efficiency Engineering Construction" GB50411—2007 on October 1, 2007 and passed and started implementing "Technical Guide for the Heat Supply Measurement and Energy-Saving Transformation of Existing Residential Buildings in Northern Heating Region" on trial in July 2008 [1]. With the development of rural economy and the improvement of farmers' living standard, the energy consumption of rural life is growing on a year-on-year basis, especially the heating energy consumption in northern area in winter. The energy-saving transformation of rural housing has a significant effect in solving the energy shortage in rural area, improving farmers' production and living conditions and protecting rural ecological environment. Hence the paper takes the residential

transformation in Balin Zuoqi area in Inner Mongolia as an example and makes a field research into the rural residential transformation, energy-saving efficiency and the correlation of various transformations.

CURRENT SITUATION OF RURAL RESIDENTIAL ENERGY-SSVING TRANSFORMATION IN NORTHERN AREA

Characteristics of Energy Consumption in Northern Agricultural and Pastoral Areas

So far, domestic and foreign scholars have made a lot of researches into the characteristics of energy consumption in agricultural and pastoral areas, with the most representative researches as follows: scholars including H. Mu[2] adopted the grey correlation analysis method to analyze and predict the consumption of biomass energy in China's rural family; scholars including Zhou Zhongren[3] took the village of Huantai County for example and analyzed the energy consumption structure of northern typical well-off rural families from 1989 to 2005; scholars including Wang Guiping[4] pointed out that the energy conservation measures including methane, wind power generation and solar energy are highly popular and significantly effective in Inner Mongolia, but factors including deficient financial resources and lagging technical level and service system are restricting the development of rural energy in Inner Mongolia; scholars including Li Chunmei[5] researched into the current situation and development countermeasures of the energy utilization in Alxa League agricultural and pastoral areas and summarized the characteristics of energy consumption in agricultural and pastoral areas; through a field investigation in Inner Mongolia, scholars including Zhou Shudong[6] found that the consumption of traditional energy takes up an absolute dominant position in the research area, animal manure, straws and coal are the main energy for family use and the usage amount and proportion of new energy is small.

Energy-Saving Transformation Measures

Residential energy-saving transformation is divided into existing residential transformation and newly-built energy efficient residence. The existing residential transformation project mainly includes outer wall, doors and windows and floor. The newly-built energy efficient residence is rebuilding the house based on the original house site and processing and implementing the thermal insulation project to houses in the rebuilding process, with main projects including external wall thermal insulation system, door and window thermal insulation system, floor thermal insulation system, roof thermal insulation system and energy-saving hollow kang.

The external wall thermal insulation system is generally divided into external wall external insulation, external wall internal insulation and external wall self-insulation, with the proportions being 84.7%, 13.6% and 1.7% respectively. The walls of original houses are built with shale bricks and the walls are 370mm thick (known as three-seven walls). The thermal performance is extremely poor and the energy waste is serious. External wall external insulation is mostly adopted in northern areas, and the technologies for external wall external insulation mainly

include external sticky benzene board (known as foam insulation board), steel wire frame polystyrene board external thermal insulation, high heat-preservation building block thermal insulation wall, sandwich structure thermal insulation wall and light steel frame precast external thermal insulation wallboard. It is concluded from the investigation on the construction market price in Beijing city that considering farmers' economic condition, to reach the same thermal insulation effect and relatively lower price compared with other thermal insulation technology, the external sticky benzene board technology is selected in rural wall transformation at present, which can minimize the loss of thermal energy from indoor to outdoor through the external wall of the building or transferring from outdoor to indoor.

At present, the energy conservation processing to doors and windows mainly includes the following aspects: first, materials with low thermal conductivity are adopted for window frame, such as PVC plastic material, aluminum alloy broken cold (thermal) bridge window frame material, glass fiber reinforced plastic material, steel-plastic composite material, as well as aluminum wood composite material and aluminum plastic composite material in high-grade products, of which, the UPVC plastic material is widely used and its raw material is high polymer material rigid polyvinyl chloride. The second is changing glass, like hollow glass and Low-E coated glass, in order to improve the resistance value of the glass. The third is selecting sealing rubber strip with high performance, which can improve the airtight performance of the doors and windows of buildings. Through market research, glass fiber reinforced plastic material is good in tightness and low in price, and to meet the energy-saving requirement and reduce pollution, the hollow glass is used in transformation.

When transforming the floor, 10cm ceramsite concrete cushion can be increased at the floor and the tile paved surface can be made on the ceramsite concrete cushion. Ceramsite is made of shale and coal ash and ceramsite is a kind of light building material that can preserve heat and insulate sounds. It is often used for floor thermal insulation, with a very good heat preservation effect.

The heat preservation and thermal insulation of roofs is one of the key points for the energy conservation of space enclosing structure. In areas with cold winter and hot summer, consideration must be given to summer and winter for the building energy conservation. The above methods all can meet the requirement of roof energy conservation to different extent. Regarding the structural condition and use of existing rural roofs, light materials with small heat conductivity coefficient are set under the roof waterproof layer for thermal insulation.

To maintain the environment quality of the living space, heating is needed in cold winter to raise the indoor temperature. From the perspective of energy conservation, the efficiency of the heating system should be improved. The traditional landing kang, also called "adobe kang", is the oldest form of heated kang with the biggest feature of being not connecting with the ground directly, and the heat is dissipated at a single side, which increased the thermal loss from the flue of the kang to the ground. Added that the thermal insulation property of the kang is not good, there exists problems like low thermal efficiency and fast cooling. Targeting the problems of traditional hollow kang, Liaoning and Jilin rural energy technicians developed a new-type hollow kang through repeated research and continuous practice, namely energy-saving hollow kang, during the period of the 7^{th} and 8^{th} Five-Year Plans. The transformation of enrgy-saving hollow kang has always been

highly praised and favored by farmers in the suburb counties of Beijing [7].

CURRENT SITUATION OF RESIDENTIAL TRANSFORMATION IN BALIN ZUOQI

Introduction of Balin Zuoqi Projects

Balin Zuoqi is located at the southeast of the southern section of Great Khingan in the north of Chifeng City, Inner Mongolia, belonging to the continental monsoon climatic region in temperate zone. Balin Zuoqi projects mainly adopt related building energy-saving technologies under the guidance of experts and select building energy conservation materials produced in the local of Balin Zuoqi to conduct energy-saving transformation to the existing residences of farmers. Through energy-saving transformation, the targets including raising indoor temperature and comfort level, lowering energy consumption and reducing environmental pollution can be achieved [8].

The paper mainly takes the energy consumption and residential energy-saving transformation of herdsmen in Balin Zuoqi, Inner Mongolia as the research object, conducts a questionnaire survey to four towns and Sumu in Balin Zuoqi area, then makes a statistical analysis on the types of transformation projects, transformation effects and herdsmen's sense of happiness and calculates the correlation of various transformation measures and indoor temperature before and after transformation by the method of contingency analysis based on the above analysis.

Situation of Energy-Saving Transformation Projects

On the whole, the use ratio of energy-saving heat preservation facilities is increased to some extent after transformation. Seen from the door and window transformation, the usage rate of plastic steel double-glass doors and windows is significantly improved and the survey samples increased to 97.09% from 1.68%, up 95.41 percentage points; from the condition of suspended ceiling, the usage rate of plasterboard suspended ceiling is increased significantly to 38.60% from 0.60%, up 38 percentage points; from the usage rate of external wall insulation, all samples are not processed with external wall insulation; from the ground condition, the ground is obviously laid with ceramic tiles and the usage rate increased to 98.25% from 39.53%, up 58.72 percentage points; from the cooking utensils in use, the survey samples are not changed much; from the heating facilities, the survey samples are also not much changed.

Doors and windows are the project that herdsmen transformed most and laying ceramic tiles on the floor is the second, followed by using plasterboard suspended ceiling. Nearly no transformation project is about stove and heating facility, and the stove is still based on the mud stove. The usage rate of the ordinary gas stove is only increased 3.25% and no herdsman adopts the energy-saving gas stove. The heating facility is still based on adobe kang, followed by the water heater. The usage rate of hollow kang did not exceed 2% before and after transformation, nearly not changed. External wall insulation is the project that is totally not transformed. It is learnt from the above transformation situation that herdsmen's acceptance to projects that can beautify indoor living environment and with relatively cheaper

price is generally higher, such as using plastic steel double-glass doors and windows, laying ceramic tiles and hanging ceilings, and their acceptance to external wall insulation, heating facility and energy-saving stove is lower. There are mainly two reasons: the first is they cannot afford it due to weaker economic conditions; the second is they know little to external wall insulation, heating facility and energy-saving stove and dare not try it easily.

Analysis on Energy-Saving Transformation Effects

THE CHANGE OF ENERGY CONSUMPTION BEFORE AND AFTER TRANSFORMATION

The rural energy consumption in the northern area can be divided into heating energy consumption and cooking energy consumption by usage classification and can be divided into coal, electricity, fuel wood/straw, coal gas, methane and solar energy by energy classification. The energy types that can be used for heating mainly include coal, electricity and fuel wood/straw. It is learnt from the statistical analysis on the questionnaire that the main energy consumption types in the area are straws, cow dung, brown coal and electricity.

It is seen from the energy consumption situation that the usage rate of straws dropped obviously before and after transformation, the usage rate of brown coal significantly grew and the usage rate of cow dung basically kept the same. The usage rate of straws declined to 14.24% from 43.17%, falling by 28.93% percentage points; the usage rate of brown coal increased to 53.56% from 20.30%, up by 33.26% percentage points; the usage rate of cow dung dropped to 31.19% from 35.79%, down 4.6%. Seen from the usage amount of coal and electricity, both are decreased to some extent after transformation, but not so obviously. The annual average usage amount of coal dropped to 2.66 tons from 2.98 tons and the monthly average usage amount of electricity declined to 70.82 degrees from 71.82 degrees.

TEMPERATURE CHANGES BEFORE AND AFTER TRANSFORMATION

Seen from the outdoor temperature, the average outdoor temperature of Balin Zuoqi in January in winter is the lowest, down to -18.06℃, and the average outdoor temperature in November is the highest, to 3.22℃. In the whole winter, the average outdoor highest temperature is -2.82℃, the average lowest temperature is -13.06℃ and the average temperature is -7.32℃. The climate is bitter cold in winter in the area and herdsmen’s living environment is harsh, so it is very necessary to conduct building energy-efficiency thermal insulation transformation.

From the point of indoor temperature, the improvement of lowest temperature in winter is better than the improvement of highest temperature in summer. It is seen from the lowest temperature in winter that the proportion of temperature of 16℃ and above increased to 97.66% from 6.30% before transformation, up by 91.36 percentage points, very significant in transformation effect. From the highest temperature in summer, the proportion of temperature of 8℃ and below rose to 31.40% to 1.75%, up by 29.65 percentage points.

Seen from indoor and outdoor temperature comprehensively, the outdoor average lowest temperature in winter is -13.06℃ and the indoor lowest temperature

for most herdsmen in winter reaches above 16℃ after transformation and herdsmen's living environment is improved significantly after transformation.

CORRELATION RESEARCH OF TRANSFORMATION PROJECTS AND INDOOR TEMPERATURE

To clarify the correlativity between various transformation projects and indoor temperature and the change of correlation before and after transformation, the paper uses the contingency analysis method to analyze the correlation between suspended ceiling types, floor types, external thermal insulation types, energy types, door and window types, stove types and temperature based on the above data statistics and analysis, aiming to uncover the influence and correlation degree of various transformations to indoor temperature before and after transformation.

Before transformation, the Pearson chi-square test of suspended ceiling types and indoor temperature and the exact test Fisher showed that the suspended ceiling types and indoor temperature are correlative and the contingency coefficients before and after transformation are 0.590 and 0.713 respectively. The correlation after transformation gets stronger than that before transformation.

Before and after transformation, the Pearson chi-square test of ground types and indoor temperature and the exact test Fisher showed that the ground types and indoor temperature are correlative and the contingency coefficients before and after transformation are 0.467 and 0.707 respectively. The correlation after transformation gets stronger than that before transformation.

Before and after transformation, the Pearson chi-square test of external wall insulation types and indoor temperature and the exact test Fisher showed that the external wall insulation types and indoor temperature are correlative and the contingency coefficients before and after transformation are 0.345 and 0.523 respectively. The correlation after transformation gets stronger than that before transformation.

Before and after transformation, the Pearson chi-square test of external wall insulation types and indoor temperature and the exact test Fisher showed that the external wall insulation types and indoor temperature are correlative and the contingency coefficients before and after transformation are 0.718 and 0.739 respectively. The correlation after transformation gets stronger than that before transformation.

Before transformation, the Pearson chi-square test of door and window types and indoor temperature and the exact test Fisher showed that the door and window types and indoor temperature are correlative; after transformation, the Pearson chi-square test of door and window types and indoor temperature and the exact test Fisher showed that the door and window types and indoor temperature are uncorrelated.

Before transformation, the Pearson chi-square test of stove types and indoor temperature and the exact test Fisher showed that the stove types and indoor temperature are correlative and the contingency coefficients before and after transformation are 0.374 and 0.612 respectively. The correlation after transformation gets stronger than that before transformation.

On the whole, except the correlation between door and window types and

indoor temperature is not significant, other indicators all have significant correlation with indoor temperature. It is seen from correlation coefficients that the correlation between transformation project indicators and indoor temperature is enhanced to some extent after transformation, which illustrated that the influence of transformation measures to indoor temperature gets strengthened after energy-saving transformation.

ANALYSIS ON HERDSMEN'S SENSE OF HAPPINESS AFTER TRANSFORMATION

Herdsmen's Satisfaction Degree

On the whole, herdsmen have higher satisfaction to Balin Zuoqi projects and are most satisfied with the transformation subsidy work, followed by the housing conditions. Those who are very satisfied with the transformation subsidy work take up 95.9% and those who are very satisfied with housing conditions take up 90.70%, from which it is known that the subsidy measure can continue to be used in the promotion of some projects in the future to improve farmers' enthusiasm. Relatively speaking, the income and public facilities are not much satisfactory. Those who are very satisfied with income take up 61.76% and those who are relatively satisfied take up 30.59%; those who are very satisfied with the public facility take up 62.21% and those who are relatively satisfied take up 37.79%. The data show that herdsmen are eager to increase income, improve living conditions and the quality of life, and hope to build more public facilities to enrich their entertainment and facilitate their lives.

CONCLUSIONS

First, it is seen from the energy-saving transformation that herdsmen made much higher transformation to doors and windows, floors and suspended ceilings and nearly did not transform the external wall insulation, heating facilities and stoves.

Second, it is seen from the types of rural energy consumption that the main energy consumption types in the area are straws, cow dung, brown coal and electricity.

Third, it is seen from the indoor and outdoor temperature that the improvement of the lowest temperature in winter is better than the improvement of the highest temperature in summer and the living environment is improved obviously.

Fourth, seen from herdsmen's sense of happiness, herdsmen are most satisfied with the transformation subsidy work, followed by the housing conditions.

At last, it is learnt from the correlation analysis that the transformation project and indoor temperature are correlative; the effect of transformation measures to indoor temperature is strengthened after the energy-saving transformation, which also illustrated that the housing energy-saving transformation is conducive to improving living environment and the quality of life; the influence of door and window types to indoor heat preservation is not obvious, which might be affected by factors including construction, for example, untight sealing leads to the effect of

door and window transformation being not obvious.

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Cover page

Title: *Litter Production in both Natural and Degraded Mangrove Forest of Peninsular Malaysia*

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ABSTRACT

Mangrove in Peninsular Malaysia is characterized of tropical forest setting. Nevertheless, the state of the mangrove forest have led to varied classification; hence natural and degraded mangrove types. The study aimed to utilize litter production potential as a means of evaluating the degree of productivity of the mangrove types while also assessing the role of seasonal variations as well. Leaf litter accounted for more than 70% of the total litter production in both natural and degraded mangrove, and December was found to be the peak period for both mangrove forest; 82.7% and 82.2%, for SHD and KSNP, respectively. However, the organic carbon (CO) content a cross the litter parts varied across the 3 season; while leaf litter recorded highest CO during the dry season, the branch and propagules showed that intermediate and wet season avail higher CO than found during dry season. Hence, the study concluded that both mangrove types in Peninsular Malaysia experience some proximity in litter production, yet the differences suggest that counter measures need to be adopted so as to protect mangroves from degradation in order to avoid productivity loss.

INTRODUCTION

Litter production is fundamental to ecosystem process due to its importance to organic matter production and decomposition cycle. From global view point, mangrove is a major productive ecosystem that is not only known for its primary productivity but is as well recognized for export of organic matter and support for variety of aquatic life [1]. Litter fall is highly required in energy and nutrients cycle in the woodland ecosystem [2]. It mitigates nutrient depletion by tree harvesting and as such do affect sustainability of land use. Whereas obtaining direct methods of measuring primary productivity in mangrove forests are technically difficult Bunt et al [3] utilized the extrapolation of litter production data for the generation of net primary production.

This is to infer that litter from mangrove swamps potentially represents a significant organic input into the sea, especially where the swamps are extensive, such as on the west coast of the Peninsular Malaysia [4]. Geographically location is even

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found to influence mangrove productivity. This is because litter production and breakdown rate do not only vary with species but also varies geographically [5]. Infact in the tropics, mangrove swamps achieve their highest structural and floristic diversity; hence litter production rates in the temperate region are less than what is obtained in the tropical setting [1]. Estimates of litter production have been reported for some mangrove forest around the global. Leaf litter production in Florida and Central America was 2 g dry wt. m^{-2} day^{-1}, [6], the total litter was 2.4 g m^{-2} day^{-1} [7]; in Queensland it ranged from 1.04 g m^{-2} day^{-1} to 5.26 g m^{-2} day^{-1} [8]; and Sasekumar and Loi [4] recorded 3.5 g m^{-2} day^{-1} to 6.72 g m^{-2} day^{-1} in mangrove forest zones of peninsular Malaysia. Despite the importance of mangrove forest, litter has been published on litter production. Similarly, none of the literature has viewed litter production from the angle of evaluating both natural and degraded mangroves. While some mangrove forests have been left untouched; hence natural, some have experienced alterations and disturbances due to anthropogenic activities like building resorts, fishing etc, thereby making them degraded mangrove forests. Both mangrove forest types characterize peninsular Malaysia. Therefore this study presents data on the litter production in both natural and degraded mangrove forests of Malaysia, and also aimed to determine the pattern of litter production across the months of seasons

MATERIALS AND METHODS

The study areas: Sungai Haji Dorani (03° 40′ N and 100°58′ E) and Kuala Selangor (03° 20′ N and 101°14′ E) are both located in the Selangor state of Peninsular Malaysia on 35 ha and 95 ha respectively. Both zones, Sungai Haji Dorani (SHD) and Kuala Selangor (KSNP) are characterized of humid tropical climate with mean annual rainfall of 1701-1710 mm. SHD experience a semi – diurnal tidal regime whereas KSNP is inundated only during the spring high tides. The study areas show consistent weather pattern defined with three climate season: the dry season (April to September), wet season (October to December) and the intermediate season (January to March). At relative humidity average of 77%, the zones experience mean annual temperature of 27.3- 27.7 ℃ while under the influence of northeast monsoon and southwest monsoon situations. These areas exhibit a great diversity in tropical vegetation varied markedly on individual species; SHD (*Avicennia marina, Bruguiera cylindrical, Excoecaria agallocha, Xylocarpus meluccensis* and *Sonneratia alba*) and KSNP(*Avicennia officinalis, Bruguiera parviflora, Rhizophora mucronata* and *Sonneratia alba*) [9].

Quadrants (1m) used were suspended with nylon ropes between trees at 2m heights from the ground level so as to avoid effects from high tides. In each of the mangrove forests, 15 quadrants were set up randomly. Sampling was undertaken from august, 2012 to the end of July, 2013. A total of 12 collections were made in each station on monthly basis.

Litter collected were oven dried at 80 ˚C for 4 days and eventually weighted. While converting total amounts for each station to daily figures, no correction were made to accommodate leaching or other losses. The ensuring total litter falls for the 12 collections were correlated with the 3 seasons that characterized peninsular Malaysia, namely; dry season, wet season and intermediate season. All samples were analyzed for organic carbon using Walkey- Black [10] method.

RESULTS AND DISCUSSION

Production of litter at both mangrove forests was observed throughout the year (Table 1). Though there was high proximity in pattern of distribution across the months, yet distinct seasonal variation was observed. Leaves and branches accounted for the largest part of the litter produced throughout the year which is typical of previous studies [1], [4]. Similarly with more 70 % of the total litter being leaf, it can be attributed to the type of tree distribution in the mangrove area which includes leafy trees like distribution was observed during the dry season (averaged 54.2%) while high proximity in distribution was found between wet season and intermediate season as their average values were 74.18 %, respectively. However evaluating the leaf litter distribution across individual months of the year, December was found to be the peak period both mangrove forest; 82.7% and 86.2%, for SHD and KSNP, respectively. However, when the natural and degraded mangrove are compared in term of leaf litter production, least value 28% was collected in the natural mangrove (KSNP) while was about 5% less the value found in the same month (July) at SHD which is a degraded mangrove forest. In SHD, the trend of leaf litter fall showed a continuous increase from September until December before decelerating, with the exception of switch between February to May.

TABLE I. LITTER PRODUCTION IN BOTH STUDY AREAS

Month	Leaves (%)		Branches (%)		Flowers (%)		Bracts (%)		Propagule (%)	
	SHD	KSNP	SHD	KSNP	SHD	KSNP	SHD	KSNP	SHD	KSNP
Aug	49.4	55.2	12.5	8.5	0.0	0.0	7.6	4.6	30.5	31.7
Sep	64.4	65.7	16.8	19.6	0.0	0.0	15.8	9.5	3.0	5.2
Oct	72.2	71.9	13.7	14.7	0.0	0.0	14.1	13.4	0.0	0.0
Nov	76.3	74.0	14.2	13.0	0.0	0.0	9.6	13.0	0.0	0.0
Dec	82.7	86.2	10.9	4.4	0.0	0.0	6.4	9.4	0.0	0.0
Jan	78.3	79.8	10.3	1.6	1.5	8.6	9.9	10.1	0.0	0.0
Feb	73.6	67.0	9.3	12.0	3.3	4.0	13.8	17.0	0.0	0.0
Mar	74.7	70.4	10.7	14.0	4.1	5.6	10.5	10.0	0.0	0.0
Apr	69.8	71.8	14.4	11.7	6.2	5.9	7.8	8.6	1.9	2.0
May	67.4	71.5	10.1	9.3	5.6	5.6	8.7	8.7	8.2	4.9
Jun	63.5	66.7	10.7	10.6	0.0	0.0	10.6	11.3	15.1	11.4
Jul	31.9	28.0	5.1	6.2	0.0	0.0	4.2	4.6	58.8	61.2

Flower litter was not part of the total litter production for both mangroves from August to December and this is attributed to the non- flowering period of the mangrove trees. However, they become part of the total litter from January and increased until April and May. Therefore presence of flower litter is highly limited to intermediate season in mangrove forests of Peninsular Malaysia regardless of natural or degraded status of the mangrove. Sequel to flowering period is seed production in trees, hence the absence of propagule in the litter from October until March is accounted from by the plant cycle.

Therefore dry season is the peak of propagule litter production in both mangrove forest; 58.8% and 61.2 % for SHD and KSNP respectively, in the July. Bracts which were the very small leaves attached to the trees, were also parts of the litter production throught the year. Peaks for bracts litter were found at the early wet season and mid intermediate season but the least distribution was found in dry season especially in July wherein 4.2 % and 4.6 % was obtained in SHD and KSNP, respectively.

Furthermore, the results of the litter production rate showed that the individual litter rate ranged from 0.08 to 6.59 g m^2 day and 0.09 to 8.82 g m^2 day for SHD and KSNP, respectively. Discrete analysis revealed that the leaf production rate for the degraded mangrove was 6.59 g m^2 day in March (Figure 1) as against 5.29 g m^2 day recorded in November for the natural mangrove (Figure 2). The result from natural mangrove is similar to Sasekumar and Lio [4] which found Malaysian mangrove to be comparable to those of Queensland where 5.36 g m^2 day can be obtained [8]. However, the higher value found in SHD may be attributed to the interferences in its existence brought about by the anthropogenic activities (resort, fishing etc) around it. The foregoing it also showed that the rate of daily leaf litter production varied between the wet and intermediate seasons for both mangrove forests. Therefore it is worthy to note that mangrove productivity may vary considerably due to nutrient conditions of the soil [11].

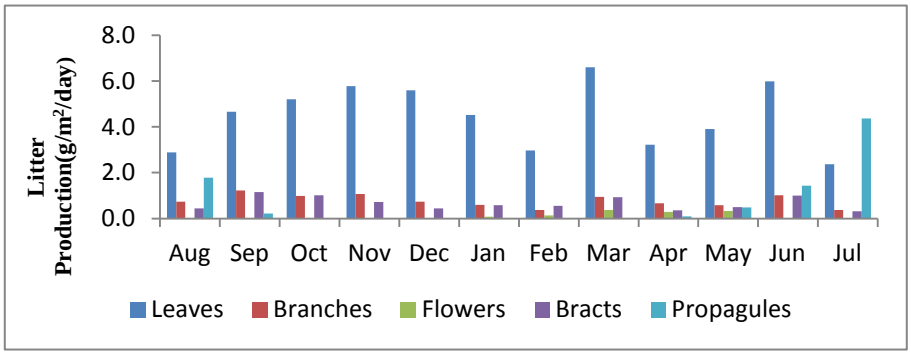

Figure 1: Litter production in SHD Mangrove Forest

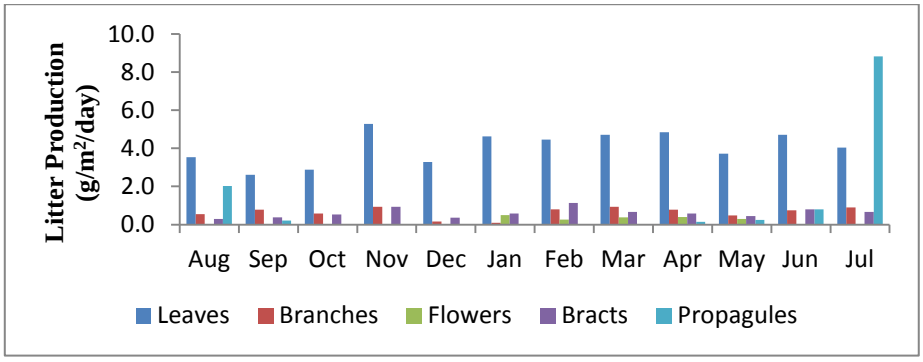

Figure 2: Litter production in KSNP Mangrove Forest

Similarly, the higher rate of propagules litter (8.82 g m^2 day) found in KSNP than in SHD (4.36 g m^2 day) might be an evidenced of an enriched nature of natural mangrove hence seed production become associated to degree of mangrove

productivity. In overall, the non- leaf litter accounted for less than 30% of total litter production in both mangrove forest.

The result of the organic carbon (CO) is represented by Figure 3. About 52% CO was found in the leaf litter of KSNP in dry season to mark the highest concentration of CO within the studied and seasons as against approximately 49% and 43% obtained in intermediate and wet season. However the trend in branch and propagules was slightly different. Highest CO was found in the intermediate season, followed by wet season before the dry season. Expectedly dry season often show that soil contains more CO than in the wet season because dry season is characterized of evapotranspiration that allows for vertical transport of organic carbon [11]. However, the high degree of organic carbon in the branch and propagules for the intermediate and wet season may be as a result of the ability of such plant parts to retain and store nutrients than in leaf where exposure to sunlight and increased surface area may be limiting factors. Organic carbon content in all the tree parts (leaf, branch and propagules) revealed that KSNP showed higher concentrations that what were found in SHD. This might be a reflection of the degraded nature of SHD unlike KSNP that is natural and have almost undisturbed vegetation; hence nutrient conservation is expectedly higher.

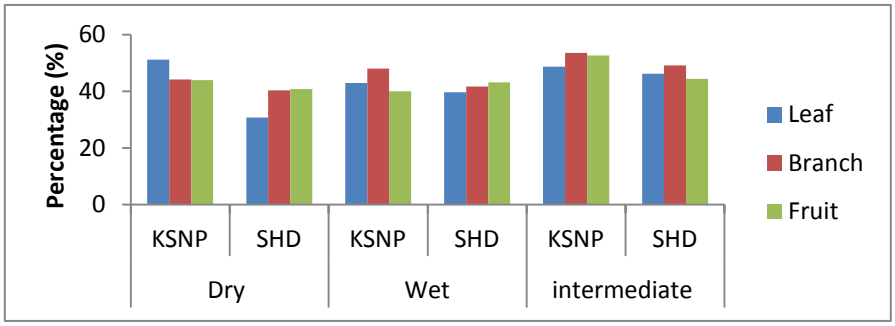

Figure 3: organic carbon for both study areas

CONCLUSION

The degree of liter production on the mangrove may project them among the most productive forest types in Peninsular Malaysia. Though season may vary but leaf litter production is pronounced year round and such can be inferred to buttress the fertile nature of the mangrove forest. However, significant differences exist between total litter production in the natural mangrove and the degraded one. Hence it becomes imperative to institute protective mechanisms that will shield existing mangrove forests from anthropogenic influences.

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Title: *Abundance and Deficiency Diagnosis of Nitrogen Nutrition Level of Lettuce Leaves at Tillering stage Based on Digital Color Images*

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ABSTRACT

Because image includes comprehensive information and is easy to be collected, nondestructive detection based on digital color images is studied in this paper. The main contents are gathering samples of lettuce leaf with multiple nitrogen level, photographing digital color images, preprocessing images and extracting features of texture and color. In order to reduce the correlations among the features, LDA and OLDA were applied respectively, to further reduce dimensional optimization features. Results showed that recognition rate of judging abundance and deficiency diagnosis of nitrogen nutrition level of Lettuce leaves using OLDA was higher than using LDA. However, it also can still be improved. Since Adaboost method can enhance classification accuracy of any given learning algorithm, the method combining Adaboost and OLAD was studied in further dimension reduction and optimization features. The experiment results showed that the classification recognition rate was significantly improved by a strong classifier constituted by multiple weak classifiers.

1. INTRODUCTION

There are many medical functions of lettuce, such as clearing heat, anti-inflammatory, analgesic hypnosis, lowering cholesterol and treatment neurasthenic, which grows in major areas of our country. Experts has paid more attention to Lettuces' planting.

Nitrogen plays an important role in crop physiological metabolism and growing development of crops and it is one of the main limiting factors for the growth of crop. Nitrogen nutrition is more required during the period of crop growth, and lacking of nitrogen can inhibit differentiation of leaves, which reduces the number of leaves and has negative influence on nutritional quality. It leads to pollution of ground water and the contamination and degradation of soil easily by using excessive nitrogen fertilizer, so it is very important to use nitrogen fertilizer reasonably for the yield and quality of crops. The research shows that nutritional deficit can not only cause leaves color and texture but also macroscopic

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characteristics of other surface change in plant physiology and plant nutrition. In recent years, nondestructive testing technology is widely used in crop nitrogen nutrition diagnosis based on computer vision diagnosis technology. This technology with the advantages of being fast convenient and non-destructive, timely providing fertilizers with needed information, has been focused on by researchers in domestic and international crop nutrition diagnosis [1]-[3], but the nondestructive detection of nitrogen in lettuce by using image technology has been seldom reported .

In this paper, the method using digital camera to directly capture picture is intuitive, convenient and inexpensive. Then feature data are extracted from the image. The descending dimension algorithms are studied carefully, such as LDA[4]-[6], OLDA[7]-[8] ,and the improved algorithm was studied to judge nitrogen level.

2. MATERIAL AND METHODS

2.1 Preparation of the Sample

The object of experiment is the lettuce named as Italian Annual heat-resistant and bolting lettuce, growing period of which is more than 50 days. Testing place is located in intelligent control greenhouse shelter of Agricultural Engineering in Jiangsu University Institute. The perlite was taken as the main culture substrate and the experiment of growing seedlings was scheduled. In order to obtain different nitrogen levels of lettuce leaves, in view of nitrogen nutrient in the lettuce nutrient solution, lettuce nitrogen levels of nutrient solution were classed as deficient nitrogen, normal nitrogen, and excessive nitrogen.

Lettuce leaves of all nitrogen levels were collected from the basin of all nitrogen levels in the field, and the images of lettuce leaves were captured with image acquisition device immediately.

Device of graph collection is made up of digital camera and bracket. The canon IXUS 500 HS digital camera is used in this study. The CCD camera's effective pixels are 10.1 million, zoom ratio is 12 times optical zoom, sensor size is 1/2.3 inch, equivalent focal length is 28-336mm and aperture range is F3.4 - F5.6. In each growth period of lettuce, color images of lettuce leaves were captured from11.am to13.pm. In this paper, image features were extracted from the 67 samples in tillering stage. Samples marked from1 to 23 were in deficiency nitrogen, and samples marked from 24 to 42 were in normal nitrogen and those marked from 43 to 67 were in excessive nitrogen. Samples (1-15, 24-37, 43-56) were selected as training samples of model, the rest as testing samples.

2.2 Image Pre-Processing

During capturing images, a white paper was put under the leaf to acquire the image clearly, and the original image is shown in Figure1. The original image has good quality so it is convenient for the preprocession of image. Images were conducted in gray scale processing, as shown in Figure 2. Because the target image of leaf is obvious, so binarization peak-valley method was chosen to complete the image segmentation, and the image after binarization segmentation is shown as Figure3. Figure 4 is gray segmentation image resulting from multiplying the pixels of

lettuce leaf black and white binary image and gray image point by point. Figure 5 is color segmentation image resulting from multiplying the pixels of lettuce leaf black and white binary image and original color image point by point.

2.3 Feature Extraction

The grey-scale texture features of leaf images were abstracted. Set gray levels of image as L, the gray histogram as h (i), i=0, 1,⋯,L-1, gray mean for m, its n order center statistical moment as

$$u_n = \sum_{i=0}^{L-1} (i-m)^n h(i) \quad \text{n=2, 3,}\cdots \tag{1}$$

In type, u_2 is the variance, as measurement index of gray contrast degree; u_3 expresses the partial slope of histogram, and u_4 describes the relative flatness of the histogram. In this paper, the following 6 texture statistical metric features were used, such as mean value $m = \sum_{i=0}^{L-1} ih(i)$, standard deviation $\sigma = \sqrt{u_2}$, smoothness $R = 1 - 1/(1+u_2)$, third moment $L_3 = u_3 / L^2$, consistency $U = \sum_{i=0}^{L-1} h^2(i)$, entropy $e = \sum_{i=0}^{L-1} h(i) \log h(i)$.

The color features of colorful leaf images were extracted as below. Assume the pixel value of the jth pixel and ith color component in color image is pij, and the number of pixels of the image is N, then the two central moments of the ith color component of RGB color is respectively one order central moment $e_i = \frac{1}{N}\sum_{j=1}^{N} p_{ij}$, and three order central moment $s_i = (\frac{1}{N}\sum_{j=1}^{N}(p_{ij} - e_i)^3)^{\frac{1}{3}}$, a total of six color features.

Fig.1 original image

Fig.2 grayscale image

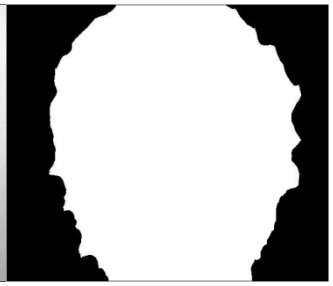

Fig.3 binarization Segmentation image

Fig.4 gray segmentation image

Fig.5 color segmentation image

2.4 Methods of Pattern Recognition

2.4.1 LINEAR DISCRIMINATE ANALYSIS (LDA) PRINCIPLES

Linear Discriminate Analysis (LDA)[6] is a kind of classic feature extraction which has been widely used in many kinds of image and spectral analysis. Orthogonal linear discriminate analysis method (OLDA) is put forward on the basis of LDA, using matrix's orthogonal transformation dimension reduction to get a kind of new algorithm[6].

2.4.2 ORTHOGONAL LINEAR DISCRIMINATE ANALYSIS (OLDA) PRINCIPLES

OLDA[8] is used to solve problems by a series of orthogonal vector of calculating diagonalization divergence matrix, which can be seen as calculating optimization problems as follows:

$$F(G) = \arg\max_{G} trace((G^T S_w G)^+ G^T S_b G) \tag{7}$$

For the solution of (7), assuming that matrix X exists, at the same time meets the following conditions:

$$X^{\mathrm{T}} S_b X = \begin{pmatrix} \Sigma_b & 0 \\ 0 & 0 \end{pmatrix} \equiv D_b \qquad X^{\mathrm{T}} S_w X = \begin{pmatrix} \Sigma_w & 0 \\ 0 & 0 \end{pmatrix} \equiv D_w \qquad X^{\mathrm{T}} S_t X = \begin{pmatrix} I_t & 0 \\ 0 & 0 \end{pmatrix} \equiv D_t \tag{8}$$

Then the matrix $G = X_q M$, in which $q = rank(S_b)$, M is arbitrary nonsingular matrix[8**]**.

According to reference[9], Adaboost method can improve any given learning algorithms accuracy rate of classification, and then the method of combining Adaboost and OLAD is adopted.

2.4.3 ADABOOST-OLDA PRINCIPLE

Adaboost[9] is the most popular deformations deriving from booting method. The algorithm constantly adds new "weak classifier", until it reaches some predetermined small enough error rate. In Adaboost method, each training sample is endowed with a weight, meaning the probability of being elected to the training set by a component classifier. If a sample point has been accurately classified, then in constructing the next focus training, its probability of being selected will reduce. On the contrary, if a sample point is not been classified correctly, and then its weight is improved. Through such iterative training, Adaboost method can focus on the more difficult classification sample.

Adaboost is combined with OLDA as follows[9,12]. The nearest neighbor classifier of OLDA feature subspace is used to get weak classifier. Adaboost-OLDA algorithm is described as follows[9,12]:

(1)Input a series of training samples $\{(x_1, y_1),...,(x_N, y_N)\}$

(2)Set initialization. $K_{init} = 0$, $K_{\max}$ and train sample's weight $W_i^1 = \frac{1}{N}$, i=1,⋯,N.

(3)Execute in cycle

①In settled good weight data train the nearest neighbor classifier of OLDA feature subspace to get weak classifier h_k

②Calculate h_k error rate $\varepsilon_k = \sum_{i=1}^{N} W_i^k$

③When $0 \langle \varepsilon_k \langle 0.5$, $K = k+1$。 If $\varepsilon_k \rangle 0.5$, terminate the algorithm.

④Make $a_k = \frac{1}{2} \ln \left[\frac{(1-\varepsilon_k)}{\varepsilon_k} \right]$

⑤ $W_{k+1} = \frac{W_k(i)}{Z_k} \times \begin{cases} e^{-a_k} & \text{If } h_k(x_i) = y_i \text{(correct classified)} \\ e^{a_k} & \text{If } h_k(x_i) \neq y_i \text{(incorrect classified)} \end{cases}$

In which $Z_k = \sum_{i=1}^{n} W_{k=1}(i)$

(4)When $k = K_{\max}$ then terminate the algorithm

(5) Output, and then use each component classifier adding weight and averaging to get the last general classification decision: $g(x) = \left[\sum_{k=1}^{K_{\max}} a_k h_k(x) \right]$

3. RESULTS AND DISCUSSION

There are 67 samples in this experiment, with 43 training samples and 24 testing samples. Experimental platform is CPU i3-2330 2.20 GHZ / 2 G hardware environment using Matlab7.11 (2010 b) to simulate the method of this paper. The experiment is divided into two parts. LDA and OLDA feature extraction algorithm respectively were used to test the sample with the nearest neighbor classifier. As for LDA feature extraction algorithm, experiment sample number n is larger than sample dimension L, so the scheme of direct calculation was used.

In experiments, the samples were projected onto the feature subspace of c-1 dimensional LDA and OLDA respectively, and the nearest neighbor classification [10]-[11] was used to carry out classification tests in the feature space.

The accuracy recognition rate of LDA is 58 %, and the accuracy recognition rate of OLDA is 75%. It can be seen that, the accuracy of OLDA recognition rate is higher than LDA and the classification effect of Orthogonal Linear Discriminate Analysis OLDA is better than the traditional LDA. However, there is also space for improvement. Using the method of combining Adaboost and OLDA algorithm again, it can be seen from following Figure 6 that accuracy recognition rate is more than 91% after three rounds iteration. Thus it can be seen that Adaboost-OLDA algorithm can improve the classification accuracy rate.

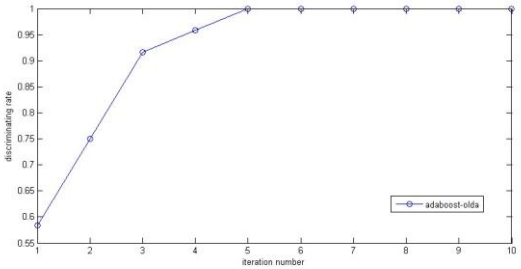

Fig 6 Adaboost-olda algorithm iterative discrimination rate

4. CONCLUSIONS

In this paper, a digital camera was used to capture lettuce leaf images of three different nitrogen levels in test shelter, the data of the image features were extracted and three kinds of algorithms of feature extraction of the LDA, OLDA and Adaboost-OLDA were compared in experiments. At last, recognition rate of Adaboost-OLDA is the highest, so this method provided an effective way to monitor accurate nitrogen status of lettuce. The experiment results showed that several weak classifiers can be constituted into a strong classifier, which can improve the classification recognition rate of the model markedly.

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Title: *Research on Intelligent Judgment of Water Deficiency of Tomato Based on SVM Algorithm*

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ABSTRACT

Adequate water is essential to healthy growth of crops. The nondestructive model of judging water deficiency is studied in this article. The tomatoes of various water levels are planted with the techniques of soilless cultivation. What's more, hyperspectral images of tomato leaves in various water levels are collected. The selected feature band images were analyzed and the image feature vectors were extracted. Then, the methods of support vector machine, Mahalanobis distance, linear classification and secondary classification were used to build model and judge water of tomato. Through the test, the results show that the support vector machine classification effect is optimal and classification correct rate is 90%.

1. INTRODUCTION

Agricultural water accounts for 70% of the whole society in our country, and only 33% of water is actual utilization of irrigation water. Water is wasted seriously. Irrigation philosophy, rapid and accurate diagnosis of crop water stress conditions and irrigation scientific precision to ensure high quality and high yield of the crop are important tasks confronting the healthy production of crops [1].

Diagnosis of crop moisture is based on the experience of producers for a long time, decisions can be made only when symptoms happen. The crop has been damaged at this time, and diagnostics have a certain degree of subjectivity, and the misdiagnosis occurs inevitably [2]. In addition, crop water diagnosis can be measured indirectly through plant leaf stomatal conductance, leaf water potential, canopy temperature, transpiration rate, plant stem diameter variation index and so on, or crop water diagnosis can be directly measured and calculated through the fresh weight and dry weight of plant samples for determination of moisture content difference to determine the plant water status [3]. The accuracy of these traditional means of testing is low, and the sampling, measurement, and data analysis need a

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lot of manpower and material resources, and timeliness is bad, which are detrimental to the application [4]. Hyperspectral image based on blade technology is quick, easy, non-destructive and provides crop information in time, which is current diagnosis technique of crop research at home and abroad.

Water shortages will affect the normal growth of tomato, and when it is up to a certain extent, abnormal growth of the crop will show the corresponding phenomena in shape, such as loss of growth and stagnation, plant stature, green, red, leaf deformities, atrophy of apical buds, such as crack, stem and root rot and so on. During the growth of tomatoes, they are different in different growth conditions for water, which can be roughly divided into water shortage and normal water. Tomatoes are selected as a research object in this article, and study on intelligent identification is done about that tomato is lack of water or not through computer image processing technology and pattern recognition algorithm.

2. CULTIVATION EXPERIMENT AND HYPERSPECTRAL IMAGE ACQUISITION

Experiments were done in Jiangsu Province of Modern Agricultural Equipment and Technology Department of the University to build a focus in Venlo type greenhouses. We finished tomato cultivation in April 2009 and September 2009.The variety of tomatoes is cooperation 906. Study has two different levels of water treatment. 1th set (W1) throughout the growing season has adequate water supplies and 2nd (W2) group of irrigation water is a standard formula of 50% concentrate. Cultivation of samples was did with Japan Yamazaki formula, and configurations of the nutrient solution were prepared with distilled water, which was to avoid different trace elements in samples of water quality and water cultivation of adverse effects.

The secondary light source containing the visible and near-infrared was selected, and V10E&N17E hyper-spectral imaging system (400-1700nm) was used to build hyperspectral image data acquisition system. When hyperspectral images were collected, first of all, the black point and white point were calibrated. The research which gets the light parts of the image with for hyperspectral is image acquisition system. The system is based on Imaging Spectrometer. The image acquisition system based on hyperspectral imaging spectrometer used "Push-broom" imaging method to get the hyperspectral image. When the data was collected, detector in optical focal plane did longitudinal arrangement and completed horizontal scan direction and you can get the target in the corresponding space and various wavelengths of each pixel in the image information; when conveyor belts move forward in the process of the system at the same time, the probe completed vertical scanning, and the target of three dimensional hyperspectral image information could be got with detection of vertical and horizontal scanning. Hyperspectral image has the particular pixel under the spectral information and the image information of a specific wavelength. The data collection of Hyperspectral image is based on SpectralCube (Spectral Imaging Ltd,Finland); the range of actual spectrum of acquisition is 871.6-1766.3nm. Spatial resolution is 62.5um and the sampling interval for 3.5nm [6][7].

3. IMAGE PROCESSING AND FEATURE EXTRACTION

Hyperspectral image processing is based on ENVIV.4 (Research System, Inc, USA) and MatlabV.7.0 (the Mathworks Co., USA) software platform. Analysis on hyperspectral image processing uses principal component analysis method, and the feature band is selected [5]. For example, in the first stage of tomato (graft-flowering), we select the characteristic bands of 1065nm, 1280nm, 1429nm. The band images under characteristic band are handled, and characteristic band image features are collected, such as mean value (Gray) feature AG, texture (Vein) feature AV and fusion (Fusion) feature AF. The part of eigenvalues of tomato samples (graft-flowering) are shown in table 1 below.

TABLE 1 PART OF EIGENVALUES OF HYPERSPECTRAL IMAGING OF TOMATOES

Sample number	The eigenvalues of feature-band image of mean value (Gray) feature AG			The eigenvalues of feature-band image of texture (Vein) feature AV			Eigenvalues of feature-band image of fusion (Fusion) feature AF		
	1065	1280	1429	1065	1280	1429	1065/1280	1280/1429	1429/1065
1	204.09	190.88	64.35	0.11	0.10	0.12	1.07	3.00	0.32
2	184.07	171.07	54.96	0.14	0.13	0.13	1.08	3.11	0.30
3	110.01	88.22	29.42	0.34	0.31	0.18	1.25	3.00	0.27
4	136.51	109.43	30.08	0.33	0.28	0.18	1.25	3.63	0.22
5	86.94	78.59	35.42	0.31	0.28	0.20	1.11	2.22	0.41
6	147.09	111.91	32.87	0.33	0.29	0.15	1.31	3.40	0.22
7	113.09	90.51	33.66	0.34	0.30	0.18	1.25	2.69	0.30
8	132.09	100.31	20.65	0.30	0.26	0.13	1.32	4.86	0.16
9	203.14	192.03	78.83	0.15	0.14	0.14	1.06	2.44	0.39
10	110.00	87.88	36.02	0.35	0.30	0.17	1.25	2.44	0.33

In the transplant - blossom period, 80 samples were collected, and the samples of normal water level was 40, and samples of water shortage was 40. The tomato sample feature library was built, which was used for subsequent modeling using judgment. 60 randomly selected samples were training samples and the remaining 20 samples were test samples.

4. TRADITIONAL CLASSIFICATION ALGORITHMS AND MODELING

4.1 The Mahalanobis Distance

Mahalanobis distance can be defined as the degree of difference between two random variables. The two random variables obey the same distribution and their covariance matrixs are Σ. If the covariance matrix is a identity matrix, then the Mahalanobis distance is simplified as the Euclidean distance. If the covariance matrix is a diagonal matrix, then it can also be referred as the normalized Euclidean distance.

(1) The Calculation of Mahalanobis distance is based on the overall sample;

(2) In the process of the Calculation of Mahalanobis distance, the overall sample number is required to be greater than the dimensions of the sample, otherwise the overall sample covariance matrix's inverse matrix does not exist.

It is not affected by the influence of dimension. In addition, the Mahalanobis distance between two points is independent of the units and measurement of raw datas; the Mahalanobis distance between two points, which is calculated by standardized data and center data (the difference between the original data and the mean) is the

same. Mahalanobis distance can also eliminate the interference of the correlation between variables.

If d_{ij} is used to stand for the distance between the ith sample and the jth sample, then for all i, j and k, d_{ij} should satisfy the following four conditions:

① if and only if i= j, $d_{ij} = 0$

② $d_{ij}>0$

③ $d_{ij}=d_{ji}$(symmetry)

④ $d_{ij}\leq d_{ik}+d_{kj}$(triangle inequality)

Obviously, the Euclidean distance meets four conditions above. There are a lot of the functions meeting the conditions above. The Mahalanobis distance used in this article is one of them.

The Mahalanobis distance d_{ij} between the ith sample and the jth sample is calculated by the following formula:

$$d_{ij}=((x_i - x_j)^T S^{-1}(x_i - x_j))^{1/2}$$

Among them, T stands for transpose. x_i and x_j are respectively the vector consisted of m-index of the ith sample and the the jth sample. The S stands for covariance matrix.

4.2 Linear Classification and two Classification

The principle of linear classification and two classification is similar to the Mahalanobis distance, and they are the extension and improvement of Mahalanobis distance.

Now, the three methods are used to determine the category of tomato:

In the first growth period of tomatos (the transplantation - blossom period), the serial numbers of 1~30 are the first class, denoted by G_1 (the normal type); The serial numbers of 31~ 60 are the second class, denoted by G_2 (the dry type).The characteristic parameters using the X_1, X_2, X_3 stand for the mean characteristics of AG of the characteristic band image characteristic values; X_4, X_5 and X_6 are used to stand for the texture features of AV of characteristic band image characteristic values; X_7, X_8 and X_9 are used to stand for the fusion feature AF of the characteristic band image characteristic value .

Processes are as follows: (1) proposed the known sample data, here of the transpose of a matrix; (2) put forward to be convicted of sample data; (3) the known sample classification; (4) the Mahalanobis distance classification; (5) linear classification; (6) two classification.

By using the Mahalanobis distance, the correct classification rate is 70%; by using linear classification correct classification rate is 75%; by using the two classification of the correct classification rate is 80%.

5. SUPPORT VECTOR MACHINE AND MODELING

SVM is based on statistical learning theory of pattern recognition, and it is mainly used in the field of pattern recognition. The realization of statistical learning theory (Statistical Learning Theory, SLT) and some important problems encountered by the new machine learning methods of the neural network and so on, such as the problem of how to determine the network structure, over learning and owe learning problems,

local minimum problem and so on makes SVM develop quickly and better. SVM shows many unique advantages in the problems of small samples, nonlinear and high dimension pattern recognition, and can be applied to the function fitting and other machine learning problems. Now SVM has achieved successful application in many fields.

The commands of the support vector machine are rich and powerful in matlab. Firstly, the svmtrain function of the classifier of the support vector machine is used to train sample sets and make them learn. Secondly, the svmclassify function of the classifier of support vector machine is used to test sample sets. The sequence minimizing parameter function svmsmoest usd by the functions of the support vector machine is designated. Next, these functions are used to distinguish the category of tomato.

In the first growth period of tomatos (the transplantation - blossom period), the serial numbers of 1~30 are the first class, denoted by G_1 (the normal type); The serial numbers of 31~ 60 are the second class, denoted by G_2 (the dry type).The characteristic parameters using the X_1, X_2, X_3 stand for the mean characteristics of AG of the characteristic band image characteristic values. X_4, X_5 and X_6 are used to stand for the texture features of AV of characteristic band image characteristic values; X_7, X_8 and X_9 are used to stand for the fusion feature AF of the characteristic band image characteristic value.

The program flow of matlab is as follows:

(1)In the table, all data of X_1... X_8 is stored in a plain text file. (2) The data which has been classified and waits to be classified is extracted. (3) The standardization of the data which has been classified.(4) The standardization of the data which waits to be classified. (5)The category label of samples which is known; (6) The separator of the support vector machine is trained. (7) The label of the support vector is returned. (8)The weight coefficient of the classification function is returned.(9) The constant term of the classification function is returned. (10)The known samples are tested and verified. (11)The rate of the wrong judgment of the known samples is calculated.(12) The sample points which are to be classified are classified.

The specific process is as follows:

i = 1…60 is used to stand for 60 tomato samples, and the value of a_{ij} is the j_{th} data of the i_{th} tomato.

The calculation of the mean vector of known 60 samples is

u =[u1…u9]=[142.017, 118.296, 36.9, 0.2819, 0.2497, 0.1573, 1.2308146, 3.4018586, 0.2556307],

The standard deviation vector of 60 sample is σ=[σ1…σ9]=[37.948, 42.221, 17.066, 0.0883, 0.07659, 0.024, 0.1058, 0.804, 0.0714996].

All the sample data is handled by the following formula of standardization: $\tilde{a}ij = (aij - uj)/\sigma j, i = 1,...60; j = 1,...9$。

Correspondingly, $\tilde{xj} = (xj - uj)/sj$ j=1,2,…9. is called the standardized variable. That is $\tilde{x} = [\tilde{x}1,...,\tilde{x}9]^{T}$.

The 60 samples of standardization which have been classified are called data row vector $b_i=[A_{i1},…,A_{i8}]$, i=1…60. The support vector machines model of the linear kernel function classification is used to classify, and the support vector is b_{14}, b_{15}, b_{17}, b_{19}, b_{24}, b_{25}, b_{27}.

Linear classification function is

$$c(\tilde{x}) = \sum \beta i\ K(bi, \tilde{x}) + b$$

$$= 0.4649K(b_{14}, \tilde{x}) + 0.0477K(b_{15}, \tilde{x}) + 0.6750K(b_{17}, \tilde{x}) + 0.5979K(b_{19}, \tilde{x})$$

$$- 0.4234K(b_{24}, \tilde{x}) - 1.2908K(b_{25}, \tilde{x}) - 0.0757K(b_{27}, \tilde{x}) + 1.0269$$

$$In\quad which: \beta_i = a_i g_i, \tilde{x} = [x1 \ldots x9], K(bi, \tilde{x}) = (bi, \tilde{x})。$$

When the $c(\tilde{x}) \geq 0$, $\tilde{x}$ belongs to the first class. When the $c(\tilde{x}) < 0$, $\tilde{x}$ belongs to the second class.

The discriminant function is used to distinguish the rest of 20 test samples, and the correct rate is 90%.

6. CONCLUSION

This article collectes hyperspectral images of the first growth stage(the transplantation - blossom period) of tomato. The feature vector is extracted by dealing with and analyzing images. The methods of support vector machine, Mahalanobis distance, linear classification and two classification are used to build models and judge whether the tomatos are water shortage or not. Through the test, the results show that the support vector machine classification is the best.

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Title: *Effect of Linseed Oil Supplementation on Performance, Carcass Quality and Fatty Acid Profile of Crossbred Wagyu Beef Steers*

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ABSTRACT

The objective of this study was to determine the effect of linseed oil supplementation on carcass quality and fatty acid profile of crossbred Wagyu beef steers. Twenty four fattening Wagyu crossbred beef steers, averaging 640±18 kg live weight (LW) and approximately 30 mo old, were stratified and randomly assigned in completely randomized design. All steers were fed approximately 7 kg/d of 12% CP concentrate with *ad libitum* rice straw and had free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control concentrate plus 200 g/d of palm oil; 2) control concentrate plus 100 g/d of palm oil and 100 g/d of linseed oil;. 3) control concentrate plus 200 g/d of linseed oil. This present study demonstrated that supplementation of LSO which rich in C18:3n-3 linolenic acid increase C18:3n3 and decrease SFA, n-6:n-3 ratio in LD and SM muscles. However, its supplementation did not affect growth performance, beef chemical composition and carcass quality.

Words: Linseed oil, growth performane, carcass quality, fatty acid, beef steers

INTRODUCTION

The health benefits of α-linolenic acid (18:3n-3) have become into the leading position of food and nutritional science research. The long-chain polyunsaturated fatty acids, especially linoleic (C18:2n6) and α-linolenic (C18:3n3) have anticarcinogenic (Parodi, 1997) and potentially cardioprotective roles in humans (Massaro et al. 1999). As a result there has been a great deal of interest in manipulating the fatty acid profile of meat fat to respond to consumers' concerns. A reduction in the consumption of SFA and an increase in the consumption of polyunsaturated fatty acids (fatty acids with more than one double bond; PUFA) is encouraged, while monounsaturated fatty acids (fatty acids with one double bond; MUFA) are generally regarded as beneficial for human health. Within the PUFA, the two main types of fatty acids are omega-3, where the first double bond occurs at the third carbon from the methyl end of the molecule and omega-6, where the first double bond occurs at the sixth carbon from the methyl end of the molecule. These apparently small differences in structure confer different metabolic properties to each fatty acid. Both types of PUFA are termed essential, i.e. they cannot be synthesised by humans and so must form part of the diet. The main sources of supplementary

fatty acids in ruminant rations are plant oils and oilseeds. Supplementation of plant oils and oilseeds rich in omega-3 fatty acids could increase omega-3 fatty acids in meat fat. Thus, the objective of the present study was to examine the effect of linseed oil supplementation on performance and fatty acid profile of Wagyu crossbred beef steers.

MATERIALS AND METHODS

Animal and Feeding

Twenty four Wagyu crossbred fattening steers (50% Wagyu crossbred), averaging 640±18 kg live weight (LW) and approximately 30 mo old, were stratified by their LW into 3 groups and each group was randomly assigned to three dietary treatments. All steers were fed approximately 7 kg/d of 12% CP concentrate with *ad libitum* rice straw and had free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control concentrate plus 200 g/d of palm oil; 2) control concentrate plus 100 g/d of palm oil and 100 g/d of linseed oil;. 3) control concentrate plus 200 g/d of linseed oil. The fatty acid compositions of feed and oils used in the present study are presented in Table I.

Sample Collection, Chemical and Fatty Acid Analysis

Feed offered and left after eating of individual steer were collected on 2 consecutive days weekly to calculate DM intakes. Sample were taken and dried at 60℃ for 48 hours and at the end of the experiment, feed samples were pooled to make representative samples for proximate (AOAC, 1995) and detergent analyses were determined using the method described by Van Soest et al. (1991) the experiment lasted for 67 days with the first 7 days as adjustment period followed by 60 days of measurement period. Feed and beef were extracted using a modified method used by Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared by the procedure described by Ostrowska et al. (2000).

Statistical Analysis

Data measurements were analyzed by ANOVA for complete randomized design using the Statistical Analysis System (SAS, 2001). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $p<0.05$ was used (Steel & Torrie, 1980).

RESULT AND DISCUSSION

Feed Intake and Animal Performance

DM and CP intakes, final weight and live weight change were unaffected by the dietary treatments (Table I). However, DM and CP intakes of concentrate tended to increase when linseed oil was added. Noci et al. (2007) and Dawson et al. (2007) reported silage DMI, total DMI, and BW gain were not significantly affected by

dietary treatment. This is probably because total energy consumption was balanced by treatments.

TABLE I. DM AND CP INTAKES, INITIAL WEIGHT, FINAL WEIGHT AND LIVEWEIGHT CHANGE OF WAGYU CROSSBRED FED PALM AND/OR LINSEED OIL

Items	200 g/d PO	100 g/d PO+ 100 g/d LSO	200 g/d LSO	SEM	P-value
DM intake (kg/d)					
Concentrate	5.70	6.46	6.32	0.23	0.062
Rice straw	2.87	2.79	2.82	0.12	0.883
Total	8.57	9.25	9.14	0.31	0.351
CP intake (g/d)					
Concentrate	623	726	705	29.3	0.051
Rice straw	81	74	68	4.9	0.230
Total	704	801	774	32.8	0.122
Initial weight (kg)	642	631	646	18	0.817
Final weight (kg)	709	703	718	16.7	0.801
Live weight change (kg/d)	1.11	1.20	1.20	0.06	0.481

PO = palm oil; LSO = linseed oil; SEM = standard error of the mean

Fatty Acid Composition of Muscle Fat

There were significant increases ($P<0.05$) in C18:3n3 and $^{C22:6n-3}$ and decreases in SFA and n-6: n-3 ratio in LD and SM muscles when LSO was supplemented. Noci et al. (2006) suggested that the potential of addition of PUFA-rich plant oils or oilseeds to concentrate rations is to increase the PUFA content of ruminant meat. In contrast, Baird et al. (2010) report no significant difference in the total C18:3n3 across treatment, as linseed supplementation increased while there was a linear increase in C18:3n3 as a proportion of total PUFA increased. The present experiment resulted in a decrease in the SFA and an increase in the PUFA proportion in both muscles. This decrease in SFA suggests an increase in the incorporation of PUFA in the muscle at the expense of SFA, due to the different proportions of fatty acids in the unsupplemented and supplemented diets. Scollan et al. (2003) proposed a negative exponential relationship between the amount of intramuscular fat and the PUFA: SFA ratio. Comparisons of the PUFA: SFA ratio across studies should therefore be made with caution because lean animals will have a greater PUFA: SFA ratio irrespective of ration composition (Raes et al., 2003). Feeding LSO decreased the n-6: n-3 PUFA ratio from 10:1 to 2:1 in the present study which confirmed the results in Belgian Blue bulls (Raes et al., 2004). The n-6: n-3 PUFA ratio of beef is of relevance in its contribution to the whole diet of humans. Despite medical advice, n-6 PUFA consumption by humans is excessive resulting in dietary n-6: n-3 PUFA ratios of 7.2:1 and 7.4:1 for men and women in the UK, respectively (Gregory et al., 1990).

TABLE II. FATTY ACID COMPOSITION OF *Longissimus dorsi* MUSCLE FROM WAGYU CROSSBRED FED PALM AND/OR LINSEED OIL

Items	200 g/d PO	100 g/d PO+ 100 g/d LSO	200 g/d LSO	SEM	P-value
No. of cattle	8	8	8		
Longissimus dorsi					
C10:0	0.12	0.13	0.13	0.01	0.744
C12:0	3.76	3.52	2.96	0.26	0.661
C14:0	5.25	4.59	5.87	0.19	0.506
C15:0	0.90	0.81	0.73	0.07	0.368
C16:0	22.76	24.99	25.78	0.32	0.493
C16:1	4.40	3.79	3.43	0.20	0.078
C17:0	1.21	1.24	1.11	0.09	0.810
C18:0	7.28	7.48	7.97	0.31	0.349
C18:1n-9	39.23	40.35	39.86	0.38	0.274
C18:1t-11	3.35^{a}	2.79^{b}	2.67^{b}	0.10	0.014
C18:2n-6	6.86^{a}	5.72^{b}	4.06^{c}	0.27	0.004
CLA c9,t11	1.05^{a}	0.77^{b}	0.62^{b}	0.06	0.007
CLA t10,c12	0.17^{a}	0.13^{b}	0.10^{b}	0.01	0.031
C18:3n-3	0.48^{c}	0.86^{b}	1.59^{a}	0.10	0.001
C20:4n-6	3.81^{a}	2.88^{b}	2.34^{c}	0.12	0.002
C20:5n-3	0.08	0.11	0.27	0.03	0.523
C22:5n-3	0.28	0.37	0.55	0.03	0.132
C22:6n-3	0.23^{b}	0.37^{b}	0.68^{a}	0.05	0.004
SFA	41.28	42.76	44.55	0.48	0.983
UFA	58.72	57.24	55.45	0.48	0.913
MUFA	43.63	44.14	43.29	0.48	0.234
n-3 PUFA	1.07^{c}	1.71^{b}	3.09^{a}	0.19	0.004
n-6 PUFA	10.67^{a}	8.60^{b}	6.40^{c}	0.32	0.002
Total CLA	1.22^{a}	0.90^{b}	0.72^{b}	0.07	0.008
n-6:n-3	9.97^{a}	5.03^{b}	2.07^{b}	0.79	0.001
UFA:SFA	1.42	1.34	1.24	0.03	0.981
PUFA:SFA	0.28	0.24	0.21	0.01	0.062

TABLE III. FATTY ACID COMPOSITION OF *Semimembranosus* MUSCLE FROM WAGYU CROSSBRED FED PALM AND/OR LINSEED OIL

Items	200 g/d PO	100 g/d PO+ 100 g/d LSO	200 g/d LSO	SEM	P-value
No. of cattle	8	8	8		
Semimembranosus					
C10:0	0.10	0.11	0.12	0.01	0.756
C12:0	3.69	3.42	3.01	0.24	0.658
C14:0	5.08	4.61	5.76	0.16	0.511
C15:0	0.87	0.83	0.75	0.07	0.351
C16:0	22.87	25.08	25.81	0.30	0.487
C16:1	4.37	3.59	3.41	0.19	0.073
C17:0	1.19	1.25	1.14	0.08	0.804
C18:0	7.39	7.41	7.87	0.28	0.351
C18:1n-9	39.23	40.60	39.96	0.34	0.268
C18:1t-11	3.46^{a}	2.83^{b}	2.74^{b}	0.11	0.013
C18:2n-6	6.91^{a}	5.82^{b}	4.23^{c}	0.26	0.003
CLA c9,t11	1.07^{a}	0.76^{b}	0.61^{b}	0.06	0.007
CLA t10,c12	0.16^{a}	0.14^{ab}	0.11^{b}	0.01	0.029
C18:3n-3	0.52^{c}	0.87^{b}	1.56^{a}	0.09	0.001
C20:4n-6	3.75^{a}	2.74^{b}	2.21^{c}	0.11	0.002
C20:5n-3	0.08	0.10	0.24	0.03	0.467
C22:5n-3	0.24	0.38	0.53	0.03	0.129
C22:6n-3	0.25^{b}	0.36^{b}	0.66^{a}	0.04	0.003
SFA	41.19	42.71	44.46	0.46	0.979
UFA	58.81	57.29	55.54	0.46	0.922
MUFA	43.60	44.19	43.37	0.46	0.228
n-3 PUFA	1.09^{c}	1.71^{b}	2.99^{a}	0.17	0.003
n-6 PUFA	10.66^{a}	8.56^{b}	6.44^{c}	0.31	0.002
Total CLA	1.23^{a}	0.90^{b}	0.72^{b}	0.06	0.007
n-6:n-3	9.78^{a}	5.01^{b}	2.15^{c}	0.73	0.001
UFA:SFA	1.43	1.34	1.25	0.03	0.978
PUFA:SFA	0.29	0.24	0.21	0.01	0.064

CONCLUSION

This present study clearly demonstrated that supplementation of LSO rich in C18:3n3 linolenic acid increased C18:3n3 and decreased SFA, n-6:n-3 ratio in LD and SM muscles. However, its supplementation did not affect intake, growth performance and beef chemical composition. LSO addition to fattening cattle's diets can increase the n3 fatty acids and decrease the n6/n3 ratio which is beneficial to consumers' health.

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Title: *Effect of Linseed Oil Supplementation on Performance, Carcass Quality and Fatty Acid Profile of Crossbred Beef Steers*

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ABSTRACT

The objective of this study was to determine the effect of linseed oil supplementation on carcass quality and fatty acid profile of crossbred beef steers. Fifteen fattening steers, averaging 337±43 kg live weight (LW) and approximately 2 years old, were stratified and randomly assigned in completely randomized design. All steers were fed approximately 4 kg/d of 14% CP concentrate with *ad libitum* fresh grass (*Pennisetum purpureum* x *Pennisetum americanum*) and had free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control concentrate plus 200 g/d of palm oil; 2) control concentrate plus 100 g/d of palm oil and 100 g/d of linseed oil;. 3) control concentrate plus 200 g/d of linseed oil. This present study demonstrated that supplementation of LSO which rich in C18:3n-3 linolenic acid increase C18:3n3 and decrease SFA, n-6:n-3 ratio in LD and SM muscle. However, its supplementation did not affect growth performance, beef chemical composition and carcass quality.

Words: Linseed oil, growth performane, carcass quality, fatty acid, beef steers

INTRODUCTION

Beef contains approximately 50% of saturated fatty acids (SFA) content, which is the result of the process of rumen biohydrogenation [14]. Saturated fatty acids (SFA) have been recognized by the international dietary authorities as primary targets for diet reduction [20]. SFA is a major factor causing chronic diseases in the Western world; cardiovascular disease and colon cancer probably [7]. The fatty acid composition of beef (including muscle and subcutaneous adipose tissue) can be influenced, at least in part, by fatty acid composition of the diet [4], [8]. Most of the research aimed at improving dietary quality of beef has been focused on manipulation of animal feed with attempts to increase the intramuscular n-3 PUFA content accomplished by feeding n-3 PUFA rich in ruminants' diets. In addition, low PUFA n-6/n-3 ratio aids in the prevention of many chronic diseases. Increasing the content of PUFA and reducing SFA with the net effect of increasing PUFA/SFA

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and reducing n-6/n-3 ratio are priorities [16]. Linseed oil is a natural source of α-linolenic fatty acid, and its may afford polyunsaturated fatty acids (PUFAs). It is also a precursor of eicosanoids, which play an important antithrombotic and anti-inflammatory role [10]. In general previous studies reported the effect of different linseed forms and concentrations on performance and on FA composition of muscle and adipose tissue in beef cattle [6], [7]. Thus, the objective of this study was to examine the effect of linseed oil supplementation on performance, carcass quality and fatty acid profile of crossbred beef steers.

MATERIALS AND METHODS

Animal and Feeding

Fifteen fattening steers (87.5% Branhman crossbred), averaging 337$\pm$43 kg live weight (LW) and approximate 2 years old, were stratified by their LW into 3 groups and each group was randomly assigned to three dietary treatments. All steers were fed approximately 4 kg/d of 14% CP concentrate with *ad libitum* fresh grass (*Pennisetum purpureum x Pennisetum americanum*) and free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control concentrate plus 200 g/d of palm oil; 2) control concentrate plus 100 g/d of palm oil and 100 g/d of of linseed oil;. 3) control concentrate plus 200 g/d of linseed oil. The fatty acid compositions of feed and oils used in the present study are presented in Table I.

Sample Collection Chemical and Fatty Acid Analysis

Feed offered and left after eating of individual steer were collected on 2 consecutive days weekly to calculate DM intakes. Sample were taken and dried at 60 ℃ for 48 hours and at the end of the experiment, feed samples were pooled to make representative samples for proximate [1] and detergent analyses were determined using the method described by [19]. Feed and beef were extracted using a modified method used by [3]. Fatty acid methyl esters (FAME) were prepared by the procedure described by [9].

Statistical Analysis

Data measurements were analyzed by ANOVA for in complete randomized design using the Statistical Analysis System [13]. Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $p<0.05$ was used [18].

RESULT AND DISCUSSION

Feed Chemical Composition

The fatty acid composition of the oils used in the experiment is show in Table I. Grass had a greater proportion of C16:0, MUFA, PUFA and n-3FA than the concentrate. The LSO concentrate had a similar proportion of n-3 FA to grass, but n-3

FA was almost absent from the palm oil concentrate. The concentrate had more C18:1, than the grass, and this accounted for the high proportion of PUFA in the concentrates. [8] Report grass has higher C16:0 and SFA than concentrate and C18:3 concentrate has a similar LSO (47.50, 49.16 g/100 gFA, respectively).

TABLE I. FATTY ACID COMPOSITION OF FEED AND OILS.

Fatty acid (% of total FA)	Concentrate	Fresh grass	Linseed oil	Palm oil
C16:0	17.78	19.42	5.56	38.30
C18:1	31.68	6.93	17.92	40.62
C18:2	20.34	19.34	16.40	13.66
C18:3	0.34	48.96	55.82	0.27
SFA	46.98	24.77	9.06	42.02
MUFA	52.44	78.37	18.05	84.30
PUFA	21.34	68.30	72.89	14.90
Total n-3	0.34	48.96	56.09	0.44
Total n-6	21.00	19.34	16.69	14.46
n-6:n-3	61.76	0.40	0.30	32.86

FEED INTAKE AND ANIMAL PERFORMANCE

Animal performances are summarized in Table II. Dry matter intake, final body weight, ADG, Energy gain, Feed:Gain ratio and dressing percent were not affected by dietary treatments. [8] Report silage DMI, total DMI, and BW gain from the beginning of the trial until slaughter were not significantly affected by dietary treatment. Because total energy consumption was balanced by treatment.

TABLE II. EFFECT OF TREATMENT ON PERFORMANCE.

Item	Treatments			SEM	Pr<F
	Control	100 g/d LSO	200 g/d LSO		
Dry matter intake, kg/d	9.31	9.24	9.56	0.15	0.702
Initial body weight, kg	336	338	338	11.17	0.998
Final body weight, kg	402	409	402	10.91	0.956
Average daily gain, kg/d	0.9	1.0	0.9	0.06	0.783
Energy gain	4.12	4.62	4.04	0.06	0.730
Feed:Gain ratio	0.15	0.13	0.16	0.01	0.606

FATTY ACID COMPOSITION OF MUSCLE FAT

The fatty acid composition of fat extracted from LD and SM muscle are presented in Table III. There were significant increase ($P<0.05$) in C18:3n3 and decreases in SFA and n-6:n-3 ratio in LD and SM muscles when LSO was supplemented. The present report confirms the result of [8] that the potential of addition of PUFA-rich plant oils or oilseeds to concentrate rations is to increase the PUFA content of

ruminant meat. [2] Report no significant difference in the total C18:3n3 across treatment, as linseed supplementation increased and there was a linear increase in C18:3n3 as a proportion of total PUFA increased. The present experiment resulted in a decrease in the SFA and an increase in the PUFA proportion in the muscles. This decrease in SFA suggests an increase in the incorporation of PUFA in the muscle at the expense of SFA, due to the different proportions of fatty acids in the unsupplemented and supplemented diets. It is recognized that the fat concentration of muscle has a major influence on the PUFA: SFA ratio because PUFA are mainly found in the polar lipid fraction, which is diluted by the growth in neutral lipid fraction as animals accrete lipid. Proposed a negative exponential relationship between the amount of intramuscular fat and the PUFA: SFA ratio [14]. Comparisons of the PUFA:SFA ratio across studies should therefore be made with caution because lean animals will have a greater PUFA:SFA ratio irrespective of ration composition (Raes et al., 2003). In the current experiment, feeding grass contributed to a high daily intake of C18:3n-3, which, despite extensive ruminal biohydrogenation, maintained the n-6:n-3 PUFA ratio largely within the recommended values (<4:1) for muscle fat.

TABLE III. EFFECT OF TREATMENT ON FATTY ACID COMPOSITION OF *LONGISSIMUS DORSI* (LD) AND *SEMIMEMBRANOSUS* (SM) MUSCLE.

Fatty acid (% of total FA)	Treatments			SEM	Pr<F
	Control	100 g/dLSO	200 g/dLSO		
Longissimus dorsi (LD)					
C16:0	31.07	30.96	30.47	0.45	0.887
C18:1	38.51	34.76	34.40	0.93	0.184
C18:2	4.06	2.45	2.80	0.49	0.616
C18:3	0.07[b]	0.14[a]	0.15[a]	0.01	0.025
SFA	60.36[a]	58.06[b]	55.34[c]	1.03	0.020
MUFA	36.04[a]	36.91[a]	38.48[b]	0.98	0.027
PUFA	3.60[a]	5.03[b]	6.18[b]	0.46	0.026
Total n-3	0.44	0.49	0.53	0.13	0.300
Total n-6	6.42[a]	3.89[ab]	3.16[b]	0.86	0.045
n-6:n-3	14.59[a]	7.93[b]	5.96[b]	1.91	0.013
Semimembranosus (SM)					
C16:0	32.54	29.84	29.60	0.52	0.081
C18:1	39.65	38.25	38.24	0.63	0.670
C18:2	4.21	3.14	1.89	0.38	0.086
C18:3	0.27	0.30	0.29	0.04	0.978
SFA	56.97[a]	52.12[b]	50.16[b]	0.87	0.016
MUFA	35.40[b]	41.41[a]	44.80[a]	0.64	0.016
PUFA	7.63[a]	6.47[ab]	5.04[b]	0.56	0.014
Total n-3	0.56[b]	0.80[a]	0.99[a]	0.12	0.039
Total n-6	6.64[a]	4.97[ab]	3.29[b]	0.53	0.025
n-6:n-3	11.86[a]	6.91[b]	3.32[c]	1.19	0.018

Feeding LSO decreased the n-6:n-3 PUFA ratio from 6:1 to less than 4:1 in Belgian Blue bulls (Raes et al., 2004). The n-6:n-3 PUFA ratio of beef is of relevance in its contribution to the whole diet of humans. Despite medical advice, n-6 PUFA consumption by humans is excessive resulting in dietary n-6:n-3 PUFA ratios of 7.2:1 and 7.4:1 for men and women in the UK, respectively [5].

CONCLUSION

This present study demonstrated that supplementation of LSO which rich in C18:3n3 lenolenic acid increased C18:3n3 and decreased SFA, n-6:n-3 ratio in LD and SM muscles. However, its supplementation did not affect growth performance, beef chemical composition. It can be clearly concluded that LSO addition to fattening cattle's diets can increase the n3 fatty acids and decreasing the n6/n3 ratio which is beneficial to the health of beef consumers

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Title: *Effects of Oleic Acid Enriched Oils Supplementation on Performance and Carcass Quality of Crossbred Brahman Steers*

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ABSTRACT

The objective of this study was to determine the effectsofoleic acid enriched oils supplementation on performance and carcass quality of crossbred Brahman steers (Brahman x Thai native). Twelve Crossbred Brahman steers, averaging 17±2 month old and 319±38 kg body weight, were stratified and randomly assigned in a completely randomized designinto three groups of four steers. The first group (control) received basal diet with approximately 6 kg of 12% CP concentrate plus 1 kg cassava meal. The second group was fed the basal diet supplemented with 200 g/d palm oil, while the third group was fed the basal diet supplemented with 200 g/d rice bran oil. All steers were also received*ad libitum* rice straw, individually housed, and fed in a free-stall unit. The experiment lasted for 104 days with the first 14 days as the adjustment period, followed by 90 days of measurement period. Feeds offered and residues of individual steers were collected on 2 consecutive days every 2 weeks. Live weights were recorded at the start and end of the study. All cattle were slaughtered at the end of the experiment. The results showedthat supplementation of oil rich in oleic acid decreased cooking loss in *Longissimusdorsi*. ($P<0.05$); however, oilsupplementation did not affect growth performance, carcass quality, and feed intakes.

Words: Oleic acid, Palm oil, Rice bran oil, Cooking loss, and Growth performance

INTRODUCTION

Currently, Thailand's farmers import beef cattle for developing breed type to produce beef in high quality but now Thailand's farmer don'tproduce high quality beef thatlead to have problem in feed management. The high beef qualityisjudge the price from intramuscular fat, calling marbling score. Many producers fed the cattle without knowledge to get high marbling score. Marbling score depends on feed management and breed type where feed management is more importantthan breed type.The fatty acid primarily responsible for soft fat in beef cattle is oleic acid (C18:1n-9). The concentration of oleic acid is also positively correlated with overall

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palatability of beef (Waldman et al., 1968; Westerling and Hedrick, 1979), which may be related to fat softness. Stearic acid (C18:0) is a primary determinant of fat hardness (i.e., lipid melting point; Smith et al., 1998; Wood et al., 2004; Chung et al., 2006b), so any dietary or production factor that enhances the conversion of stearic acid to oleic acid will increase fat softness. There are three fatty desaturases in animal tissues, Δ5,Δ6, and Δ9 desaturase. Of these, only the Δ9 desaturase acts upon saturated fatty acids (SFA) to convert them to their respective monounsaturated fatty acids (MUFA). The most abundant fatty acid in beef is oleic acid produced by the Δ9 desaturation of stearic acid. The Δ9 desaturase, which is encoded by the stearoyl-CoA desaturase (SCD) gene, also converts *trans*-vaccenic acid (TVA) to its corresponding conjugated linoleic acid (CLA) isomer, *c*-9,*t*-11 CLA. Approximately 5% of the total fatty acids in beef are comprised of polyunsaturated fatty acids (PUFA), by far the most abundant of which is linoleic acid. As is the case for MUFA and *c*-9, *t*-11 CLA, PUFA contain a double bond at the Δ9 position; however, this is introduced into the fatty acid structure by the plant from which the linoleic acid was derived, and is not produced by Δ9 desaturation in animal tissues. There are three major factors that affectfatty acid composition of beef: 1) age of animal; 2) diet; and 3) breed type. Age of animal and breed type specifically affect concentration of MUFA in beef by affecting SCD gene expression and activity. However, incomplete biohydrogenation oleic acid will pass to small intestine and associate develop fat cells. Monounsaturated fatty acids accumulate in adipose tissue in cattle fed high-concentratefinishing diets. This coincides with an increase in SCD gene expression and/or catalytic activity, which is exaggerated in Korean Hanwoo and Japanese Black cattle. These cattle have not onlya greater genetic tendency to produce more MUFA, but also fed for longer periods of time. When cattle are grazed in pastures or are fed hay, their beef contains less marbling, much less MUFA, but contains slightly more n-3 PUFA. In addition, any production practices that elevates *t*-10,*c*-12 CLA in bovine adipose tissue will reduce intramuscular adipose tissue development. In overall, of the three factors that affectbeef total fat and fatty acid composition, breed type has the smallest effect.

MATERIALS AND METHODS

Animals and Treatments

Twelve crossbred Brahman steers (Brahman x Thai native) averaging 17±2 month old and 319±38 kg body weight werestratified by their LW into 3 groups, and each group was randomly assigned to three dietary treatments. The first group received basal diet with approximately 6 kg of 12% CP concentrate plus 1 kg cassava meal(Control). The second group was fed the basal diet supplemented with 200 g/d palm oil, while the third group was fed the basal diet supplemented with 200 g/d rice bran oil. All steerswere also received*ad libitum* rice straw, individually housed, and fed in a free-stall unit. The experiment lasted for 104 days with the first 14 days as the adjustment period.

Feed Intake, Carcass Collection, and Analysis

Feeds offered and residues of individual steers were weighed for two consecutive days of each period, and samples were taken and dried at 60°C for 48 hour to calculate

feed intake. At the end of feeding trial, the animals were weighed to calculate average daily gain. All steers were transported to a commercial abattoir and then slaughtered at NakornRatshasima slaughterhouse, NakornRatchasima, Thailand, following procedures outlined in Jaturasitha (2004). All experimental procedures were carried out following the animal welfare standards of Department of Livestock Development, Ministry of Agriculture and Coopperative, Rayal Thai Government. The carcasses were weighedafterward to calculate hot carcass percentage. The muscle samples were cut from outside *Longissimusdorsi* (LD) muscle and *Semimembranosus* (SM) muscle on the left side of each carcasstodetermine pH at 45min and 24h. Back fat was determined by using venire caliper at 12-13th ribs.

All samples were placed in plastic bags, placed on ice, and chilled at 4 ℃ for 48 h. Meat samples were determined for color and shear force. These samples were measured beef color by Chroma meter (Minolta Co., Ltd) and then L* (lightness), a* (redness) and b* (yellowness) value were measured in six locations. Shear force was done with a Warner-Bratzle shear attachment by Texture analyzer (TA-TX2 Texture Analyzer, Stable Micro Systems, UK).

STATISTICAL ANALYSIS

Data measurements were analyzed by ANOVA for completely randomized design using the Statistical Analysis System (SAS, 2001). Significant differences among treatment means were assessed by Duncan's new multiple range test. A significant level of $p<0.05$ was used (Steel andTorrie, 1980).

RESULTS AND DISCUSSION

Animal performances are summarized in Table 1. Dry matter intake, initial body weight, final body weight, body weight gain, and daily gain were similar among the treatments. These might due to all treatments were balanced feed energy.He et al.(2012),Dawson et al. (2009), and Noci et al. (2007) reportedoil supplementation atlow level has no effects on dry mater intake and body weight gain from the beginning of the trial until slaughters

TABLE1: EFFECT OF SUPPLEMENTED OLEIC ACID ENRICHED OILS ON FEED INTAKE AND PERFORMANCEOF STEERS

Parameter	Treatment[1]			SEM	P-value
	Control	Palm oil	Rice bran oil		
DMI (kg/d)					
Concentrate	5.6	5.6	5.6	-	-
Rice straws	3.8	3.6	3.4	0.221	0.514
Total	9.4	9.2	9.0	0.221	0.562
Body weight change					
Initial BW (kg)	329.8	318.5	309.8	21.0	0.800
Final BW (kg)	412.0	399.8	390.3	18.6	0.717
BW gain (kg)	82.3	81.3	80.5	4.34	0.960
Daily gain (kg/d)	0.91	0.90	0.89	0.05	0.952

[1]Treatments were: palm oil 200g/d; rice bran oil 200 g/d, BW: body weight, SEM = standard error of mean

Carcass qualityisreported in Table 2. The supplemented oil rich in oleic acid has no effect on hot carcass weight, hot carcass percentage, back fat, and pH (P>0.05) The supplementation ofrice bran oil has lesscooking loss than other 2 groups. This is dueto water loss from rice bran oil-supplemented beef was less than others.

TABLE2: EFFECT OF OLEIC ACID ENRICHED OILS SUPPLEMENTATION ON CARCASS QUALITY

Parameter	Treatment[1]			SEM	P-value
	Control	Palm oil	Rice bran oil		
Hot carcass weight (kg)	219.8	225.5	217.0	15.016	0.922
Hot carcass (%)	53.4	56.3	55.3	1.828	0.529
Backfat (cm)	0.47	0.46	0.42	0.046	0.744
pH at 45 min					
LD	7.36	7.23	7.21	0.154	0.763
SM	7.25	7.43	7.09	0.116	0.109
pH at 24 hr					
LD	5.21	5.57	5.62	0.131	0.098
SM	5.38	5.48	5.36	0.107	0.702
Cooking loss (%)					
LD	36.4[b]	37.3[b]	35.1[a]	0.286	0.001
SM	35.1	33.4	35.3	0.920	0.337

[a,b]means without same letters on the same column are significantly different (P<0.05)
[1]Treatments were: palm oil 200g/d; rice bran oil 200 g/d
LD = *Longgisimusdorsi*
SM = *Semimembranosus*
SEM = standard error of mean

CONCLUSION

This present study demonstrated that supplementation of rice bran oil rich in oleic acid decreased cooking loss in *Longgisimus dorsi* muscle. However, its supplementation did not affect growth performance. It can be clearly concluded that rice bran oil addition to fattening cattle's diets can decrease cooking loss without affecting growth performance.

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Cover page

Title: *The Place of Agriculture in Economic Growth*

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ABSTRACT

Agriculture - economic growth relationship is the subject of many studies, with different methodology, content and perspectives. In this context, the paper presents an econometric analysis of macroeconomic level whose aim is to measure the contribution of agriculture to economic growth equation and evaluating the causal relationship with industry. The econometric analysis undertaken, using OLS method (Ordinary Least Square or the method of least squares) and TSLS method (Two Stage Least Square or the method of least squares in two-stage). Contribution of agriculture is defined in terms of the agricultural GDP (gross domestic product) and the economic growth in terms of evolution of real GDP.

The results reveal that agriculture occupies a secondary place in economic growth. Regarding the links between agriculture and industry, they are in favor of industry. Why? Because, in the agricultural GDP equation, the industrial GDP coefficient has a negative sign (-), and in the industrial GDP equation, the agricultural GDP coefficient is positive (+). Therefore, the industrial sector has greater benefits from industry-agriculture relationship and by effect of agriculture-growth relationship.

Key words: agriculture, economic growth, gross domestic product, benefits

INTRODUCTION

The objective of the paper is the answer to the questions:

- What is the role of agriculture in economic growth?
- What is the causal relationship of agriculture – economic growth and agriculture - industry?

MATERIAL AND METHOD

This paper uses econometric analysis as research method. The econometric analysis undertaken, uses OLS method (Ordinary Least Square or the method of least

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squares) [8] and TSLS method (Two Stage Least Square or the method of least squares in two-stage) [8].

The used material for the calculation of indicators was taken from the National Statistics Institute of Romania (INS) [9] and field studies. The field studies were performed on the economy of Dorna Basin Region-Romania.

RESULTS AND DISCUSSION

Dorna Basin is located in northern Romania. It has an area of 222.19 km^2 representing 0.63% of the country surface. Within this zone are covered 9 communes with 49 villages and an urban center represented by Vatra Dornei City. This localities are administratively part of Suceava County. The economy is dominated by tourism, food industry, wood industry and agriculture. Agriculture is specific to mountain areas in Europe that is based on forage crops and livestock [5].

To achieve the work objective, the authors considered the region's economy as a sum of results that form the GDP equation [2]. The GDP was calculated on the total region and to simplify the econometric analysis, it was considered that GDP has only two incorporation sources, namely the agriculture and industry (by industry being understood all branches of the local economy, except agriculture).

Therefore, the sectoral economic performance in terms of GDP (y) were evaluated through the report that is created between capital (K) and labor (L).

$$Y_{j,i} = F(k_{j,i}, L_{j,i}) \quad (1)$$

j = agriculture

i = industry

Capital and labor were considered as a limited given (as a resource) defined by an opportunity cost [7]. The opportunity cost answer the question: Which cost of the possible costs multitude is optimal to perform? For example, the capital has a yield in agriculture and other in industry. The same for the labor.

Starting from these considerations, capital and labor were considered mobile across sectors. This can be seen by considering the total derivative of the equation [8]:

$$dy_j = \frac{\partial F}{\partial K_j} dk_j + \frac{\partial F}{\partial K_j} dl_j$$
$$\frac{dy_j}{y_j} = \frac{\partial F}{\partial k_j} \frac{K_j}{y_j} \frac{dk_j}{k_j} + \frac{\partial F}{\partial L_j} \frac{L_j}{y_j} \frac{dL_j}{L_j} \quad (2)$$

or, in terms of estimated parameters:

$$\dot{y}_j = \beta_k \dot{k}_j + \beta_l \dot{l}_j, \quad (3)$$

where:

$\dot{y}_j$, $\dot{k}_j$ și $\dot{l}_j$ are the growth rates of sector j output, respectively, the growth rates of capital and labor used in sector j.

B_s - is the input elasticity coefficients.

In order to highlight the links between agriculture and industry it were used the relationships:

-for the agricultural GDP,

$$\dot{y}_a = \beta_0 + \beta_k \dot{k}_a + \beta_l \dot{l}_a + \beta_i \dot{y}_i + \beta_t \dot{l}_t + e \quad (4)$$

where: coefficient β_0 is the constant term and the other β_s coefficients are the elasticities of variable to be estimated.

- for the industrial GDP

$$\dot{y}_a = \alpha_0 + \alpha_k \dot{k}_i + \alpha_l \dot{l}_i + \alpha_a \dot{y}_a + \alpha_t \dot{y}_t + e \quad (5)$$

where: α_0 is the constant term and α_s represent the elasticities of variable to be estimated.

The main estimated variables were:

- agricultural GDP (InGDPagr);
- industrial GDP (InGDPind);
- labor employed in agriculture (InLagr);
- labor employed in industry (InLind);
- gross capital formation in industry (InGCFind);
- gross capital formation in agriculture (InGCFagr).

According to methodological benchmarks, the analysis led to the following results:

InGDPagr = +0.301 InGCFind – 0.446 InLagr – 0.297InGDPind + 0.152 dummy + 4.289 C – 0.253 AR (6)

$$R^2 = 0.80 \quad (7)$$

$$DW = 1.13 \quad (8)$$

InGDPind = +0.135 InGDPagr + 0.158 InGCFind + 0.096 InLind + 0.016 dummy + C – 0.253 AR (9)

$$R^2 = 0.94 \quad (10)$$

$$DW = 1.02 \quad (11)$$

The estimates results made by TSLS (the method of least squares in two stages) for both agricultural GDP, as well as industrial GDP are shown in the table below:

TABLE I. ESTIMATES OBTAINED USING THE TSLS METHOD - 1

Agricultural GDP		Industrial GDP	
Capital	0.475	Capital	2.365
Labor employed in agriculture	-0.429	Labor employed in agriculture	1.847
Industrial GDP	-0.829	Industrial GDP	1.511
Free term	2.440	Free term	-6.476
Dummy	0.366	Dummy	0.744 (1.818)
R^2, DW	0.67; 1.96	R^2, DW	0.65; 2.10

Source: own calculations

According to the data in Table I, that presents the equation of agricultural GDP and industrial GDP into a systemic approach, we note that the sign of the coefficients for all considered variables are kept and generally, the results obtained using this procedure (TSLS method) confirmed those obtained using the OLS method.

Follows:

- The capital (+0.475 in agricultural GDP and 2.365 in industrial GDP) is the main factor of economic growth. The variable "labor" (-0.429 in agricultural GDP and +1.847 in industrial GDP) also has a positively influences on GDP growth, but at a slower rhythm. Hence, results the fact that labor relative to capital has a secondary role in economic growth (capital determines investment, investment leads to technical progress, technical progress determines competitiveness and economic growth);
- The variable labor employed in agriculture has a negative sign in each estimated equation, which means that an increase of employment in agriculture is no longer justified. Moreover, agriculture's GDP growth is inversely related to employment growth (-) and directly related to capital increase (+).

Analyzing the impact of agricultural GDP and industrial GDP on total GDP growth, using the methodology described above, resulted the following (tab. II):

- The sign of the coefficient is positive for the industry and negative for agriculture;
- Total GDP is positively influenced both industry and agriculture;
- The industrial GDP growth influence the total GDP growth at a rate higher than the increase of agricultural GDP (the ratio is 0.755 / 0.198);
- The relative importance of agriculture decreases as the total GDP increase;
- The relative importance of industry increases as the total GDP increase.

TABLE II. ESTIMATES OBTAINED USING THE TSLS METHOD - 2

Total GDP	
Industrial GDP	0.755
Agricultural GDP	0.198
Free term	0.047
R^2, DW	0.85; 1.84

Source: own calculations

The results showed that the industrial sector benefit more from relationship industry - agriculture.

Agricultural development through investments in capital (capital is the main engine of economic growth) increase the demand of agriculture for industrial goods and by effect, the industry develops faster.

Thus, agricultural growth deserves priority positions since the growth in this sector help industry to grow further. However, capital investment in agriculture frees farm labor that often is not absorbed by the economy and rural poverty worse. Thus, by encouraging industrial development through the improving of agriculture, the position of rural poverty may increase.

This fact indicates that agricultural development should not be curbed in favor of concentrating resources for industrial development and must be found an optimal balance between industry and agriculture, so the rural poverty to be alleviated and the economy to register a sustainable growth.

Basically, the results obtained in this paper represent information that substantiates the investments guidance policies in economy and that fight against underdevelopment. Thus, defining objectives and strategies of Dorna Basin Region development should focus on [5]:

I. Increasing of income and improving of life quality by promoting diversification of economic activities, namely by stimulating and promoting investment in the area and development of capital markets (cheaper credit).

II. Transforming agriculture in a efficient and diverse activity by stimulating the investment in the field.

III. Attracting and retaining the young people in rural areas, to reduce the average age of the rural population, to ensure optimal human resources in rural areas.

IV. Developing human potential through continuous training, support and involvement of local people in the process of economic development.

V. Supporting the development of technical and social infrastructure through investment and "disability compensation".

VI. Providing alternative income through the efficient use of local resources and stimulating tourism activities and crafts.

CONCLUSIONS

1. Dorna Basin is located in northern Romania. It has an area of 222.19 km^2 representing 0.63% of the country surface. Within this zone are covered 9 communes with 49 villages and an urban center represented by Vatra Dornei City. This localities are administratively part of Suceava County.

2. The capital (+0.475 in agricultural GDP and 2.365 in industrial GDP) is the main factor of economic growth. The variable "labor" (-0.429 in agricultural GDP and +1.847 in industrial GDP) also has a positively influences on GDP growth, but at a slower rhythm.

3. Results showed that the industrial sector benefit more from relationship industry - agriculture. The agricultural growth deserves priority positions since the growth in this sector helps industrial and service sector to grow faster (the multiplier effect is higher from agriculture to industry and services, and not vice versa).

4. Based on the game of economic growth determinants in Dorna Basin Region were formulated measures that may underlie the development.

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Cover page

Title: *Effect of Tidal Operation on Pilot Scale Horizontal Subsurface Flow Constructed Wetland for the Treatment Groundwater Contaminated by Monochlorobenzene*

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ABSTRACT

Three different flow regimes were undertake in a pilot scale horizontal subsurface flow constructed wetland (planted with *Phragmites australis*) treating groundwater contaminated with MCB (monochlorobenzene). The three regimes were continuous flow, 7 days cycle discontinuous flow and 2.5 days cycle discontinuous flow. The results shows intensify tidal regime (2.5 days cycle) enhanced MCB removal significantly before 2 m from inlet. The increase of oxygen concentration in pore water through intensify tidal operation gives the explaination of better MCB removal performance. Thus, intensify tidal operation was suitable for increasing MCB removal.

1. INTRODUCTION

Constructed wetlands (CWs) have been set up as an alternative to the conventional intensive treatment systems due to their low external energy requirements, easy operation and maintenance [1]. They have been widely used in various types of wastewater treatment [2]. In generally, conventional CWs are affected by poor wastewater distribution on the wetland surface and a low oxygen transfer rate [3], which restrict the performance of treatment wetlands. With this intention, tidal flow operation strategy was developed. It refers to an operation that repeatedly allows wetland matrices to be filled with wastewater, and then to be completely drained. A maximum pollutant biofilm contact will be established while the matrices are filled. As the wastewater drains, air is drawn from the atmosphere into the matrices, to reinforce the biofilms with oxygen. During cycle of ‘wet’ and ‘dry’ periods, the wastewater acts as a passive pump to repel and draw oxygen into the wetlands. Thus, the tidal flow operation has the potential of improving treatment efficiency through enhanced aerobic microbial decomposition and pollutant biofilm contact [4].

Chlorinated solvents are among the most common pollutants of groundwater. monochlorobenzene (MCB) is one of the low chlorinated hydrocarbons found in contaminated groundwater systems. It can be biodegraded by indigenous microbial degradation mechanisms. Aerobic MCB degradation has been well studied and found

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to be efficient [5]. MCB is generally regarded as persistent in anaerobic environments. However, isotopic data revealed that the incorporation of ^{13}C from labelled MCB into long chain fatty acids of bacteria is causing an *in situ* MCB biodegradation to occur in an anoxic aquifer [6].

In the present study, an investigation covering three growing seasons (from April 2009 to December 2011) was carried out in a pilot-scale HSSF CWs to treat groundwater contaminated with MCB. The system was run under there different phases: phase I: continuous flow during 20th April, 2009 to 20th July, 2010; phase II: 7 days cycle from 21st July, 2010 to 12th April, 2011; phase III: 2.5 days cycle during 13th April, 2011 to 6th December, 2011. The objective of this study was to (1) compare the effect of different tidal regime on the removal of low chlorinated hydrocarbons; (2) test the function of different tidal regime for the control of sulfide problems in HSSF CWs.

2. MATERIALS AND METHODS

2.1. Setup of the Experimental Pilot Scale Constructed Wetlands

The HSSF CW was established in March 2003 in Bitterfeld, Germany. The segment consisted of a container with 6 m length, 1 m width and 0.6 m height. The HSSF CW was filled with the local aquifer material to a height of 0.5 m. The filling material consists of gravel (25-47%), sand (50-67%) and silt/clay (2-8%) with a hydraulic conductivity (k_f value) of 2.1×10^{-3} m/s and porosity of 0.28. The HSSF CW was planted with common reed (*Phragmites australis*), and were continuously supplied with contaminated groundwater at a flow rate of 5.0 L/h. Water levels were adjusted at 0.4 m by a float valve in an open water compartment after a flow path of 5.5 m. The outflow water volumes pumped off were recorded by flow meters continuously. The main pollutant concentrations in the inflow are listed in Table 1.

TABLE 1. CONCENTRATIONS OF MAIN POLLUTANTS IN THE INFLUENT (2009 – 2011, N=45)

	Mean	standard deviation
PCE (mg/L)	1.9	0.4
MCB (mg/L)	7.8	2.5
2-Chlorotoluene (mg/L)	0.03	0.01
1,2-DCB (mg/L)	0.03	0.01
1,4-DCB (mg/L)	0.29	0.05
NH_4^+ (mg/L)	53	11
NO_3^- (mg/L)	1.2	0.9
Fe^{2+} (mg/L)	0.5	0.2
SO_4^{2-} (mg/L)	863	54
S^{2-} (mg/L)	0.5	0.6
Cl^- (mg/L)	320	89
PO_4^{3-} (mg/L)	4.3	2.5
Eh (mV)	50	87
pH	6.6	0.1
Electrical conductivity (mS/cm)	3.1	0.3

With the intention to increase MCB removal efficiency in the HSSF CW, two tidal operation regimes were done. The first regime is 7 days cycle regime, which is 2 hours of fast outflow flushing, by this the water level decreased fast from 40 cm to 15 cm; The subsequent refilling (5.0 L/h) again to the water level of 40 cm lasted about 34 hours; then followed the resting phase of 132 hours until a new cycle started. The second regime has the resting phase of 24 hours, which was used for taking samples at the resting phase. Therefore, the system can be divided into three phases. Phase I: continuous flow during 20th April, 2009 to 20th July, 2010; phase II: 7 days cycle from 21st July, 2010 to 12th April, 2011; phase III: 2.5 days cycle during 13th April, 2011 to 6th December, 2011.

2.2 Sampling and Analysis

In total, 20, 9, and 16 sampling actions were done during phase I, II, and III, respectively. Inflow water samples were taken directly before the inflow stream which enters the CWs; pore water samples in the HSSF CW with discontinuous outflow flushing were taken at the end of the resting phase (132 hours or 24 hours) prior to the next fast outflow flushing (of only about 2 hours).

The pore water samples were pumped out through a lance by a peristaltic pump with a sampling rate of 78 mL/min. The dissolved oxygen was measured in the field in a flow through mode using an optical oxygen trace sensor system (polymer optical fiber, sensor spot FTC-PSt6 and instrument FIBOX-3-trace, PreSens) when taking the samples. For organic analysis, 10 mL water samples were collected in 20 mL glass flasks, sodium azide solution was added into the samples to inhibit microbial activity, and sealed with Teflon-lined septa. The concentrations of MCB was measured by headspace gas chromatography which has been described elsewhere [7].

2.3 Data Analysis

The contaminant load rates were calculated based on water volume flow rates and contaminants concentrations. The decrease of organic load along flow path was calculated assuming that the water loss followed a linear change. The area specific load was calculated after 4 m from inlet with surface area of 4 m^2.

T-test was performed based on the loads to compare the treatment performance between three different phases. Non-parametric Mann-Whitney U-tests were carried out when normal distribution could not be assumed according to one sample Kolmogorov-Smirnov tests [8]. All the tests were done by SigmaPlot 11.0 programme, and the differences were regarded as significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Tidal Effect on MCB Removal

In general, the MCB removal load decreased with the decrease of inflow load (Fig. 1). Both tidal regimes (7 days and 2.5 days cycle tidal) shows insignificant difference on MCB load removal efficiency after 4 m compare with the continuous flow (Fig. 1). However, intensify tidal regime 2.5 days cycle regime enhanced the MCB removal significantly before 1 m compare with continuous flow and before 2 m compare with 7

days cycle tidal regime ($p<0.05$). Anyhow, both tidal regimes didn't increase the MCB removal efficiency significantly after 3 m ($p>0.05$), but the 2.5 days cycle tidal regime seems to increase MCB removal efficiency after 3 m compare to other two flow models. These proof that intensify tidal flow do have benefits for the removal of MCB. The improve of MCB removal only happened before 1 or 2 m from the inlet gives a hint that oxygen limitation in the first part of the system.

The vertical profile shows that better MCB removal performance was reached in the upper layer (30 cm) than in the deeper layer (50 cm) (Fig. 1). Significant difference of MCB concentrations between two depths was found under continuous flow after 2 m from the inlet ($p<0.05$). MCB concentration difference between two depths was found even at 0.5 m when the system under 2.5 days tidal regime ($p<0.001$). The 2.5 days cycle tidal regime do increases the MCB removal efficiency (mean of depths), but it especially increase MCB removal in the deeper layer (50 cm) (Fig. 1). This might be explained by the microbial adaption, which means more oxic prefer microorganism were established by the more frequent tidal regime, in particular in the deeper layer (50 cm).

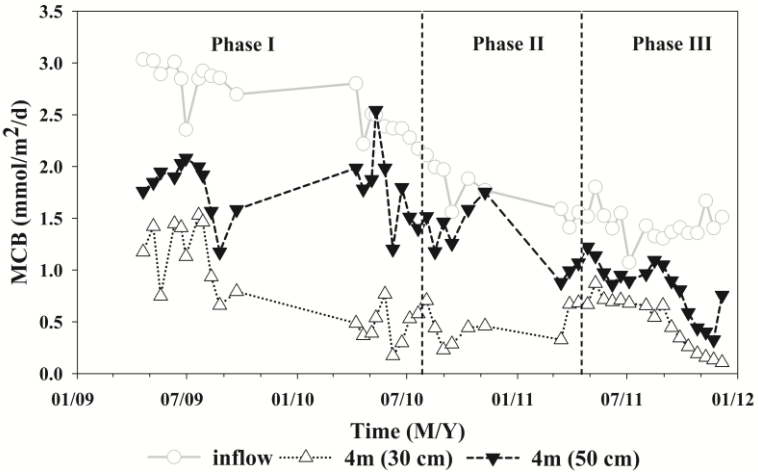

Figure 1. Inflow loads and removed loads of MCB after 4 m (30cm and 50 cm in depth) during the investigation period (2009-2011)

3.2 Oxygen Condition

In general, HSSF CW is an oxygen limited system. The oxygen concentration in pore water was less than 0.2 mg/L under the normal continuous flow model (Fig. 2). However, with the operation of 7 days cycle regime discontinuous flow, the oxygen concentration in pore water increased to 0.97 mg/L at 0.5 m in the upper layer (Fig. 2). Furthermore, higher oxygen concentration was found in pore water both at 30 cm (2.1 mg/L) and 50 cm (0.5 mg/L) under the operation of 2.5 days cycle regime discontinuous flow (Fig. 2). These confirms that more frequent tidal procedure do increase the oxygen input in the HSSF CW. With the better oxygen condition achieved by 2.5 days cycle regime tidal operation, the removal efficiency of MCB was enhanced significantly, especially before 1 m, where higher oxygen concentration was observed before 1 m. Meanwhile, the increased oxygen concentration at 50 cm also gives an explaination for the better MCB removal at 50 cm (Fig. 1).

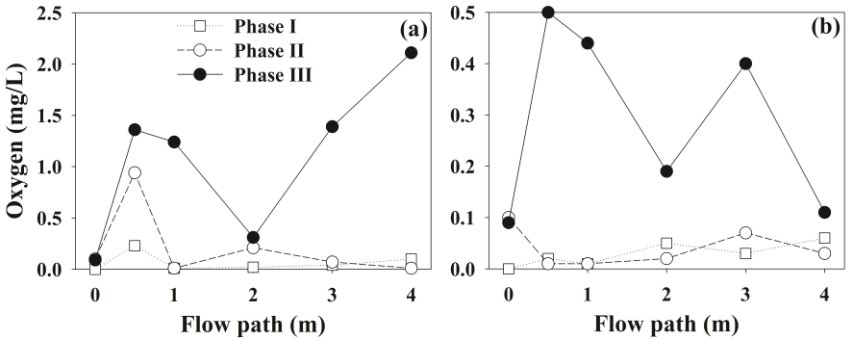

Figure 2. Oxygen concentrations in pore water at two depths (a: 30 cm, b: 50 cm) along the flow path at the three different phases

4. CONCLUSIONS

Intensify tidal operation (2.5 days cycle regime) increase the oxygen concentration in pore water, thus, it enhanced MCB removal efficiency in the HSSF CW significantly before 2 m from inlet. Therefore, the intensive tidal operation is a useful option to increase the removal efficiency of chlorinated hydrocarbons in the HSSF CW.

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Cover page

Title: *Study on the Maturity Modeling of Seed Industry Systems*

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ABSTRACT

Taking advantage of the idea of capability maturity model(CMM), this paper elaborates the maturity of seed industry systems that is composed of five levels, builds assessment systems of seed industry development status, puts forward thinking framework and process approach of maturity evaluation of seed industry systems, and sets forth the algorithm model of comprehensive assessment. This assessment research of seed industry systems based on CMM is expected to have significance in theory and practice to the construction of new-style seed industry systems in China.

INTRODUCTION

As agricultural lifeblood and the strategic and fundamental core industry in China, seed industry plays a crucial role in the development of agricultural industry. However, compared with other foreign countries, currently the development status of seed industry in China is still in primary stage [1]. According to related studies, the commercialization rate of seed industry research achievements in China is only 30%-40%, greatly lagging behind developed countries with the rate reaching to 70%-80% [2].

Seed industry systems in China could not meet the requirements of modern agricultural development, thus making it urgent to establish new-style systems. In order to achieve the strategic goal, we must deeply explore each important parts including top-level design, general planning, route map and schedule time-table, of which an indispensable basic task is the assessment of seed industry system that is worthy of being given enough attention and deep research whether from theoretical or practical perspective. The existing research papers lack the research about assessment systems of seed industry development status, which is explored in this paper based on the Capability Maturity Model [3,4].

CMM originated from the research about standardized production of software products in the middle of 1980s. Software Engineering Institute of Carnegie Mellon University (CMU-SEI) published an influential CMM for software development capabilities [3]. Kerzner (2001) put forward Project Management Capability Maturity Model (K-PMMM), which closely links maturity assessment of project management

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with strategic management [4]. In addition to involving nine knowledge systems in project management, it also takes key factors in enterprises and institutions into consideration, thus making it applicable in many industries. However, to our knowledge, there is no related research of CMM in the field of agricultural seed industry.

Taking advantage of the idea of capability maturity model, this paper builds a hierarchy structure index system of seed industry system assessment and provides a thinking framework and process approach of maturity evaluation of seed industry systems. CMM-based seed industry system assessment will promote the completion and operation of Chinese commercialized breeding system, of great significance to the enhancement of seed industry scientific and technical innovation capabilities and national seed industry's international competiveness.

MATURITY MODELING FRAMEWORK OF SEED INDUSTRY SYSTEMS

General View of Maturity Level of Seed Industry Systems

On the basis of the characteristics of seed industry, seed industry system maturity level is divided into five levels (see Figure 1). The advance of seed industry system maturity level is from building industry environment & infrastructure to the direction of improving technological management system.

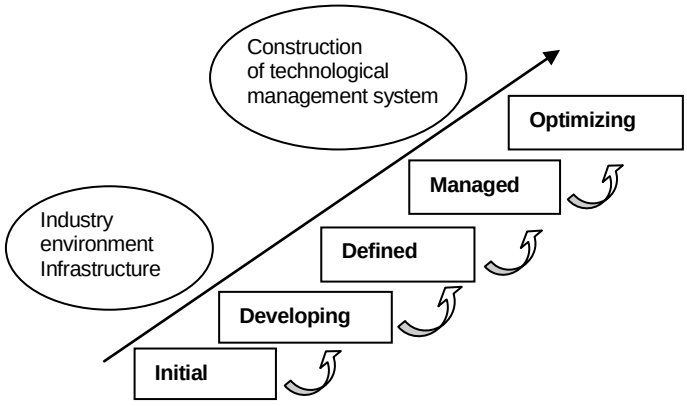

Figure1. General view of the maturity level of seed industry system

Interpretation of Maturity Level of Seed Industry Systems

MATURITY LEVEL 1: INITIAL CAPABILITY

Government administrative intervention usually happened and the industry is lack of supervision. Both seed and breed management suffered low efficiency. Transformation rate of scientific achievements is low. There is lack of effective cooperation among the main body of the industrial chain, as the research institutes and seed enterprises are often working separately. This situation also happened in university-industry cooperation. The market concentration degree is low and there is lack of sustainable innovative seed enterprises with international competitiveness.

MATURITY LEVEL 2: DEVELOPING CAPABILITY

Government pays more attention to building better policy environment and realizes the importance of the marketization and industrialization. Enterprises established a integrate value chain and the market concentration get continually improved. The process of seed production and quality control is more regular and powerful, marketization of varieties promotion continues to accelerate, there for, transformation rate of scientific achievements get improved. A new seed industry system is coming into being.

MATURITY LEVEL 3: DEFINED CAPABILITY

Seed Industry operate stable. Each participant has clear property rights. Fundamental breeding and commercial breeding are rational separated, university return to fundamental breeding research and commercial breeding work is responsible by the enterprises with an integrate supply chain. Germplasm resources gain more attention; marketilization of varieties promotion get improved and transformation rate show a significant increase. A new seed industry system has already formed.

MATURITY LEVEL 4: MANAGED CAPABILITY

Information within the system get fully exchanged; resources be used efficiently; transformation rate remains a high level; each participant of the industry such as government, enterprises and institutions can position themselves clearly and cooperate closely. Management services of government is institutional, regular and standardized; germplasm resources are well protected and get better exploitation; universities and research institutes have developed high level skills of fundamental breeding. Seed enterprises take the leading position in innovation and they are actively involved in commercial breeding research and development. A new seed industry system has continually developed and improved.

MATURITY LEVEL 5: OPTIMIZING OR INTEGRATED CAPABILITY

Seed System operates in a stable and efficient way; main participants perform their duties well. The process of building policy environment and management services system is going well. Market concentration is high; service capability of industry associations is strong; investment in seed breeding remains a high level; seed enterprises take the leading position in innovation. Fundamental breeding promoted by the government and commercial breeding drove by the market; transformation rate is high. The innovation not only brings new air to the industry but also promotes the optimal allocation of resources; seed industry system constantly improved along with the environment change. New innovative organizations push the new scientific and technical innovation seed industry systems to improve and optimize.

HIERARCHICAL STRUCTURE OF MATURITY ASSESSMENT OF SEED INDUSTRY SYSTEMS

Referencing to the maturity modeling in [5] and combining with the characteristics of seed industry, based on the research work of our research group we

build a framework of seed industry system maturity evaluating indicator, as shown in Table I.

Seed Industry system is divided into three subsystems: the first is category management system; the second is seed management system and the third is Participants system[6]. Each sub- system includes a number of key areas, such as category management system includes “variety certification and protection”, “seed variety promotion” two key areas; seed management system includes "seed breeding ", "seed trade" two key areas; Participants system includes “government”, “seed enterprises and association”, “institutions and university”, “relationship between value chain of the industry” four key areas. Each area is composed of several key variables. System maturity evaluation methods are described at next section.

TABLE I. HIERARCHICAL STRUCTURE OF MATURITY ASSESSMENT

Leverage domains	Key domain areas	Critical variables
Breed management system	Variety certification and protection	The efficiency of variety certification (0.35)
		The efficiency of certification process(0.35)
		Protection and utilization of germplasm resources（0.30）
	Breed variety promotion	The degree of market-driven approach(0.2)
		Extent of developing marketization management(0.3)
		Transformation Rates of the achievement in seed industry(0.5)
Seed management system	Seed breeding	Degree of market orientation breeding(0.4)
		Standardization of seed production process(0.3)
		Extent of building quality certification system(0.3)
	Seed trade	Degree of market openness(0.5)
		Regulation of market rules(0.25)
		Standardizing trade regulation(0.25)
Behavioral agents system	Government	Administrative management standardizing level(0.3)
		Development of the industry investment rules(0.3)
		Degree of building policy environment (0.4)
	Seed Enterprises and Association	Market concentration of seed enterprises(0.2)
		Seed enterprises as the prime of innovation(0.3)
		Seed breeding investment level(0.3)
		Service providing capability of industry association(0.2)
	Institutions and university	Self positioning of institutions(0.5)
		R&D level of fundamental seed breeding(0.5)
	Cooperation of agents at links of industrial chain	Compactness between the industry chain(0.4)
		Cooperative effect among Policy, Manufacturing, Education, Research & Application(0.6)

PROCESS APPROACH OF MATURITY ASSESSMENT OF SEED INDUSTRY SYSTEMS

Maturity Evaluation of Critical Variables

There are many ways such as industry statistics, literature research and interviews with experts to collect the quantitative data related to the critical variables. Considering the leading level both here and abroad and also the opinions of experts, reprocess the data to make sure their value is between 0 and 1. Then, define the level

of the key variables by their value. As we all know, the process has subjectivity, so according to the inherent law of decision making, we can also take the Pareto Law and Golden Section Method as reference when defining the level of key variables, refer to the table II.

Table II. DIVISION OF MATURITY LEVEL OF CRITICAL VARIABLES

Level 1	Level 2	Level 3	Level 4	Level 5
[0, 0.2)	[0.2, 0.382)	[0.382, 0.618)	[0.618, 0.8)	[0.8, 1.0)

Maturity Evaluation of Key Domain Areas

Key variables have weighed which relative to key areas, which is mean that for the same key area all key variables' weight sum to 1. According to the relative importance of variables or through experts' opinions, the weight of key variables defined. The weight of key variables in this study is shown in Table I. Once the level of the key variables is determined, the score of the key areas can be easily calculated.

Comprehensive Assessment of Seed Industry Systems Maturity

The maturity level of the whole seed industry system can be inferred from the maturity level of all key variables. Each key area is very important, and plays a significant role in different stages of development. There for, the simple calculate method is no longer appropriate. Referencing to [5], there is no need to give key areas weight. The comprehensive assessment algorithm of seed industry maturity level is shown in Table III.

Table III. COMPREHENSIVE ALGORITHM OF SYSTEMS MATURITY

Leverage Domains	**Key domain areas**	**Maturity level**				
		Initial	**Developing**	**Defined**	**Managed**	**Optimizing**
Breed management system	Breed certification and protection			2	3	4
	Breed variety promotion		2	3	4	5
Seed management system	Seed breeding		2	3	4	5
	Seed trade			2	3	4
Behavioral agents system	Government		2	3	4	5
	Seed Enterprises and Association		2	3	4	5
	Institutions and university			3	3	4
	Cooperation of agents at links of industrial chain				3	4

The comprehensive assessment algorithm given in Table III is explained as follows. If the seed industry system cannot meet the requirements of initial level, it will be regarded as "initial". If the maturity level of the main key areas "seed variety promotion", "seed breeding", "government" and "seed enterprises and association" reach to 2, the industry system will be regarded as "repeatable". When the maturity level of the main key areas "seed variety promotion", "seed breeding", "government", "seed enterprises and association" and "institutions and university" reach to 3, "variety certification and protection" and "seed trade" reach to 2 or above, the industry system will be regarded as "defined". When the maturity level of the main key areas "seed

variety promotion", "seed breeding", "government" and "seed enterprises and association" reach to 4, "institutions and university" "variety certification and protection", "seed trade" and "relationship between value chain of the industry" reach to 3 or above, the industry system will be regarded as "managed". When the maturity level of the main key areas "seed variety promotion", "seed breeding", "government" and "seed enterprises and association" reach to 5, "institutions and university" "variety certification and protection", "seed trade" and "relationship between value chain of the industry" reach to 4 or above, the industry system will be regarded as "optimizing". Different maturity level may have different main key areas. After the maturity level of the seed industry system has been defined, the score of the current level is equal to the lowest one of former level.

CONCLUSIONS

This paper explores the situation assessment of seed industry systems development by taking advantage of the idea of capability maturity model (CMM). The maturity level of seed industry systems is divided into five levels: Initial Capability, Developing Capability, Defined Capability, Managed Capability, and Optimizing or integrated Capability. The hierarchical structure of maturity assessment of seed industry systems is composed of leverage domains, key domain areas, and critical variables, hence constitute an evaluation index system. To assess the maturity level of seed industry systems, the maturity of critical variables should be evaluated at first, then the maturity of key domain areas, thus the maturity level of seed industry systems can be determined with the maturity results of key domain areas by using of the algorithm model of comprehensive assessment. This assessment research of seed industry systems based on CMM is expected to have significance in theory and practice to the construction of new-style seed industry systems in China.

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Cover page

Title: *Biofloc Technique, Applicable to Zero-Water Exchange and Intensive Culture Systems for the Shrimp Litopenaeus Vannamei*

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ABSTRACT

The massive effluent discharge from shrimp *Litopenaeus vannamei* culture systems has limited its sustainable development. Biofloc technique is demonstrated to be effective for limited and zero-water exchange culture. We compared the variation of biofloc in *L. vannamei* culture systems under different carbohydrate addition percentages, and systems with different bottom substrates of oyster shell powder, sugarcane bagasse and their mix. The variation of dissolved organic carbon and total organic carbon were also examined. Our results showed that 75% of the theoretical quantity was the best among carbohydrate addition treatments for biofloc production. Compared to carbohydrate addition, the improvement of bottom substrates was more beneficial to biofloc production. Additionally, biofloc contributed mainly to the total carbon whereas not dissolved organic carbon. We first indicate that bottom substrate improvement is effective for biofloc production.

INTRODUCTION

The culture of the shrimp *Litopenaeus vannamei* is the major aquaculture industry in China, however the discharge of nutrient-rich effluent from culture ponds has limited its sustainable development due to the severe impact on the environment [1]. Studies have demonstrated that the utilization of bio-floc technique (BFT) can culture *L. vannamei* intensively under limited or zero-water exchange and realize more responsible and sustainable aquaculture [2-5]. Through using BFT high density of flocculated particles can be produced in the culture water column that are rich in bacteria and phytoplankton and beneficial to absorb excess nutrients from the water

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and thus maintain high water quality [2, 3, 5]. These particles are also the potential food source for shrimp and can increase the feed conversion ratio [4].

Our study showed that adding carbohydrate source into the culture system can realize *L. vannamei* intensive culture without water change as well as the water quality and shrimp growth performance can be improved compared to the control without carbohydrate addition [1]. Our results are consistent with the previous studies [2-4], whereas 110-day culture period and super intensive shrimp densities fully demonstrate the applicability of BFT to *L. vannamei* zero-water exchange systems. In addition to t-he investigation of carbohydrate addition effects to the water column [1], we also focused on the improvement of bottom substrates of *L. vannamei* culture system by using oyster shell powder (OSP) and sugarcane bagasse (SB) [6]. These two materials are demonstrated to be effective for the improvement of bottom substrate of shrimp culture system. However, knowledge about the biofloc variation under different carbohydrate addition and bottom substrates are limited. In this study, we compared the biofloc variation together with the dissolved and total organic carbon.

MATERIALS AND METHODS

Experimental Design

The experiment of the carbohydrate addition was conducted according to Gao [1]. Briefly, sucrose was added as the carbohydrate into *L. vannamei* culture tanks at five levels, i.e., 0, 25%, 50%, 75% and 100% of the theoretical quantity of carbohydrate addition, which were expressed as Control, S25%, S50%, S75% and S100%, respectively. The theoretical adding quantity of sucrose was calculated based on the formula proposed by Avnimelech [3]. The experiment of the improvement of bottom substrates was conducted according to Shan [6]. Oyster shell powder (OSP), sugarcane bagasse (SB) and their mix were added on the surface of bottom substrate.

Determination of Experimental Parameters

Biofloc was evaluated using the concentration of particulate organic carbon (POC), which was followed by Gao [1]. Dissolved organic carbon was determined by an element analyzer, whereas the total organic carbon (TOC) was referred to as the plus of POC and DOC.

RESULTS

1. Variation of Biofloc in Carbohydrate Addition Systems

During the culture period the variation of biofloc, which was expressed as POC, presented an increasing trend in all the treatments (Fig. 1). In the late period (from 70th day), biofloc concentrations in the treatments with sucrose addition were above 30 mg/L and significantly higher than that of the control without sucrose addition. Additionally, biofloc concentration of 75% treatment among the sucrose addition ones was the highest among all the treatments in the end of the culture (Fig. 1).

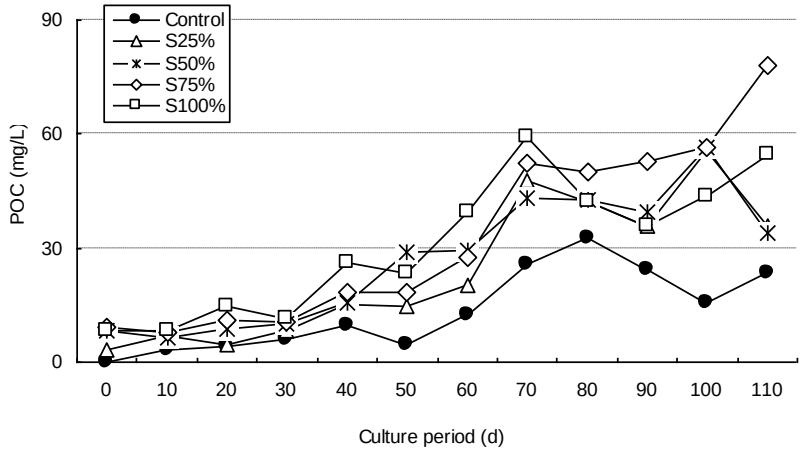

Fig. 1 The variation of particulate organic carbon (POC) in the water column during the culture period. Control indicated the culture system without sucrose addition. S25%, S50%, S75% and S100% indicate the treatments with 25%, 50%, 75% and 100% of the theoretical quantity of carbohydrate addition, respectively.

2. Variation of DOC and TOC in Carbohydrate Addition Systems

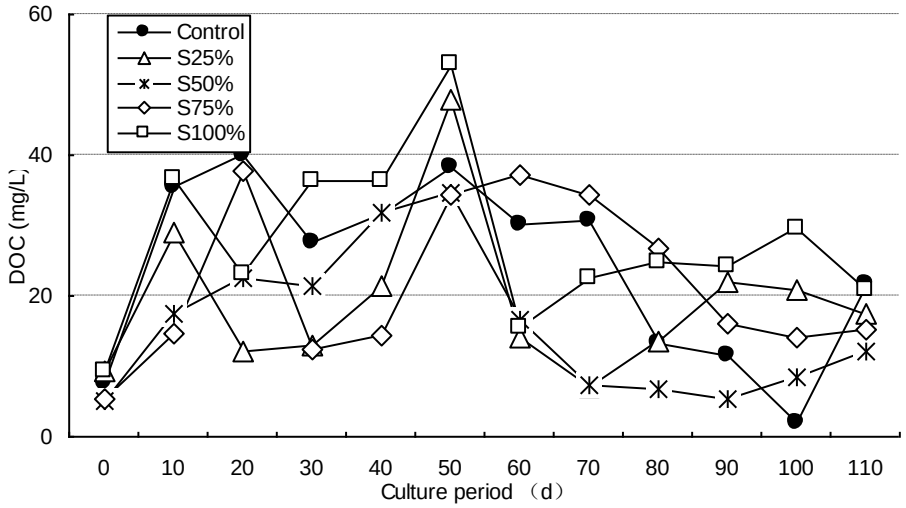

Fig. 2 The variation of dissolve organic carbon (DOC) in the water column during the culture period. Control indicated the culture system without sucrose addition. S25%, S50%, S75% and S100% indicate the treatments with 25%, 50%, 75% and 100% of the theoretical quantity of carbohydrate addition, respectively.

During the culture period, the concentrations of DOC were mostly below 40 mg/L and no obvious differences were found between the treatments of sucrose addition and the control (Fig. 2). Also, no differences were examined among the treatments of different percentages of sucrose addition. For the TOC concentrations, the variation of sucrose addition treatments showed the obvious increasing trend during the culture period and mostly above 60 mg/L for S75% (Fig. 3). Compared to this, no increasing trend was found in the control and TOC concentrations were below 60 mg/L (Fig. 3).

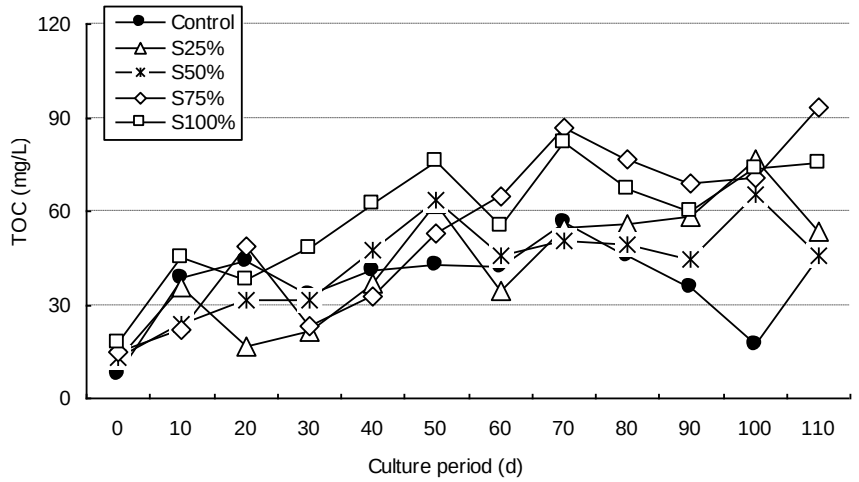

Fig. 3 The variation of total organic carbon (TOC) in the water column during the culture period. Control indicated the culture system without sucrose addition. S25%, S50%, S75% and S100% indicate the treatments with 25%, 50%, 75% and 100% of the theoretical quantity of carbohydrate addition, respectively.

3. Variation of Biofloc in Bottom-Substrate Improved Systems

Biofloc, which was expressed as POC, presented an obvious increasing trend in the treatments with improved bottom-substrate and their concentrations were mostly above 30 mg/L (Fig. 4). In contrast, no obvious increasing trend was found in the control (fresh soil as bottom substrate) and the concentrations were below 30 mg/L. From the mid-late culture period, biofloc concentrations in improved bottom-substrate treatments were significantly higher than those of the control. Additionally, the bottom substrate of BS produced relatively higher biofloc concentrations.

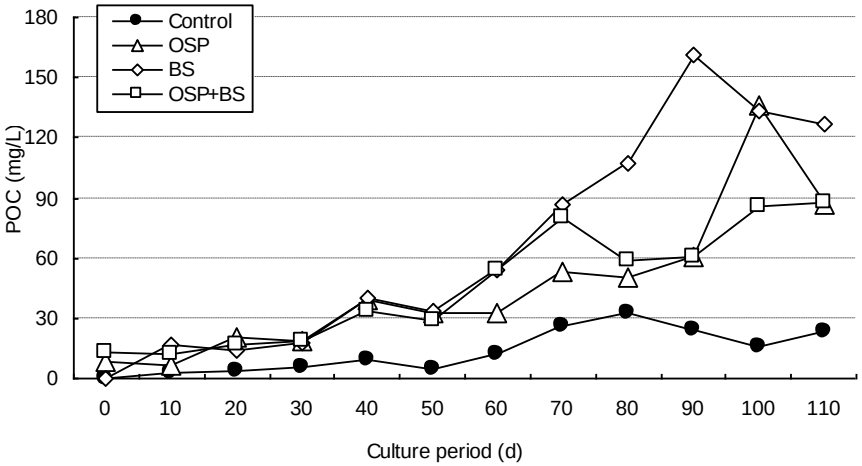

Fig. 4 The variation of particulate organic carbon (POC) in the water column during the culture period. Control indicated the culture system with the bottom substrate of fresh soil. OSP, BS and OSP+BS indicated the culture systems with the bottom substrates of oyster-shell powder, sugarcane bagasse, and the mix of oyster-shell powder and sugarcane bagasse, respectively.

4. Variation of DOC and TOC in Bottom-Substrate Improved Systems

The concentrations of DOC among all the treatments did not show obvious differences and were mostly below 40 mg/L during the whole culture period (Fig. 5). The concentrations of TOC presented the similar trend as those of POC (Fig. 6), i.e., TOC presented an obvious increasing trend in the treatments with improved bottom-substrate and their concentrations were mostly above 60 mg/L. In contrast, no obvious increasing trend was found in the control and the concentrations were below 60 mg/L. Among the TOC concentrations in OSP, BS and OSP+BS, those in BS were relatively higher in the late culture period, indicating BS was beneficial to produce biofloc (Fig. 6).

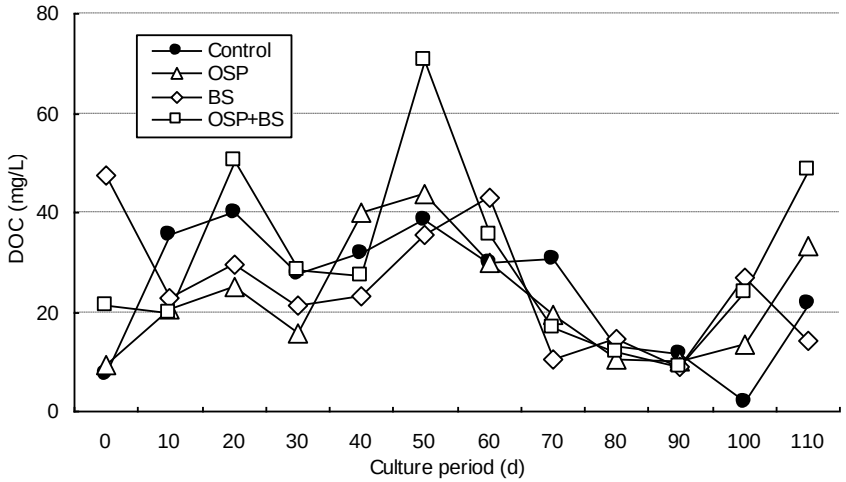

Fig. 5 The variation of dissolved organic carbon (DOC) in the water column during the culture period. Control indicated the culture system with the bottom substrate of fresh soil. OSP, BS and OSP+BS indicated the culture systems with the bottom substrates of oyster-shell powder, sugarcane bagasse, and the mix of oyster-shell powder and sugarcane bagasse, respectively.

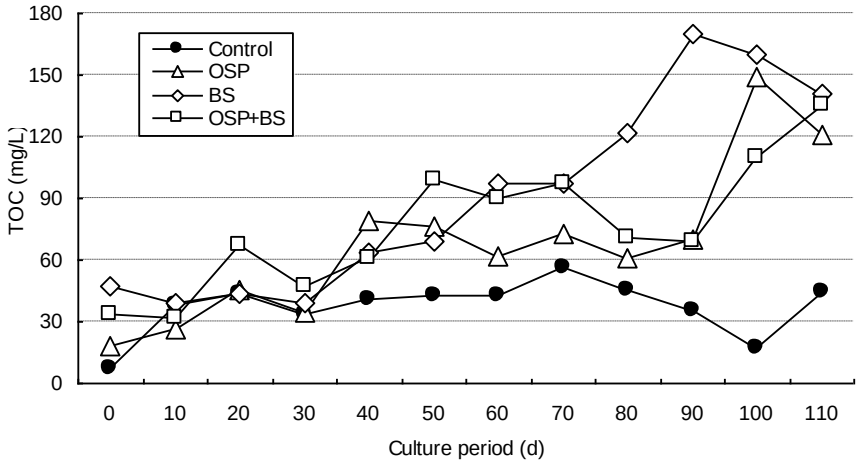

Fig. 6 The variation of total organic carbon (TOC) in the water column during the culture period. Control indicated the culture system with the bottom substrate of fresh soil. OSP, BS and OSP+BS indicated the culture systems with the bottom substrates of oyster-shell powder, sugarcane bagasse, and the mix of oyster-shell powder and sugarcane bagasse, respectively.

CONCLUSIONS

To limit the water exchange for shrimp culture systems, studies have investigated water treatment effects of many techniques of which water-recirculating and bio-floc techniques are focused on [1-5, 7, 8]. Compare to water-recirculating technique, more and more studies in recent years have demonstrated that biofloc technique under carbohydrate addition is beneficial to intensive and zero-water exchange system of *L. vannamei* culture [1-5]. Our previous study also demonstrated that bottom-substrate improvement is beneficial to improve water quality and shrimp growth performance [6]. In this study, we found that 75% of the theoretical quantity was the best among carbohydrate addition treatments for biofloc production. Compared to carbohydrate addition, the improvement of bottom substrates was more beneficial to biofloc production. Additionally, biofloc contributed mainly to the total carbon whereas not dissolved organic carbon. We first indicate that bottom substrate improvement is effective for biofloc production. This study was supported by National Undergraduate Training Programs for Innovation and Entrepreneurship of China (No. 201211117033), Jiangsu Aquaculture Innovation Project (Y2013-40) and National "Twelfth Five-Year" Plan for Science & Technology Support of China (No. 2011BAD13B03).

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Title: *Productive Performance and Egg Quality of Laying Hens Kept under Different Rearing Systems*

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ABSTRACT

An experiment was carried out to determine the effects of different rearing systems on the productive performance and egg quality of laying hens. A total of 276 hens, 30-wk-old ISA Brown laying hens were randomly allocated into 3 treatments: conventional battery cage, floor pen and free-range system. In conventional battery cage, birds were reared with 4 birds per cage (0.046 m^2 per bird). In floor pen, birds were housed with 5 birds per m^2 (0.2 m^2 per bird). In free-range system, birds were housed in a similar floor pen; in addition, they also had an outdoor grass paddock (2.0 m^2 per bird). All birds were provided with the same diet during the experimental period. The results showed that the different rearing systems did not affect to egg production, egg weight and FCR. However, the yolk weight of laying hens kept in floor pen and free-range system was lower than laying hens kept in conventional battery cage.

Key words: rearing system, productive performance, egg quality, laying hens

INTRODUCTION

The egg industry still focuses on production volume, lead to conventional cage rearing system that kept laying hens in the battery cages. This system has permitted a reduction in labor requirements and improved both hygiene and health of laying hens [1]. However, keeping hens in cages has led to lack of possibilities for birds to perform behaviors such as nesting, perching, and using litter material, as well as a lack of space [2, 3]. Therefore the alternative systems have been sought to improve the animal welfare of the laying hens. In addition the consumer's desire for healthier foods has increased the demand for animal products from natural processing and animal welfare. Recently the segmentation market for value added egg production by rearing system become popular, for example, barn laid egg, free-range egg, and organic egg. Because of the restrictions of organic rearing system such as Genetically Modified Organism (GMOs) free feed stuffs, and only organic feedstuffs are allowed. Therefore, free-range system becomes possible especially in Thailand.

The previous studies showed contradictory results. There was no difference in productive performance among the different housing systems (floor vs. cage), however

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keeping hens in cages had higher feed efficiency than keeping hens in floors [4]. While Singh et al. [1] found that there was no significant deference in feed intake and feed efficiency among the different housing. Yakubu et al. [5] reported that hens kept in deep litter had lower egg production than hens kept in cage. Floor pen rearing laying hens trend to laid more quality egg such as increasing albumin weight [4] and egg thickness [1]. Thus the objective of this study was to study the effects of different rearing systems on the productive performance and egg quality of laying hens.

MATERIALS AND METHODS

A total of 276 hens, 30-wk-old ISA Brown laying hens were randomly allocated into 3 treatments: conventional battery cage, floor pen and free-range system. Each treatment was represented by 3 replications. In conventional battery cage, birds were reared with 4 birds per cage (0.046 m^2 per bird). In floor pen, birds were housed with 5 birds per m^2 (0.2 m^2 per bird). In free-range system, birds were housed in a similar floor pen; in addition, they also had an outdoor grass paddock (2.0 m^2 per bird). The experimental diets were formulated to meet the nutrient requirement of laying hens [6]. All birds were provided with the same diet during the experimental period (TABLE I).

TABLE I. INGREDIENT COMPOSITION AND CALCULATED ANALYSIS OF THE EXPERIMENTAL DIETS.

Ingredients	**Amount (%)**
Corn	48.33
Rice bran	10.00
Soybean meal	24.00
Fish meal	3.00
Soybean oil	4.30
Calcium carbonate	9.00
Dicalcium Phosphate (P21)	0.65
DL-Methionine	0.12
Salt	0.35
Mineral-vitamin premix	0.25
Calculated nutrient composition	
Crude protein (%)	17.00
Metabolizable energy (kcal/kg)	2,900

Data Collection and Analytical Determinations

Hen-day egg production, egg weight, and feed consumption were measured throughout the experiment. Every 2 weeks of experiment, eggs were randomly selected for egg quality measuring included yolk weight, albumin weight, albumin height, shell weight and shell thickness.

Statistical Analyses

Data collected in completely randomized design (CRD) were subjected to an ANOVA, and treatment means were compared using Duncan's multiple range test. The level at which differences were considered significant was $P<0.05$. SPSS for windows (Release 10) (SPSS Inc., Chicago, IL) was used for statistical analyses.

RESULT AND DISCUSSION

Productive Performance

The effect of different rearing systems on productive performance of laying hens is shown in TABLE II. There was no significant difference among treatments for hen day egg production, egg weight and feed conversion ratio (FCR) ($P>0.05$). These results agree with previous study [1] who found that the difference between housing types had no effect on egg production. But in contrast with another study [5] who found that hens kept in battery cage had significantly higher egg production and egg weight compared with those on deep litter because they were relatively guarded against air pollution and pathogenic organisms. In the present study, feed intake of birds kept in floor pen was higher than that of birds kept in cage ($P<0.05$) but no difference when compared with birds kept in free-range system ($P>0.05$). These are possibly related to the greater mobility of the bird kept in floor pen and free-range system resulting in more energy utilization, thus bird was consumed more feed to maintain the body energy. Moreover free-range laying hens with outdoor accessed can consume the energy from forages, and worms, which available in the pasture [3].

TABLE II. EFFECT OF DIFFERENT REARING SYSTEMS ON THE PRODUCTIVE PERFORMANCE OF LAYING HENS.

Treatment	Egg production (%)	Egg weight(g)	Feed intake (g/b/d)	FCR
Cage	88.83	59.09	113.17^{b}	2.21
Floor pen	87.86	58.03	117.48^{a}	2.36
Free-range	87.51	59.32	116.66^{ab}	2.27
P-value	0.86	0.28	0.02	0.10
SEM	1.00	0.32	0.63	0.02

TABLE III. EFFECT OF DIFFERENT REARING SYSTEMS ON EGG QUALITY.

Treatment	Yolk weight (g)	Albumin weight (g)	Albumen height (mm)	Shell weight (g)	Shell thickness (mm)
Cage	15.22^{a}	35.31^{b}	7.32	7.26	0.37
Floor pen	14.65^{b}	35.68^{b}	7.63	7.33	0.39
Free-range	14.50^{b}	36.83^{a}	7.79	7.43	0.39
P-value	0.04	0.03	0.13	0.23	0.33
SEM	0.153	0.0927	0.138	0.037	0.000006

Egg Quality

The effect of different rearing systems on egg quality is shown in TABLE III. There was no significant difference among treatments for albumen height, shell weight and shell thickness ($P>0.05$). This result is in contrast with previous study [1] who reported that egg shell quality was better in floor pens than in cages likely because increased activity may benefit calcium metabolism. The yolk weight of birds kept in floor pen and free-range system was lower than that of birds kept in cage ($P<0.05$). While the albumen weight of birds kept in free-range system were higher than that of birds kept in floor pen and cage ($P<0.05$). These observations are in agree with previous studies [1, 4] who found that yolk weight of birds kept in floor pen was lower than that of birds kept in cage. This result could explain that the free-range and floor pen rearing hens have more possibilities to move and to perform behaviors, tends to more energy utilization that can decrease fat deposition in egg yolk.

CONCLUSION

The different rearing systems did not affect to egg production, egg weight and FCR. However, the yolk weight of laying hens kept in floor pen and free-range system was lower than laying hens kept in conventional battery cage. The further experiments are needed to examine the effect of different rearing systems on fatty acid composition and cholesterol content in egg yolk.

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Cover page

Title: *Agricultural Intelligent Greenhouse Management System Based on Z-Wave Wireless Sensor Network*

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ABSTRACT

In view of the inefficiency of traditional greenhouse manual monitoring and high cost of cable monitoring, an agricultural intelligent greenhouse management system of low power, low cost and high reliability is designed. As a result of combination the Z-Wave wireless technology with sensor technology, the system realizes the real-time acquisition of environmental information. The core intersection node gateway unit transmits the information to the expert system wirelessly and after summary and analysis, the user can view data from the expert system graphical interface and control the external devices. The system is a truly wireless real-time monitoring system, and satisfies the needs of the management of the agriculture intelligent greenhouse.

FOREWORD

The greenhouse technology has been occupied an important position in the development of Chinese agriculture, but the traditional manual acquisition and control is time-consuming. In recently years, the greenhouse using field bus technology appeared. However, the complex wiring and high cost make the cable connection is inconvenient to use. And the problem will be difficult to accurately positioned, which causes difficult maintenance, poor reliability etc[1].

The wireless sensor technology is thought to be the best way to meet the demand. In this paper we integrate sensors to the wireless transmission network by use of the Z-wave wireless technology, and design an intelligent agricultural greenhouse management system which makes up the defects of the existing wired system. The proposed system realizes the automatic management of crops by more accurate and dynamic method, and finally enhances the utilization of resources and productivity.

1. SYSTEM DESIGN

As shown in Figure 1, the whole system is composed of the following parts:

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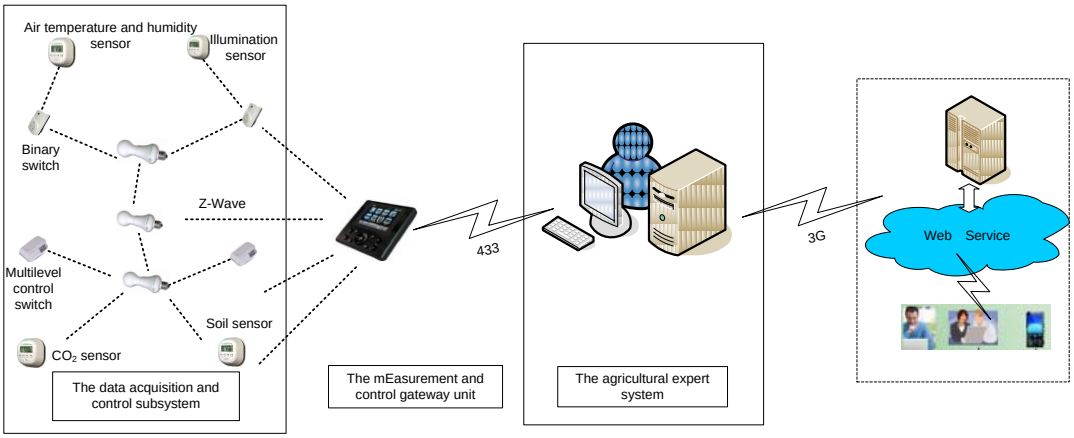

Figure 1. Z-Wave module structure

The data acquisition and control subsystem. The data acquisition nodes are used for environmental parameters collection and transmission. The control nodes drives equipments such as fans, spray systems and window switches by receiving and executing the control instructions issued by the agricultural expert subsystem.

The measurement and control gateway unit (Hereinafter referred to as the gateway unit) manages and controls the greenhouse Z-wave network. It receives and stores environment parameters from the data acquisition nodes. After interaction with the expert subsystem, the gateway unit obtains the control instructions and configuration parameters and completes the electric drive of relevant control nodes.

The agricultural expert subsystem (hereinafter referred to as the expert system) receives the greenhouse environmental data from the gateway unit and after analysis in the expert knowledge base according to different season and crop growth period, the expert subsystem gets the decision results and issues configuration directives.

In addition, the expert system can communicate with the background service system, release data and suggestions to users through web technology.

2. DATA ACQUISITION AND CONTROL SUBSYSTEM DESIGN

2.1 Data Acquisition and Control Subsystem Hardware Design

The data acquisition node is composed of a sensor module, a power module and a Z-Wave module. The sensor module includes a sensor, ADC, a counter and interfaces. The one-way information flow module can accomplish the extraction and quantification of specific physical quantities. The power module provides the required DC power supply to entire sensor node. The Z-Wave module achieves data transmission and control command downlink transmission. As shown below, a Z-Wave module is mainly composed of a CPU(8051), a 128B SRAM, a 32KB Flash, interfaces, a repeater and an antenna. The SRAM caches data acquired by sensor module and control commands returned by the gateway, and the Flash is used to store node IDs given by a Z-Wave network and run software for nodes. In interface, the Z-Wave module supports SPI, UART and I2C interface.

The control node is composed of a Z-Wave module and a control module. The user's control commands which received by Z-Wave module wirelessly are

transmitted to the control module by SPI/UART interface. This system uses two module: binary switch control module and complex control module, the binary switch control module is used to control the simple units which are generally only two or several status, such as pumps, atomizers, etc. The complex control, such as dimmer control use standardized protocols defined by enterprises.

2.2 Data Acquisition and Control Subsystem Software Design

Data acquisition and control subsystem software mainly achieves two functions of sensor data acquisition and transmission in the Z-Wave network. Acquisition nodes，control nodes and the gateway node form a mesh Z-Wave network with independent network address(Home ID). The Z-Wave protocol supports networks of up to 232 nodes, which can be freely shared between two different types of network nodes: Controller and Slave. A Node ID is an 8bits value and it is assigned to slave and controller nodes by a Primary Controller. There are many types of controller nodes in the network, but there is only one Primary Controller[2]. In this system, the gateway node is the Primary Controller, it polls each acquisition node, acquires environmental data and sets control parameters to control nodes.

3. MEASUREMENT AND CONTROL GATEWAY UNIT DESIGN

3.1 Measurement and Control Gateway Unit Hardware Design

The gateway unit is an embedded system with Samsung S3C2440A chip and Linux operating system. The S3C2440A is developed with ARM920t core, 0.13um CMOS standard cells and a memory compiler. Its low power, fully static design is particularly suitable for cost and power-sensitive applications[3]. The S3C2440A is connected with the Z-Wave300 module and the 433 wireless module by URTA interfaces. With the point-to-point control distance of up to 30meters, the Z-Wave300 module is used to communicate data between the gateway unit, acquisition and control nodes. And the 433 wireless module is used to achieve data transmission between the gateway unit and the expert system. The unit also integrates a 64M SDRAM and a 64M NAND Flash. The Flash is used to store the Boot-Loader, Linux file system, the kernel and applications, while the SDRAM loads the program to execute[4].

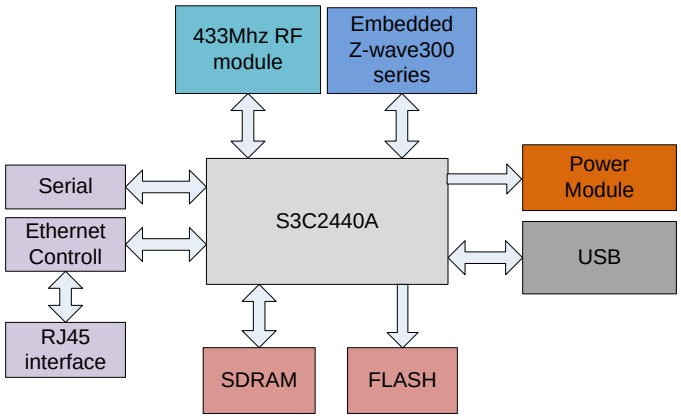

Figure 2. Gateway unit hardware structure

3.2 Measurement and Control Gateway Unit Software Design

The gateway unit software can be divided into three parts primarily: communication with the Z-Wave network, data and commands transmission to the expert system and data storage, processing and task management[5-6].

The communication between the gateway CPU and the Z-Wave300 adopts the Z-Wave serial communication protocol, while the communication between the Z-Wave300 and acquisition and control nodes follows the Z-Wave wireless protocol. Acquisition nodes are in dormant status when idle, and wake up when met the setting time. Then they send messages to the gateway to notify that they have been in working status and wait the gateway to query[7]. After powered up, the gateway unit creates a SerialProcFunction thread to receive messages uninterruptedly sent from each sensor node, send down the query message and storage received acquisition data in the message pool. The ZProcFunction thread extracts and parses data constantly from the message pool. According to different Node ID, the function Update() updates the acquisition data in database.

By building the physical layer on 433MHz radio frequency, the communication between the gateway and the expert system obtains the UHF electromagnetic features of strong penetration and anti-decay ability which can better against the challenge of electromagnetic signals hybridization in complex communication conditions. As shown below, the 433 frame format is defined above the expert system protocol. The gateway creates a Serial433ProcFunction thread to receive 433 data and store the actual data for further processing .

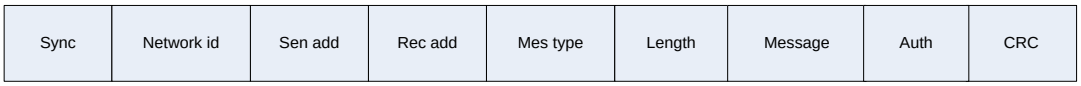

Figure 3. 433 frame format

The data interface between the gateway and the expert system is message. When they communicate, they need to establish a connection. The expert system sends the Channel_setup message to the gateway, if successful, the gateway returns the Channel_status message, otherwise Channel_error. When they receive inconsistent data, the connection will be closed and reestablished. In addition, we use Info_read message to read the acquisition data, use Info_write message to write the configuration information to the gateway and use Status_inquire message to query the node status.

The thread WProcFunction calls function ProcMessage to extract and analyze these messages. The function sends the acquisition data and device status information stored in gateway database to the expert system and configures the control field of nodes in database to achieve the purpose of the system control.

4. THE AGRICULTURAL EXPERT SYSTEM DESIGN

The expert system feeds the greenhouse environment information into the expert knowledge bases for analysis and then provides the decision results and displays all of them through graphical interface. Therefore, the function of the expert system is divided into the following categories: expert setting, communication control, data extraction, parameter display, external device control and graphical display.

The main process of expert system is shown in Figure 4:

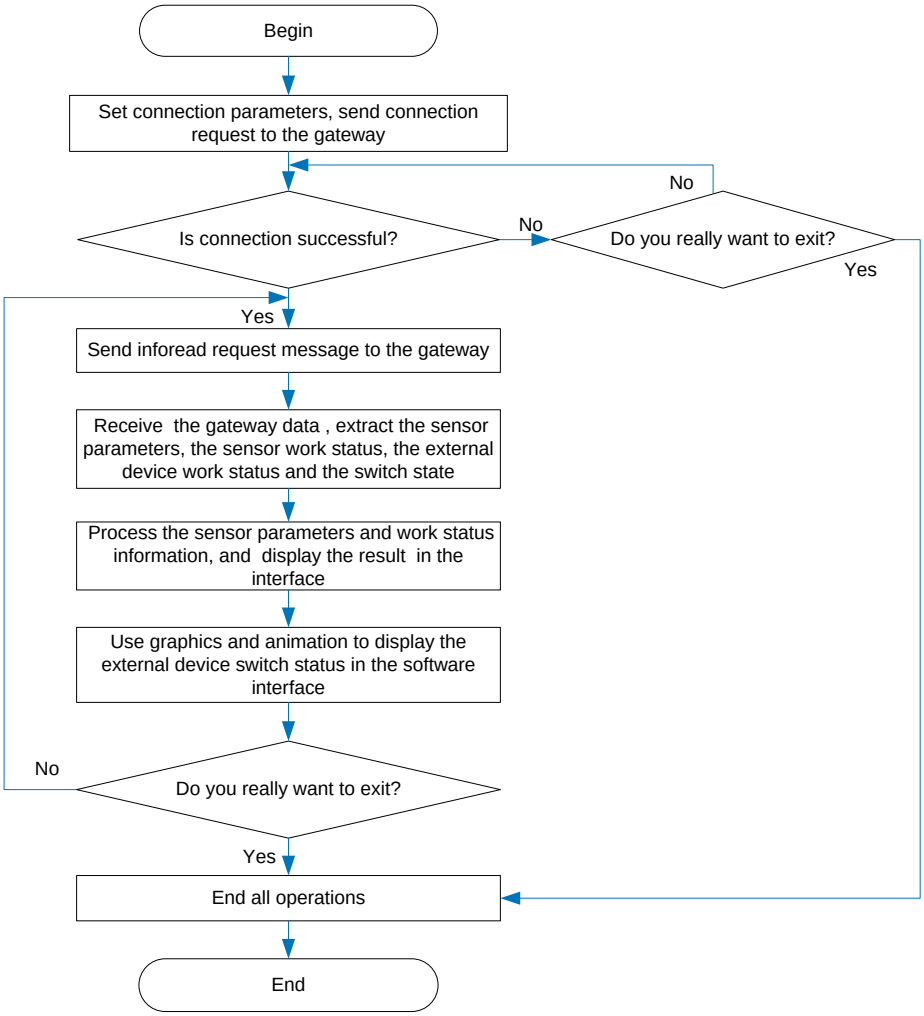

Figure 4. Flow chart of the expert system

The main interface schematic diagram shown in Figure 5:

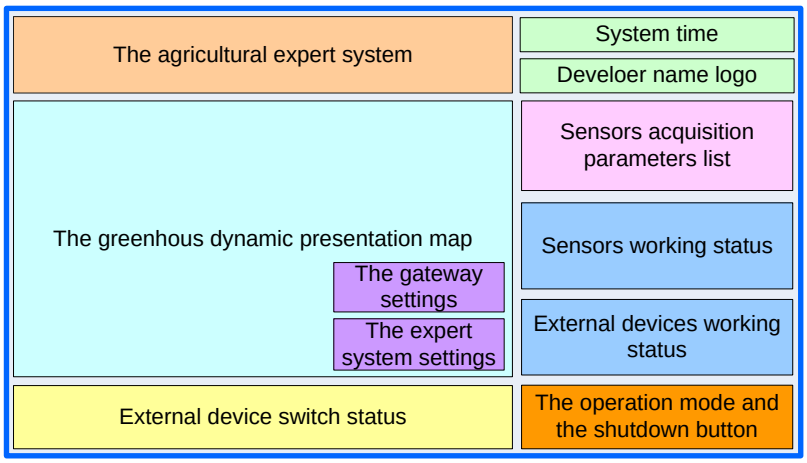

Figure 5. The expert system main interface

CONCLUSION

The agricultural intelligent greenhouse management system based on the Z-Wave wireless technology can realize the real-time monitoring of the environment and automatic control of the equipments in the greenhouse. By extension, it also can achieve remote monitor. Z-Wave wireless technology using in system is a completely different new wireless network technology compared with the traditional ones. It has the features of low power consumption, low cost, convenient installation and high transmission security. Therefore, the application of Z-Wave technology used in the agricultural greenhouse is a good solution to traditional monitoring problems and is also referential to the current designs.

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Cover page

Title: *A Retrospect and Prospect on Researches of Light Pollution*

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ABSTRACT

"Light pollution" has been became a new urban environmental pollution, not only harmful to human vision, human health, but also endanger the survival of the human environment. In this paper, light pollution laws and regulations, as well as light pollution monitoring and evaluation techniques are discussed. A series of problems, which are on the unreasonable laws and evaluation system, are put forward. At the end, some measures are put forward to restrain and improve light pollutions, including increase regulatory agencies, monitor personnel training, improve awareness of light pollution prevention. This article provides theoretical support and reference frame for the research of China's light pollution, and for establishes its laws and regulations.

Keywords: light pollution; light pollution monitoring and evaluation techniques; light pollution laws and regulations; existing problems

1. INTRODUVTION

Now, with living standards improved and industrial development, "light pollution" is becoming more serious, at home and abroad "light pollution" incidents have occurred frequently. For example, in August 1996, in China area Shandong province, Mr.li could not tolerate the light reflected from the opposite building's glass curtain wall and roof of metal decoration ball, which lasted more than ten hours in a day. The long time exposure leads to indoor temperature too high. Not only restless of the family, but also the plaintiff and his wife's hypertension exacerbations. To this end, the "light pollution" as a reason put some companies in Shandong to the court. Verified by the court, the building's glass and its two metal decoration balls are really reflected lights. But whether reach the standard of "light pollution" or not, shall be liable any responsibility, there is no law, his rights has no security. Consequently, the plaintiff's claims were rejected by the court [1]. Along with the light pollution accidents happened frequently in China, then the regulations of light were added in "the Constitution", "the general principles of the civil law", "environmental protection law", and" the property law".

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In other countries, light pollution accidents often happen. A motor vehicle company in France installed neon advertising signs nearby the residential, radiating an orange light, so the surrounding residents draw the curtains, it affects human's working and living. To this end, the surrounding residents sued, the court made the company to withdraw its neon advertising signs (completed within a month after the verdict), and compensate for the losses of 25000 francs [2].

Domestic and foreign light pollution cases show that abroad are early than domestic on the "light pollution" and the relevant knowledge, and put more emphasis on the hazards of "light pollution". The first draft law "multi-party collaborative meeting on environmental issues "which is about light pollution prevention regulations, appears in 2007.

2. LIGHT POLLUTION MONITORING AND EVALUATION RESEARCH PROGRESS

Light pollution is a new kind of environmental pollution, and its connotation and denotation have not yet been recognized as a scientific definition. At present, the light pollution has a lot of specific definitions, which are shown in table 1. Although the concept of light pollution has not been determined, the dangers of light pollution has been more recognized and valued, because it has not only confined to the influence on astronomical observation, and has affected people's health and normal life, so light pollution cases began to appear and increase gradually. A growing number of light pollution cases exposed a problem that the shortage of research and evaluation technology on light pollution prevention, controlling and monitoring.

TABLE 1 LIGHT POLLUTION DEFINES

scholar	Defines
Linyong	Light pollution is the result of visible light, invisible light into the environment. And exceeded the normal biological indices can withstand a variety of species, caused the deterioration of ecological environment and hazards, which affect people's normal production and life. [3]
Riesel K W.	Light pollution refers to a variety of lights, which has impact on the natural environment, on human normal life work, rest and recreation adversely. Damaged people's ability to observe objects, causing discomfort and damage human health. [4]
Zhang Shijun	Light pollution is spilling light, reflected light and glare.etc. Which modern urban architecture and night lighting produced and interference or negative to humans, animals and plants. [1]
Gaolei. etc.	Light pollution is caused by the artificial light that contrary to human physiological and psychological needs or detrimental to physical and mental health. Including glare pollution, radiation pollution, flood light, visual monotony, strobe. [5]
Terrel Gallaway	Light pollution commonly refers to excessive or obtrusive artificial light caused by bad lighting design. [6]

2.1 Light Pollution Monitoring and Evaluation Research Status

As an academic concept, the definition of light pollution not only has a scientific basis and logical rigor, but also has measurable technical indicators. Detected and quantified the adverse consequences of light and a set level to determine the extent of light pollution to ensure its operability. [7]

The growing urban nighttime light pollution problems have been studied, scientists proposed overall evaluation indicators of light pollution. Environmental

luminance partitions, lights-out time, light and color control, regional pitch control, the incident light ratio, brightness balance as the evaluation and monitoring technical index, some scholars proposed urban light pollution assessment procedures, for establish and improve the urban light environmental planning, evaluation and support system. [8]

From controlling light pollution's and protecting the dark sky resource's perspective, took Tianjin night sky for instance discussed the reasons of for the sky bright and its quantitative evaluation, the corresponding results are analyzed. In view of the urban night sky brightness monitoring methods and evaluation methods, scientists have carried on the preliminary study. [9]

The light pollution of glass curtain wall's environmental impact evaluation system has been discussed by taken the case of two constructions. Offered glare features and discomfort glare evaluation level as the evaluation criteria, at the same time, determined the scope of pollution by calculating and control measures. [10]

Based on the analysis of the urban light pollution on driving safety impact assessment, combined with relevant evaluation index system, cited Ruike scale method to construct safety evaluation scale, and based on matter-element theory to evaluate urban light pollution on driving safety. [11]

C. Chalkias, etc. are used geographic information systems (GIS) and remote sensing (RS) techniques to simulate light pollution, and on the basis of obtaining parameters for the Athens area light pollution evaluation. [12]

Astronomy way was used into monitor light pollution of the whole day. Studies show that, with the help of the classic optically celestial wide-angle camera, light pollution can be measured, and gives the relationship between B, V, R and light pollution. [13]

2.2 Existing Problems

Based on the analysis of the current light pollution monitoring and technical evaluation, there are still some shortcomings: 1) The current degree of light pollution of quantitative classification refinement and integration in the residential area is not specific enough, and compared residential areas' light pollution degree of subjective evaluation results with the result of grading, looking for different degrees of light pollution quantitative indicators corresponding to the degree of people's subjective feelings. 2) There are few researches on glare, spill light, violate light from different climate, geographical environment. Three types of light pollution degree formula should be corrected by some coefficients. 3) Lack of using the theory of evaluation to study residential interior glare pollution, light pollution on the degree of light pollution in residential areas of the overall contribution rate. 4) Evaluation of light pollution, one should consider the different types of light sources influencing factors, expanding research object of quantitative evaluation light pollution. 5) The weights of indicators ensure the completeness of the evaluation still needs further study.

3. THE CONSTRUCTION PROGRESS OF LIGHT POLLUTION LAWS AND REGULATIONS

Increasing light pollution incident has exposed the absence of the environmental legislation of light pollution. Light pollution accidents occurred in recent years, due to

lack directly applicable legal basis, we can see the court once involve light pollution will result that the victim unable to maintain the legitimate rights and interests. Therefore, it is necessary for our country to analysis of domestic and foreign light pollution laws and regulations; it also will provide the necessary reference for China to establish a suitable light pollution laws and regulations.

3.1 The Development Status of Light Pollution Laws and Regulations

China's relevant laws and regulations in light pollution have no separate establishment, they are generally described in the relevant documentation, in some rules and regulations, and there are separated provisions on lighting equipment.

3.1.1 NATIONAL LAWS AND REGULATIONS

"Constitution" Article 26, paragraph 1: "The State protects and improves the living environment and the ecological environment; prevents pollution and other public hazards." Country provides the general policy on environmental protection. Light environment is an integral part of the overall environment, light pollution is one of the specific forms, and therefore "the Constitution" with the terms of Article 26 shows that China has a responsibility to protect the light environment, including prevention of light pollution. [14]

"Environmental Protection Law" Article 16 provides that: "Local governments at all levels should be responsible for the environmental quality of the area and take measures to improve environmental quality." Article 22 stipulates: "Urban planning should confirm environmental objectives and tasks, which are protection and improvement of the environment."Which refers to the improvement and protection of the environment should also be taken the light environment into account. Article 24 states: "we should take effective measures to prevent the production of construction or other activities which are gas, waste water, waste residues, dust, malodorous gases, radioactive substances, noise, vibration and electromagnetic radiation and other environmental pollution and hazards."[15]

3.1.2 THE LOCAL ENVIRONMENTAL PROTECTION LAWS AND REGULATIONS

"Shandong Provincial Environmental Protection Regulations" Article 10 stipulates: "the city government should be in accordance with national environmental remediation quantitative assessment indicators, the development of the region's comprehensive environmental objectives and measures to strengthen the waste water, waste gas, dust, solid waste, noise and light pollution prevention ";" Zhuhai City environmental Protection Regulations" Article 32 stipulates:" enterprises, institutions and individual industrial and commercial households produce gas, waste water, waste, noise, dust, malodorous gases, radioactive substances, vibrations, electromagnetic radiation, light pollution that harm to the environment, must clear environmental responsibility and take effective measures to prevent environmental pollution. "." Xiamen building exterior decoration Interim Provisions "Article 14 stipulates: the surrounding environment of the glass curtain wall light pollution or metal walls, etc. should be coated low-E or polished metal plate, and not use of mirror

glass, metal plates or other materials that harmful to the light environment. "These regulations have been made clear provisions on light pollution. [16]

3.1.3 LIGHT POLLUTION ENVIRONMENTAL STANDARDS

(1) Architectural Lighting

"Civil lighting design standards" summed up our civil lighting design experience, reference to the relevant international and foreign advanced standards, according to scientific research, experimental verification, the relevant units advice, included libraries, theaters, office buildings and shops, etc. illumination standards, and provides illumination uniformity, glare limitation, reflectance and illuminance ratio, for the lighting design of a certain basis.[17] "Lighting design standards of industrial enterprises" primarily restrict the lighting of industrial sites, including two parts of indoor and outdoor lighting. It also provides a corresponding illumination standards, glare limitation, reflectance and illuminance ratio, etc., and "civil lighting design standards" make that distinction. [18]

While "Architectural Lighting Design Standards" published simultaneously, the original "industrial enterprises lighting design standards" (GB50034-92) and the "civil lighting design standards" (GBJ133-90) shall be repealed. The standard is more specific, added the light environment that involved terms and lamps and attachment selection criteria, such as libraries, theaters, office buildings and stores the required standards of illumination, as well as the illumination uniformity, glare limitation, reflections and an illuminance ratio than before are more detailed requirements.[19]

(2) Site-specific lighting

"Dance hall lighting and light pollution limited standard “for the dance halls of the special place provides its lighting standards; "Stadium lighting design and testing standards” provides design standards and testing standards, making the lighting more rationalized, saving a variety of resources. [20-21]

(3) Road lighting

"Urban road lighting design standards" provide the classification of road lighting and lighting various classifications corresponding standard values, proposed road lighting evaluation. "Urban road lighting engineering construction and acceptance procedures" mainly on the overhead lines, low-voltage cable lines, transformers, and box-type substation, distribution equipment and control, security, and lights installed these parts were described. [22-23]

3.2 Global Light Pollution Laws and Regulations Promulgated by the Case [24-25]

The Czech Republic is the first country that wrote light pollution prevention provisions into national law, and on March 25, 2003 in the "Environmental Law” made provisions for the urban light pollution control. Czech pioneered the light pollution explicitly written into national law which has been widely praised. In regulations specified the way of light use of any organs and personal , do not allow using the scattering lighting devices, above light bulbs must be used the lamp to prevent the scattering of light into the night sky, and offenders will be fined. In addition, the Act also provides the prevention of light pollution public obligations and enjoys the rights of no light pollution.

Sweden, "Environmental Protection Law" (1969 No. 387, as amended in 1995) details a number of cases that caused by environmental pollution, which will contain light pollution.

British "2005 Act neighborhoods and environmental purification" of light pollution is defined as a statutory nuisance, provisions that local environmental officer can deal with the region light pollution problem that affect people's lives and sleep according to the law, which is the UK "Environmental Protection Law" amended. In the "Environmental Protection Law" Article 79, paragraph 1 stipulates that added one of statutory nuisance types: artificial light of buildings that may cause damage to health or cause nuisance.

Light violations in France are also identified as a type of tort nuisance neighbor, belongs to the scope of neighboring relations. October 2008 France issued a new "Environmental Law", states that if artificial light on human, animal, plant or hazardous environment, resulting in waste of energy, or hinder the activities on observing the night sky, then the light will be restricted.

3.3 Problems and Shortcomings

At present, China's national legislation has involved in the control of light pollution, but the light pollution is not explicitly included in the national "Environmental Protection Law", light pollution for complaints and lawsuits can only be based on property rights protection, and demanded an end to violations and reparation; local regulations (e.g. several provinces environmental regulations) has referred to prevent light pollution, but there are no specific management measures, for light pollution laws define "Who management" and "How to management " are not clearly, in violation of environmental regulations would cause light pollution damage, however, how to penalties there are no laws to obey. Basically, domestic light pollution prevention and control are still in the level of lighting design and technical specifications. "Constitution" is only mentioned from the height of responsibilities, but not from the human rights' perspective to mentioned ecological environmental protection of China's light industry technical specification and part of the urban landscape lighting pollution control norms in the light of relevant content. More fragmentally, the content is not uniform, the main purpose of lighting design and technical specifications is to make urban lighting to meet urban safety, traffic, residents' daily outdoor activities’ demands. Light pollution content just involved a little.

Furthermore, the lack of individual technical specifications light pollution for the measurement and evaluation and also makes light pollution’s daily supervision lack of technical basis. In addition, various departments the level of awareness of light pollution prevention is not enough. This will cause the phenomenon of evading responsibilities and rights or competitive situation, and also influence the efficiency of the sector. [25]

In other countries, the Czech Republic carries out light pollution prevention and control work in a large system of environmental law. Advantageously, in the event of light pollution infringement of the law is a legal basis to follow; while its inadequacies are that the system is too complex, and produce light pollution prevention disputes prone multi-standard, leading to cases of final judgment produce different results. Britain and France two countries have enacted laws, including the problem of

protection against light pollution, belong to the same mode of tort law system, and must be two adjacent properties is a necessary condition, but light pollution are not necessarily occurs in adjacent relationship, there are certain limitations.

4. PROPOSALS AND MEASURES

Based on the research on light pollution, as well as analyzed the present laws and regulations. We can make some adjustments as follows:

1) To raise public awareness of light pollution prevention and control. China should prevent and control light pollution from the fundamental. It is necessary to raise public awareness of light pollution prevention and control. In densely populated areas, we can hair leaflets, in schools and other related units, students and staff can be organized to learn the relevant knowledge on light pollution, in doing so can make the public aware of the hazards and prevention of light pollution rights.

2) Establish and improve the environmental regulatory system. In the legislative process to build an environmental monitoring system; to develop light environment unified technical standards. Technical standards can be used to judge whether light pollution would constitute infringement. It also can make clear light pollution prevention and control specific elements and departmental responsibility to develop light pollution technical specifications as well as light pollution local regulations.

3) Strengthen urban construction, design management. Because most of the light pollution in the city is generated by unreasonable construction, such as the white light pollution was caused by the glass curtain wall, artificial daylight was caused by excessive night lighting, light pollution from the business district, etc. was caused by the frequent use of colored light sources, therefore, we must control from the source, and manage light pollution strictly in urban construction.

5. CONCLUSIONS

Light pollution is a new pollution accompanied by industrial and urban development. China on the light pollution monitoring, evaluation techniques, laws and regulations has made certain achievements. However, on light pollution monitoring, although borrowed abroad experience, and does not match our actual light pollution, there is to be further improved; on light pollution evaluation techniques to establish a light pollution evaluation system, but too simple to comprehensive analysis of light pollution to the actual situation; on light pollution laws and regulations lacking of regulatory agencies, making light pollution prevention achieved success.

For these situations, we want to create light pollution regulators, increase management control efforts, and take measures actively to control and manage the light pollution, stepping up the improvement of legislation and norms, while strengthening the "light pollution" public awareness, improve the control of light pollution awareness of pollution and damage to a minimum.

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Coverpage

Title: *Effects of Transglutaminase on the Properties of Rice Gel*

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ABSTRACT

In order to improve the texture and stability of the rice gel, the effects of transglutaminase (TG) on the characteristics of the rice gel, such as rheological, thermal, crystal properties and microstructure, were investigated in this study. After the treatment of TG, the rice protein was catalyzed with cross-linking, and the microstructure of the rice gel became more compact and smoother. The X-ray diffraction pattern of rice gel had altered, and its relative crystallinity decreased. The onset temperature and change in enthalpy during endothermic process had increased due to the strengthened interaction forces between molecules. The storage modulus and loss modulus recorded in the dynamic rheological tests declined, the reason was starch granules were wrapped by cross-linked proteins that disturbed the interactions of starch molecules. Thus, the elasticity and viscosity of rice gel decreased. Storage stability (4 ℃) of rice gel was improved with optimum addition of TG (0.10 %, 0.15 %).

INTRODUCTION

The rice gel is generated from rice flour gelatinizes with limit moisture. The rice gel products, such as rice noodles, rice cakes, rice puddings, have unique texture, flavor, and nutrition [1, 2]. Starch is the major component which contributes to the formation of the rice gel. The gelatinization of starch occurs after the granules are heated in an aqueous medium. During the gelatinization, a three dimensional network is formed due to the interaction of the leached out amylase and clumps of swollen amylopectin [3]. During the gelatinization, protein, fat and other polymers' chains stretch and react with water, and generate a complex structure. This kind of structure is favorable for chewiness and flexibility [4, 5]. Texture is an important property of rice gel products. During storage under low temperature, the combinations between polymers' chains with water decline, the polymers' structure is shrinkage. All of these changes will induce uprising of the hardness, disappearance of the flexibility, and decrease of digestibility of the rice

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gel [6]. The texture of rice gel is influenced by water [7], temperature [8], starch composition [9], and lipid [10] et al. Its texture can be improved with other products, such as enzyme [11], low molecular sugar [12], or emulsifier [13], etc.

The textual properties of food materials can be modified by protein cross-links. Transglutaminase (TG) (EC 2.3.2.13) can catalyze an acyl-transfer reaction between ε-amino group of lysine residues and γ-carboxyamide group of glutamine residues, so the covalent bond of ε–(γ-Glu)–Lys is formed and cross-links with other proteins [14]. TG has been widely used in meat [15], aquatic [16], dairy [17], and bakery products [18], to improve the qualities of the products. TG can catalyze the cross-linking of their proteins, thus the quantities of free amino acids decrease [19], and meanwhile the capacities of water retention and thermal stability are improved [20]. Although rice contains about 8% protein which is relatively rich in glutamic but deficient in lysine, but studies showed that TG also catalyzed cross-linking of rice protein [19]. The purpose of this study was to investigate the effects of TG on the rheological properties, thermal properties, microstructure and crystal characteristics of rice gel to provide some evidence for the quality control of rice gel.

MATERIALS AND METHODS

Materials

Rice flour was produced from Zhongza (non-waxy indica) in Huanggang Dongpo Oil Group Co., Ltd., Hubei, China. The rice flour had moisture, starch, crude protein, crude fat and amylase of 4.98±0.01%, 85.51±3.15%, 5.95±0.3%, 1.94±0.03%, 33.44±0.304%, respectively. The TG was from Taixing Enzyme Co., Ltd., Wuxi, China. All reagents were of analytical grade.

Preparation of Rice Gel

TG was mixed with water first. Then some rice flour was added in to make sure the ratio of rice flour and water was 1:1.2 (w/w). Rice slurry was added into the aluminum disc with a diameter of 7.5cm after stirring sufficiently. The aluminum molds were put into the steaming cabinet for 6 min, when its inner temperature reached 100 ℃. The molds were placed upside down to prevent the evaporation of water for 20 min at room temperature. Rice gel was packaged with plastic wrap.

Dynamic Rheological Measurements [20]

Temperature ramp tests were determined with a rheometer (AR 2000 ex, TA instruments, New Castle, DE) using parallel-plate geometry (40 mm plate diameter). Rice slurry was placed between parallel plates. The gap was adjusted to 1 mm and the excess rice slurry was removed. A solvent trap coated with liquid paraffin was to cover the exposed samples in order to prevent drying at the edges. Temperature sweep tests were performed from 25 to 100 ℃ and 100 to 4 ℃ at a rate of 5 ℃/ min. The oscillation frequency and stain were 1 Hz and 2 %.

Differential Scanning Calorimetry Measurements

The measurements were carried out using a differential scanning calorimeter (204 F1, Netzsch Co., Selb, Germany). Rice slurry was placed in refrigerator at 4 ℃ for 12 h before measurement. Samples of 10.00 mg (accurately weighed to 0.01 mg) were hermetically sealed in the aluminum sample pans, and set aside for 1 h at 37 ℃ before measurements. An empty aluminum pan was used as the reference to balance the heat capacity of the sample pan. All samples were heated from 30 to 100 ℃ at a rate of 5 ℃/min.

X-Ray Diffraction

Rice gel was freeze-dried for 24 h, stored in refrigerator at 4 ℃ for 3 days, ground and sifted through a 100 mesh sieve. The X-ray diffraction patterns of these samples were determined using an X-ray diffractometer (Bruker AXS, Inc., Germany) with Cu Kα radiation at 40kV, 50 mA and a scan rate of 15°/min. The diffraction angle (2 θ) ranged from 10 to 60°. Data processing and analysis were performed using MDI-Jade 5.0 software.

Microstructure

Rice gel was freeze-dried for 24 h, ground and sifted through a 100 mesh sieve. The rice gel powder was gold-coated. The scanning electron microscopy (SEM) images were obtained by a JSM-6390 scanning electron microscope (NTC, Japan).

Statistical Analysis

Statistical analysis was conducted using Microsoft Office Excel 2007 and MDI Jade 5.0 software (Materials Data inc., Livermore, CA, USA).

RESULTS AND DISCUSSION

Effects of TG on the Formation of Rice Gel

Based on the formation of rice gel, it could be characterized into four different stages. Trends of storage modulus (G') and loss modulus (G") with different levels of TG were the same. The changes of G' and G" of rice gel treated by 0.10% TG along with time were showed in Figure.1. ① Starch granules didn't gelatinize when the temperature was from 30 to 60℃. G' and G" values both remained low. ② Starch gelatinized when the temperature was from 60 to 79 ℃. Starch began to absorb massive amounts of water. Amylose leached out from the irreversibly swollen starch granules, intertwined and formed a tight network with swollen starch granules which led to a sharp rise in G' and G". ③A subsequent heating from 79 to 100 ℃ resulted in the collapse of swollen starch granules and a rapid decrease in G' and G". ④During the cooling stage from 100 to 4 ℃, molecules moved slowly, and the molecular chains became more rigid as temperature went down. Amylose began to bind with amylopectin through hydrogen bonds, so that a complex network

structure formed, and then G’ and G” increased again.

The parameters of rheological characteristic of rice gel by TG were showed in Table I. It indicated that the temperatures of initial gelatinization of rice slurry, and peak values of G ', G” had increased with TG treatment. On the contrary, the peak values, and values at 100 ℃ and 4 ℃ of G', G" decreased along with the increasing of TG. This might due to the cross-linked proteins catalyzed by TG wrapping around starch granules that disturbed the swelling of starch granules and leaching of amylase [2]. Thus, starch granules required higher temperatures to collapse. This structure also disturbed interactions among starch molecules, and combined more water molecules in the network [19]. Therefore, the hardness and viscosity of rice gel were reduced. In the cooling process, as temperature was below the gelatinization temperature of starch, G' and G" increased significantly when TG is 0 and 0.05%. Rice gel with 0.10 % and 0.15 %TG showed slight changes in G' and G". It was presumed that proper amount of TG (0.10 %, 0.15 %) could improve the storage stability of rice gel.

Effects of TG on the Thermal Properties during Rice Gel Formation

DSC analysis can provide information concerning the fusion of starch crystal and denaturation of protein during the formation of rice gel [21]. The DSC curves of rice gel and effects of TG on its thermal parameters were shown in Figure.2 (50 to 90℃) and Table II. All the DSC curves had only one endothermic peak which indicated the process of gelatinization of starch and denaturation of protein in rice slurry. TO, Tp, Te, and ΔH increased with the addition of TG. Rice flour contained about 85% starch and 6% protein. Starch granules absorbed large amounts of water, and swelled irreversibly around 70 ℃, and rice protein denatured at the same time [22]. During gelatinization of starch granules, their crystal structure disappeared. For starch granules were wrapped by the cross-linked proteins catalyzed by TG, they were difficult to swell. Stretch of molecular chains, leach of amylase and fusion of starch crystal were also disturbed, so that more energy was required to break the forces among molecules. Thus, TO and ΔH increased eventually.

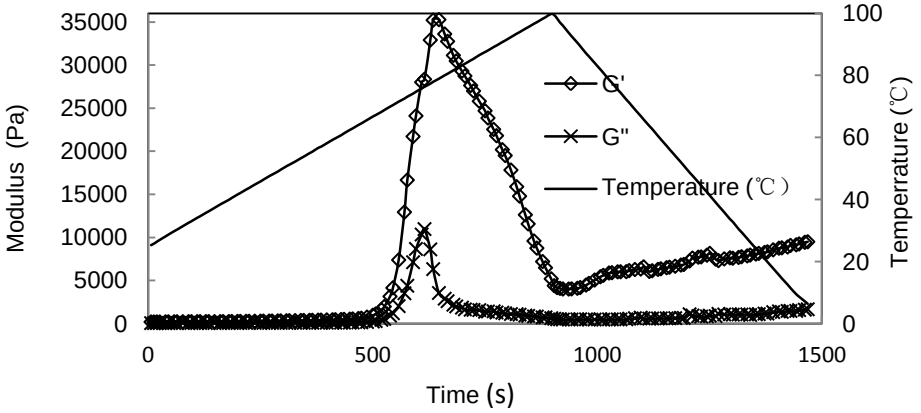


Figure.1 Effects of TG on the forming of rice gel (TG 0.10 %)

TABLE I. EFFECTS OF TG ON THE RHEOLOGICAL PARAMETERS OF RICE GEL

TG/%	T_O/℃	G'				G"			
		T1/℃	T2/Pa	T3/Pa	T4/Pa	T1/℃	T2/Pa	T3/Pa	T4/Pa
0	62.5	73.7	116700	24350	45090	73.2	24990	1924	14010
0.05	65.6	75.8	66470	15050	55420	74.7	12532	1604	21030
0.10	66.7	78.9	35260	5181	9479	76.3	10950	608	1642
0.15	68.2	78.3	27480	3396	7581	75.7	3640	445	995

T_O was the onset temperature of the endothermic process; T1 was the peak temperature; T2 was the peak value; T3, T4 was the value when the temperature reached 100 ℃ and 4 ℃, respectively.

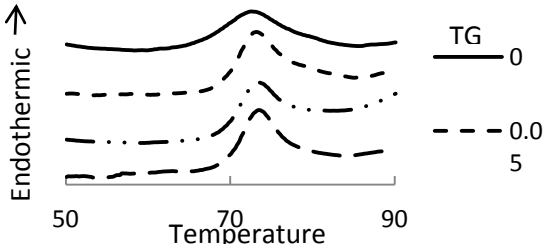


Figure.2 DSC curves of rice gel

TABLE II.EFFECTS OF TG ON THE THERMAL PATAMETERS OF RICE GEL

TG/%	TO/℃	Tp/℃	Te/℃	ΔH J/g
0	67.9	72.4	76.2	1.543
0.05	70.3	73.2	76.0	2.188
0.10	70.2	73.4	76.4	2.350
0.15	70.4	73.5	76.5	2.500

T0, Tp, and Te represented the onset, peak, and final temperature of the endothermic process, respectively. ΔH represented the change in enthalpy.

Effects of TG on the Crystal Characteristics of Rice Gel

The X-ray diffraction curves of rice gel (stored at 4 ℃ for 3 days) treated with different levels of TG were showed in Figure.3. Its crystal parameters were showed in Table III. Crystal structure of starch disappeared after complete gelatinization with excess moisture. Starch recrystaled due to the formation of double helix structure of amylose molecules and the rearrangement of outside short chains of amylopectin during storage (4 ℃) [2，23]. According to Figure.3 and Table III, there were three strong diffraction peaks at 2θ = 17 °, 20 °, 22 °of rice gel without TG after stored 3 days. After the treatment of TG, the peak at 2θ = 22° disappeared, simultaneously, X-ray diffraction pattern of the rice gel was changed, and its FWHM had decreased. W_x declined with the increase of TG. These revealed that TG had altered the inner structure and crystallization of rice gel, improved the regularity of crystal, and inhibited the recrystallization of starch.

Effects of TG on the Microstructure of Rice Gel

The effects of TG on the microstructure of rice gel were showed in Figure.4. Rice gel without TG had more holes compared with the ones treated with TG.

Treatment of TG led to fewer holes, a more compact structure and smoother surface of rice gel. Tseng and Liu [24] had reported the effects of TG on the microstructure of low-salt chicken meat-balls. Their results revealed that the cross-linking of protein catalyzed by TG contributed to a more compact and steady structure. Wu and Corke [25] also reported that noodles had less holes, a more compact structure and smoother surface because of the cross-linking of gluten catalyzed by TG. These results were consistent with the effects of TG on the rheological and thermal properties of rice gel, thus, the cross-linked protein catalyzed by TG contributed to a more compact and smoother structure which caused an increase in TO, ΔH and water retention of rice gel.

CONCLUSIONS

In order to improve the texture and stability of the rice gel, TG was added in the rice gel to catalyze cross-linking of rice protein. Microstructure of rice gel and corresponding X-ray diffraction pattern were investigated. The thermal properties, TO and ΔH were explored by the differential scanning calorimetry measurements. The cross-linked proteins had altered the microstrcture of the rice gel. The rice gel became more compact and smoother, which expained the increases in TO and ΔH. The cross-links structure wrapped around the starch granules and held more water in the compact network, inhibiting the recrystallization of starch. Thus, the G' and G" decreased in the dynamic rheological measurements study. The elasticity and viscosity of rice gel decreased. It could say that the trend of storage stability of rice gel was better according to G' and G" values measured by the dynamic rheological instrument at low temperature.

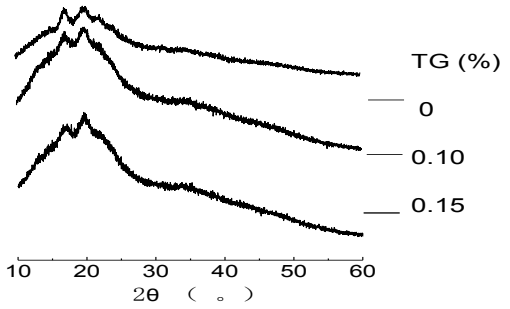
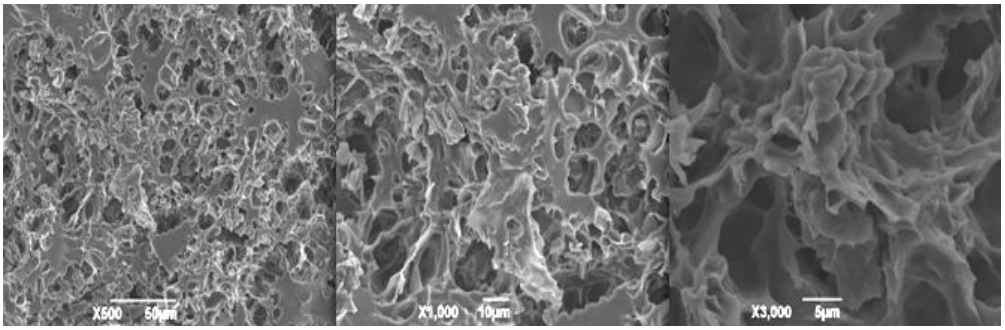


Figure.3 X-ray diffraction curves of rice gel

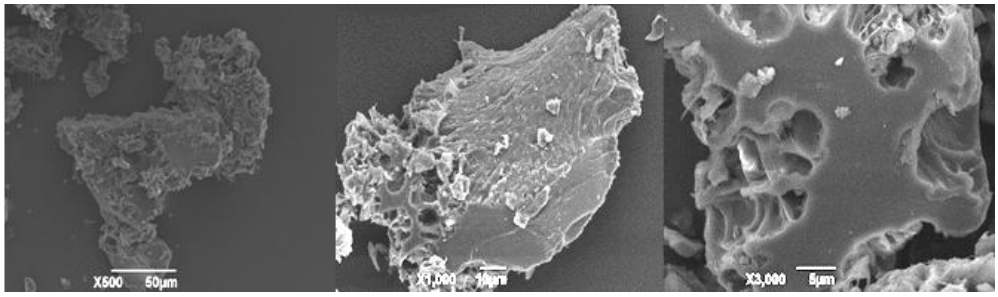
TABLE III.EFFECTS OF TG ON THE CRYSTAL PARAMETERS OF RICE GEL

TG/%	P/°	d/A°	FWHM/°	RI/%	W_x/%
0	16.783	5.278	1.131	86.32	15.34
	19.885	4.461	0.954	75.85	
	22.075	4.023	0.918	52.23	
0.10	16.626	5.327	1.032	86.94	11.61
	19.627	4.519	0.866	100.00	
0.15	17.120	5.175	0.796	55.34	10.10
	19.884	4.461	0.928	84.46	

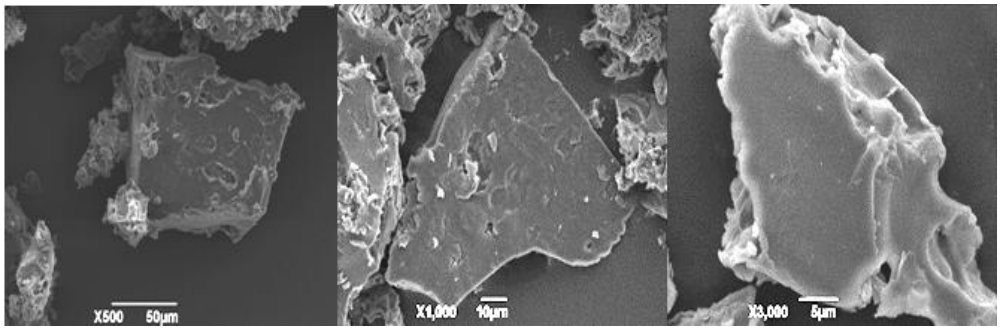
P, d, FWHM, RI, and W_x represented the position of X-ray diffraction peak, interplanar spacings, full width at half maximum, relative intensity and relative crystallinity, respectively.



TG 0 %



TG 0.10%



TG 0.15%

Figure.4 Effects of TG on the microstructure of rice gel

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Title: *The Effect of N^+ Ion Implantation Mutagenesis on the Streptomyces Aureochromogenes NJYHWG 66382*

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ABSTRACT

In order to obtain a polyoxins high-yield, stable production strain. N^+ ion implantation was introduced to mutate *Streptomyces aureochromogenes* NJYHWG 66382 with an energy level of 30 keV and dose ranging from 50 to 300 $\times 10^{15}$ions/cm^2. One screened high-yield strain was achieved and through five-generation investigation, the production of polyoxins increased more than 35%. Meanwhile the fermentation time was shortened from 120 to 72 h.

1. INTRODUCTION

In 1986, Institute of Plasma Physics collaborated with Institute of Rice of China for a program of mutation breeding for rice by low-energy ion beam [1]. Since then, mutagenesis technique grabbed some serious attention in breeding of crops, plants, and microorganisms [2]. Ample proof demonstrated that ion beam mutation had a wide range of biological effects, including extracellular and intracellular DNA damage [3, 4], amino acid and nucleotide decomposition in aqueous solution [5], inactivation of viruses, microbes [6] and aberrant chromosomes in wheat [7].

The polyoxins, are antifungal antibiotic agents and nucleotide antibiotics which are produced by *Streptomyces cacaoi* var. *asoensis*[8,9]. In 1967, Isono found polyoxins A-N to be a new class of peptide nucleosides, they are effective toward certain phytopathogenic fungi especially of rice-plant diseases, because polyoxins are powerful inhibitors of chitin synthesis which are particular component of insect cuticle and fungal cell wall. [10,11]. Polyoxins are not toxic in crop, vertebrates and mammalian; as a result of they do not contain chitin.

2. MATERIAL AND METHODS

2.1 Microbe and Media

Streptomyces aureochromogenes NJYHWG 66382 is stored in our lab.

Slant medium: Potato 200 g/L, Sucrose 20 g/L and agar 20 g/L;

Plate medium: the same as slant medium;

Seed media: Glucose 10 g/L, soybean 10 g/L, KH_2PO_4 1 g/L, NaCl 1 g/L, $CaCO_3$ 3 g/L.

Fermentation medium: Potato 200 g/L, Sucrose 20 g/L.

2.2 Cultivation Conditions

The slant medium was cultured for 7 days at 28 ℃. Prepared spore suspension (OD_{600}=0.25) and inoculated in 250 ml flasks (working volume 50 ml). The flasks were incubated on a rotary shaker at 180 rpm and 28 ℃ for 36 h. Then transferred seed media to fermentation medium, and inoculation quantity was 3.0%. Fermentation broth was subjected to centrifugation (10000 rpm, 15 min, 4℃) to determinate the concentration of polyoxins by using HPLC analysis.

2.3 Preparation of Spore Suspension

The strain was activated on agarslantculture-medium. After 10ml sterile water was added, the spores were washed down and transferred into a triangular flask with some micro glass beads. The flask was stirred to form a spore suspension liquid for use.

2.4 N+ ion Implantation

0.1ml suspension liquid was transferred onto the sterile plate. After painted averagely and dried by sterile wind, it would form a strain pellicle. Microscopic examination was conducted to select the nonoverlapping to be N^+ implanted. In this experiment, 30keV N^+ was implanted with different quantity. The pressure of target chamber is 10^{-3}Pa. Every pulse lasted 10s and rested for 50s. The dose was 50×10^{15}ions/cm^2-300×10^{15}ions/cm^2. There was a control group in each condition. After N^+ implantation, the strain was eluted with 0.5ml sterile water and painted on plating medium. The culture temperature was 28℃.

2.5 Screening Conditions

The HPLC assay was performed on DIONEX P680, at the following conditions: at a flow rate 0.6 ml/min, 263 nm, Kromasil C18 (250mm×4.6mm×5μm), 30 ± 0.1 ℃, V(methanol): V(water)=10:90, sample size was 5μm.

2.6 Computing Methods

Survival rate (%) = the survival of the colony count with ion implantation / the control samples without ion implantation

Positive mutation rate (%) = the concentration of polyoxins (over 10% after mutation)/ the original concentration of polyoxins

Negative mutation rate (%) = the concentration of polyoxins (under 10% after mutation)/ the original concentration of polyoxins

3. RESULTS AND DISCUSSION

3.1 The Survival Rate

In our experiment, we chose N^+ as ion source and the energy was set at 30 KeV, 50×10^{15}, 100×10^{15}, 150×10^{15}, 200×10^{15}, 250×10^{15}, 300×10^{15} ions/cm^2 dose. The dose of nitrogen ion radiation to *Streptomyces aureochromogenes* NJYHWG 66382 cells on survival rate is shown in Fig. 1. The figure clearly showed that firstly in the range of 0-100 $\times10^{15}$ ions/cm^2 dose, with an increasing implantation dose, the survival rate rapidly decreased; then when the implantation dose increased from 100 $\times10^{15}$ to 150 $\times10^{15}$ ions/cm^2, the survival rate increased from 12.5% to a maximum of 41.7%; finally the survival rate decreased with the implantation dose increased. The survival curve exhibited a clear ‘‘saddle shape’’ curve with increasing of dose, which is endemism with the implantation dose [12].

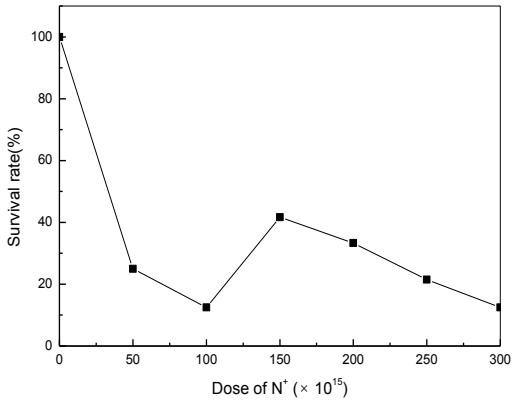

Fig. 1 The survival rate curve by N^+ beam implantation

Yu and colleagues [1] proposed that the interaction between low-energy ions and the strains was characterized by energy deposition, momentum transfer, mass deposition and charge neutralization. According to this theory, at the first period(0-100 $\times10^{15}$ ions/cm^2 dose), energy deposition caused cell surface damage and etch, and DNA, biofilm and other biological macromolecules are damaged by a large number of free radicals at the same time. With increasing dose (100 $\times10^{15}$ to 150 $\times10^{15}$ ions/cm^2), certain repair mechanisms (called SOS reaction) within the cell are activated, causing the survival rate to rebound. However, with the continuous increase of the implantation dose (up to 150 $\times10^{15}$ ions/cm^2), it will not cause permanent damage and can not be repaired, and the survival rate decreases. Therefore, we chose 150 $\times10^{15}$ ions/cm^2 as the appropriate dose for further mutation.

3.2 Effect of Different N+ beam Implantation Dose

Single colonies implanted in different doses of N+ ions were randomly chose and cultured with a shake flask (5 days), then the titer was measured. Calculated the mutation rates and graphically displayed in Fig. 2. In the implantation dose ranging from 50 $\times10^{15}$ to 300 $\times10^{15}$ ions/cm^2, we can found that the negative mutation rate is also higher than the positive mutation rate, which indicated the broad mutation spectrum. When the injected dose was 150 $\times10^{15}$ ions/cm^2, the negative mutation rate

and the positive mutation rate reached the maximum, so this dose was appropriate dose to obtain mutation strains needed.

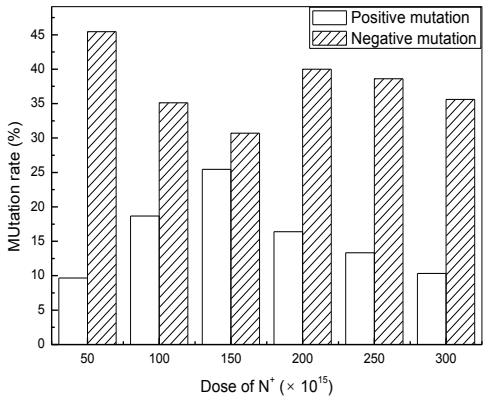

Fig. 2 Effect of N^+ beam implantation dose on mutation rate of *Streptomyces aureochromogenes* NJYHWG 66382

These results were consistent with the characteristics of plants and microorganisms ion implantation, which also had high mutation rates and the broad mutation spectra [13].

3.3 Screening Method for Strains with High-Yield

High-yield mutants were obtained at an energy level of 30 keV and dose of 150 × 10^{15}ions/cm^2. After screening, we got one high-yield strain. The yield of polyoxins was increased from 712 μg/ml to 1025 μg/ml. Meanwhile the fermentation time of mutant is shortened from 120 h to 72 h.

3.4 Stability Test of the High-Yield Strain

We obtained high-yield mutants which needed a good genetic stability. In order to evaluate the genetic stability of the screened high-yield laccase mutant strains, the strain was successively passaged for five generations, and the titer of polyoxins and the dry weight were shown in Fig. 3. As the Fig. 3 shows, after continuous passaging for five generations, the titer and dry weight were still remained at a higher level.

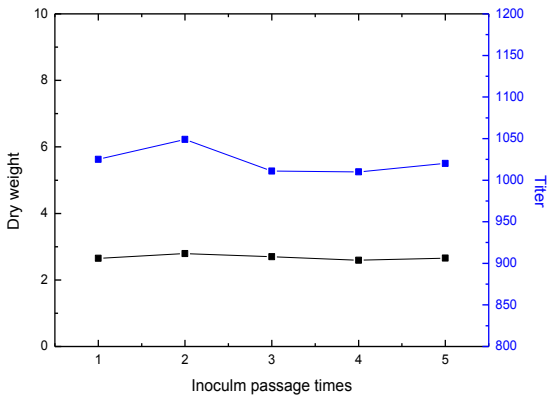

Fig. 3 The passage stability of mutant strain

3.5 The Fermentation Characteristics of the High-Yield Strain

The strain was transferred to fermentation medium (50/250ml), and the characteristics were determined every 24 h (shown in Fig. 4). It can be seen that during the whole fermentation stage, pH continues to rise; after 96 h, titer of polyoxins reached its maximum and remained stable; at initial stage, dry weight was relatively low and after 96 h cell weigh increased quickly.

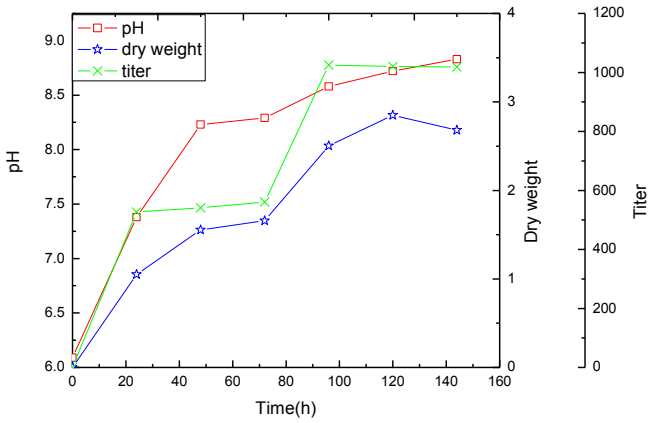

Fig. 4 The characteristics of high-yield strain

4. CONCLUSIONS

In our study, we chose the dose of 200 $\times 10^{15}$ ions/cm2 and energy of 30 keV as an optimal point, and one high-yield strain was gained. The concentration of polyoxins produced by the mutant strain was 1025 μg/ml higher than the original strain. After continuous passaging for five generations, the titer and dry weigh maintained at a stable level.

ACKNOWLEDGEMENTS

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Title: *The Effect of N^+ Ion Implantation Mutagenesis on the Streptomyces Aureochromogenes NJYHWG 66382*

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ABSTRACT

In order to obtain a polyoxins high-yield, stable production strain. N^+ ion implantation was introduced to mutate *Streptomyces aureochromogenes* NJYHWG 66382 with an energy level of 30 keV and dose ranging from 50 to 300 $\times 10^{15}$ions/cm^2. One screened high-yield strain was achieved and through five-generation investigation, the production of polyoxins increased more than 35%. Meanwhile the fermentation time was shortened from 120 to 72 h.

1. INTRODUCTION

In 1986, Institute of Plasma Physics collaborated with Institute of Rice of China for a program of mutation breeding for rice by low-energy ion beam [1]. Since then, mutagenesis technique grabbed some serious attention in breeding of crops, plants, and microorganisms [2]. Ample proof demonstrated that ion beam mutation had a wide range of biological effects, including extracellular and intracellular DNA damage [3, 4], amino acid and nucleotide decomposition in aqueous solution [5], inactivation of viruses, microbes [6] and aberrant chromosomes in wheat [7].

The polyoxins, are antifungal antibiotic agents and nucleotide antibiotics which are produced by *Streptomyces cacaoi* var. *asoensis*[8,9]. In 1967, Isono found polyoxins A-N to be a new class of peptide nucleosides, they are effective toward certain phytopathogenic fungi especially of rice-plant diseases, because polyoxins are powerful inhibitors of chitin synthesis which are particular component of insect cuticle and fungal cell wall. [10,11]. Polyoxins are not toxic in crop, vertebrates and mammalian; as a result of they do not contain chitin.

2. MATERIAL AND METHODS

2.1 Microbe and Media

Streptomyces aureochromogenes NJYHWG 66382 is stored in our lab.

Slant medium: Potato 200 g/L, Sucrose 20 g/L and agar 20 g/L;

Plate medium: the same as slant medium;

Seed media: Glucose 10 g/L, soybean 10 g/L, KH_2PO_4 1 g/L, NaCl 1 g/L, $CaCO_3$ 3 g/L.

Fermentation medium: Potato 200 g/L, Sucrose 20 g/L.

2.2 Cultivation Conditions

The slant medium was cultured for 7 days at 28 ℃. Prepared spore suspension (OD_{600}=0.25) and inoculated in 250 ml flasks (working volume 50 ml). The flasks were incubated on a rotary shaker at 180 rpm and 28 ℃ for 36 h. Then transferred seed media to fermentation medium, and inoculation quantity was 3.0%. Fermentation broth was subjected to centrifugation (10000 rpm, 15 min, 4℃) to determinate the concentration of polyoxins by using HPLC analysis.

2.3 Preparation of Spore Suspension

The strain was activated on agarslantculture-medium. After 10ml sterile water was added, the spores were washed down and transferred into a triangular flask with some micro glass beads. The flask was stirred to form a spore suspension liquid for use.

2.4 N+ ion Implantation

0.1ml suspension liquid was transferred onto the sterile plate. After painted averagely and dried by sterile wind, it would form a strain pellicle. Microscopic examination was conducted to select the nonoverlapping to be N^+ implanted. In this experiment, 30keV N^+ was implanted with different quantity. The pressure of target chamber is 10^{-3}Pa. Every pulse lasted 10s and rested for 50s. The dose was 50×10^{15}ions/cm^2-300×10^{15}ions/cm^2. There was a control group in each condition. After N^+ implantation, the strain was eluted with 0.5ml sterile water and painted on plating medium. The culture temperature was 28℃.

2.5 Screening Conditions

The HPLC assay was performed on DIONEX P680, at the following conditions: at a flow rate 0.6 ml/min, 263 nm, Kromasil C18 (250mm×4.6mm×5μm), 30 ± 0.1 ℃, V(methanol): V(water)=10:90, sample size was 5μm.

2.6 Computing Methods

Survival rate (%) = the survival of the colony count with ion implantation / the control samples without ion implantation

Positive mutation rate (%) = the concentration of polyoxins (over 10% after mutation)/ the original concentration of polyoxins

Negative mutation rate (%) = the concentration of polyoxins (under 10% after mutation)/ the original concentration of polyoxins

3. RESULTS AND DISCUSSION

3.1 The Survival Rate

In our experiment, we chose N^+ as ion source and the energy was set at 30 KeV, 50×10^{15}, 100×10^{15}, 150×10^{15}, 200×10^{15}, 250×10^{15}, 300×10^{15} ions/cm^2 dose. The dose of nitrogen ion radiation to *Streptomyces aureochromogenes* NJYHWG 66382 cells on survival rate is shown in Fig. 1. The figure clearly showed that firstly in the range of 0-100 $\times10^{15}$ ions/cm^2 dose, with an increasing implantation dose, the survival rate rapidly decreased; then when the implantation dose increased from 100 $\times10^{15}$ to 150 $\times10^{15}$ ions/cm^2, the survival rate increased from 12.5% to a maximum of 41.7%; finally the survival rate decreased with the implantation dose increased. The survival curve exhibited a clear ‘‘saddle shape’’ curve with increasing of dose, which is endemism with the implantation dose [12].

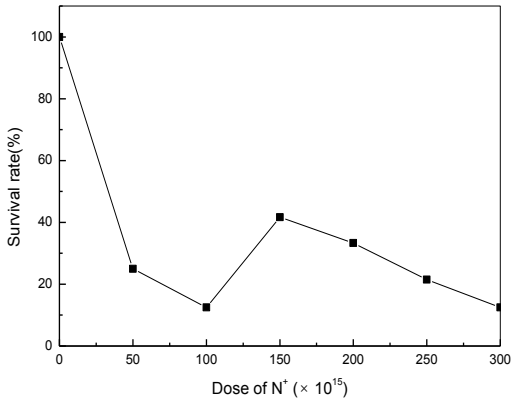

Fig. 1 The survival rate curve by N^+ beam implantation

Yu and colleagues [1] proposed that the interaction between low-energy ions and the strains was characterized by energy deposition, momentum transfer, mass deposition and charge neutralization. According to this theory, at the first period(0-100 $\times10^{15}$ ions/cm^2 dose), energy deposition caused cell surface damage and etch, and DNA, biofilm and other biological macromolecules are damaged by a large number of free radicals at the same time. With increasing dose (100 $\times10^{15}$ to 150 $\times10^{15}$ ions/cm^2), certain repair mechanisms (called SOS reaction) within the cell are activated, causing the survival rate to rebound. However, with the continuous increase of the implantation dose (up to 150 $\times10^{15}$ ions/cm^2), it will not cause permanent damage and can not be repaired, and the survival rate decreases. Therefore, we chose 150 $\times10^{15}$ ions/cm^2 as the appropriate dose for further mutation.

3.2 Effect of Different N+ beam Implantation Dose

Single colonies implanted in different doses of N+ ions were randomly chose and cultured with a shake flask (5 days), then the titer was measured. Calculated the mutation rates and graphically displayed in Fig. 2. In the implantation dose ranging from 50 $\times10^{15}$ to 300 $\times10^{15}$ ions/cm^2, we can found that the negative mutation rate is also higher than the positive mutation rate, which indicated the broad mutation spectrum. When the injected dose was 150 $\times10^{15}$ ions/cm^2, the negative mutation rate

and the positive mutation rate reached the maximum, so this dose was appropriate dose to obtain mutation strains needed.

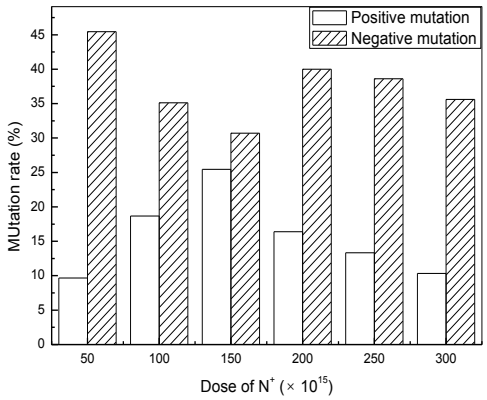

Fig. 2 Effect of N^+ beam implantation dose on mutation rate of *Streptomyces aureochromogenes* NJYHWG 66382

These results were consistent with the characteristics of plants and microorganisms ion implantation, which also had high mutation rates and the broad mutation spectra [13].

3.3 Screening Method for Strains with High-Yield

High-yield mutants were obtained at an energy level of 30 keV and dose of 150 × 10^{15}ions/cm^2. After screening, we got one high-yield strain. The yield of polyoxins was increased from 712 μg/ml to 1025 μg/ml. Meanwhile the fermentation time of mutant is shortened from 120 h to 72 h.

3.4 Stability Test of the High-Yield Strain

We obtained high-yield mutants which needed a good genetic stability. In order to evaluate the genetic stability of the screened high-yield laccase mutant strains, the strain was successively passaged for five generations, and the titer of polyoxins and the dry weight were shown in Fig. 3. As the Fig. 3 shows, after continuous passaging for five generations, the titer and dry weight were still remained at a higher level.

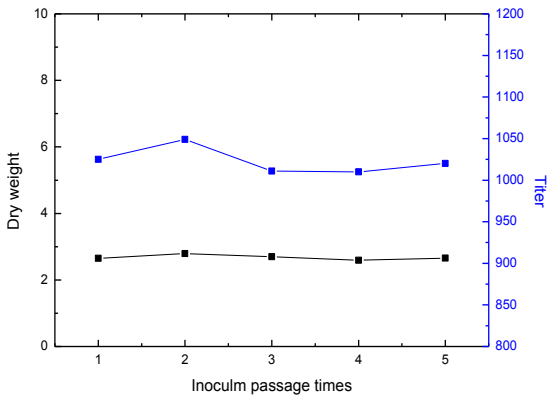

Fig. 3 The passage stability of mutant strain

3.5 The Fermentation Characteristics of the High-Yield Strain

The strain was transferred to fermentation medium (50/250ml), and the characteristics were determined every 24 h (shown in Fig. 4). It can be seen that during the whole fermentation stage, pH continues to rise; after 96 h, titer of polyoxins reached its maximum and remained stable; at initial stage, dry weight was relatively low and after 96 h cell weigh increased quickly.

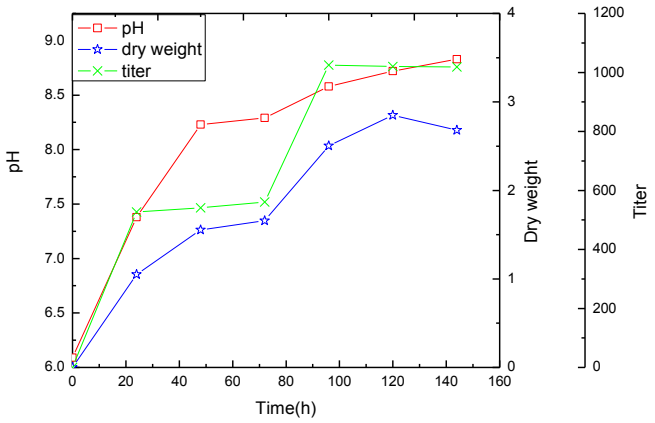

Fig. 4 The characteristics of high-yield strain

4. CONCLUSIONS

In our study, we chose the dose of 200 $\times 10^{15}$ ions/cm2 and energy of 30 keV as an optimal point, and one high-yield strain was gained. The concentration of polyoxins produced by the mutant strain was 1025 μg/ml higher than the original strain. After continuous passaging for five generations, the titer and dry weigh maintained at a stable level.

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Title: *Use Technological Innovation to Promote the Interactive Development of Industrialization, Urbanization and Agricultural Modernization*

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ABSTRACT

Industrialization, urbanization and agricultural modernization have become the basic social and economic development trend. The three aspects influence reciprocally, promote mutually, and develop jointly. The development of industrialization, urbanization and agricultural modernization has always been inseparable from the important role of scientific and technological progress. The weak technological innovation ability is an important constraining factor in their interactive development. There are important practical significance for the further implementation of the interactive development of China's industrialization, urbanization and agricultural modernization if we increase the technological investment and strengthen the technological innovation system.

1. SITUATION ANALYSIS OF INTERACTIVE DEVELOPMENT OF CHINA'S INDUSTRIALIZATION, URBANIZATION AND AGRICULTURAL MODERNIZATION

The meaning of the industrialized roughly into two categories: one category is narrower definition that industrialization is the industry (especially in manufacturing) as well as in the development of secondary industry employment increase in the proportion of the working population [1] ; another is a relatively broad definition, such as our famous economist Professor Zhang Peigang's definition that industrialization is "a series of essential economic production function (or a combination of factors of production) occurred in succession from lower to higher groundbreaking process of change."[2]. He thoughts the most important characteristic of it is the industry and agriculture's mechanization and modernization, is a technology revolution and the social productive forces unprecedented transformation; simultaneously it also contain the production organization and the national economy structure multi-level corresponding adjustments and the change and the share of agriculture gradually reduce in the national economy. Industrialization process will generally be divided into beginner,

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intermediate and advanced three phases. From 1949 to 1978, China relies on primitive accumulation way of unequal agro-industrial exchange and the centrally planned economic system, starting from more than 20 billion yuan, quickly build up around 500 billion yuan of state-owned enterprise fixed assets and laid the basis for industrialization [3], which represent that China has completed the primary stage of industrialization; Since 1979 carries out reform and open policy until now, Chinese industrialization entered the intermediate development phase, the industry and the entire national economy experienced a rapid growth process, the industrial structure is adjusted constantly and tend to coordination and the market economy system gradually formed; With the process of industrialization speeding up, China is expected will enter the advanced development stage of industrialization around the year 2020, which indicates China's industrialization task be fulfilled. Meanwhile, China's countryside industrialization process also include sudden appearance of township enterprises in the vast rural areas in 1980s later, which has profound significance to accelerate the process of urbanization and agricultural modernization.

Urbanization is a process that people in rural areas move into urban areas, rural areas evolve into urban areas and the urban population has been growing. Meanwhile the mode of production and lifestyle have transformed, which caused by the improvement of productivity in the rural areas [4].Based on the special national conditions, China should not blindly copy the urbanization model of developed countries and should develop small towns. Urbanization development patterns will be of great significance for china [5]. Because of the industrial cluster effect, industrialization and urbanization usually develop synchronously. Industrialization process in general will be accompanied by the transfer of agricultural labor force to the industrial, the rural population to urban. In order to form industrial chain and reduce facilities costs, industrial enterprises in geographically tend to be concentrated, and produce an aggregate economic efficiency, which promotes the emergence and development of the city. The urbanization is often the result of industrialization. In general, due to policy that before the reform and opening in China, the progress of urbanization is slow. Urbanization lags behind industrialization, and from 1952 to 1978 China's urbanization level increased from 12.5% to 17.9%, the average increase of only 0.21% every year. After reform and opening up, China's urbanization process is accelerating. From 1978 to 1998, urbanization rate increased to 30.4% from 17.9%, the average annual growth of 1.25%; from 1998 to 2007, China's urbanization rate increased from 30.4% to 44.9%, average annual growth of 1.61%. After that, urbanization rate basically stable at about 0.90%, China's urbanization rate is expected to reach around 52% in 2015 [6].

2. FUNCTIONS AND EXISTENT PROBLEMS OF TECHNOLOGICAL INNOVATION IN THE INTERACTIVE DEVELOPMENT OF INDUSTRIALIZATION, URBANIZATION AND AGRICULTURAL MODERNIZATION

Generally, the progress and innovation of technology play a decisive role in the interactive development of industrialization, urbanization and agricultural

modernization, which can be shown in the following aspects. Firstly, the progress of modern science and technology directly push forward the development of industrial and accelerated the process of industrialization, promote the interactive development between industrialization and urbanization. Secondly, the progress of science and technology and innovation is promoting the interactive development between industrialization and modernization of agriculture. On the one hand, the progress of technology can not only improve the level of industrialization, but also the level of agricultural mechanization, extensive application of industrial technology in agriculture has opened up a broad road for the modernization of agriculture. On the other hand, the extensive application of modern science and technology in modern agriculture and the pursuit of the fine varieties automation, scientific management, operating corporatization and standardization, all put forward more new topics for industrial development and technological progress and expand a broad space for the expansion of industrialization connotation and higher standards. Thirdly, modern science and technology directly promoted the development of industrialization, urbanization and agricultural modernization. Modern agriculture is the industry which built on the foundation of increasingly developed industrialization, not only the biotechnology, computer technology, electronic technology, laser technology, remote sensing technology, information technology, atomic energy of modern high-tech technology that urban industrial give impetus to is widely used in agriculture, but there are also a large number of urban industrial production of industrial and agricultural production, the development of new agricultural technology sector and industrialization and modernization of agriculture to development activities.

China's science and technology problem in promoting interactive development among agricultural modernization, urbanization and industrialization include the following several aspects. First, China's industrialization model is more extensive, technological innovation capability is not strong. At present, the traditional industries' proportion is large but the high-tech industries' is small in China's industrial system. China has more serious pollution and high energy consumption industry and china's extensive feature of industrialization is very significant; industrial enterprises lack of technological innovation ability and there is a common phenomena existing in industrial enterprises that they rely on high investment return for high output.

Secondly, China value speed over quality in the process of urbanization, urban construction and economic development has low-tech. Since reform and opening up, especially after 1990s, urbanization speed and scale have experienced a fast development phase. However, China's urbanization process has also revealed many contradictions and problems, and focus on the following areas: on one side, speed and quality of urbanization serious lack of coordination that paying attention to quantity but despising quality, excessive expansion of large cities, small cities and small towns deficiency, land urbanization faster than population urbanization. On the other side, the urban economy growth way is extensive, the development efficiency is low, the resources bottleneck restraint strengthens day by day, all of which will directly impact on the improvement of urban industrial competitiveness and comprehensive competitiveness.

Thirdly, agricultural modernization technical support system is imperfect and the agricultural labor force's cultural and technical quality is low. On one hand,

china still has a big gap compared with developed countries in the agricultural technology aspect. China's contribution rate of agricultural science and technology is only 40% while developed countries is about 80%; China's agricultural science and technology achievements transformation rate is about 30%~40% whereas the developed countries reach 60%~80%; china also has a noticeable gap compared with developed countries in the grain yield, crop seed coverage, utilization of fertilizers, irrigation water use efficiency, feed conversion rate and other aspects. On the other hand, China's agricultural labor force as a whole has low culture and technical quality which increase the difficulty of agricultural modernization construction. Furthermore, China's lower agricultural information lever and imperfect information service system have restricted the development of modern information agriculture Compared with foreign advanced level.

3. STRENGTHENING INNOVATION OF SCIENCE AND TECHNOLOGY TO FURTHER PROMOTE CHINA'S INDUSTRIALIZATION, URBANIZATION AND AGRICULTURAL MODERNIZATION INTERACTIVE DEVELOPMENT

(1)Choose the new development of industrialization and to push industrialization, urbanization and agricultural modernization interaction and coordinated development. The so-called new industrialization pattern is to take the industry technological innovation as a method, update the industrial structure, labor allocation, the level of urbanization is promoted to the target, Change the mode of industrial development as soon as possible. Using high technology to transform traditional industries, eliminate backward technology, high energy consumption and serious pollution industries .With the development of new types of low energy consumption, low pollution, low emissions and high efficiency industry to achieve the development of high technology industry and low carbon. We should rely on technological progress to improve industrial production and management level and realize the sustainable development of industrial economy. At the same time, new industrialization pattern is also process of the urbanization and agricultural modernization, the key is the ability to improve the carrier capacity of the industry. Through the promotion of urbanization expand employment, through the transformation and absorption of a large number of rural surplus labor force speed up the process of agricultural modernization. So we must increase our investment in science and education, implement strategy of invigorating the country through science, technology and education actively, improve the broad masses of workers' cultural and technical quality, constantly improve the technological innovation system and promote high-tech research and effective conversion.

(2) Developing the high and new technology industry positively to achieve industrialization, urbanization and agricultural modernization interaction development by technical system innovation impetuses. At present, China is undergoing a historical process which of rapid industrialization, urbanization and agricultural modernization and is faced with the pressure of resources, environment and population. It is hard to continue the traditional technology system to promote the extensive mode of development. We need to use technological innovation system as the driving force and high-tech as the support to accelerate the strategic transformation of development to achieve sustainable economic and social

development through the intensive development pattern of industrialization, urbanization, and agricultural modernization. The information technology which is represented by photonics, micro-electronics, computers, networks, software and communications technology and the high and new technology which is represented by bio-engineering technology life technology, nanotechnology, spatial technology, remote sensing technology, new material and new energy technology and so on are widely used in developed countries' progress of resource exploration and development, environmental monitoring, governance, industrial and agricultural production and rural economic development process, and improve the productivity and quality of economic development greatly. Secondly, lead the urbanization by technology and innovation and promote industrialization and agricultural modernization by urbanization. Industrialization needs to be based on urbanization and agricultural modernization needs urbanization to push. Speeding up urbanization can solve the problems from the second, the three industrial development and "three agriculture" better, pulling the agriculture surplus labor force transfer to the towns, and enhance the effects of urbanization to rural areas. Therefore we must strengthen the technical element and the technology content of urbanization, promote the quality of urbanization, speed up the construction of high-tech Industrial Park, Industrial Park, and Science and Technology Park Construction, foster urban Strategic emerging industries and new and high-tech industries. Adhere to the way which is based on science and technology and make the most use of the function from urbanization to industrialization and agricultural modernization.

(3) Developing information technology and service industry vigorously to lead the Interactive development of industrialization, urbanization and agricultural modernization. First, it is needed to realize the integration and penetration between informationization and industrialization. With the formation of a buyer's market, many industry products encounter sales dilemma. If enterprise can grasp the trend of informatization and actively improve the level of enterprise informatization and timely understand the technical and market information and apply related information technology to industrial product design, construction, management and service, it will seize the opportunities and enhance business competitiveness in the market. Secondly, we should continuously improve the urban information level. It is necessary to continuous increase the city's information industry input, improve information and communications infrastructure in cities and towns, reinforce software and hardware construction and work hard to build the city "information superhighway". Thirdly, we should raise the level of agricultural information and actively develop modern information agriculture. Our country must strengthen the countryside information infrastructure construction, perfect countryside information database, establish and perfect agricultural water quality monitor and plant disease preventing and controlling, improve rural labor force employment guidance information service system and so on so as to provide good information infrastructure conditions for agricultural production and management[7]. Simultaneously Our country should speed up raises the needed talent in information technology, management and services areas , promote information service in rural and speed up the pace of transition from traditional agriculture to modern agriculture and finally lead the development of agricultural modernization, urbanization and industrialization by strengthening the agricultural informatization construction.

(4) Developing modern agriculture technology vigorously and enhancing the cultural and technical quality of the farmers. First of all, we should identify the basic features of modern scientific agriculture, take practical measures, raise the level of agricultural science and technology and implement the science and technology strategy [8]. At the international level there is a large gap between china and developed counties in agricultural technology inputs, agricultural technology research capacity, technology reserves, and agricultural technology results' transformation rate and contribution rate at present. We must increase investment in agricultural science and technology, establish innovation system of it and intensify agricultural research and introduction of high-tech jobs, by all of these to promote the development of agricultural modernization. At the national level, at present China's agricultural modernization lag behind the industrialization and urbanization significantly modern science and technology play a due role in the development of agriculture. Therefore, it is needed to actively implement the science and technology strategy, further improve agricultural mechanization equipment level. Secondly, it is necessary to strengthen technical training of the agricultural labor force and gradually increase the peasants' cultural and technical quality. Only the agricultural science and technology achievements be available to farmers can it to be translated into reality productivity. The agriculture modernization requests the worker to grasp the advanced modern agriculture science and technology knowledge and has scientific farming, cultivation and management ability. In view of the fact that the overall quality of our country's agricultural labor force is somewhat low, the government must continue to increase investment in technical training to farmers, conduct various forms of agricultural technology, vocational skills and labor force training.

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Cover page

Title: *Content Determination of Total Saponins from Opuntia*

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ABSTRACT

Objective: To establish for content determination of total saponins in *Opuntia*. Methods: Total saponins was determined by UV-VIS spectrophotometry after extracts of the sample had been coloured. Results: The methods was linear in the range of 0.05~0.30mg (r=0.9987), and the average recovery was 98.47%, RSD was 3.29% (n=5). Conclusion: The methods is sensitive, reliable, simple and reproducible for the determination of the contents of saponins in *Opuntia*.

INTRODUCTION

Opuntia dillenii Haw. is a herbal plant belonging to genus *opuntia* in cactaceae family. The plants of *Opuntia* have a wide distribution in the world. In China, its stems are used as a folk medicine for the treatment of diabetes, gastric ulcer, mastitis, parotitis and several other diseases[1]. More and more present pharmacological studies have revealed that the plants of *Opuntia* had a lot of interesting biological effects such as anti-inflammatory, anti-diabetes[2]. *Opuntia dillenii* Haw. contains a variety of more complex chemical composition, they are organic acids, sterols, flavonoids, sugars, fatty acids, alkaloids, and terpenes[3]. Saponin compounds in the Cactus is one of its active ingredients[4], have significant analgesic effect[5].

In this paper, on the bases of previous research of total of *Opuntia* saponins and the facilities of the laboratory, a colorimetric method for quantitative analysis of total of *Opuntia* saponins. Given there are no statutory reference substance saponin constituents of a cactus, we picked oleanic acid to the reference substance, using spectrophotometric method for determination of total saponin content of cactus, so as to lay the foundation for use of in-depth development of cactus.

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APPARATUS AND MATERIALS

Appartus

Electronic balance (Shanghai Balance Instrument Factory, FA2104), Centrifugal machine (BECKMAN company), Vacuum rotating evaporation instrument (Shanghai Senco machinery Institute, R-501), Thermostat water bath (Shanghai Senco machinery Institute, R501), ULtrascan2000 UV-vis spectrophotometer(Amersham Pharmacia Biotech), DRAGON Pipette (large Dragon medical equipment (Shanghai) limited), Small vacuum pump, Buchner funnel, filter, dryer, etc.

Reagents and Materials

Oleanolic acid (Abbreviation OA, purity >95%, National Institute for the Control of Pharmaceutical and biological Products, lot number: 110709-200304), industrial alcohol; methanol (Tianjin NO.3 Chemical Reagents Factory), perchloric acid (70%~72%) (Shanghai Taopu Chemical Industrial Factory), vanillin (Tianjin Kemiou Chemical Reagent Co., Ltd.), glacial acetic acid (Tianjin NO.3 Chemical Reagents Factory), petroleum ether (60~90℃) (Tianjin NO.3 Chemical Reagents Factory), n-butanol (Tianjin NO.3 Chemical Reagents Factory), Cactus collected from Kunming, deionized water.

METHODS AND RESULTS

Extracting Total Saponins from *Opuntia*

The dried cactus powder(5.0 g) were placed in flasks of 250 mL, soaked for 12 hours with 100mL 70% aqueous EtOH, extracted for three times at 80 ℃ in a water bath, 2 hours each. The combined solution was concentrated under reduced pressure to about 5 mL. The residue was and transfered to separatory funnel, and extracted with petroleum ether (60~90℃) for three times(per 10 mL) to give petroleum ether extract. The petroleum ether extract was washed a small amount of water, discarded the ether layer, merged wash water and water phase to give water extract. The water extract was enriched to approximately 5 mL. The concentrated liquid was put in a separatory funnel, using water saturated butanol to extract till colorless, discarded water layer to give n-butanol extract. The n-butanol extract was concentrated in water bath. The residue was dissolved with methanol to transfer 25 mL volumetric flask, constant volume, shake, give 200mg/mL ethanol extract of cacti.

Determination of Total Saponins Content

PREPARATION OF REFERENCE SOLUTIONS

The reference substance oleanic acid（10.0 mg）were placed in a 50 mL volumetric flask, dissolved in methanol, diluted to scale and shaked to give the concentration of 0.20 mg/mL oleanic acid reference substance solution.

DETERMINATION OF WAVELENGTH CHOICE

0.20 mg/mL oleanic acid reference substance solution(0.25mL) and 200mg/mL samples ethanol extract of cacti (0.10mL) were put in tube with plug, waved dry solvent on water bath, joined 0.2 mL new preparation of 5% vanillin-ice acetate solution, dissolved and joined 0.8 mL perchlorate, shaked uniform, heated 15 min at 60℃ water bath. Then the tube was removed and cooled with water to suspended reaction. 5 mL glacial acetic acid was joined in the tube, shake uniform, accompanying blank reagents was regarded as comparison, was scaned for 400-700 nm wavelength range. Oleanic acid reference substance at 548 nm had maximum absorption, samples has not maximum absorption at 548 nm, but has strong absorption, so choosed 548 nm as the determination of the wavelength. The measured results were calculated total saponin content as oleanic acid for the baseline.

PREPARATION OF STANDARD CURVE

Oleanic acid reference substance solution was be measured accurately 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 mL were put in 10 mL 7 tube with plug respectively. Methanol was volatilized at water bath, again joined 0.2 mL new preparation of 5% vanillin-ice acetate solution and 0.8 mL perchlorate, shaked uniform, heated 15 min at 60 ℃ water bath, removed, cooled by water to room temperature, again joined glacial acetic acid 5 mL, shake uniform, accompanying blank reagents was regarded as comparison, measured absorbency by the ULtrascan2000 UV-vis spectrophotometer at 548 nm, then drawed standard curve to give regression equations: A=6.425 C-0.0016, r=0.9987 (n=7). The results showed that the linear relationship is right in the range 0.05~0.30 mg/mL.

METHODS OF STUDY

Stability test

Oleanic acid solution and sample solution were drawed 0.25 mL respectively. Absorbance values were measured under the same conditions depending on the method of preparation of standard curve, accompanying blank reagents was regarded as comparison, measured absorbency at 548 nm, each 10 min determination once absorbency, total 100 minutes, the results are shown in table 1. The results indicated that the generated of colored materials were stability which the color reagents and oleanic acid and samples ethanol extraction solution reacted in 100 minutes. Operation quickly could be used as quantitative analysis, and reliable degree is high.

TABLE 1 THE RESULTS OF STABILITY EXPERIMEBNT

Color Time(min)	0	10	20	30	40	50	60
Standard sample Absorbency	0.303	0.302	0.299	0.309	0.307	0.302	0.300
Sample Absorbency	0.320	0.320	0.318	0.314	0.319	0.320	0.318

Precision test

Oleanic acid solution and sample solution were drawed precision 5 pieces, each 0.25 mL. Absorbance values were measured under the same conditions depending on

the method of preparation of standard curve. The results are shown in table 2, precision RSD<5%, indicating good instrument precision.

TABLE 2 THE RESULT OF PRECISION EXPERIMENT

NO	Sample Volume (mL)	Absorbency	RSD (%)	NO	Sample Volume (mL)	Absorbency	RSD (%)
1	0.25	0.304		1	0.25	0.330	
2	0.25	0.302		2	0.25	0.320	
3	0.25	0.298	2.09	3	0.25	0.305	2.82
4	0.25	0.296		4	0.25	0.316	
5	0.25	0.288		5	0.25	0.318	

Reproducibility test

5 same medicine powder (5.0 g) were extracted by ethanol according with 2.1 to give cactus sample solution. Accurately imbibed 0.25 mL sample solution, absorbance was determinated in the light of the standard curve, the results are shown in table 3, reproducibility RSD=4.06% (n=5).

TABLE 3 THE RESULT OF REPETITION EXPERIMENT

NO	Sample Volume(mL)	Absorbency	Average	RSD(%)
1	0.25	0.330	0.326	4.06
2	0.25	0.326		
3	0.25	0.320		
4	0.25	0.344		
5	0.25	0.308		

Recovery test

Precision drawing on a known concentration of the sample solution 5 parts, each part was 100 μL, precise joined 50,100,150,200,250 μL of standard solution respectively. Then, absorbance values were measured by standard curve method at 548 nm, results are shown in table 4. The results indicated that the average recovery rate reach to 98.47%, RSD=3.29% (n=5).

TABLE 4 THE RESULTS OF RECOVERY RATE EXPERIMENT

No	Total quantity of saponins in sample(μg)	Added quantity(μg)	Analysis result (μg)	Recovery rate(%)	Average (%)	RSD (%)
1	21.0	10.0	31.25	102.50		
2	21.0	20.0	39.83	94.15		
3	21.0	30.0	50.36	97.87	98.47	3.29
4	21.0	40.0	59.86	97.15		
5	21.0	50.0	71.35	100.7		

Sample size determination

5.0 g Cactus powder was extracted by ethanol to give sample solution according to 2.1. Absorption was mersured by standard curve method, the contents of total quantity of saponins in sampLe was calculated, the results are shown in table 5.

TABLE 5 THE CONTENTS OF TOTAL QUANTITY OF SAPONINSIN SAMPLE

No	Total quantity of saponins in sample (mg/g)	RSD(%)
1	0.254	
2	0.265	
3	0.250	3.06
4	0.251	
5	0.244	

DISCUSSION

Determination of total saponins had spectrophotometry and HPLC method, due to instrument is expensive in the method of HPLC, measurement methods is complex. Spectrophotometric is easy, operation is simple, reproducible and suitable for field determination and routine analysis.

Currently, because there is no statutory reference substance about saponin constituents of a cactus, so the structure and selection of Oleanic acid triterpene saponin from cactus near could be as reference substances. The total saponin content in cacti was measured using Spectrophotometric method. While test results had a little difference, their principle of showed colour was consistency, therefore, the method is still a better choice, in the case of lack of standards provisional.

Extracts used in the test, after degreasing by petroleum ether and excepting small molecules of sugar by n-butanol extraction, the reduced the interference of impurities. Because of vanillin-glacial acetic acid reagents is more sensitive with the color reaction of saponins from *Opuntia.* Chromogenic reagent is prepared prior to the use. But experimental results is affected by temperature and time, thus rendering time and temperature should be strictly controlled. Before joining the glacial acetic acid and determination of absorbency, test tubes must be with water cooling to keep the timely termination of reaction and determination in a timely manner. Before the heating components to show color, tubes must be shake, or experimental error is more. Test tubes must be fully dry, otherwise results are disturbing.

Determinated of total saponins in the samples, the color is stability within 30~60min, linear range is 0.05~0.30mg (r=0.9987), RSD of precision test is 2.09%, an average recovery rate is 98.47%, which is indicatd that these photometric precision, reproducibility, stability is better, and the saponins is without larger losses in extract purification, you can ensure that the final determination of the validity of the results.

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Cover page

Title: *The Study of Using both Ultrasonic Method and Fluid Shear Method to Disrupt Sludge*

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ABSTRACT

The effect of the sludge disrupted by ultrasonic method or fluid shear method separately under the same energy consumption shows that: the fluid shear method has high efficiency in the early stage of the sludge disruption, but the ultrasonic method has higher efficiency in the later stage. Instead, it has lower efficiency when using fluid shear simultaneously with ultrasonic. A comparing to using the ultrasonic method or the fluid shear method separately shows that, using the joint method-fluid shear in first 1 minute followed by the ultrasonic -will significantly improve the efficiency of sludge disruption.

INTRODUCTION

Sludge is the product of sewage treatment. At present, according to the statistics of whole sewage flow in china, experts estimate that it will reach to twenty million tons of sludge flow per year in 2015[1]. Sludge includes a lot of water, causative agent, heavy metal and zooglea consisting of microorganism and so on. It is very helpful to wipe off the causative agent, toxic and harmful matter and stink by making proper treatment and disposal on sludge[2]. The sludge treatment and disposal must follow two principles: one is making full use of the organic matter and all kinds of nutrient elements, the other is realizing the energy recycling. The resource of excess sludge exists in the zooglea. Sludge disruption can release the organic matter and reback it to the soil. This makes it possible to enhance the efficiency of anaerobic digestion, which recycles the energy in the sludge expediently. So excess sludge disruption is a necessary way to realize the hazard-free treatment, innocuity and reclamation during sludge treatment[3,4]. At present, there are many methods to realize sludge disruption, such as physical methods, mechanical methods, chemical methods, biological methods and joint methods[5]. This article provides a joint method to disrupt sludge by both ultrasonic method and fluid shear method. The article also compares this joint method with using ultrasonic method or fluid shear method separately, and makes it possible to find a way to disrupt sludge efficiently to reduce energy consumption.

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THE STUDY OF THE EFFECT OF SLUDGE DISRUPTED BY ULTRASONIC METHOD OR FLUID SHEAR METHOD SEPARATELY

The sludge in this research takes from Shenshuiwan sewage treatment works in Shenyang Province. In order to comparing the efficiency of using ultrasonic method or fluid shear method at one time, this research takes a contrastive experiment using a same batch of sludge, in which the MLSS(mixed liquor suspended solids) is 14248mg/L, and the average median diameter is about 120um. This research uses GM-1200D ultrasonic processor provided by Shunma equipment Co. Ltd. The ultrasonic form of this equipment is pulse mode, and the power is from 0 to 1200W. When using ultrasonic method to disrupt the sludge, firstly the 500ml activated sludge was poured into a 1000ml beaker, and then the amplitude transformer was extended into the mixed liquor about 1.5cm to the level. This research takes an organization compactor as the fluid shear equipment, which is produced by Waring company in America and provided by Lingchu scientific LLC. The type of this equipment is HGB550, the rotate speed of it is 2400r/min, and the capacity of it is 2000ml. In this equipment there are several rapidly rotating vanes that general strong shear stress during shearing the sludge to achieve sludge disruption. The current-detected by a Frank 512 ammeter-is 1.8A when using this organization compactor to disrupt 500ml sludge. As a result the power consumption should be 400W. The frequency of the ultrasonic processor is 20kHz and the pulse ratio is 3:1. The rate of energy and ultrasonic power conversion of this ultrasonic processor is 80%, then choose the ultrasonic power of 320W. Based on the above, the power consumption is also 400W when using this ultrasonic processor to disrupt 500ml sludge. In the same conditions of the energy consumption of these two kinds of sludge disruption methods, this research finished sludge disruption by ultrasonic method or fluid shear method separately. The breaking time of those two methods is 1 min, 2 min, 3 min, 5 min and 10 min. Then analyzing the COD, protein, TP, nitrate, the concentration of nucleic acid and peptidoglycan and the DD_{COD} [6]of the supernatant of the disrupted sludge which centrifuged by the centrifugation. These can be showed by the following formula: $DD_{COD} = \left[\frac{COD_{Ultrasound} - COD_0}{COD_{NaOH} - COD_0} \right] \times 100\%$ [7]，Where $COD_{Ultrasound}$ is the SCOD of the disrupted sludge, COD_0 is the SCOD of the sludge before disrupted, COD_{NaOH} is the alkaline hydrolysis deliquescent COD. The comparative result of the concentration and the DD_{COD} of the sludge disrupted by ultrasonic and fluid shear stress separately has been shown in Table 1.

In Table I, it is seen that the effect of sludge disrupted by fluid shear in 1 to 3 minutes is better than that of sludge disrupted by ultrasonic; while the effect of sludge disrupted by ultrasonic in 3 to 10 minutes is better than that of sludge disrupted by fluid shear. This means that using fluid shear to disrupt the larger particles zoogloea into smaller particles has a significant efficiency. Excess sludge disrupted by ultrasonic has two steps: destruction of sludge flocs and cell lysis[8]. They occur simultaneously at initial time, but the destruction of the sludge flocs is the dominant role. The analysis of the process of sludge disruption shows that the efficiency of excess sludge disrupted by fluid shear followed by ultrasonic will be improved.

TABLE I. THE COMPARISON TABLE OF EACH INDEX OF SLUDGE DISRUPTED.

Breaking time/min	COD density/mg/L	DD_{COD} /%	TP density /mg/L	Protein density /mg/L	Nucleic acid density/mg/L	Brotland density /mg/L	NitrateN Density/mg/L
ultrasonic 1	702.5	2.1	43.5	168.0	68.8	194.7	6.1
fluid shear 1	802.5	3.0	61.2	211.3	83.4	236.5	8.1
ultrasonic 2	815.0	3.1	69.7	294.0	116.1	312.9	11.8
fluid shear 2	927.5	4.1	64.2	315.3	123.3	338.3	10.5
ultrasonic 3	1202.5	6.6	72.3	453.3	157.4	424.7	15.9
fluid shear 3	1077.5	5.5	75.0	388.7	150.5	389.2	13.1
ultrasonic 5	1690.0	11.1	87.3	686.7	233.1	590.1	23.7
fluid shear 5	1227.5	6.9	75.2	324.0	110.1	267.4	11.0
ultrasonic 10	2027.5	14.2	103.6	793.3	263.2	651.0	34.1
fluid shear 10	1315.0	7.7	74.6	437.3	162.4	429.2	17.6

THE EXPERIMENTAL STUDY OF FLUID SHEAR COMBINED WITH ULTRASONIC TO DISRUPT EXCESS SLUDGE

The same batch of excess sludge is taken to do a couple of comparative experiments. The excess sludge is contrastive by three methods: fluid shear alone, ultrasonic alone and a joint method-one minute of fluid shear followed by ultrasonic. The experimental conditions of fluid shear and ultrasonic are consistent with the experimental conditions above. The liquid supernatant of excess sludge disrupted by the three methods are measured to obtain the value of various indexes. They are listed in Table II.

TABLE II. THE VALUE OF VARIOUS INDEXES OF THE LIQUID SUPERNATANT.

Breaking time /min	COD Density /mg/L	DD_{COD} /%	Brotland density/mg/L	Protein density/mg/L	Nucleic Acid density/mg/L	TP Density /mg/L
ultrasonic 3	1202.5	6.6	424.7	453.3	157.4	72.3
fluid shear 3	1177.5	6.4	389.2	308.7	150.5	75.0
fluid shear 1 + ultrasonic 2	1302.5	7.5	347.4	452.7	138.6	66.7
ultrasonic 7	1465.0	9.0	448.3	609.3	188.0	88.6
fluid shear 7	1240.0	7.0	320.1	640.3	137.3	79.5
fluid shear 1 + ultrasonic 6	2327.5	16.9	580.1	668.0	230.5	110.5
ultrasonic 10	2027.5	14.2	651.0	686.7	263.2	98.2
fluid shear 10	1427.5	8.7	429.2	324.0	162.4	74.6
fluid shear 1 + ultrasonic 9	2377.5	17.4	829.2	874.0	297.0	103.2

In table II, the result of excess sludge disrupted by fluid shear followed by ultrasonic in three minutes is better than that of excess sludge disrupted by both fluid shear alone and ultrasonic alone. The components of excess sludge disrupted by fluid shear is mainly micro-particles. Sludge is more homogeneous and more continuous.

This facilitates the transmission of ultrasonic and the full use of energy. Simultaneously, micro-bacteria micelles and bacteria are easier to fill up interaction space of ultrasonic bubbles uniformly because of the homogeneous of excess sludge, so the cavitation effect has been effectively used. The excess sludge disrupted by fluid shear can greatly enhance the efficiency of ultrasonic cavitation.

THE EXPERIMENTAL STUDY OF FLUID SHEAR SIMULTANEOUSLY WITH ULTRASONIC TO DISRUPT EXCESS SLUDGE

In order to investigate whether it has a better effect that fluid shear simultaneously with ultrasonic to disrupt excess sludge or not, a couple of comparative experiments were did: ultrasonic to break sludge alone and a joint mothed- fluid shear simultaneously with ultrasonic to break sludge . The experimental conditions are consistent with the experimental conditions above. When making the experiment, the ultrasonic probe of ultrasonic cell disruption equipment was fixed on foothold. A side of probe was put in organization compactors, then the both two machines started to disrupt excess sludge. The two machines had the same electrical power. The breaking time was 2, 4 and 6 minutes when ultrasonic was used to disrupt excess sludge alone and that was 1, 2 and 3 minutes when the joint method was used to disrupt excess sludge. The value of various indexes of broken excess sludge are listed in Table III.

In Table III, it is seen that the effect of excess sludge broke by fluid shear simultaneously with ultrasonic is worse than that of excess sludge broke by ultrasonic alone. The reason of the poor effect is that growth and collapse of the cavitation bubbles need a certain time, the cavitation bubbles have not enough time to growth and collapse because of the strong fluid shear stress caused by fluid shear, so the ultrasonic cavitation effect is poor.

TABLE III. THE VALUE OF VARIOUS INDEXES OF BROKEN EXCESS SLUDGE.

Breaking time /min	COD/mg/L	Ammonia N Density/mg/L	Protein density/mg/L	Nucleic acid density/mg/L	Brotland /mg/L	Nitrate N /mg/L
ultrasonic 2	915.0	7.2	191.3	60.7	84.7	10.4
both 1	840.0	6.4	158.0	52.8	65.6	11.6
ultrasonic 4	1015.0	9.3	344.7	97.9	163.8	15.1
both2	927.5	6.8	198.0	58.1	72.0	13.8
ultrasonic 6	1452.5	10.5	440.7	135.2	205.6	18.7
both3	1227.5	9.4	236.7	78.1	113.8	17.5

CONCLUSIONS

(1) By comparing the effect of excess sludge disrupted by fluid shear or ultrasonic in the same conditions of energy consumption, it is obvious that the effect of

excess sludge disrupted by fluid shear is better than that by ultrasonic in the first three minutes. Fluid shear has a high efficiency when breaking large sludge flocs.

(2) By comparing the effect of excess sludge disrupted by three methods as followed: fluid shear followed by ultrasonic, fluid shear alone and ultrasonic alone. The first method can improve the breaking efficiency.

(3) By comparing the effect of excess sludge disrupted by three methods as followed: fluid shear simultaneously with ultrasonic, fluid shear alone and ultrasonic alone, the first method has a low breaking efficiency.

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Title: *An Assessment of Farmers' Response to Price Factor: Evidence From Sierra Leone Rice Cultivation*

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ABSTRACT

The aim of this paper was to intensify the awareness of the specification and evaluation of rice acreage response and to offer an apparatus for agricultural policy assessment. The ordinary least squares (OLS) procedure was applied to determine the coefficients of acreage response models for specific rice varieties. The magnitudes of the coefficients of both the ROK and NERICA lagged acreages were found positive and highly significant, which indicates that farmers' adjustment rate was very low. Concerning lagged actual price for both the ROK and NERICE varieties, the short-run price elasticities were lower than long-run, which is suggesting a long term adjustment of the acreage under the crop.

Nevertheless, the seeming recommendations for policy modification are to open farm gate prices and to reduce government's involvement in agricultural sector particularly in the acquisition of agricultural inputs. Forthcoming research need to be focus on how this might be better realized. Necessary support should be made available to private sector by means of curtailing price volatility. In agreement with structural reforms, it is necessary to transfer output prices to cultivators with minimum distortion. There is need to eliminate price subsidies and control, which create distortions in the market as well as huge financial costs.

Keywords: Acreage response, Nerlovian model, rice varieties, Sierra Leone

INTRODUCTION

Agricultural sector is the backbone of most emerging nations and is the major crops producer, and therefore plays an essential role in the development of these nations. It accounts for a substantial share of the gross domestic product[1], creates job opportunities for the labor force and it is the main source of livelihood of the farming communities in most developing countries.

However, there are several views to support the conception that most farmers in emerging countries such as Sierra Leone failed to respond to price incentives.

Current studies for most of these countries at the farm-crop level have relatively arrived at analogous conclusion that the supply response is however, less elastic. The causes cited for the poor response are financial limitations, infrastructure, irrigation,

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agricultural policies among others.

Newbery [2] and Anderson[3] studied farmers' response concerning resource allocation in some emerging nations(Sierra Leone inclusive) to the eventual changes in prices. Their findings disclosed that farmers had been determinedly adjusting their resource allocation such as land as well as capital among crops in response to corresponding changes in prices and appropriately supported the hypothesis that farmers' do response to price incentives in their traditional agricultural activities. Similarly, studies based on cross sectional data both by Hayami [4] and Peterson[5], suggested that most efficient allocation of several agricultural inputs are not properly managed by most farmers for increasing agricultural production through the reallocation of specific resources.

Sierra Leone 's agricultural sector has not observed a significant growth during the past three decades both in the factor along with product markets, instead it has observed many unsuccessful policy changes, particularly changes in the structure of the market incentives which farmers consider very challenging.

Nonetheless, several of these variations have to do with crop specification since there have been prevalent disparities in major changes in the incentive scheme. Equally, the performance of some crops in a particular period simulated wide discrepancy, conceivably in response to varying incentives. Therefore, this study attempts to evaluate the performance of rice crop in Sierra Leone concerning cultivated area in addition to the contributions of market incentives relating to the problem under consideration. Nevertheless, the unsatisfactory status of the crop's cultivation in Sierra Leone is supported by numerous problems regarding the flared supply along with the demand for the crop within the country.

Subsequently, this has prompted the government to use huge money on importation of the grain that should have been locally cultivated with less amount.

In the mid 1960s and late 1970s, Sierra Leone was rice grain self-sufficient and it was even exporting some key agricultural crops such as cocoa, coffee and rice to receive foreign exchange. However, negligence by state authorities in the mid 1980s lead the country to the over reliance in rice importation. Consequently, there exist a wide food supply-demand difference and increasing food importations expenses.

Despite that, there exist a huge empirical literature concerning agricultural supply response. Some of the scholars that have made major researches on agricultural supply response include Huang[6],Peterson[7], Gardner[8], Shonkiler[9], Bockstaell[10] and Bond[11], Thus, the aim of this study is to strengthen the awareness of the specification and evaluation of rice acreage response and to offer a gadget for the agricultural policy review. The precise objectives of this research are as follows: to measure the acreage response of rice within the period 1980-2011, utilize a realistic new dynamic procedure to solve the problem under consideration, estimate in addition to compare both short and long-run price elasticities, and lastly to identify the factors that have been distressing crop supply response in Sierra Leone within 1980-2011.

DATA SOURCE AND MODEL SPECIFICATION

Data Sources

Data on yield, area and prices of some crops during the years were collected from the Food and Agricultural Organization database (FAOSTAT, 2012).Others were

collected from government agencies in Sierra Leone such as, the Ministry of Agriculture, Forestry and Food security, the Sierra Leone Agricultural Research Institute (SLARI) and Statistics Sierra Leone (SSL). Furthermore, the study sourced information from published papers in reputable journals. The data set covers form 1980-2011 period.

Model Specification

In this subsection, the sources, nature and limitations of the data along with specification concerns are considered. Additionally, the empirical evaluation of this study will be supplemented with a sample of annual data for the rice crop varieties for the period 1980-2011.

Here, two rice crop varieties are considered and these include the NERICA and ROK varieties, which are disseminated to all farmers by experts from the Sierra Leone Agricultural Research Institute (SLARI). These varieties have specific advantages ranging from the high yielding potential, short life span, and high protein content to palatability.

However, there are a few additional crops that compete with rice in many specialized rice crop growing areas within the country on account of technical restrictions and agro climatic environments. Nevertheless, there are several areas where rice crop faces strong challenge from other crops. Furthermore, there is competition between NERICA and ROK varieties concerning land use and other resources similarly in specific rice growing areas.

Besides the financial aspect as revealed by the prices of certain rice grains, several other factors such as the agricultural inputs, infrastructural development, support from various institutions, economic advantage of challenging crops, etc., influence on farmers' allocation of existing resources to a particular crop. Several estimated models, utilized the area cultivated by the crops as the dependent variable. However, area cultivated has been preferred to production, since farm production is equally influenced by climatic situations that cannot be controlled by farmers. Also, yield is an issue of random variation than cultivated area because of specific factors that cannot be managed by farmers.

However, based on the general literature review, meetings with agricultural specialists and experienced farmers abetted to classify the succeeding issues impacting on farmer's allocation of the cultivated area by a specific crop and these issues comprise: prices of a crop received by farmers during the preceding years, yield of a precise crop acquired in preceding year, yield of competing crops as a substitute for the opportunity cost, and farmers' talents and experience concerning rice cultivation.

Conversely, during the planting period, farmers are unsure of the prices of their crops during the harvest period regardless of the declaration of support prices that are proposed to offer a levelling ground to the market price as a result of scanty institutional system for implementation. A close assessment of the preceding prices data has presented a significant variation between the market as well as support prices of several crops. Subsequently, prices received by farmers in the preceding season were employed as an independent variables. Nonetheless, the actual prices; precisely, market prices which are deflated by the GDP deflator rather than the nominal prices were utilized to neutralize the potential effect of inflation in this basis.

It has been recognized that yield is a significant factor of the profitability of specific crops in a particular cropping period. But since the yield of a specific crop at its planting period is unidentified, farmers focus their hopes of profitability for a specific crop on the yield achieved in the present year. The lagged area is as well employed as independent variable with the expectation of capturing the impacts of farmers' talent and experience for a specific crop.

The NERICA acreage can be expressed as follows:

NERICA acreage = Ƴ (actual price of NERICA at time t-1, NERICA yield at time t-1/ROK yield at time t-1, area cultivated to NERICA at time t-1)

ROK acreage can be expressed as follows:

ROK acreage = Ƴ (actual price of ROK at time t-1, ROK yield at time t-1/ NERICA yield at time t-1, area cultivated to ROK at time t-1)

Nevertheless, since farmers are inept to get the prevailing information completely, at this point, a lagged price response model is conceivably rational. The lagged actual prices (nominal prices that are deflated by GDP deflator in 2002, $ 16/ kg) are employed in the model.

MATERIALS AND METHODS

For an empirical evaluation on how the quantity of a specific crop supplied responds to conforming changes in its prices along with other related variables considered formerly, it is essential to employ a statistical model to realize our goal. We transform all variables of inclusion in logarithmic forms for acceptance of mathematical operations in addition to direct estimation of both the short and long-run price elasticities. For this study, the suggested model is elucidated by employing the Nerlovian model[12] that defines the fluctuating features of agricultural supply by integrating price expectations and the adjustment expenses. Hence, the functional model can be specified as:

$$Q_t = \theta + \lambda P_t^* + \mu W_t + \varepsilon_t. \tag{1}$$

Where, Wt symbolizes supplementary exogenous factors and ε_t an error term. As expected price is unobservable, all expectations are assumed to obey:

$$P_t^* = P_{t-1} + \kappa\left(P_{t-1} - P_{t-1}^*\right) \tag{2}$$

$$0 \prec \kappa \leq 1. \tag{3}$$

Where, P_t designates real price at time t and κ is a coefficient of expectation. If κ tends to 0, then, it does not seem to be a difference between both the current year's expected price and previous' year actual price, and if κ = 1, then, the expected price is equal to previous year actual price. However, '(2)' proposes that cultivators adjust their expectations of future price regarding previous experience, and that they can now learned from their past errors. Substituting '(2)' into '(1)' and simplifying gives:

$$Q_t = \kappa\theta + \kappa\lambda P_{t-1} + \kappa\mu W_{t-1} + (1-\kappa)\varepsilon_{t-1} + G_t \tag{4}$$

Where,

$$G_t = \varepsilon_t - (1-\kappa)\varepsilon_{t-1} \tag{5}$$

Hence, form '(4)', it can be appreciated that the current year expected price is a proportion of both last years' expected and actual price[13]. So, price expectations are weighted moving average of past prices[14] in which the weights reduce geometrically.

Equation (5) is the adaptive expectation model[12]. Also, noting that the partial adjustment model with the hypothesis that the desired area Qt is really a function of price (Pt) along with other exogenous factors (Wt):

$$Q_t^* = \theta + \lambda P_t + \mu W_t + \varepsilon_t \tag{6}$$

Since the desired area under cultivation was undefined, the partial adjustment hypothesis becomes:

$$Q_t - Q_{t-1} = \pi(Q_t^* - Q_{t-1}) \tag{7}$$

$$0 \prec \pi \leq 1 \tag{8}$$

Where, π is the estimated rate of area adjustment coefficient between desired and actual area[15] in the preceding time. If π tends to 0, area remains constant from one year to the other, and if λ = 1, then, adjustment is instant. Usually, adjustment to the desired level is feasible to be imperfect as a result of physical and institutional restrictions, fixed capital, etc. It is important to note, that λ offers the connection between the short and long-run elasticities. Substituting '(7) 'into '(6)' gives the partial adjustment model, which is:

$$Q_t = \pi\theta + \pi\lambda P_t + \pi\mu W_{t-1} + (1-\pi)Q_{t-1} + \pi\varepsilon_t \tag{9}$$

Combining '(1)' and '(6)' gives:

$$Q_t^* = \theta + \lambda P_t^* + \mu W_t + \varepsilon_t \tag{10}$$

Where the desired area level (Q^*_t) in addition to expected price (P^*_t) are unobservable.

Substituting '(2)' and '(7)' in '(10)' and simplifying, reduces the result to the estimating equation '(11)'.

$$Q_t = \pi\theta + \pi\lambda P_t + \pi\mu W_{t-1} + (1-\pi)Q_{t-1} + \pi\varepsilon_t. \tag{11}$$

Where,

$$\theta_0 = \kappa\pi\theta \tag{12}$$

$$\theta_1 = \kappa\pi\lambda \tag{13}$$

$$\theta_2 = (1-\kappa)+(1-\pi) \tag{14}$$

$$\theta_3 = -(1-\kappa)(1-\pi) \tag{15}$$

$$\theta_4 = \pi\mu \tag{16}$$

$$\theta_5 = -\pi\mu(1-\kappa) \tag{17}$$

$$G_t = \pi\varepsilon_t - \pi(1-\kappa)\varepsilon_{t-1} \tag{18}$$

RESULTS AND DISCUSSION

In this section, the estimated results of both NERICA and ROK models and their associated statistics are presented and discussed. We first try to evaluate the plausibility of these results regarding the economic concept and rationality, a priori expectations of signs of the estimated coefficients[16] and their sizes. Subsequently, we try to discuss the estimated models in the succeeding sections.

ESTIMATES OF RICE ACREAGE RESPONSE MODELS

There are two varieties of rice crop estimated models and theses are submitted and their results are successively discuss below in the next subtitles

ESTIMATES OF ROK ACREAGE MODEL

Table I depicts all coefficients of the estimated model for ROK acreage with the associated statistics.

The estimated coefficients pertaining to all the included variables have the expected signs and thus are acceptable. Basically, most of the tests concerning model assessment are satisfactory. The R^2 value is 0.611, and it suggests a well-fitting model.

F-ratio test is statistically significant, which indicates that the joint significance of all the parameters. The D-W is 1.604, and in accordance with Table II the corresponding Durbin(H) statistics is 0.481, signifying that there are no serial correlations[17]. A simple Reset test was used to test for misspecification in the functional[18] and it was just less than the critical value, therefore, the null hypothesis concerning the exact functional form of this model cannot at all be rejected. In accordance with this result, Jarque[19] test for establishing the normality of the residual distribution revealed a value less than the critical, thus, the null hypothesis concerning the normal distribution of this residuals cannot at all be rejected.

THE LAGGED ACTUAL PRICE:

The Specific price elasticity is found to be positive and insignificant for the short

run. Conversely, in the long run, the magnitude is moderately higher. In line with this findings, the ROK acreage response is 0.188, which proposes that an increase of 1% in price of ROK variety is liable to bring about 19% in acreage expansion. Hence, the corresponding estimated long-run price elasticity is 0.362(Table V).

THE RATIO BETWEEN ROK AND NERICA VARIETIES YIELD

ROK and NERICA varieties compete with each other for acreage as well as other resources and this happens in some regions. Because of this, the ratio between ROK and NERICA varieties yields was incorporated in the function to determine the effect of higher harvest of one variety on the other. Though the coefficient of the variable concern is positive, nonetheless it is marginally significant. Therefore, there is specific evidence of higher ROK yield as compared to NERICA variety in competing for expansion in its acreage, which might happen to the detriment of NERICA variety.

LAGGED ROK ACREAGE

The coefficient of ROK lagged acreage is found to be positive and highly significant. Therefore, farmers' experience and talent in rice agribusiness and its traditional activities have significant bearing on its production. Once more, the magnitude of the coefficient is high, demonstrating a very sluggish rate of adjustment of cultivators.The magnitude of the estimated coefficient of the lagged dependent variable is 0.643. Hence, the adjustment coefficient is relatively large, signifying faster adjustment of the ROK cultivators to several stimuli in addition to incentives.

ESTIMATES OF NERICA ACREAGE MODEL

Table III depicts both the coefficients of the estimated NERICA acreage model and related statistics. The results of diagnostic tests for the acceptability of the model are satisfactory. The R^2 displays 96% of the variation in the dependent variable[20] are explained by all the independent variables. F-ratio is as well high and significant, presenting the general goodness of fit. The D-W test statistic is 1.911, signifying no serial correction[17].

Table IV indicates that the estimated Durbin (H) statistics as well confirms that there is no evidence of serial correlation[21]. The Reset test for misspecification in the functional[18] also carries a value that is less than the critical value[22], confirming to the accuracy of the estimated functional form[18].

Jarque[19] test for determining the normality in the residual distribution obtaining a value less than the critical value[22], therefore, the null hypothesis concerning the normal distribution of this residuals cannot at all be rejected. Below is a discussion regarding the estimated coefficients of this model.

THE RATIO BETWEEN NERICA AND ROK VARIETIES YIELD

The estimated coefficient of ratio between NERICA and ROK varieties is 0.281 and it is positive although not significant. Therefore, increase in NERICA yield with respect to that of ROK did not appear to support farmers' in its acreage expansion, and this could be that the cultivation of NERICA has been restricted to specific areas.

LAGGED NERICA ACREAGE

The coefficient of the lagged NERICA Acreage is establish to be positive and significant, and the magnitude of its coefficient is also very high (0.936), suggesting farmers' adjustment rate is very low. The rate at which cultivators adjust the acreage under a specific crop in response to the changes on the factors deliberated previously, could be understood from the values of the adjustment coefficients (table V).

Nevertheless, a very slow rate of adjustment was observed, suggesting that acreage was more influenced by institutional along with technological difficulties and that price incentives functioned sluggishly.

Concerning lagged actual price, the short-run price elasticity is lower than the long-run[23], suggesting a long term adjustment of the acreage under the crop.

POLICY IMPLICATIONS

The Nerlovian model was used to estimate the acreage response of rice crop varieties to changes in their corresponding prices in addition to other related factors in Sierra Leone. The rice crop data from 1980-2011 period was utilized and the coefficients of the acreage response models for all rice varieties were estimated using the OLS procedure. However, the responses of these rice varieties to changes in their respective prices, as discovered in their respective short and long run price elasticities and also supplemented by their adjustment coefficients are recorded in Table V.

ROK VARIETY

Rice cultivation needs an improved technical skill as well as experience which is expected to be imparted by agricultural experts to all Sierra Leone farmers. Generally, specific types of soil in various rice growing eco-zones in addition to climatic environments do not encourage progressive cultivation of some other crops, and therefore limiting farmers' selection of crops to be grown. This appears to be real for the acreage where ROK variety cultivation is dedicated as the estimated coefficient of the price elasticity was 0.187 and it was slightly significant. On the other hand, the estimated coefficient of the long run price elasticity was 0.362 as the coefficient of adjustment was 0.466 for ROK variety.

NERICA VARIETY

NERICA is a long grain aromatic rice variety, which is mostly cultivated in the Northern Province of Sierra Leone. It is equally challenging regarding its pre as well as post-harvest management needs. Though, the short run price elasticity of NERICA acreage has been estimated to be small (0.133), however, its long run was large (0.975). The coefficient of the price elasticity was as well significant. In short, these findings highlight the part played by the price factor in influencing acreage dedicated to NERICA farming. The coefficient of adjustment for NERICA variety has been found to be 0.131, suggesting a slow rate of adjustment. Hence, among others factors, this possibly caused by the specific atmosphere of the crop as well as its management measures.

CONCLUSIONS AND RECOMMENDATIONS

The seeming recommendations for policy modification are to open farm gate prices and reduce government's involvement in agricultural sector particularly in the acquisition of agricultural inputs.

Forthcoming research need to focus on how this might be better realized. Necessary support should be made available to private sector by means of preventing price explosion. Economic efficiency as well as incentive structures existing in the rice sector in Sierra Leone is exhibiting the capability to utilize the necessary advantage of market circumstances. Similarly, it is possible that a decrease in distortions in the local markets may increase the cultivation of NERICA variety in Sierra Leone, which successively will improve the livelihood of the cultivators. One significant necessity is that cultivators should be given the chance to respond to market indicators to translate the challenges of the international market into better vision. Sierra Leone should however, accept and practice sustainable agricultural policies by efficiently utilizing its available resources as well as using a proper blend of government policies and market chances.

In agreement with structural reforms, it is necessary to transfer output prices to cultivators with minimum distortion. There is need to eliminate price subsidies and control, which create distortions in market along with huge financial costs.

TABLE I THE RESPONSE MODEL OF THE ESTIMATED COEFFICIENTS OF ROK RICE VARIETY (1980-2011)

Variable	Coefficient (λ)	Std. error	T-statistic	Prob
Constant	0.810	2.324	0.421	0.635
Actual price of ROK at t-1	0.188	0.156	1.311	0.265
ROK yield t-1/NERICA yield t-1	3.096	1.865	1.573	0.214
Acreage planted to ROK at t-1	0.643	0.165	3.742	0.004
R^2	0.611	Mean dependent variable	-	8.002
Adjusted R^2	0.598	S.D. dependent variable	-	0.241
S.E. of regression	0.096	Akaike info criterion	-	-1.713
Sum squared residual	0.264	Schwarz criterion	-	-1.542
Log likelihood	31.002	F-statistic	-	6.949
Durban Watson stat	1.604	Prob (F-statistic)	-	0.000

Source: Authors' data analysis, 2013, dependent variable is Ln (ROK Acreage), variables are in logarithm.

TABLE II DIAGNOSTIC TESTS

DH statistic	0.481
Jarque	4.61(0.102)
Reset test	1.13 (0.307)

Source: Authors' data analysis, 2013

TABLE I. THE RESPONSE MODEL OF THE ESTIMATED COEFFICIENTS OF NERICA RICE VARIETY (1980-2011)

Variable	Coefficient (λ)	Std. error	T-statistic	Prob
Constant	-0.027	1.976	-0.004	0.954
Actual price of NERICA at t-1	0.134	0.153	1.101	0.299
NERICA yield t-1/ ROK yield t-1	0.281	2.664	0.107	0.909
Acreage planted to NERICA at t-1	0.936	0.078	9.407	0.000
R^2	0.960	Mean dependent variable	-	7.654
Adjusted R^2	0.932	S.D. dependent variable	-	0.244
S.E. of regression	0.081	Akaike info criterion	-	-2.121
Sum squared residual	0.133	Schwarz criterion	-	-1.764
Log likelihood	34.126	F-statistic	-	95.053
Durban Watson stat	1.911	Prob (F-statistic)	-	0.000

Source: Authors' data analysis, 2013, dependent variable is Ln (NERICA Acreage) variables are in logarithm

TABLE II.DIAGNOSTIC TESTS.

DH Statistic	0.31
Jarque	0.63 (0.83)
Reset Test	0.05 (0.96)

Source: Authors' data analysis, 2013

TABLE III.THE SHORT AND LONG RUN PRICE ELASTICITIES ESTIMATES AND THEIR ADJUSTMENT COEFFICIENTS.

Rice crop variety	Short run elasticity	Long run elasticity	Adjustment coefficient
ROK	0.187	0.362	0.466
NERICA	0.133	0.975	0.131

Source: Authors' data analysis, 2013

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Title: *Farmers' Awareness of Modern Rice Cultivars in African Environment — Evidence from Northern Sierra Leone*

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ABSTRACT

Although several rice cultivars (RC) exist today, few are cultivated because researchers failed to consider Farmer Awareness Preferences (FAP) during the developmental stage of the rice cultivar. Many farmers still depend on low-yield landrace RC, resulting in production shortfalls to meet growing demands of rice-consuming countries. This study presents FAP in terms of yield, drought stress and management practices as inputs to rice breeding research. The study is conducted in the Northern Province of Sierra Leone using the Participatory Action Research (PAR) technique. A total of 120 farmers are randomly, individually and collectively interviewed in ten farming communities belonging to three ecological zones in the Province. The study shows that FAP for rice yield, drought stress and management practices as production restraints vary considerably across ecological zones. Although farmers in irrigated eco-zone prefer high-yield, long-duration RC, those in rainfed lowland and upland eco-zones prefer tall, short-duration RC. Also in rainfed lowland and upland eco-zones, farmers prefer white and red long-grain RC, respectively. However, farmers in irrigated eco-zones are non-sensitive to grain color. The most favored tall RC by farmers in rainfed lowland and upland eco-zones are different form the "green revolution" dwarf RC promoted in Asia. It is concluded that yield, drought stress and cultivation efficiency preferences of RC in different eco-zones should be promoted to enhance overall global rice production. Irrespectively, high-yield rice cultivation increases rice production, which will sequentially benefit millions of the people who grow and consume rice for various purposes.

Keywords: eco-zone; drought stress; farmer awareness preference; participatory action research; rice cultivar, Sierra Leone.

INTRODUCTION

Rice, the staple and leading food grain of a large proportion of the world population, is cultivated under rainfed and irrigated lowland, upland and floodplain ecologies. Under rainfed conditions, rice cultivation largely depends on the patter (intensity and frequency) of rainfall. In the floodplains of the Great Scarcies of Sierra

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Leone, rice is cultivated in three main ecological zones (eco-zones). Recently in Sierra Leone, there is visible increase in rice production due to expansions in cultivated areas in the floodplains of the Great Scarcies. Rice cultivation has increased after the end of the civil war in 2002 because of the return of the war-displace/refuges to their farming communities[1].With this development, there is now the need to develop and organize high-yield, drought/disease-resistant rice cultivars to meet consumption demand of the fast-growing population.

Rural-urban migration in developing nations accounted for 60% of urban growth during the 1970s[2], apparently increasing urban rice consumption/production. Since it is rural population that mainly engages in farming, the rural-urban shift created a production-consumption gap that most developing countries (including Sierra Leone) today continue to grapple with. This production shortfall could drop if farmers grow modern high-yield cultivars that are adaptable to different production practices and eco-zones. This implies that rice production could increase without necessarily expansions in cultivated land areas. Production could also increase if researchers successfully identify and redress farmers' adoption issues related to modern high-yield rice cultivars. Another critical issue that needs redress is cultivation practice (e.g., cropping time and scheme), which differs greatly across cultivated rice eco-zones. Rice yield is generally low in Sierra Leone because farmers depend largely on low-yield landrace rice cultivars. Modern high-yield rice cultivars are hardly available on the market or to farmers. The low adoption of modern rice cultivars in Sierra Leone could be due to low awareness, lack of capital, non-access to extension agents and markets, and high fertilizer and labor costs.

Disconnectedness of researchers from farmers and other stakeholders further compound efforts in identifying Farmer Awareness Preferences (FAP) for improved modern rice cultivars. Because farmers lack cultivars that meet their preferences, they continue to cultivate landrace and other inefficient cultivars. However, this problem could have been avoided if both researchers, farmers and other stalkholders do participate in the farming process, especially in the selection of high yielding cultivars and eco-zones.

In a Participatory Plant Breeding (PPB) research in Brazil, Sambatti[3] noted that most farmers plant landrace rice cultivars regardless of accessibility of improved modern cultivars. The integration of farmers' views into different propagation techniques could increase crop adoption series. This is efficient in terms of adoption costs and skills of desired cultivars. Reyes[4] noted that in terms of cost, PPB approaches are not significantly different from non-PPB approaches in Beans farms. Also Reyes[4] noted that at the same cost, PPB offers more information than non-PPB in Beans farms. Parlevliet [5] observed that farmer preferred wheat cultivars are more efficient in terms of agronomic traits and disease resistance. In another study, Astudillo [6] reported that breeders in Andean used male and female farmers' selection criterion to select and improve modern maize, quinoa, bean, sweet potato and wheat cultivars. In China, Wang et al.[7] used farmer participatory tests to determine site-specific nitrogen efficiency in irrigated rice farms. These studies demonstrate the effect of FAP on large-scale adoption of modern farming practices.

In regions with rice-sorghum systems (where sorghum is a drought-resistant crop and ROC-5 manually harvested), tall cultivars are preferred over dwarf cultivars. In communities where rice grain is the general diet high amylase rice cultivars are favored. These preferences drive farmer's decisions on cultivated crop cultivars.

The aim of this study was to determine FAP for rice yield, drought stress and management practices in Africa, using Sierra Leone as a case study. The tested research hypothesis was that for efficient rice production in Sierra Leone, variety development and adoption should correspond with FAP. For this purpose, the Participatory Action Research (PAR) method was used. The findings of the study could enhance rice production in Sierra Leone, Africa and in other rice production/consumption nations across the globe. This resolution will benefit the millions of rice growers and consumers for various purposes.

MATERIALS AND METHOD

The Study Area

The study was piloted in the Northern Province of Sierra Leone[8], which is on the northern flank of Sierra Leone that borders with Guinea. In terms of land area, the Northern Province accounts for almost half the size of Sierra Leone. It is comprised of five districts, which are generally the largest districts in Sierra Leone. The Northern Province borders the Western Area (where Freetown is, the capital city of Sierra Leone) on the West, the Republic of Guinea on the north-east, the Eastern and Southern Provinces on the south and east[9], respectively.

The study area lies in the transitional semiarid climate region with an average annual precipitation of 1500 mm. It covers some 35 936 km^2 of land area and has a total population of 1.7 million people [10]. In the study area, rice is grown largely under rainfed conditions in lowland and upland eco-zones. Irrigated rice cultivation in the study area is very limited. The region accounts for about 75% of cultivated rice and over 83% of lowland rice production in Sierra Leone. The Northern Province therefore presents rice cultivation conditions that are considered representative of those across Sierra Leone.

Sampling Techniques

The study was conducted in May, 2013 in collaboration with the Sierra Leone Agricultural Research Institute (SLARI), which is a division of the Ministry of Agriculture and Food Security (MAFFS) in Sierra Leone. Following endorsement by authorities in the selected farming communities in the study area, the principal researchers along with community personnel and SLARI extension agents in each of the farming communities discussed the farmers' selection criteria for the study. Then a list of 25 farming communities with well-established rice farmers in the Province was provided by SLARI officials. From the list, 10 farming communities were randomly selected for the study. In all, a total of 120 farmers (65 males and 55 females) were interviewed (Table I).

TABLE I. INDIVIDUAL FARMER SCORES BY GENDER IN THE 10 RANDOMLY SELECTED FARMING COMMUNITIES

Farming communities	**Male**	**Female**	**Total**
Kambia	6	5	11
Rokupr	10	6	16
Port Loko	12	4	16
Robuya	2	4	6
Makeni	8	4	12
Sanda	6	7	13
Kabala	8	7	15
Korobola	4	7	11
Magburaka	3	3	6
Makali	6	8	14
Total	65	55	120

Data Collection

For the field work, the survey team consisted of the project manager, SLARI representative, social gender expert for farming community and WARDA (West Africa Rice Development Association) field workers. The staff of the rainfed upland rice program developed the interview methods and guidelines for the data collection (Table II).

TABLE II. METHODS AND GUIDELINES USED IN THE DATA COLLECTION

Basin Rice Farming Data		
Question & Iteration	Observation, Question & Iteration	Pair-wise Rank Performance
Rice eco-zone		
Rice sowing period	Rice sowing method	
Land preparation		
Farmers' union		
Loan access		
Fertilizer use		
Rice varietal awareness		
Farmer-ext. agent		
Required Drought Management Skills		
Crop Growth Stage	Drought Stress Set-in	Planting Method
Crop device in drought	Planted variety	Preferred variety and why
Drought crop yield	Cultivated land area	
Non-drought crop yield	Cultivated land area	
Farmer awareness issue		

The survey team reviewed the conceptual research environment to ensure reliable data collection during which period interpersonal interaction with farmers in the data collection phase was emphasized. For cultural reasons, female workers were also included into the survey team to encourage rural women farmers' participation. The

survey team was educated on sensitive cultural norms before embarking on the data collection process in the selected farming communities[11]

The PAR research team was trained in quality data collection attributes, including creating the enabling atmosphere, allowing sufficient time for would-be contributors, listening and learning about the issues raised by the farmers. The PAR team also took specific interests in community matters and treated farmers in accordance to basic cultural norms such as greeting farmers in their local dialects.

During the interview, the PAR team used such skills as personal observations, indirect probing, cordial iterations, preference rating and pair-wise ranking. Iteration here means seeking the same information in several different ways. This technique ensured that the data collected were sufficiently reliability [11]. Probing strengthened the confidence of respondents in answering the tailored questions more precisely. It formulated the responses of the respondents and ensured desired treatments of relevant issues. The how, what, where, why, who and when questions served as a mode of farmer-agent interaction. In establishing cultivated regions, for instance, farmers were asked about the area of land planted and the quantity of harvested crops. This was subsequently used to compute the crop yield, which was sequentially related to the cultivated area. Observation in this context means moving around farm lands or direct inspection of storage and other farm/crop-related facilities.

For farmer preferences, pair-wise ranking was used. Farmers also recorded significant traits of current and previous rice cultivars they cultivated. Through probing and iteration, all the traits were ranked in order of significance. To determine why one variety was favored over another, pair-wise analysis was performed on the recorded rice cultivars and traits. The field survey enabled the PAR team to determine the traits farmers used to distinguish rice cultivars. Then the Chi-square tool was utilized to test the actual association of the cultivators' preferences for rice traits with the diverse eco-zones in the study area.

Apart from group discussions, farmers were individually interviewed using structured questionnaires. This allowed farmers to articulate individual views without undue influence from other farmers. In the interpersonal interviews, farmers expressed sincere opinions about major issues regarding their farming activities. For example, the research team asked the farmers' questions, such as, how many 25kg bags of rice were harvested[12]. This was utilized to estimate the actual harvested grain[13]. In verifying the collected data via both the group and interpersonal approaches, farm trips were made to four randomly selected individual and collective farms. Also random measures of cultivated area were recorded in some farms for verification purposes. Storage facilities/services were also visited to examine the amount and quality of rice grains in storage.

Results and Analyses

Based on the study, most of the farmers grow rice in two or three eco-zones in the floodplains of the Great Scarcies of Sierra Leone. Respectively, 62.5%, 25.8% and 11.7% of the farms are under rainfed lowland, rainfed upland and irrigated tidal-swamp conditions (Table III). While female farmers dominate in lowland and irrigated tidal-swamp farming, male farmers preferred upland rice cultivation.

TABLE III. SURVEYED FARMERS IN THE 10 RANDOMLY SELECTED FARMING COMMUNITIES OF THE ECO-ZONES

Farming communities	Rain-fed lowland	Rain-fed upland	Irrigated tidal-swamp	Total
Kambia	2	8	1	11
Rokupr	10	4	2	16
Port Loko	14	2	0	16
Robuya	0	4	2	6
Makeni	8	3	1	12
Sanda	12	1	0	13
Kabala	14	0	3	17
Korobola	5	4	2	11
Magburaka	3	2	3	8
Makali	7	3	0	10
Total	75	31	14	120

Farming and Rice Traits

Chi-square analysis shows that farmer-favored rice traits in the different eco-zones surpasses the aggregate total at the 1% and 5% levels of significance (Table IV).

TABLE IV

Rice trait	Eco-zone				Statistic	
	Rain-fed[1] lowland llowland	Rain-fed[2] uupland	Irrigated tidal-swamp	Mixed	Total	Mean
Tall plant (TP)	71	74	19	59	223	55.75
Short period (SP)	51	83	30	55	219	54.50
High-yield (HY)	32	30	97	36	195	48.25
Long period (LP)	37	8	29	38	112	28.00
Long red (LR)	33	58	5	34	130	32.50
Long white (LW)	50	6	5	30	91	22.75
High tiller (HT)	12	3	19	31	65	17.25
Medium period (MP)	21	8	22	24	75	18.75
Short plant (SP)	11	6	2	8	27	6.75
Nerica	15	6	—	-	21	10.50
War-77	6	4	—	4	14	4.67
ROC-5	—	—	2	1	3	1.50
IR-64	5	—	6	-	11	5.50
Total[X]	339	286	236	320	1181	

Note that "Total[X]" is percent total in excess of 100 due to multiple response recording, Lowlad[1] and Upland[2] DF is the degree of freedom (DF = 12). Calculated Chi-square value at 329.08 and DF. at 12 and at tabulated values of 1%, 5% and 10% significance levels of 24.51, 19.00 and 36.10, respectively.

The Chi-square calculated score for cultivators' preferred rice traits in diverse eco-zones surpasses the tabular scores (DF = 12) at the 1% and 5% levels of significance. This suggests that farmers favored rice traits significantly differ among the

eco-zones. About 37% of the farmers favored long duration, high-yield and long red-grain rice cultivars. Only about 2% of the farmers favored traits characteristic of ROC-5, War-77 and IR-64 rice cultivars (Table IV).

At the ecological phase, differences also exist among farmers for preferred rice traits such as grain quality, smell, color and size. Farmers in rainfed uplands rank short duration traits before tall and grain color as well as high yielding traits. Except for the long red varieties, all other varieties traits were of less significant to approximately 9% of the farmers in rainfed upland (Table IV). Also a small portion of rainfed lowland farmers were bothered about grain quality traits apart from the color and length. This set of farmers rank plant height over plant duration. Approximately 30 % of them regarded high yielding potentials, long red grain as well as long duration as significant. All farmers in irrigated tidal-swamps preferred high-yield traits. Less than 30% of the farmers in other eco-zones preferred traits other than high-yield. Another 30% and 29% of the farmers in irrigated tidal-swamps favored short and long duration rice cultivars respectively. Approximately 25% of the farmers regarded high tillering, medium duration and tall cultivars as critical traits in their selection process. Conversely, only 12% and 3% of the farmers in rainfed lowland and rainfed upland eco-zones respectively, were bothered about tillering traits of rice.

Drought and Rice Farming

In each eco-zone, drought-resistance is critical at both the vegetative and reproductive stages of rice growth. While lowland and upland rice eco-zones show drought stress at vegetative stage, irrigated tidal-swamp rice shows drought stress at reproductive growth stage (Table V). Visible differences in phenotypic traits begin to emerge among rice cultivars during drought spells. In each eco-zone, a relatively small number of farmers stop rice cultivation when drought sets in during sowing season. The highest output in typical drought stress period is in irrigated tidal-swamps, followed by lowland and then upland eco-zones (Table V). On the average, rice grain yield drops by 54.5% due to drought stress. Table V shows similar effects of drought stress on rice grain yield across the eco-zones (calculated χ^2 at 1.83, with tabulated value of 5.13, DF of 2 and at 5% significance level).

TABLE V. FARMERS WHO NOTICED DROUGHTS AT VEGETATIVE AND REPRODUCTIVE CROP-GROWTH PERIODS AND ESTIMATED GRAIN YIELDS FOR THE ECO-ZONES IN THE NORTHERN PROVINCE, SIERRA LEONE[8]

Eco-zone	Farmers with drought (%)		Estimated grain yield (kg/ha)		
	Vegetative period	Reproductive period	Non-drought condition	Drought condition	Yield difference
Lowland	60	28	840	390	53.6%
Upland	70	12	762	376	50.7%
Irrigated	33	78	1102	450	59.2%

Calculated chi-squared at 1.83; DF at 2; and at tabular value of 5.13 at the 5% levels of significance. Also percent yield difference is calculated in relation non-drought condition.

In each eco-zone, a fraction of the farmers (about 38%) failed to take drought effect minimizing actions on crop yield. However, majority of the farmers use some other measures such as growing early maturity cultivars (Table VI). Also some farmers

build clay mounds around plots to retain water around crops. Early cultivation is the least favored (2%) management practice against drought stress.

TABLE VI. PERCENT OF FARMERS WITH DROUGHT MANAGEMENT APPROACHES IN THE NORTHERN PROVINCE, SIERRA LEONE[8]

Drought management approach	Percent farmers
Early growing variety	22
Plausible variety	10
None adopted drought management	38
Early cultivation	2
Farm hurdling	6
Adapted local variety	10
Adapted modern variety	12

Problems faced by farmers during the awareness phase of modern rice cultivars are summarized in Table VII. The table suggests that 62.5% of the farmers lack investment capital, 7.5% cannot afford labor cost and 4.0% struggle with fertilizer cost. These issues limit the rate of farmers' adoption of modern rice cultivars. About 17% of the farmers cite access to market as major obstacle to adopting modern rice cultivars. Table VII also shows that about 9% of the farmers have no access to extension agents, thus limiting awareness about modern rice cultivars.

TABLE VII. MODERN RICE VARIETY AWARENESS PROBLEMS ENCOUNTERED BY FARMERS IN THE NORTHERN PROVINCE, SIERRA LEONE[8]

Problem	Frequency	Percent total
High fertilizer cost	5	4.16
High labor cost	9	7.50
Non-contact with extension agents	11	9.17
Lack of market to sell produce	20	16.67
Lack of capital	75	62.50
Total	120	100.00

Farmers obtain information on modern rice cultivars from extension personnel, radio programs, friends, village committee and other farmers (Table VIII). Table VIII shows that about 42% of the respondents get information about modern rice cultivars from extension personnel, 5% from radio programs, 16% from friends, 8% from village and 29% from other farmers. The analysis shows that most of the farmers (42%) get information about modern rice cultivars from extension agents. This implies that in terms of cultivated modern rice cultivars in Sierra Leone, extension agents are the main sources of information. Thus more frequent contacts with extension agents increases farmers' awareness about modern rice cultivars, and thus increases the chance of adoption of new cultivars that will eventually enhance production.

TABLE VIII. FARMERS' INFORMATION SOURCES FOR MRC IN THE NORTHERN PROVINCE, SIERRA LEONE[8]

Information source	Frequency	Percent of total (%)
Radio	6	5.00
Village committee	10	8.33
Friends	19	15.83
Other farmers	35	29.17
Extension agent	50	41.67
Total	120	100.00

Table VIII shows that a reasonable number (42%) of farmers get information about modern rice cultivars from the extension agents. This implies that extension agents are in frequent contact with farmers in the study area. A small number (5%) of farmers get information through radio programs, indicating some level of poverty or low education in the farming communities in the study area. Farmers could not afford radios because of poverty or lack the will to have radios because of low education.

Table IX indicates that another small a number of farmers (15%) have access to credit. This could also generally limit farmers' awareness about modern rice cultivars in their environment. Farmers' access to credit is a critical factor as high-capital farmers are more likely to seek new technologies, including new rice cultivars. Table IX also shows that not very many farmers (28%) take interest in memberships to farmers' unions. This could be because farmers hardly share information with others about cultivation processes such as modern rice cultivars, fertilizers and credit access; which generally enhance farming activities.

TABLE IX. DISTRIBUTION OF RESPONDENTS ON THE BASIS OF ACCESS TO CREDIT AND FARMERS' UNIONS

Access	Frequency	Percent total
Credit		
Yes	18	15.00
No	102	85.00
Total	120	100.00
Farmers' union		
Yes	32	26.70
No	88	73.30
Total	120	100.00

Cultivation Practices

In the northern region of Sierra Leone (the study area), rains start in April, peak in July-August and cease in September. Some farmers in rainfed uplands start planting rice in June, but most (78%) do so in July. In rainfed lowlands, panting starts in May and is largely (63%) completed by end of the month. For irrigated tidal-swamps, cultivation starts in late May to mid-June (70%). Some of the rice farmers also grow millet and sorghum, the planting times are quite flexible. So even though some crops

fall behind the most suitable planting seasons, failure of one crop could be compensated by the success of another.

Almost all the rice farmers are engaged in mono-cropping and only about 28% practice mixed-cropping. Rainfed upland rice cultivars (such as DJ11-509, IRAT-10, DJ12-519 and IRAT-13) are mostly cultivated with millet and maize. Access to capital, labor or land determines the cultivation methods used. Although the dibbing and drilling methods of rice cultivation are labor intensive, they maximize use of available lands compared with the broadcasting method. On the other hand, broadcasting is a common planting method in the study area. This is particularly the case in rainfed lowland eco-zones (77%) where labor is scarce but plant growth is fast due to highly wet soil conditions. Some 13%, 24% and 63% of rice cultivation across the eco-zones are done via drilling, dibbing and broadcasting. Fertilizer use also varies with the eco-zones. The highest use is in rainfed upland (71%), followed by rainfed lowland (19%) and then irrigated tidal-swamp (10%) eco-zones. While about 15% of the farmers weed three times, 60% weed twice and 25% weed only once during rice cultivation period.

DISCUSSIONS

Gender and Rice Farming

In most cases, women grow rice in irrigated tidal-swamp and rainfed lowland eco-zones. This is because women are more inclined towards vegetable production than rice production and these eco-zones serve this purpose well. By and large, vegetable crops are grown after rice harvest. Over 50% of the harvests are sold in local markets to meet family financial commitments. Unsold vegetables are internally consumed by the producing families or preserved as planting materials. Generally, more men do rainfed upland rice farming than women. This farming condition is more suitable for millet, sorghum, maize and cassava than for vegetable crops. Men are better suited to cultivating these crops than vegetable crops. The dominance of female farmers in specific eco-zones (e.g., rainfed lowland and irrigated eco-zones) has also been noted in other regions of the world. For instance, in Nigeria, Ezumah[14] noted more women in rice cultivation than men. This suggests that female PPB could better reveal farming obstacles in women communities. There is now a popular recognition of women's awareness in promoting self-reliance and strengthening women's roles in rural and nation development[15]

Preferred Cultivars

Farmers prefer short, medium and long duration cultivars, which directly allow alternating crop production. Farmers in rainfed lowland and upland eco-zones favored short duration crops. This is because short duration crops such rice could be harvested before the droughts get severe. Furthermore, farmers could use the same piece of land to grow other crops during the year. Almost all farmers favored tall plants over short ones. This is because of difficulties in harvesting short crops like the ROC-5; which demands much bending, slanting and twisting. Also the long leaves of tall plants can be mixed with mud to construct farm houses, pavilions and huts.

The green revolution in Asia focuses on short-gene rice cultivars. Even so, tall-gene crops are immensely enhancing green revolution across the continent. Some farmers in rainfed upland conditions favored long, red grain rice deemed it more attractive and edible with simple source. This reduces farm household expenditure compared with white grain rice that requires fairly rich source to improve palatability. This suggests that the breeding of red pericarp rice should be promoted in Sierra Leone. Farmers in irrigated tidal-swamps generally preferred high-yield cultivars, possibly because of its easy accessibility. The main cultivation obstacles in the study area include drought stress, low-yield crops and lack of inputs such as information from experts, equipment and fertilizer availability. This means that emphasis should be put on breeding cultivars that are more drought resistant, high-yielding and do well without fertilizers. Across the eco-zones, almost all farmers preferred tall, short duration and high-yield cultivars. Crop breeding scientists in the region should refocus research efforts on these farmers' preferred traits to improve food security in Sierra Leone and in the sub-region.

Knowledge of the diversity of farmers' preferences for rice traits across the eco-zones could help scientists to adopt more eco-zone-based approach to crop breeding. This could meet farmers' needs on the basis of eco-zone, which synergy could boost farmers' income and food security in the country. The research approach could require connecting not only PPB with stakeholders in rice farming communities, but also with agricultural extension agents and the government. This line of action will integrates the perceptions of farmers, researchers and the government for increase food production. This could eventually lead to the development of cultivars that are broadly acceptable to both farmers and consumers of different communities.

Drought Stress

Lack of rains could limit food scarcity and lead to famine, particularly in rainfed upland eco-zones, where rains are unpredictable, irregular and concentrated in the wet season. While water stress affects every trait, rice spike weight in addition to length are negatively related with drought vulnerability index[16]. Also, drought at vegetative growth stage delays flowering, limiting dry-matter cultivation and tiller number[17]. As reported by Bonsu[18], farmers in rainfed lowland and rainfed upland eco-zones use early crop cultivars with short duration. Tall rice cultivars are preferred in these eco-zones because of the high grain yields and root water uptake at deep soil horizons[19]. Carvalho [20]showed that under nutrient deficiency, dense rooting crops are more efficient in soil nutrient and water uptake. Since rice is manually harvested with knives or sickles, tall cultivars are preferred. This is even so when women harvest with babies tied on their backs. Flowering occurs in September to December when daytimes are short and sometimes water reserves are low. This leads to irrigation rationing, which sequentially affects grain formation and yield.

Based on the study, farmers ignore drought management practices because of either lack of knowledge or capital. Drought management practices such as drilling water wells and installing irrigation pipes require substantial capital input and operational knowledge. This suggests that the state extension divisions and non-governmental organizations (NGO) should reinforce the promotion of appropriate drought management packages to ensure food security.

Management Practices

Few farmers make use farm animals to clear the fields in the study area. Broadcasting rice seeds/seedlings is a much faster planting method that requires less labor and skill than transplanting. This is the reason why rainfed lowland and irrigated tidal-swamp eco-zone rice farmers concurrently grow two or more rice cultivars. However, upland rice farmers grow only one cultivar because of the use of different sowing methods. Generally, seeding rate is 60–80 kg/ha in broadcasting and 40–50 kg/ha under drilling or dibbling method[21]. This suggests that rice seed availability is another critical factor particularly in rainfed lowland and irrigated tidal-swamp eco-zones. It is therefore important to give seed aids and seed management techniques to farmers in the study area.

Based on farmers' participatory research in Vietnam, it was revealed that decrease in the rate of seeding, fertilizer dosage and minimal use of pesticide in rice cultivation had a negative impact on output but substantially increase farmers' income[22]. Most upland rice farmers use fertilizers to make up for the short land fallow periods. Lowlands and irrigated tidal-swamps are more fertile than upland fields. These eco-zones are less prone to erosion, the process by which nutrients are lost in upland fields. Farmers are therefore hardly use fertilizers in lowland and irrigated tidal-swamp fields. For farmers to adopt other rice cultivars, it is essential that fertilizer use is promoted. Also with enough sunlight, farmers can take home more grains when fertilizers are used.

To enhance yield, farmers prefer mono-cropping over mixed-cropping. Farmers in lowlands and irrigated tidal-swamps weed two or three times during the cultivation season because of high weed growth. Upland farmers normally weed only once due to low weed growth. For this reason, it is also critical that farmers use cultivars that out-compete weeds on farmlands.

CONCLUSIONS

This study shows that early maturity and tall rice cultivars are amongst the most critical traits that farmers search for in rainfed systems in Northern Sierra Leone. Farmers in rainfed lowland and upland eco-zones are also sensitive to rice grain color and yield. For farmers in irrigated lowland or tidal-swamp eco-zones, high-yield and late maturity cultivars are preferred. However, more farmers use fertilizers in rainfed upland than in rainfed lowland and irrigated tidal-swamp eco-zones. These differences cause the variations in grain harvest among these eco-zones. Drought stress at vegetative and reproductive growth stages is common across the eco-zones. This stresses the need for developing and promoting more drought-resistant cultivars in the study area. In summary therefore, it is important for farmers to adopt efficient eco-specific cultivars to boost productivity and food security. This could benefit farmers and consumers beyond the study area and the millions more in the sub-region.

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Cover page

Title: *GIS Analysis of Pedological Data and Measures for Improvement and Protection of Soils*

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ABSTRACT

The aim of this research was to determine soil erosion and associated factors that influence its quality and establish measures to improve it using GIS methods. Main results obtained from this study are: In the analyzed area, the surface erosion "limited" soil quality over an area of 1600 hectares, 26.36% of the total agricultural area. The aggressiveness of this process is amplified by the action of other factors (land unevenness, carrying capacity, compactness, depth erosion, slope, etc), which, generally manifests with an increasing intensity once surface erosion intensity also increases. In 30.78% of cases, surface erosion is associated with 6 other limiting factors, in 38.46% of cases some 5 factors manifest, into 23.07% of soil units 4 factors operate and only 7.69% of the cases studied, surface erosion is accompanied by 3 other limiting factors. Measures to prevent soil erosion were set differently, depending on the intensity of erosion processes and limiting factors that are the erosion is associated with.

INTRODUCTION

Surface erosion is one of the most aggressive phenomena that manifest in almost all temperate areas [2], implicitly in the area analyzed in this paper. This phenomenon, in most cases, is associated with other limiting factors that act as a complex, resulting in reduction of soil quality. Based on these considerations, establishing measures to improve the quality of the edaphic environment should be viewed as a comprehensive, holistic approach.

MATERIALS AND METHODS

Demarcation was performed using specific software sites Geographic Information Systems - GIS [7] and coincides with the administrative-territorial limit.

The materials used in this research were analogue maps (topographic, pedological, scale 1:50000) processed by GIS techniques. Data taken from the Office for Soil and Agrochemical Studies Timisoara (Timisoara OSPA) have been processed and completed by own data. Each soil unit was vectorized and taxonomically classified according with the Romanian System of Soil Taxonomy (SRTS 2012).

Limitations factors and degradation processes were based on existing data at Timisoara OSPA, complete with own observations. There were selected only ground units in the area affected by surface erosion, for each of these units other degradation processes were identified, processes associated with surface erosion. Processing and interpretation of data using GIS software enabled territorial location of the units affected by soil erosion and its associated factors. Soil units with varying degrees of surface erosion were regrouped according to other factors which "accompany" the process of soil degradation, being thus obtained 4 classes, identified in the territory through specific GIS methods.

To improve the qualities of agricultural land have been established measures to prevent and combat surface erosion and other limiting factors associated with it.

The methods used in this paper are in accordance with Romanian standards in the field of pedology and soil improvers.

RESULTS

Traian Vuia administrative territory is located in the Bega river basin overlaping Lugoj Hills Făgetului Depression and Bega Plain. Physical and geographical conditions specific for each subzone, prompted major differences in the formation and evolution of soils, which are classified to 5 classes of soil [8], divided, down to the lowest taxonomic level, into 76 territorial units, with space distribution and percentage of various ranges [6], shown in Figure 1.

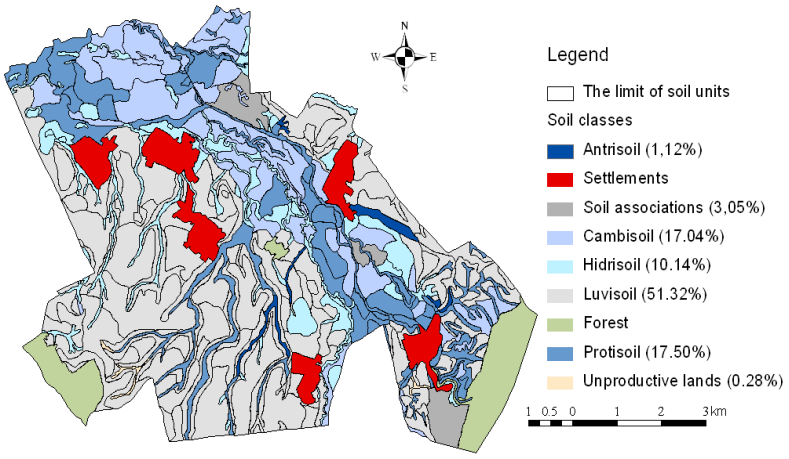


Figure 1 Pedologic map of the administrative territory Traian Vuia

Quality and hence the productive capacity of the soil is reduced under the influence of internal factors (some characteristics of soils) and/or external (relief, parent rocks, erosion, etc). Since the main objective of this paper is to analyze the "involvement" of erosion processes associated with other factors, in degradation of soil quality and establishing prevention and control measures, we will further analyze the situation of agricultural land, found under the influence of these processes. In the analyzed area, the surface erosion "limited" soil quality over an area of 1600 hectares, 26.36% of the total agricultural area. Erosion occurs with different intensities (Figure 2).

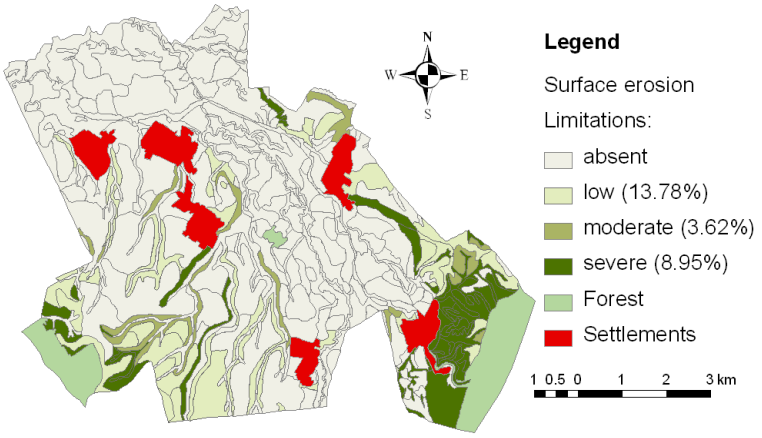
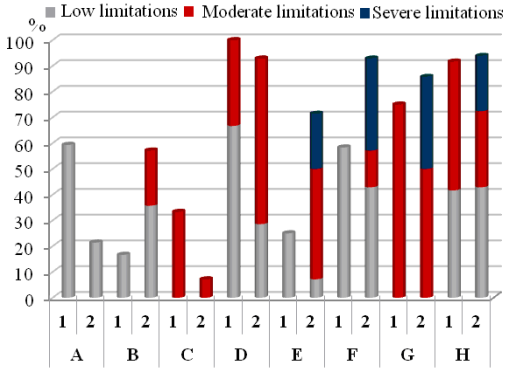


Figure 2 Surface erosion intensity [6]

Associated with the surface erosion, other limiting factors act, intrinsic or extrinsic, such as: excess moisture on the surface, land unevenness, carrying capacity, compactness, depth erosion, slope, low reserves of humus and soil acid reaction (Figure 3). To capture qualitative aspects, soil units were grouped into two categories, namely agricultural land affected by surface erosion of low intensity and respectively agricultural land that this process occurs with moderate to severe intensity (Figure 3).



1 Surface erosion with low intensity
2 Surface erosion with moderate and strong intensity
A - excess moisture on the surface
B - land unevenness
C - carrying capacity
D – compactness
E - depth erosion
F – slope
G - low reserves of humus
H - soil acid reaction

Figure 3 Correlation erosion surface with other limiting factors

Analysis of Figure 3 allows the making of the following statements:

- excess moisture on the surface occurs with low intensity, especially on soils where erosion is also at low intensity; the increase in intensity of the erosion requires, in most cases, the existence of steep slopes which determines the excess moisture on the surface to be reduced and therefore it's frequency decreases
- land unevenness "contributes" to the decrease of the productive capacity of the soil for 17% of soils with reduced limitations due to erosion; however, for a landscape with higher altitudes, on terrains "limited" by moderate and strong erosion, the intensity of the expression the nonuniformity also increases
- carrying capacity manifests with moderate intensity in combination with erosion, in both cases, especially when the intensity of the latter is low, due to the fact that a large proportion of the soils in the studied area are luvisoils (Figure 1)

• in land with low erosion intensity, compactness occurs as a limiting factor with mild to moderate the increase in the intensity of erosion is found and an increase in the incidence of moderate compactness

• depth erosion is observed with reduced limitations on soils with low surface erosion, but it intensifies on those where surface erosion acts with moderate and strong limitations, 21% are severe limitations

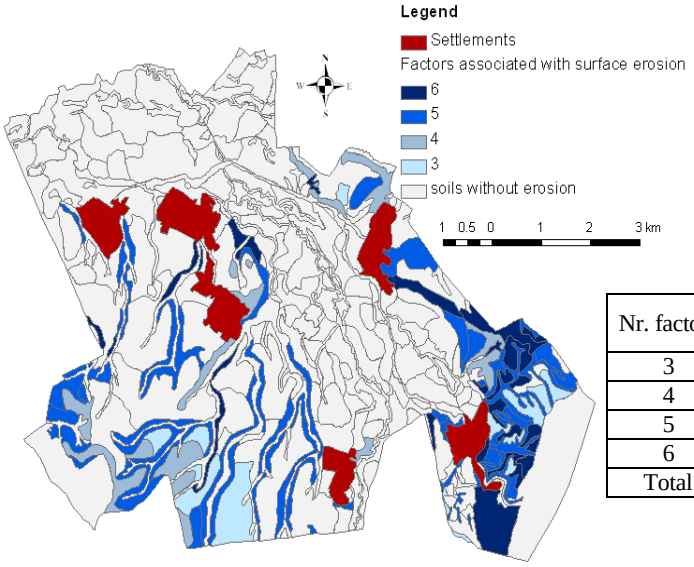
• slope, one of the determinants factors of erosion processes, associated with reduced limitations in the first case and with high intensity in the second case (35% severe limitations)

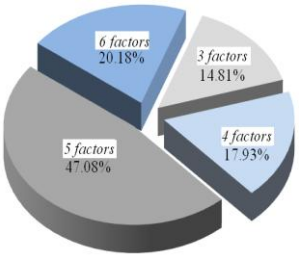
• low humus reserve affects, with moderate intensity, over 70% of soils with reduced limitations due to erosion, the intensity of this factor is more pronounced in moderately and strongly eroded soils, 90% of them being under the influence of this factor (35% are severe limitations)

• acid soils reaction "limits" about. 90% of soils subjected to surface erosion; the intensity of which is manifested in greater in the case of a more intense erosion.

Thus, the aggression of surface erosion, regarded as limiting factor, is supported by other factors, which, in general, act with an increasing intensity as the intensity of surface erosion also enhances (Figure 3).

On each soil unit subject to surface erosion, the number of factors, that "accompany" this phenomenon (Figure 4) in the process of degradation of soil quality, is different, depending on the specific environmental conditions.

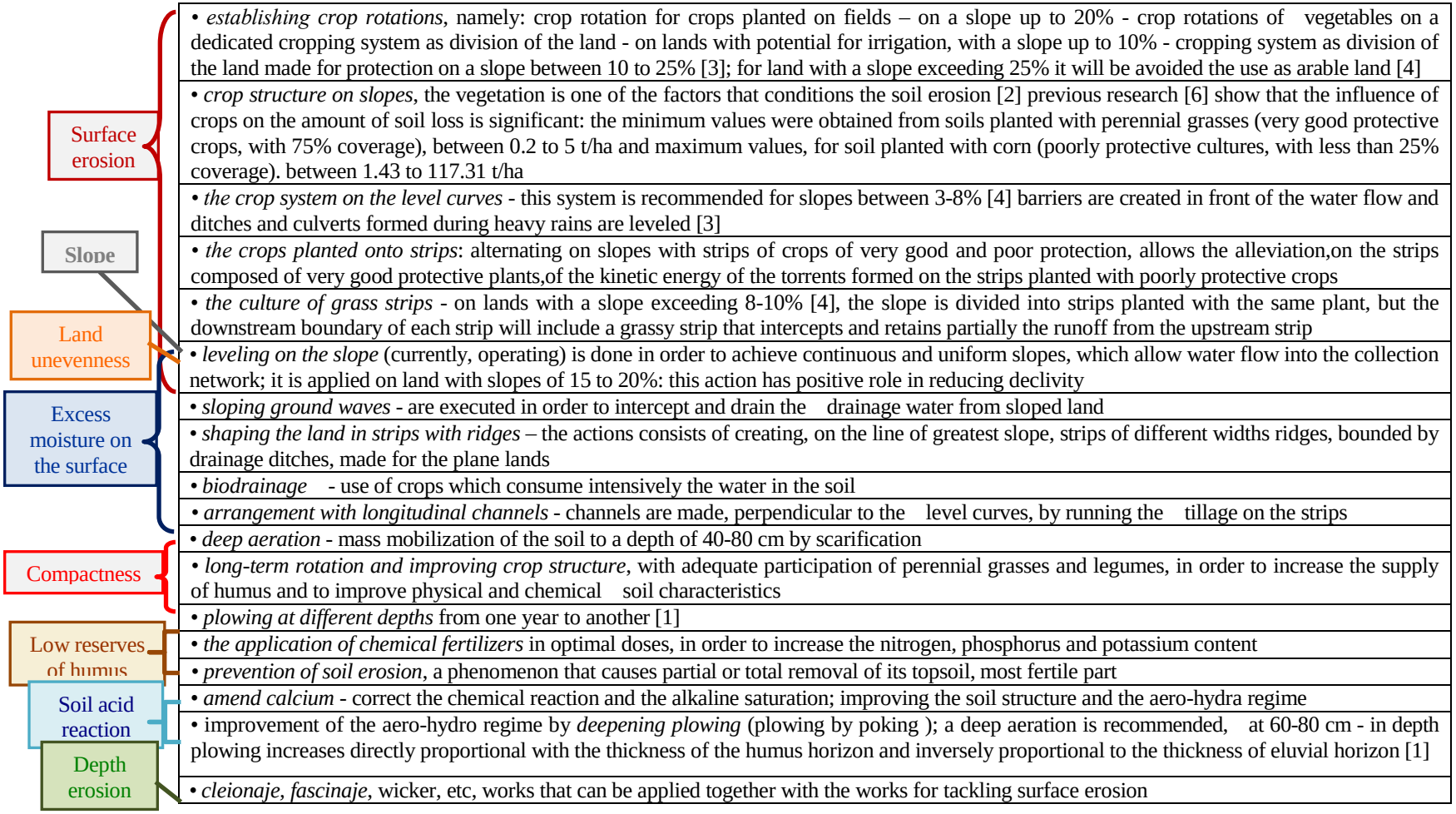




Nr. factors	Nr. of cases		Affected area	
	total	%	ha	%
3	2	7,69	237	14,81
4	6	23,07	287	17,93
5	10	38,46	753	47,08
6	8	30,78	323	20,18
Total	26	100	1600	100

Figure 4 Limiting factors of agricultural productivity associated to surface erosion

The data summarized in Figure 4 show that on a soil unit may operate, in parallel with surface erosion, even six other limiting factors. Of the total surface on which erosion processes occur, 47% is affected by another five factors limiting (Figure 4). This hierarchy emphasizes the idea that taking measures to improve the quality of agricultural land should be considered as an overall action, to eliminate the effects of each limiting factor, so it requires a holistic approach.



Scheme 1 Measures to improve the soil erosion in the area associated with other limiting factors

In order to improve the quality and to increase the productive capacity of agricultural land, based on the data presented above some measures have been established to prevent and combat soil erosion, in different ways, depending on the intensity of erosion processes and limiting factors and/or processes of degradation with which these are associated with. For improvement of soil quality, agronomical-pedological-ameliorative measures were proposed, agro-technical and simple hydro-technical measures that can be applied without significant economic efforts and which can be done on small areas of land, given the fragmentation of arable land, after the land restitution to individuals (scheme 1). All measures to improve soil quality, regardless of the factors and processes of degradation which reduce agricultural productivity, must be accompanied by fertilizing the land. After applying organic fertilizers and / or chemicals, in addition to intake of nutrients and humus, the physical, chemical and hydrophysical characteristics of the soil are improved soil (structure, aero-hydro regime, nutrient reserves, etc).

CONCLUSIONS

In the investigated area, the surface erosion acts as a limiting factor on approx. 30% of the total agricultural area. The aggressiveness of this process is amplified by the action of other factors (excess moisture on the surface, land unevenness, carrying capacity, compactness, depth erosion, slope, low humus reserves and soil acid reaction), which, generally manifests with an increasing intensity once surface erosion intensity also increases. In 30.78% of cases considered, surface erosion is associated with 6 other limiting factors, in 38.46% of cases some five factors manifest, onto 23.07% of soil units four factors operate, and only 7.69% of the cases studied, surface erosion is accompanied by three other limiting factors. This hierarchy emphasizes the idea that taking measures to improve the quality of agricultural land should be considered as an overall action, which can eliminate the effects of each limiting factor.

Measures to prevent and combat soil erosion were set differently, depending on the intensity of erosion processes and limiting factors and / or degradation processes that are the erosion is associated with.

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8. ●●● Arhiva Oficiului pentru Studii Pedologice şi Agrochimice Timişoara

Cover page

Title: *The Response of Hot Pepper (C apsicum anmuum L.) in Water Use Efficiency and Soil Environments to Mulching Practices under Greenhouse*

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ABSTRACT

Soil mulch is considered an agriculture management mothed for conserving soil moisture and improving soil micrenvrionment. This study aimed to sdudy hot pepper (*Capsicum anmuum* L) fruit yield, water use efficiency (WUE) and soil characters under mulch practices in greenhouse. A field experiment across 3 years was carried out with four types of mulch (no mulch [CK], wheat straw mulch [SM], plastic film mulch [FM], and combined mulch with plastic film and wheat straw [CM]). FM and CM treatments improved soil moistures and temperature in comparison to CK, SM increased soil water content and decreased soil temperature. CM treatment increased soil organic matter content and hydrolysis of the nitrogen content, reduced the available phosphorus content in soil, increased crop uptake of soil phosphorus and phosphorus utilization, reduced soil bulk density and increased soil porosity, improved soil structure. CM is the recommended practice for hot pepper cultivation in greenhouse due to improving soil micrenvironmental factors, and getting higher marketable yield and WUE.

1. INTRODUCTION

Soil mulch is defined as the application of various kinds of cover materials to the soil surface. It is an agriculture management mothed for conserving soil water and improving soil micrenvrionment. This practice is popular to be used in wheat [1], maize[2], cotton[3], and vegetables[4] production. It benefits crop growth and development, and conserves soil moisture, as well as improving soil physical, chemical, and biological properties, especially in dry year [1, 5]. Some scientists [6-7] found that maize stalk mulching increased soil moisture in sandy loam soil. Some Other benefits of mulching such as weed control, reduction of soil runoff and erosion, and improvement of plant earliness have also been recognized [3].

Hot pepper (*Capsicum anmuum* L.) is one of the important vegetables in China due to its nutritional and economic values. However, its cultivation is confined to

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warm and semi-arid countries where water is often a limiting for production [8]. Due to improving soil moisture and temperature and the overall positive effect on crop growth and development, mulch is recommended as a component within an integrated crop management (ICM) programme for hot pepper [9]. Mulching effects have been studied mainly in open-field condition; however, little information is available on the response of pepper to different mulching materials in greenhouse. The objectives of this research was to assess the effects of three mulches of different composition (straw and plastic film) on soil moisture, soil temperature, and as well as the effect on marketable yield, water-use efficiency, and environment in hot pepper crop under planted greenhouse.

2. MATERIALS AND METHODS

2.1 Experimental Design

The trials were carried out in the greenhouse of Northwest A&F University in Yangling (34°12′～34°20′ N; 108°～108°7′ E, elevation 560 m), Shaanxi, China, from May to September in 2008, 2009, and 2010. The annual mean air temperature is 12.9℃. The soil bulk density was 1.26 g cm^{-3} and the organic matter content was 15.0 g kg^{-1}; available N was 25.0 mg kg^{-1}; available P was 36.4 mg kg^{-1}; and available K was 144.0 mg kg^{-1}. Hot pepper cultivar Nongcheng 2 were transplanted on 15 May 2008, on 28 May 2009, and on 25 May 2010 with a density of 40,000 plants ha^{-1}. Plot size was 2.5 m long and 2.4 m wide, and four rows of pepper plants with a between-row spacing of 50 cm were transplanted in each plot in three years. The plastic membrane was set underground about 100 cm depth to prevent interpenetration of water. Fertilizers were applied with 150 kg ha^{-1} N (Urea) and 100 kg ha^{-1} P_2O_5 (Diammonium Phosphate) for each plot on 14 May and 3 July 2008, on 27 May and 10 July 2009, and 24 May and 11 July 2010, respectively.

There were four treatments including control (CK, no mulch), straw mulch (SM, 5 cm length of wheat straw with 5,000 kg ha^{-1}), plastic film mulch (FM, the first step was to cover 0.01 mm transparent polythene film, then to dibble on film and transplant pepper plants in holes), and combined mulch (CM, plastic film covered in planting row and wheat straw with 25,00 kg ha^{-1}covered in operation row). A completed random block design with four replications was used. Treatments were established on 5 June 2008, 19 June 2009, and 21 June 2010, respectively. Soil moisture (0-40 cm layer) was maintained 75-90% of field moisture capacity (FMC) with drip irrigation system.

2.2 Sampling and Plant Measurements

2.2.1 SOIL MOISTURE AND SOIL TEMPERATURE

Soil moisture (0-40 cm) was recorded with TDR (Time Domain Reflectometry) measurement. Soil temperature in each plot was measured for the entire observed periods at 5 cm, 10 cm, 15 cm, and 20 cm depths, respectively, with portable LCD soil temperature meter (Mod. TPJ-21, Zhejiang Top Instrument Co., Ltd., China).

2.2.2YIELD AND WATER USE EFFICIENT (WUE)

Fruits of pepper were harvested completely on 21st, 20th, and 25th September in 2008, 2009, and 2010, respectively. Water use efficiency for the cropping season was calculated based on total yield. No deep drainage or surface runoff was considered. Yield water use efficiency (WUE) was calculated as fresh bell pepper yield divided by total seasonal water used, respectively [10].

2.2.3 SOIL PHYSICAL AND CHEMICAL PROPERTIES

Soil physical and chemical properties were determined after harvest in 2010. Soil samples were collected at 5 points in S style in 0-20 cm and 20-40 cm soil layers. Each sample was dried at laboratory room temperature (25 ℃) to a constant weight and sieved (0.25 mm) to eliminate coarse soil particles. Soil organic matter content was determined by potassium dichromate titrimetric method; hydrolysable nitrogen content was determined by NaOH-hydrolyzation diffusion methods; soil available phosphorus was determined by NaHCO-Extraction (Ammonium Molybdate-Tartaric Emetic-Ascrbic Acid Colorimetry) method. Soil bulk density was measured by round knife method; soil porosity was calculated by function: soil porosity=(1-soil bulk density/soil specific gravity)×100%.

2.3 Statistical Analysis

The database was subject to analyses of variance (ANOVA) using SAS software package 9.1 (SAS Institute, 2003) with PROC MIXED procedure. Comparison among treatments was performed using Tukey's multiple range tests at the 0.05 probability level. The time-repeated measures analysis (repeated ANOVA) was used to determine the influences of mulching on parameters of interest during the whole observed period in 2009, and 2010, respectively, with the SAS PROC MIXED procedure [11].

3. RESULTS

3.1 Fruit Yield and Water Use Efficiency (WUE)

Mulching reduced water used, and increased the fruit yield and water use efficiency **(**WUE) (table 1). In comparison to CK control, water used in SM, FM, and CM treatments reduced by 8.3%, 16.8% and 17.2% in 2008; 32.6%, 28.2%, and 31.7% in 2009; 42.2%, 33.5%, and 41.4% in 2010. The yield was improved by 82.3%, 65.0%, and 111.5% under SM, FM, and CM conditions in 2008; 38.1%, 17.4%, and 46.5% in 2009; and 14.3%, 6.5%, and 19.6% in 2010. Accordingly, yield WUE was increased by 68.3%, 98.4%, and 155.5% in 2008; 104.8%, 63.6%, and 114.4% in 2009; and 97.9%, 60.1%, and 104.0% in 2010.

TABLE 1 WATER USED, YIELD AND WATER USE EFFICENCY (WUE) OF HOT PEPER UNDER MULCHING TREATMENTs

Treat	2008			2009			2010		
	Water used ($kg\ m^{-2}$)	fruit yield ($kg\ m^{-2}$)	WUE ($kg\ m^{-3}$)	water used ($kg\ m^{-2}$)	fruit yield ($kg\ m^{-2}$)	WUE ($kg\ m^{-3}$)	water used ($kg\ m^{-2}$)	fruit yield ($kg\ m^{-2}$)	WUE ($kg\ m^{-3}$)
CK	185b	2.43c	13.2c	110a	1.55c	14.1d	150a	2.45c	16.3d
SM	200a	4.43b	22.1b	74c	2.14a	28.9b	87c	2.80a	32.6b
FM	154c	4.01b	26.1b	79b	1.82b	23.4c	100b	2.61b	26.1b
CM	153c	5.14a	33.6a	75c	2.27a	30.3a	88c	2.93a	33.3a

The different letters indicate significant difference among treatments at $p \leq 0.05$ level based on Turkeys test.

3.2 Soil Physical and Chemical Properties in Response to Mulching

Mulch soil led to increment in soil organic matter content and hydrolysis of the nitrogen content with respect to control, but reduced the available phosphorus content in soil, increased crop uptake of soil phosphorus and phosphorus utilization, reduced soil bulk density and increased soil porosity, improved soil structure (table 2). Among mulch treatments, the lowest value was obtained under SM and the highest under CM conditions in soil organic matter content and soil porosity, and gotten the inverse results in soil bulk density.

TABLE 2 EFFECT OF MULCHING ON SOIL PHYSICOLCHEMICAL PROPERTIES AFTER HARVEST IN 2010

Treat	Soil	Hydrolysis	Available	Soil	soil
	Organic matter	N content	P content	bulk density	porosity
	(%)	($mg \cdot 100g^{-1}$)	($mg \cdot 100g^{-1}$)	(g cm-3)	(%)
CK	1.492d	9.275d	5.497a	1.324a	50.04d
FM	1.542c	9.625c	4.163d	1.272b	52.00c
SM	1.658b	12.425b	5.001b	1.244c	53.06b
CM	1.790a	13.437a	4.519c	1.228d	53.66a

The different letters indicate significant difference among treatments at $p \leq 0.05$ level based on Turkeys test.

3.3 Soil Moisture in Response to Mulching

Soil moisture under the different mulching was influenced strongly by the composition of the mulch material employed in trials (table 3). In comparison to CK, the average mean soil moisture was increased by 22.1%, 32.8%, and 44.1% under SM, FM, and CM conditions, respectively, in 2008; 18.9%, 22.4%, and 21.3% in 2009 for the entire growth season.

TABLE 3 EFFECT OF MULCHING ON SOIL MOISTUER IN 208 AND 2009 (%)

Treat	2008				2009			
	day after transplanting				day after transplanting			
	30	50	70	90	30	50	70	90
CK	6.5 c	8.3 b	9.9 c	11.3 c	11.7 b	10.9 b	11.9 b	14.8 b
SM	10.7 b	10.6 a	10.8 b	14.1 b	13.4 a	13.4 a	15.6 a	16.1 a
FM	12.6 a	11.1 a	11.7 a	15.5 a	14.7 a	14.7 a	14.2 a	16.6 a
CM	13.6 a	11.7 a	12.2 a	16.2 a	14.0 a	14.5 a	14.7 a	16.5 a

The different letters indicate significant difference among treatments at $p \leq 0.05$ level based on Turkeys test.

3.4 Soil Temperature

Soil temperature was affected significantly by mulching in three years (table 4). In all case, it was higher under plastic film mulch (FM) and combined mulch with plastic film and straw (CM), but lower under straw mulch (SM) in comparison to without mulch (CK). At the later stage of pepper growth, the difference among treatments decreased gradually.

TABLE 4 EFFECT OF MULCHING ON SOIL TEMPERATURE IN 2010 (℃)

Treat	0-10cm				10-20cm			
	day after transplanting				day after transplanting			
	30	50	70	90	30	50	70	90
CK	26.38	27.40	23.46	22.82	24.49	26.65	22.27	22.28
SM	23.69	25.70	21.75	21.41	23.43	25.47	21.76	21.51
FM	28.65	29.73	24.90	24.05	26.81	28.30	23.54	23.26
CM	26.05	28.30	23.28	23.02	24.82	27.20	22.61	22.69

4. DISCUSSION

The mulch layer over the soil can prevent water exchange between the soil and air leading to reduction in evaporation and ineffective water consumption [10]. CM was most effective in soil water conservation. It seemed that FM was better than SM in conserving soil moisture, but FM consumed too much soil water due to increasing the most soil temperature. Combined mulch with plastic film and straw was more favorable either in preserving soil water or improving soil temperature. Mulch is practically beneficial in hot pepper production and may be related to favorable soil moisture and optimal temperature, which is positively correlated to yield [12]. In this experiment, soil moisture in FM treatment was better than un-mulched control resulting in improvement in fresh fruit yield. SM could increase pepper marketable yield although soil temperature was also lower than control. Mulch soil led to increment in soil organic matter and hydrolysis of the nitrogen content, reduced the available phosphorus in soil, increased crop uptake of soil phosphorus, reduced soil bulk density and increased soil porosity, improved soil structure. Among mulch treatments, the lowest value was obtained under SM and

the highest under CM conditions in soil organic matter content and soil porosity, and gotten the inverse results in soil bulk density.

5. CONCLUSION

Combined much with plastic film and straw was more pronounced for improving soil environment than plastic film and straw mulches alone. The lowest value was obtained under SM and the highest under CM conditions in soil organic matter content and soil porosity, and gotten the inverse results in soil bulk density. This indicates that combining plastic film with straw mulch works well to facilitate soil condition, and marketable yield of hot pepper in greenhouse condition.

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Title: *Management of Rotylenchulus Reniformis and Beneficial Nematodes with Sunn Hemp*

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ABSTRACT

Sunn hemp, *Crotalaria juncea*, is known to suppress plant-parasitic nematodes such as reniform nematode, *Rotylenchulus reniformis,* while enhancing beneficial nematodes involved in nutrient cycling. A laboratory experiment was conducted to determine the required amount of sunn hemp for nematode management. *Rotylenchulus reniformis* infested soil was collected from a pineapple field. Dry sunn hemp foliage powder was incorporated on *R. reniformis* infested soil at 0%, 0.5% and 1.0% (w/w) and kept for one week. At the end of the experiment, nematodes were extracted through baermann funnel technique. Soil incorporation of dry sunn hemp foliage powder at 1.0% followed by 0.5% reduced *R. reniformis* number, but the result was not significant ($P > 0.05$). Significantly higher numbers of beneficial nematodes were found in 1.0% followed by 0.5% sunn hemp foliage power incorporated soils ($P < 0.05$). Farmers are recommended to incorporate at least 0.5% sunn hemp in their fields to enhance beneficial nematodes.

INTRODUCTION

Both, plant-parasitic and beneficial nematodes are the dominant nematode fauna in most soil communities. In Hawaii, reniform nematode, *Rotylenchulus reniformis* Linford & Oliveria, is a main pest of pineapple, *Ananas comosus* [1, 2, 3]. This nematode can reduce pineapple marketable yield by up to 26.8% at first crop [3] and by 50% in ratoon crops [4]. On the other hand, beneficial nematodes take role in soil nutrient cycling and soil health improvement. A good nematode management program in Hawaiian pineapple should include a strategy for suppressing *R. reniformis* and enhancing beneficial nematodes.

Sunn hemp, *Crotalaria juncea* L., is a tropical cover crop known to suppress *R. reniformis* and enhance beneficial nematodes after soil incorporation of its foliage [5, 6]. However, the required amount of sunn hemp for effective nematode management is questionable. Thus, the objectives of current research were to find out the required amount of sunn hemp for 1) *R. reniformis* suppression, and 2) beneficial nematodes enhancement.

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MATERIALS AND METHODS

A laboratory experiment was conducted at Kauai Community College, Lihue, HI in summer, 2012. *Rotylenchulus reniformis* infested soil was collected from a pineapple field at Kauai Agricultural Research Center, Kapaa, HI. The soil collected was Oxisol (Haliiseries; Typic Gibbsihumox, clayey, ferritic, isothermic). Initial population density of *R. reniformis* (Pi) was 274 /100 cm^3 soil. Collected soil was well mixed before potting into 7.62 cm-diameter plastic pots. Each of 12 pots was filled with 300 cm^3 soil and immediately incorporated with dry sunn hemp foliage powder at 1) 0%, 2) 0.5%, or 3) 1.0% (w/w). Dry sunn hemp foliage powder used for soil amendment was prepared from field-grown sunn hemp plants, oven dried to a constant weight, and ground with a commercial blender (Winsted Conn, Waring Products Co., CT) to powder form [6, 7]. The experiment was replicated four times and arranged in the randomized complete block design (RCBD).

Nematode assay

Soils were extracted for nematodes at starting (100 cm^3 soil) (Pi) and termination (50 cm^3 soil) (Pf) of experiment by baermann funnel technique. Extracted nematodes were counted at 100 × magnification levels using an inverted microscope (Fluovert, LeitzWetzlar, Germany). Nematodes were categorized into *R. reniformis* and beneficial nematodes.

Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) using the general linear method (GLM) procedure in Statistical Analysis System (SAS Institute, Cary, NC). Nematode numbers were log-transformed [log (x+1)] prior to ANOVA to normalize data. Untransformed arithmetic means of data are presented. Means were separated by Waller-Duncan *k*-ratio (*k*=100) t-test.

RESULTS

Effects on *Rotylenchulus Reniformis*

Soil incorporation of sunn hemp at 1.0% followed by 0.5% reduced *R. reniformis* population trend. However, the result was not significant ($P > 0.05$) (Fig. 1).

Effects on Beneficial Nematodes

Higher numbers of beneficial nematodes were found in 1.0% followed by 0.5% sunn hemp incorporated soils ($P < 0.05$) (Fig. 2). Beneficial nematodes were bacterivorous, fungivorous, omnivorous and predaceous in nature (data not presented).

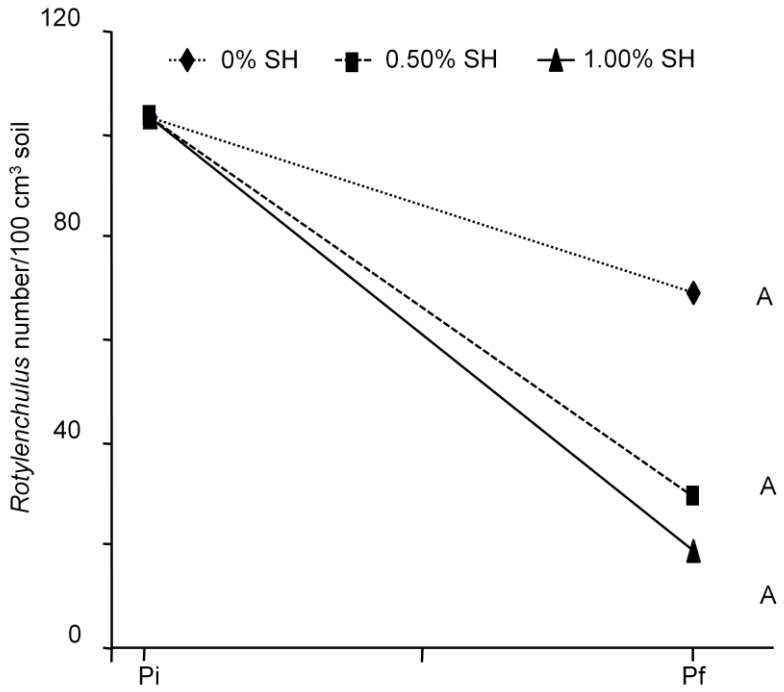

Fig. 1. Effects of soil incorporated sunn hemp at 0%, 0.5% and 1.0% (w/w) on abundance of *Rotylenchulus reniformis* at starting (Pi) and termination (Pf) of the experiment. Means are average of 4 replications. Means followed by same letter(s) do not differ according to Waller-Duncan *k*-ratio (*k*=100) *t*-test based on log(*x*+1) transformed values.

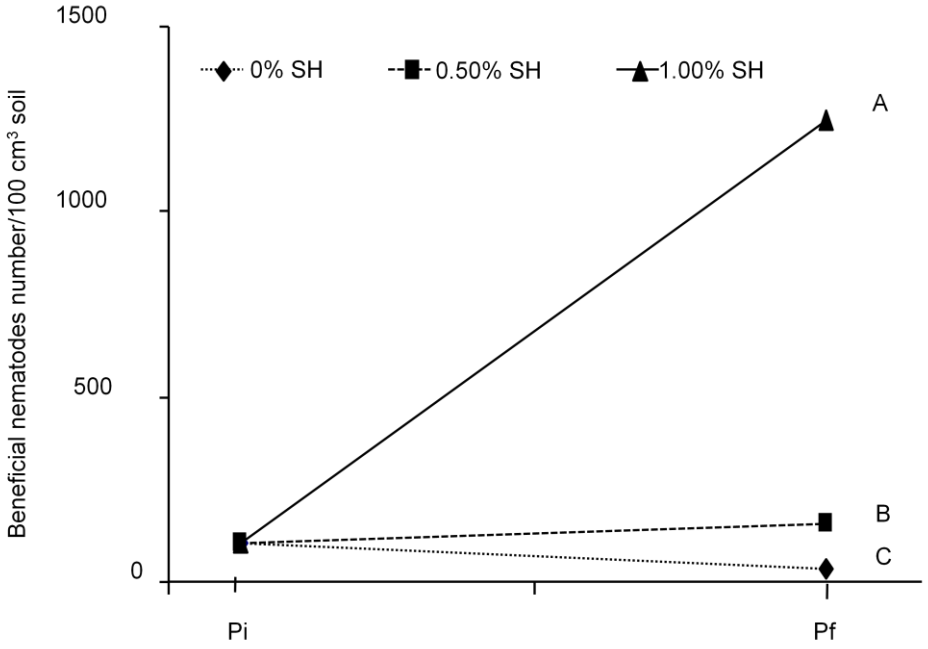

Fig. 2. Effects of soil incorporated sunn hemp at 0%, 0.5% and 1.0% (w/w) on abundance of beneficial nematodes at starting (Pi) and termination (Pf) of the experiment. Means are average of 4 replications. Means followed by same letter(s) do not differ according to Waller-Duncan *k*-ratio (*k*=100) *t*-test based on log(*x*+1) transformed values.

DISCUSSION

Current results are consistent with the earlier findings where sunn hemp has suppressed *R. reniformis* and enhanced beneficial nematodes in cowpea [5] and cucumber [6] cultivated fields. Nematode population trend on sunn hemp incorporated pots of current research has indicated the nematode management potentiality of sunn hemp in pineapple fields. Moreover, current research has shown that the higher the amount of sunn hemp, the greater the effects on nematodes.

CONCLUSION

Farmers are recommended to incorporate at least 0.5% sunn hemp in their fields to enhance beneficial nematodes. The implication of current research is that sunn hemp cover cropping on both, low and high biomass production seasons followed by soil incorporation of sunn hemp foliage is helpful for *R. reniformis* and beneficial nematodes management.

ACKNOWLEDGMENTS

This project was supported by grants from the National Center for Research Resources (5P20RR016467-11) and the National Institute of General Medical Sciences (8P20GM103466-11) from the National Institutes of Health. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health. Special thanks to Frank Matsuno, Kauai Agricultural Research Center, Kapaa, Hawaii, USA.

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Title: *Integration of Psidium Cattleianum Leaf and Aqueous Leaf Extract Into Soil for Broadleaf and Grassy Weed Management*

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ABSTRACT

Chemical interference has been suggested as a mechanism which facilitates exotic strawberry guava (*Psidium cattleianum*) plant invasion in native plant communities in Hawai`i. Allelopathy, which may give *P. cattleianum* a decided advantage as "[Hawai`i's] highest priority weed for control" has potential to be utilized by Hawai`i's growers for its inhibitory effects on seedling emergence as an alternative to imported chemical herbicides. The aggressive behavior of *P. cattleianum* in indigenous forests and disturbed areas has potential to provide a sustainable solution replacement for synthetic and ecologically altering pre-emergent herbicides in preemptive integrated pest management. In this study, we will look for evidence for plant phytotoxicity in *P. cattleianum* through two experimental trials: a field trial and a screenhouse test. In the field trial, the leaf aqueous extract of *P. cattleianum* was applied to newly tilled, weed seed-infested fallow plots at three-day intervals to assess weed seed coverage against water. In the screenhouse test, *Zea mays* seeds were sown into potting soil having different percent masses of *P. cattleianum* leaves to determine if strawberry guava leaf allelochemicals affect seedling vitality and vigor. When tested, leaf aqueous extract of *P. cattleianum* had a positive effect on grassy weed seed inhibition, when compared to the control. In contrast, prepared heterogeneous mixtures of chopped *P. cattleianum* leaves in potting soil had no significant effect on seedling emergence but suggested growth suppression of *Z. mays* seedlings when tested. Although *P. cattleianum* shows promise for reduction of weeds, more research is necessary to improve efficacy and ease of application before *P. cattleianum* is used for weed control.

INTRODUCTION

Psidium cattleianum Sabine var. *cattleianum* [*P. littorale* Raddi var. *longipes* (O. Berg) Fosberg], also known as strawberry guava or *waiawi* locally, has been extremely successful in invading and dominating natural and disturbed habitats since its 1825 introduction to Hawai`i as a fruit tree and as an ornamental [1]. Since

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P. cattleianum specimens have escaped cultivation in Hawai`i and become weeds in natural areas, expenditure of public funds is required to protect native plant communities, natural areas, and the watershed. Rapid growth, prolific reproduction, highly successful vectors, and the lack of natural enemies undoubtedly contribute to its success as an invader, but biochemical defenses found in *Psidium guajava* tissues [2] suggest another potential mechanism of invasion for related *P. cattleianum* [3]. Through the process of allelopathy (Greek, *allelo-*, of one another; *patheia*, suffering) [4], the "negative effects of one plant on another by the release of chemical compounds into the environment," [5], strawberry guava may inhibit seed germination or growth of other species when competing for space and resources. According to Smith et al. [6] "allelopathy may contribute to [*P. cattleianum's*] success" through production of toxic chemicals in the fallen leaves that prevent the growth of other plant species. Because allelochemical phytotoxins provide advantages in plant-plant competition, development of new herbicides could result from increased knowledge of these chemical relationships between plants [7].

While direct observation of forest understory and limited allelopathy evidence provide a foundation for understanding the extent of strawberry guava invasion, scientific experiments and statistical data analysis are necessary to accelerate our understanding of how *P. cattleianum* phytotoxic activity can benefit sustainable weed management. In this study, the allelopathic effects of the invasive tree *P. cattleianum* on seedling emergence were investigated in two field trials (Trial I and II) and two screenhouse tests (Test I and II). Specific objectives of the current research were to: 1) study the effects of spraying an aqueous solution of *P. cattleianum* onto newly tilled areas for suppression of broadleaf, grassy or sedge-type weeds, and 2) examine impacts on *Zea mays* seedlings when integrating *P. cattleianum* leaves into soil.

MATERIALS & METHODS

In order to investigate potential allelopathic interactions between *P. cattleianum* and other plants, green leaf material was collected from Lawa`i, Kaua`i, Hawai`i. Collected leaves were cleaned of debris, then placed into transparent plastic bags and kept refrigerated overnight. The following day, two separate experiments were conducted at Kaua`i Community College, Puhi: a field trial and a screenhouse test.

Prior to the first field trial (Trial I), the selected experimental plot was thoroughly tilled and cleaned so that a uniform, aerated soil remained. Tilled soil was smoothed of divots and uneven areas that could provide reservoirs where the sprayer was used. Each plot was randomized, then sprayed with its respective treatment, prepared *P. cattleianum* leaf aqueous solution or water, the control.

To make the leaf aqueous solution, 200 g of *P. cattleianum* leaves were added to one liter of tap water, brought to a boil, and reduced for 15 min. After the solution cooled, it was poured through a sieve into a backpack sprayer, then filled with enough additional water to reach the 15 L-line on the backpack sprayer. Liquid contents were gently swirled and applied equally with a flat spray nozzle tip between the four treated plots. The backpack sprayer was triple rinsed and then filled with water, the control. Using the same method, water was sprayed on control-labeled plots. New leaves were collected every 3 days to formulate a fresh *P. cattleianum* solution prior to spraying.

This process was repeated bi-weekly until seedling emergence occurred after thirty days, when recordings of weed type and coverage percent were logged. After completion, the field trial was repeated in a different area (Trial II), using the same preparation and application procedures.

Plots were individually scored on total weed coverage of broadleaf, grassy, or sedge-type weeds based on the Horsfall and Barratt scale [8]. Two independent scores were obtained for the coverage and weed type per plot and then analyzed. Statistical analysis was performed using one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure, employing the statistical analysis system (SAS).

The second experiment, a screenhouse test (Test I), examined *Z. mays* seedling vitality and vigor sown in different percent *P. cattleianum* leaves mixed in potting soil. In the screenhouse tests, *P. cattleianum* leaves were roughly chopped into pieces using a knife on a cutting board. To 200 g of potting soil, 2 g (1%) of chopped leaves were added to a bowl, mixed, then placed into 50 mm square seedling pots. This process was repeated with chopped leaves of *P. cattleianum*, at the rates of 5%, 10% per w/w potting soil. A control composed of potting soil without the addition of *P. cattleianum* was also made. Within each treatment, five *Zea Mays* seeds were sown into the potting soil mixtures. With five replicates per treatment, a total of fifteen heterogeneous mixtures of three concentrations and five controls were obtained.

After randomized complete block design was applied, treatments were placed on a daisy tray in the screenhouse, where they received overhead irrigation. All treated mixtures were kept on a screenhouse bench with an average temperature of 26 ºC at Kaua`i Community College. After thirty days, *Z. mays* seedling emergence and height was evaluated. This experiment was then repeated (Test II) using the same materials and methods.

ANOVA, the GLM procedure, and SAS, were employed to determine if there was a measurable relationship between *P. cattleianum* soil incorporation and *Z. mays* seedling germination and height.

RESULTS

In the field trials, Trial I and II, application of aqueous solution to tilled areas reported positive effect on reducing grassy weed control with a significance level of 10%. (Fig. 1). In addition, broadleaf type weeds were unaffected when *P. cattleianum* solution was applied to soil.

In the screenhouse tests, Test I and II, no significant differences were found between *Z. mays* seeds germination in soil mixed with *P. cattleianum* leaves and those made from potting soil alone (data not presented); however, a visible trend can be noted in the trials completed where *Z. mays* seedling height was reduced with all treated specimens (Fig. 2). Also, there was no effect on germination of *Z. mays* seeds with the incorporation of *P. cattleianum* leaves in potting soil.

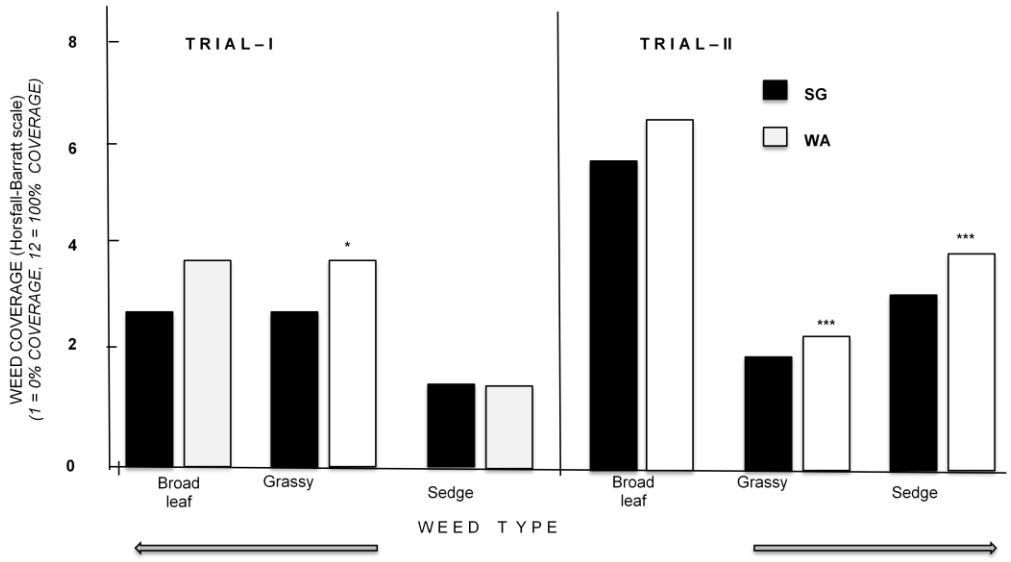

Fig. 1: Effects of strawberry guava, *Psidum cattleianum*, (SG) and water (WA) on weed growth at three weeks after starting the field experiment in Trial-I and Trial-II. Means ± standard error of mean (SEM) are average of 4 replications. Significant differences according to *F*-test result between treatments SG and WA are indicated by * and *** for $P < 0.1$, and $P < 0.01$, respectively.

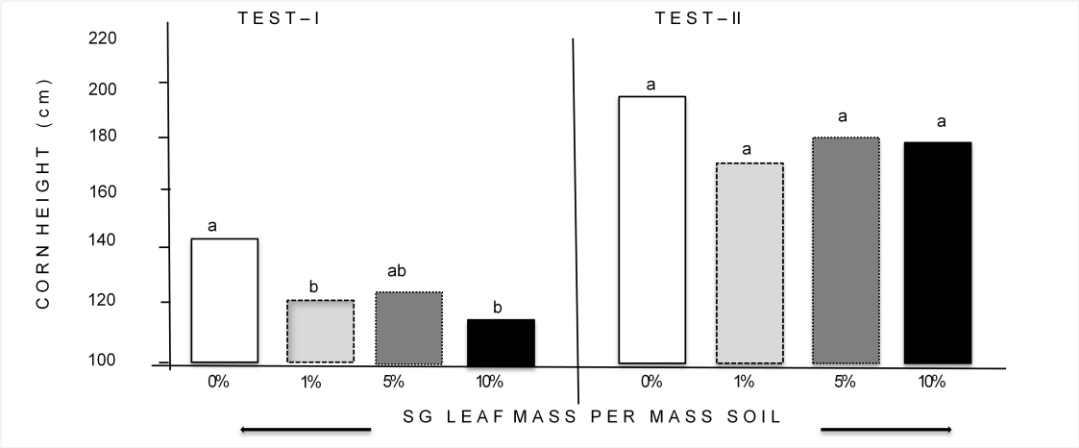

Fig. 2: Allelopathic effect of strawberry guava, *Psidum cattleianum*, (SG) on corn, *Zea mays*, in Test – I and Test - II. Means ± SEM (standard error of mean) are average of 5 replications. Means in a column between treatments followed by the same letter(s) do not differ according to Waller-Duncan *k*-ratio (*k= 100*) *t*-test.

CONCLUSION

Significant differences on the control of grassy weeds in field trials coupled with reduced *Z. mays* plant height in screenhouse tests indicate that chemical mechanisms may play an important role in competitive plant-plant toxicity. Determination of actual phytotoxicity will require quantification of chemical compound levels, in-situ experiments, soil analysis and further studies of *P. cattleianum* including more comprehensive trials using the compound on broadleaf, grassy, and sedge-type plants.

Taken together, the results indicate that allelopathy could be an important mechanism to take into account in determining how growers in Hawai`i can utilize natural resources to manage weeds. However, this study also suggests that the real significance of the allelopathic effects of *P. cattleianum* cannot be assessed and quantified without isolating the inhibitory allelochemical compounds in *P. cattleianum* or with more extensive studies and analyzation of allelopathic inhibition as a competitive attribute of *P. cattleianum.*

ACKNOWLEDGMENTS

This project was supported by grants from the National Center for Research Resources (5P20RR016467-11) and the National Institute of General Medical Sciences (8P20GM103466-11) from the National Institutes of Health. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health. Special thanks to Prof. Brian Yamamoto, Chair, Science and Math Division, Kauai Community College, Hawaii, USA.

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Title: *Mathematical Models for Predicting the Growth Trend of Microbial in Bioleaching*

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ABSTRACT

This paper consider prediction of the growth trend of microbial in the heap leaching of Uranium. Two mathematical models are constructed for predicting the growth trend based on the Gompertz and the Logistic functions, respectively. The parameters of the proposed models are estimated by the general global optimization method. The simulations show that the mathematical model based on the Gompertz function is optimal for predicting the growth of microbe in heap bioleaching technology. And we find that 30℃ was the optimal temperature for heap bioleaching.

1. INTRODUCTION

As a new direction of research and application in Hydrometallurgy, the bioleaching technology has been a hot research topic since the 1950s. And it has succeeded in extracting copper, uranium and gold. The main mechanism is using bacteria biochemical reactions to enhance the leaching efficiency of target minerals. As to the mechanism of bacteria leaching, many researchers have proposed a number of ideas. The main idea is that the function of bacteria can be divided into two categories, direct action and indirect effects[1]. And bacterial growth status will affect the result of the microbial heap leaching.

Since there are so many factors contribute to the growth of bacteria, we extracted some mixed bacteria to analyze bacterial growth from the tail liquid. Taking ambient temperature into consideration, we elicit a bacterial predictive growth model after Mathematical fitting. Furthermore, we analyze how ambient temperature affect the growth status of the hybrid strain by adjusting parameters of the mathematical model. And the optimal temperature spectrum of the bacterial growth is discussed[2].

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2. LABORATORY MATERIALS AND METHODS

In this section, we describe the main process of laboratory experiments in brief. First, we extrated some mixed bacteria from the tail liquid, mix it with 20% inoculum into a 250ml conical flask, divide the solution into 10 samples. Then, we take 5 constant temperature vibration tables, and those tables were set as 20 ℃, 25 ℃, 30 ℃, 35 ℃, 40 ℃ respectively. Each table was put two samples, which one is for a test, the other for a parallel control. Lastly, we record bacterial chemical indicators at regular intervals. The Ferrous content is determined by using EDTA titration method. The end of the test is the time when the percentage of ferrous iron oxidation is over 95%.

3. ANALYSIS OF EXPERIMENTAL RESULTS

3.1 The Oxidation Rate of Ferrous at Different Temperature Conditions

By measuring the consumption of ferrous at different temperature conditions, we drawed a diagram according to the data as follows:

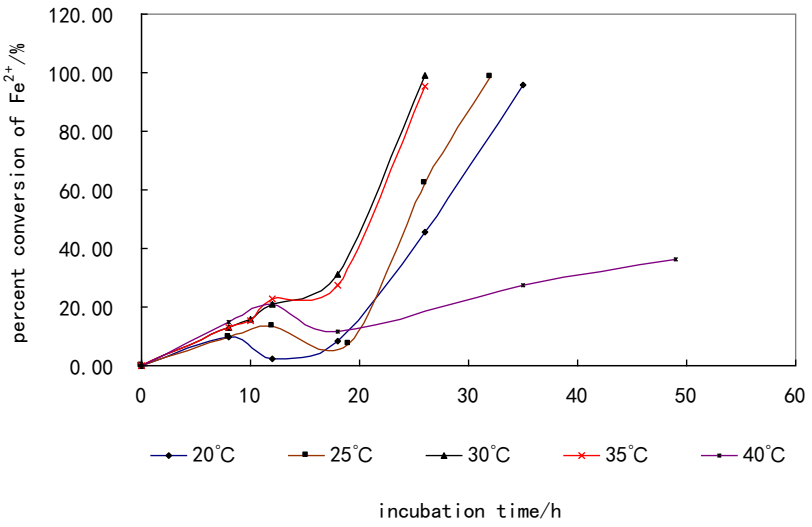

Figure 1. Consumption of Ferrous and temperature.

The graph shows how the rate of Ferrous Oxidation changed within 50 hours with different temperature surroundings. The transformation rate of ferrous oxidation increased fluctuately with the time passed. The temperature which contributes the fastest conversion rate of Ferrous Oxidation is 30℃. And the bacteria in this temperature had high activity and oxidizing ability.

3.2 The Primary Predictive Growth Model

A growth chart is used to be shown microbial growth, which is made up of three different parts: lag phase, exponential phase, stationary phase. In addition,

environment variable and growth stage will have an impact on the growth of microorganisms. Roughly speaking, the primary predictive growth models include linear model, Gompertz model and Logistic function in recent years.

Some researchers believe that Gompertz model and Logistic function is favorable to describe the development law of population growth in suitable temperature conditions. And those models can be expressed as follows.

(1) Gompertz model

$$L(t) = A + C \cdot e^{-e^{-B(t-M)}} \tag{1}$$

Where L(t), A, C, M, B, t represents the Logarithm of the number of bacteria (logCFU/g), initial number of bacteria (logCFU/g), maximum amount of bacterial growth (logCFU/g), time that Maximum rate of bacterial growth spent(h), growth rate of time M (logCFU/g/h) and the time(h), respectively.

(2) Logistic model

$$\frac{dN}{dt} = rN(1 - \frac{N}{N_{\max}}) \tag{2}$$

Where N, t, r, N_{max} refers to the amount of bacterial, time, speed of bacterial growth and the maximum number of bacteria respectively.

We fitted growth curves with Gompertz equation and Logistic equation, and their Correlation Coefficient is both 0.98. It indicates that the curve-fitting is efficient. Therefore, we compared RMSE with SSE, and showed in **Table 1**. It was conclude that Gompertz model was suitable for describing the growth of bacteria rule. Furthermore, we listed the parameters of Gompertz model in **Table 2**.

TABLE 1. A COMPARATIVE RESULT OF RMSE AND SSE BETWEEN TWO MODELS AT DIFFERENT TEMPERATURE

	Gompertz		Logistic	
Temperature (Degrees Centigrade)	RMSE	SSE	RMSE	SSE
20	0.03	0.01	0.03	0.02
25	0.02	0.02	0.05	0.03
30	0.04	0.01	0.06	0.04
35	0.02	0.01	0.05	0.03
40	0.06	0.05	0.07	0.09

TABLE 2. PARAMETERS OF GOMPERTZ MODEL AT DIFFERENT TEMPERATURE

	Temperature (Degrees Centigrade)				
Parameters	20	25	30	35	40
A	0.86523	0.95431	0.91676	0.88514	0.91186
C	0.18300	0.18484	0.18888	0.18125	0.0471
B	0.010654	0.015048	0.016625	0.016596	0.00183
M	30	28	26	26	35

3.3 The Secondary Predictive Growth Model

Usually, the secondary predictive growth model shows that how the changes of environmental factors affect the parameters of primary predictive growth model like A, B, C and M of Gompertz model. The secondary predictive growth model commonly included Square-root model, Arrhenius relationship and Response surface model.

The secondary predictive growth model was established based on a response surface model by using the Marquardt Method and the General Global Optimization Method. Further, the secondary Model can be expressed as follows.

$$B = -0.0752 + 0.0065T - 0.0001T^2$$

$$M = 82.4857 - 3.9543T + 0.0686T^2$$

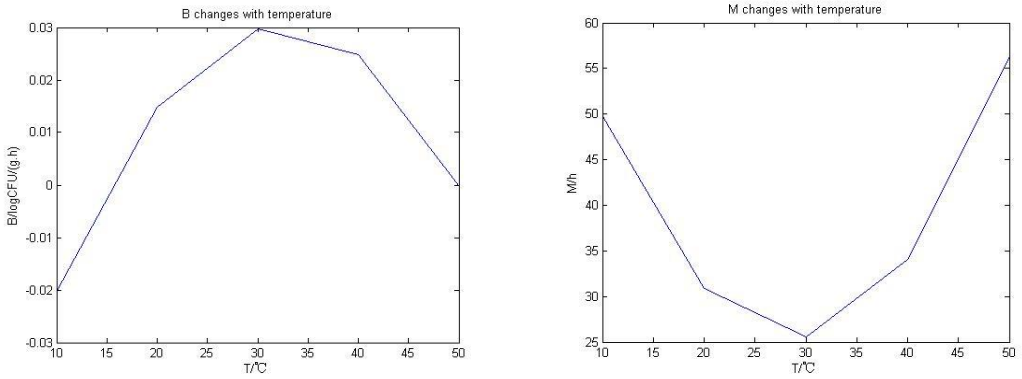

Figure 2. The parameters B and M at different temperature.

Figure 2 illustrates changes in the amounts of parameters B and M in a spectrum of temperature between 10 and 50 degrees centigrade. The maximum growth rate of bacteria (parameter B) increased gradually with the temperature increased from 10 to 30 degrees centigrade, while the interval between bacterial maximum growth rates (parameter M) fell dramatically from 50 to approximately 25 hours. When we gave a rise in temperature from 30 to 50 degrees centigrade, parameter B declined slowly, and parameter M has a rapid leap to over 55 hours. Overall, thirty degrees centigrade was a climax for parameter B, and a minimum for parameter M in the chart.

4. CONCLUSION

In this article, we extracted some mixed bacteria from the tail liquid in a science laboratory. In the process of determining a mathematical model, we have considered two main aspects. One is that we assumed the age of cells are not the same, and it will have an effect on the parameter of mathematical model. The other is the initial growth surroundings may adapt bacteria for the new environment, and change the time what the bacteria reproduce need.

Nowadays, the Gompertz model and Logistic model are usually used in the field

of microbial prediction. And the secondary predictive growth model is used to describe how the environmental factors affect the parameters of primary model. The key point of bioleaching technology consists in understanding the microorganism's law clearly.

In order to ensure comparability, we kept the same initial conditions to the five temperature vibration tables. The experiment shows that the curve-fitting method with Gompertz equation and Logistic equation was efficient. However, the Gompertz model is more suitable to describe the biological growth law after we compared RMSE and SSE carefully. And the Gompertz model has been widely used in the population projection. Further, each of its parameters has some biological significance. It was proved by laboratory experiment that the secondary model not only coheres with the bacteria growth, but also can predict its trend with high accuracy. Therefore, the model may play a significant role in the bioleaching processes.

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Cover page

Title: *Preparation of Granular Media by Using Construction Wastes and Their Performance on Hospital Wastewater Treatment by Biological Aerated Filter (BAF)*

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ABSTRACT

Construction wastes have characteristics of large reverses and unwieldiness [1]. In this experiment, a new filter material was developed with the principal raw material of construction wastes and used as granular media of biological aerated filter (BAF) to treat hospital wastewater. The influence of different hydraulic retention time (HRT), gas-water ratio (A/L) and height of media layer on the removal efficiency of COD and NH_4^+-N was investigated. Experimental results indicated that best removal efficiency achieved when HRT was 6h, A/L was 5:1and the height of media layer was 80cm. The treatment effect of two kinds of filter media which were prepared through different conditions was compared so as to determine the better preparation condition of granular media. Taking construction wastes as water purification material to treat hospital wastewater embodied the concept of recycling of resources. Moreover, this experiment also enriched the varieties and theoretical content of filter media and provided technical guidance for treatment of hospital wastewater.

INTRODUCTION

The construction waste is a kind of intractable solid garbage and how to deal with it is a major difficult problem that our country facing today [2]. The sources and compositions of the hospital wastewater are quite complicated and wastewater from different types of hospital is different. Hospital wastewater has intricate composition and extreme strong potential toxicity [3], so it can lead to large-scale pollution. Consequently, how to deal with it is a considerable problem that our country facing today.

BAF is a new type of biofilm wastewater treatment craft which developed in Europe and United States in the late 1980s and achieved greater development in the early of 1990s[4]. Comparing to conventional activated sludge process, BAF has the features of small floor area, high organic load, low operation costs and good treatment efficiency and so on [5]. As BAF technology is applied to wastewater treatment, the selection of granular media play an important role in maintaining a high amount of active biomass and a variety of microbial-populations[6]~[7]. In this study, we used construction wastes as filter media of BAF to treat wastewater from the second

Shanping Li, Xiaolong Ma, Xiaohong Cao, Yanyang Jiang, School of Environmental Science and Engineering of Shandong University, No.27, Shanda Roud(South), Licheng District, Jinan, China 250100

hospital of Shandong university and investigated the optimum preparation condition of filter media as well as the best operating conditions of BAF.

EXPERIMENTAL MATERIALS AND METHODS

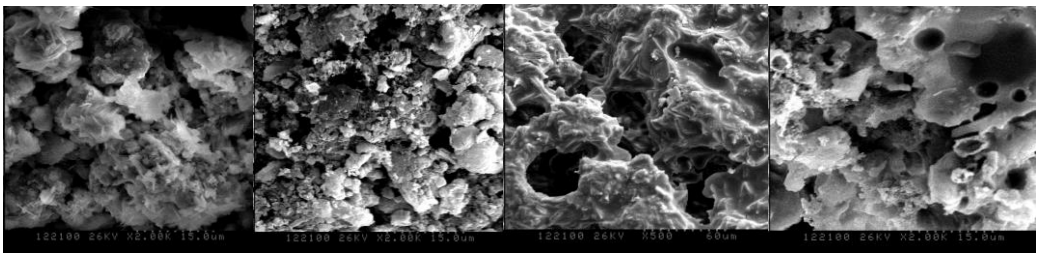
The preparation of granular media

RAW MATERIALS AND THE PREPARATION PROCESS OF GRANULAR

Medium selection is important for BAF to achieve effluent quality requirements [8]. In this experiment, we took construction wastes as main raw material and cement as the adhesives to manufacture spherical granular media. Construction wastes were retrieved from construction site and cement was bought from Jinan building materials market, Shandong. The construction wastes and cement were mixed with the ratio of 3 to 1 and the content of adhesive was about 8%, then we agitated and mixed them completely to make raw spherical objects which had diameters of 4mm ~ 6mm by balling machine. A new type of filter media could obtained by curing them about 5d in the way of natural conservancy, and calcining them for 15 minute at 1100℃could get another type [9].

THE MICROSCOPIC ANALYSIS OF CERAMISITE

Fig.1 shows that the filter media which were calcined had rougher surface and smaller holes in the internal of it than not. That means the former have higher surface adhesion and larger total surface than the latter. Larger surface roughness has stronger ability to re-distribution for influent, therefore, it creates a favorable inner environment for the mixing and contacting between microorganism and granular media [10]. Moreover, roughness surface also promotes the accumulation of biofilm in the surface of granular media.



(a) (b) (c) (d)

Figure 1 The analysis of ceramisite by SEM

The ordinary ceramisite before reaction (a) and after reaction (c)
The caclined ceramisite before reaction (b) and after reaction (d)

Experimental Apparatus

The two BAF were made of plexiglass with a diameter of 10cm. The reactors had an up-flow configuration, a height of 120cm containing 10cm of supporting layer and

80cm of filter media. In order to prevent water overflow and filter media flow out of BAF, the buffer was set at the top of BAF and its height was about 20cm. In addition, water distribution area was set at the bottom of BAF for the purpose of distributing water homogeneous and its height was about 10cm. Ports, at intervals of 16cm from the base of the columns, allowed samples to be drawn at specific intervals so that substrate removal profiles could be examined. Peristaltic pump was used to control water flow and velocity of inlet pipe as well as rotameter was used to control air flow and velocity. Of the two BAFs, one was packed with ordinary ceramisite (BAF_1) and the other with calined ceramisite (BAF_2).

The Characteristic and Quality of Hospital Wastewater

Wastewater from the Second Hospital of Shandong University has the features of intricate ingredient, high organic content and so on. According to the data from water quality monitoring station in the hospital and environmental monitoring department in the city, we determined main wastewater pollution parameters: COD, NH_4^+-N and BOD_5 is 400mg/L, 50mg/L and 200mg/L, respectively.

Analysis Methods

During the experiment, samples of influent and effluent were taken from different sample ports. The COD and NH_4^+-N were analyzed according the standard method [11]. The operating temperature of the whole system was between 20~30.

RESULTS AND DISCUSSICION

The Effect of HRT on Removal of COD and NH4+-N in BAF

Hydraulic retention time (HRT) is an important parameter to the removal efficiency of BAF [12]. In this part, 2h, 4h, 6h, 8h were selected as different HRTs for testing while keeping A/L at 5:1. The adaption time of BAF was about 3d after changing HRT. The concentrations of NH_4^+-N and COD of 1st and 2nd BAF were measured after the system could stable operating. Under every HRT, there was a two-week monitoring period. As shown in fig.3, the removal efficiencies of COD and NH_4^+-N at different HRTs.

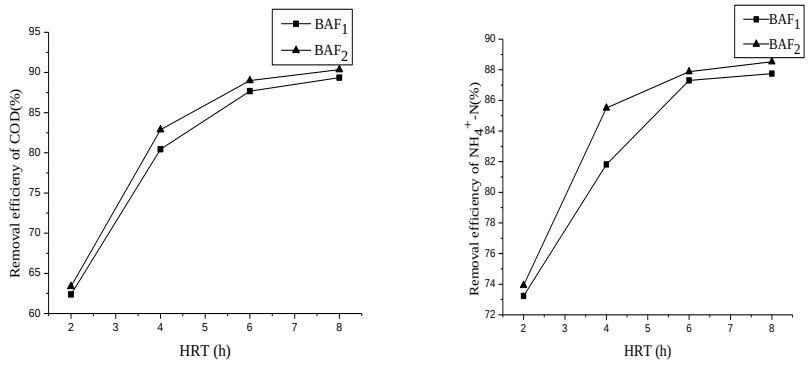


Figure 3 Influence of HRTs on average removal efficiencies of COD and NH4+-N

Fig.3 presents that the removal efficiencies of COD and NH_4^+-N increased with HRT increasing. It was because the contact time of organic matter and microorganism increased with increasing HRT [13]. The average removal efficiencies of COD were 89.4%, 87.7%, 81.4%, 62.4% in 1st and 90.3%, 88.9%, 82.9%, 63.3% in 2nd BAF, corresponding to HRTs of 8h, 6h, 4h, 2h，respectively. At the same HRTs, the average removal efficiencies of NH_4^+-N were 87.8%, 87.3% , 82.2%, 73.5% in 1st and 88.6%, 87.7%, 83.8%, 74.1% in 2nd BAF, respectively. This indicated that the removal efficiencies of COD or NH_4^+-N of 2nd BAF were better than the 1st at same HRTs and the calcined granular media were better than ordinary one in the aspect of degradation of organic compounds. Removal efficiencies of COD and NH_4^+-N changed little when HRT changed from 6h to 8h. Considering the conditions of reducing cost and saving time, 6h was selected as the best HRT.

The Effect of A/L on Removal of COD and NH4+-N in BAF

Gas-water ratio (A/L) is not only relating to the removal efficiency of BAF, but the issue of cost in the actual operation. Therefore, A/L is an important parameter to the operation of BAF. In the experiment, A/L was adjusted by changing gas flow and A/Ls of 3:1, 4:1, 5:1 and 6:1 were selected for testing. The adaption time of BAF was about 3d after changing A/L. The concentrations of NH_4^+-N and COD of 1st and 2nd BAF were measured after stable operating. Under every A/L, there was a two-week monitoring period. As shown in fig.4, the curves of removal efficiencies of COD and NH_4^+-N against A/L.

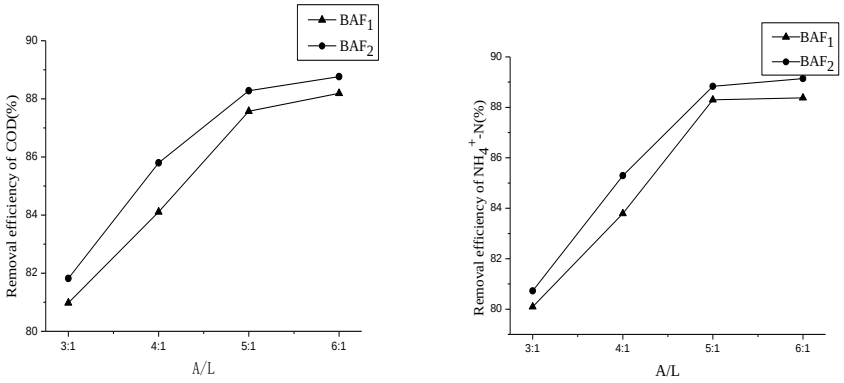


Figure 4 Influence of A/Ls on average removal efficiencies of COD and NH_4^+-N

Fig.4 presents that the removal efficiencies of COD and NH_4^+-N increased with increasing A/L. This was due to the increased of dissolved oxygen (DO) and biological activity of aerobic and facultative microorganism with increasing A/L. The average removal efficiencies of COD were 88.2%, 87.5%, 84.1%, 80.9% in 1st and 88.7%, 88.2%, 85.8%, 81.8% in 2nd BAF, corresponding to A/Ls of 6:1, 5:1, 4:1, 3:1, respectively. At the same A/Ls, the average removal efficiencies of NH_4^+-N were 88.3%, 88.1%, 83.7%, 80.0% in 1st and 88.2%, 87.8%, 85.3%, 80.7% in 2nd BAF, respectively. The removal efficiencies of COD and NH_4^+-N of 2nd BAF were better than the 1st at same A/L. Therefore, we also could conclude the calcined filter media were more suitable for wastewater treatment. Removal efficiencies changed little

when A/L changed from 5:1 to 6:1. Considering the conditions of reducing cost and saving time, 5:1 was chosen as best A/L.

The Effect of Granular Media Height on Removal of COD and NH4+-N in BAF

Sampling ports, at the height of 16cm, 32cm, 48cm, 64cm and 80cm of the columns, allowed samples to be drawn at specific intervals so that substrate removal profiles could be examined. Compared the upper with the bottom of media layer, the number of biofilm， the species of microorganism and removal efficiency of organics were different slightly. The influence of filter media height on removal efficiency of organics was investigated by monitoring the changing of COD and NH_4^+-N in different granular media heights. There was a two-week monitoring period in the experiment. As shown in fig.5, the curves of removal efficiencies of COD and NH_4^+-N against filter media height.

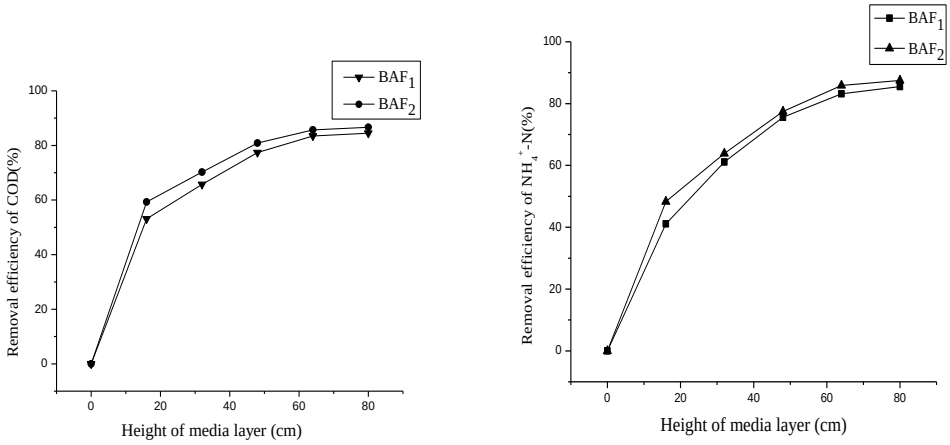


Figure 5 The removal efficiencies of NH4+-N and COD at different granular media heights

Fig.5 presents that the removal efficiencies of COD and NH_4^+-N increased with the increasing filter media height , but the rate of change became smaller and smaller. The average removal efficiencies of COD were 84.5%, 83.5%, 77.4 %, 65.7%, 53.1% in 1st and 86.7%, 85.6%, 80.9%, 70.2%, 59.3% in 2nd BAF, corresponding to heights of media of 80cm, 64cm, 48cm, 32cm, 16cm, respectively. At the same heights, the average removal efficiencies of NH_4^+-N were 85.5%, 83.2%, 75.6.7%, 61.1%, 42.1 in 1st and 88.0%, 83.7%, 76.7%, 61.2%, 42.6% in 2nd BAF, respectively. Consequently, 80cm was selected as best media layer height. The removal efficiencies of COD or NH_4^+-N of 2nd BAF were better than the 1st at same filter media height, it was indicated the calcined granular media were better than not.

CONCLSIONS

When the gas-water ratio (A/L) was 5:1, hydraulic retention time (HRT) was 6h and the granular height media was 80cm, the removal efficiencies of COD and NH_4^+-N reached the maximum and hospital wastewater discharge standard in Shandong Province. This experimental results showed that the calcined granular media were better than not and they had higher removal efficiency for organics. Wastewater

treatment granular media which were made by construction wastes had features of good rigidity, large total surface area and biological stability and so on. This study achieved the goal of waste control by waste, so it was worthy of large-scale using and popularized.

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