Bioethanol Production from Cassava (Manihot esculenta)

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Submission date: 07-Jun-2023 11:33AM (UTC+0700)
Submission ID: 2110788745
File name: enta_Peel_Using_Yeast_Isolated_from_Durian_Durio_zhibetinus.pdf (1.35M)
Word count: 3412
Character count: 18183

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IOP Conf. Series: Journal of Physics: Conf. Series **1095** (2018) 012016 doi:10.1088/1742-6596/1095/1/012016

Bioethanol Production from Cassava (*Manihot esculenta*) Peel Using Yeast Isolated from Durian (*Durio zhibetinus*)

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Abstract. The process of ethanol production generally involves pretreatment, hydrolysis of lignocellulosic biomass to fermentable sugars followed by fermentation of such sugars to ethanol. Waste of the cassava peel (*Manihot esculenta*) was hydrolysed by using sulphuric acidThis research aimed to produce of bioethanol as an alternative source of fuel using cassava peels as raw materials.. Yeast isolated from Durian fruit (Durio zhibetinus) was used in the experiment for fermentation and the concentration of sulphuric acid of hydrolysis process was fermented by yeast for 1 ; 2 ; 3 ; 4 ; 5 ; 6 ; 7; and 8 days. 50 ml of Sodium Hydroxide NaOH was prepared to be added at this step to adjust the pH of the slurry until 5 and the temperature was kept at 25 ℃. Nine samples were prepared at different three hydrolysis times at 121℃ for 30 minutes, 45 minutes and 60 ninutes. For glucose consumption and ethanol product analysis, 2 ml of the sample were *taken* out at *every* 2 days *interval until* 8 days. During this fermentation process, sugar consumption was measured by DNS method, while quantification of ethanol was analyzed by Gas Chromatography. The result of this study obtained that the best time of hydrolysis process was 45 minute, where the result of concentration of glucose was 11.189 %. By virtue of that, fermentation process was influenced by shaking incubator at 6 days. the optimum concentration of sulphuric acid of the hydrolysis process was 30 minute, and duration time of fermentation process by shaking incubator was 8 days, while the concentration of bioethanol for the highest of hydrolysis and fermentation process was obtained 1.63 % ethanol.

Keywords : Bioethanol, Fermentation, Yeast, *Durio zhibetinus*

1. Introduction

The world's economy today highly depends on fossil energy sources such as coal, oil, natural gas which are used to produce fuels, electricity, chemicals, and other goods. The increase of both human population and industrial prosperity will simultaneously be increase of global energy consumption. The utilization of these conventional fossil energy sources in the long run cause depletion of energy source and is not sustainable. Thus, we should provide raw material for the industry and human needs in sustaible way, and this is the greatest challenges for us. Furthermore, fossil fuels is non-renewable energy sources, limited stock, and have a considerable negative environment impact.

Bioenergy from renewable resources becomes an alternative replacing or supplement for fossil fuels. The raw material for alternative renewable bioenergy source is available and abundant the raw materials. Almost all petroleum-based fuels can be replaced by renewable fuels produced from biomass such as bioethanol, biodiesel, biohydrogen, etc [1-3].

Bioethanol is one of prominent bioenergy which has some advantages such as becaused it has a high octane fuel, and it reduces polluting emission. It is a clean and renewable biofuel with major environment benefit, burning of the oxigenated the fuel mixture consisting of ethanol and gasoline results more completely burnt and reduces polluting emissions.

Today, almost all the current fuel ethanol in first generation is generated from edible sources contain sugars and starch such as corn, cassava, potatoes etc. In the second generation, lignocellulosic biomass has drawn much attention in recent times. However, efficient technology development to convert lignocellulosic biomass into fermentable sugars are the key areas of development in second generation bioethanol production [4], such as utilization of simultaneous saccharification and fermentation (SSF) with a batch and fed-batch methods [5].

Cassava peels is abundant waste food in the developing country, where the high number harvest of cassava so more and more high waste of cassava peels. It has been acknowledged worldwide that cassava peel is one of the best choice to replace edible sources for fuel ethanol production, without endangering food security.

In conversion of carbohydrate into bioethanol, microbial agent plays an essential role especially in fermentation step. A common yeast used in fermentation process producing ethanol used yeast *Saccharomyces cerevisiae* as microbial agent [6-7]. This research, yeast isolated from durian (*Durio zhibetinus*) fruit was applied in fermentation of cassava peels.

2. Methodology

2.1. Sample and Material

Cassava peel was obtained from a 26 *ilir* traditional market located in Palembang. Chemicals used in this study consisted of, YPD medium (1% yeast extract, 2% peptone, 2% glucose), fermentation medium (2% yeast extract, 2% ammonium sulfate, 2% magnesium sulfate, and 4% potassium dihydrogen phosphate), bacto agar, sulfuric acid, and sodium hydroxide, 2,5-dinitro salicylic acid (DNS), and sodium potassium tartrate tetrahydrate.

2.2. Preparation of Sample

The cassava peels was thoroughly washed with tap water and cut into smaller pieces. Then cassava peels was dried in oven at 80℃ for 5 days. Once dried, the cassava peels was grinded using the grinding machine to get pul. The cassava peels sample was sealed in the seal bag or poly bag and stored in room conditions.

2.3. *Pretreatment and Hydrolysis*

Pretreatment and hydrolysis of cassava peel were conducted as protocol described in [8] with small modification. 30 grams of cassava peel flour was weighted into separate conical flasks and mixed with 150 ml of distilled water. The mixtures were covered with alumminium foil and then sterilized in an autoclave at 121℃ for 1 hour and allowed to cool and remove water. After that diluted 100 ml of 1% Sulfuric acid into each sample then autoclave in three different times at 121℃ for 30 min, 45 min and 60 min.

2.4. Inoculum preparation

Yeast isolated from Durian used for inoculation was grown in 15 ml reaction tube with 10 mL of YPD medium containing 1% yeast extract, 2% peptone, 2% glucose. After incubating in a room temperature with shaking 150 rpm for 1 day, the cell culture was aseptically transferred into fermentation medium to reach OD = 0.2 to start the fermentation.

2.5. Determination of glucose content using DNS method

Glucose content was determined using DNS method according to [9] with small modification. Both 0.3 mL of pre-heated substrate solution in 0.1 M Na-acetate and 0.3 mL of enzyme solution at 50^{o}C for 5 min)were mixed, and incubating it at 50^{o}C for 10 min. The mix solution was then added with 0.9 mL of 3,5 Dinitro salicylic acid (DNS) for 5 min, cooled at room temperature, and the absorbance was measured at 540 nm.

2.6. Fermentation

Fermentation was performed according to [10] with modification. The fermentation media was prepared as follows, 2 g yeast extract, 2 g ammonium sulfate, 2 g magnesium sulfate, and 4 g potassium dihydrogen phosphate dissolved completely in 500 ml water in a conical flask then autoclave at 121℃ for 15 min. 25 ml of this mixture media was added to each of the samples. 50 ml of 0.1M sodium

hydroxide was prepared to be added to adjust the pH of the slurry until 4.5 – 5 and the temperature was kept at 25 ºC.

2.7. Determination of ethanol content using Gas Chromatography

Ethanol content produced from fermentation process was measured by Gas Chromatography (GC 2010 Shimadzu) complemented with flame ionization detector (FID), column size was 30 m and 0.25 mm for length and diameter, respectively. The condition of experiment was rate of carrier gas, N2 136.3 mL/min, 100 kPa, temperature of injector 150^{o}C, column 60-240^{o}C, detector 200^{o}C.

3. Results and Discussion

3.1. Preparation of Cassava peel sample

Preparation of cassava peel covered washing, cutting, drying and grinding as shown in Figure 1. The cassava peels were washing with trap water to eleminate contamination. The cleaned samples were cut in order to easier in drying. The dried samples were grinded to obtaine the cassava peel powder. In powder form can increase degradation of lignin during pretreatment process, increase interaction between sample with sulfuric acid during hydrolisis process, and increase access enzymatic reaction during fermentation process.

Figure 1. Preparation of cassava peel sample consisted of washing, cutting, drying and grinding process.

3.2. Pretreatment and hydrolysis of cassava peel sample

Cassava peel is one of lignocellulosic material, mainly contains carbohydrate such as cellulose and hemicellulose, and non carbohydrate material lignin. Lignin should be reduced before fermentation of hydrolysate carried out. Pretreatment of cassava peel was conducted by physical method which is autoclaving 20% cassava peel – water mixture at 121^{o}C for 1 hour. This physical method was choosed because it is easier and make pH alteration significantly. Unfortunately, quantitative analysis of lignin content was not determined.

Pretreated cassava peel was hydrolyzed using 1% sulfuric acid to produce a reducing sugar, such as glucose. In this experiment, hydrolysis was conducted in variation time 30, 45 and 60 minutes which produce different results (Figure 2). The highest reducing sugars was produced in 60 min hydrolysis

time, 11.189% compared with 30 and 45 min hydrolysis time, and the latter producing the least amount. The result of the hydrolysis process of cassava peel was prepared as many as 12 samples to be fermented at room temperature and pH 5. Bioethanol from each sample in the fermentation process was collected at the 2nd, 4th, 6th, and 8th day each sample. Results showed that the optimum time of hydrolysis process was 60 min, which resulted 11.189%. Determination of glucose from hydrolysis process was done by DNS method. Principle of this method was reducing sugars have the property to reduce many of the reagents. Fermentation process by using durian isolate yeast which could convert glucose to bioethanol.

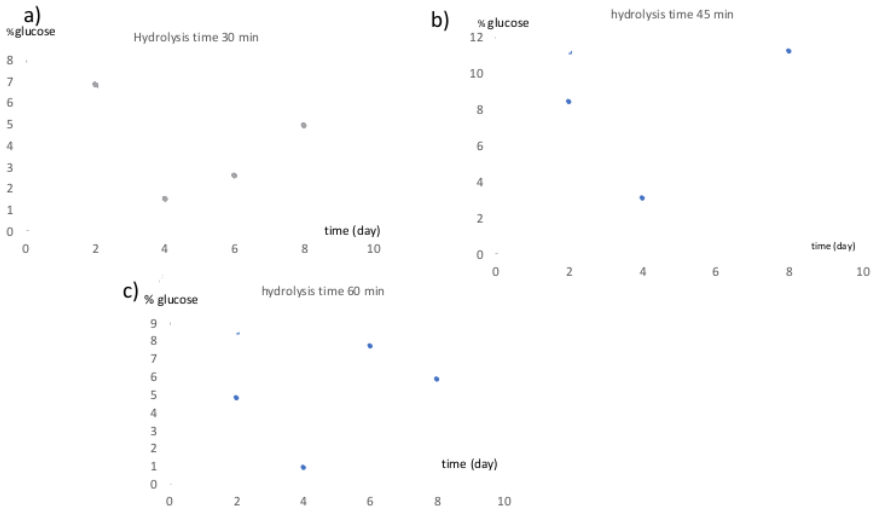

Figure 2. Glucose contents generated from hydrolysis of cassava peel with hydrolysis time variation 30 min, 45 min, and 60 min.

Comparison of the amount of reducing sugars expressed as hydrolyzed feedstock per 30 g raw feedstock, produced during the hydrolysis of the different times using 1% Sulfuric acid and 8 days shaking incubator process displayed the glucose content is start from high to low concentration, then continue increasing again in the last day of fermentation. So the result was not optimum concentration. This may be due to the disability of the pretreatment process did not convert cellulose and hemicellulose to glucose as well as it could be. Soaking in a aqueous ammonia treatment increased surface area and the pore size, and it lead to higher ethanol production [11].

The low amount of reducing sugars obtained from cassava peel during pretreatment may be attributed to the preparation procedure since they were not crushed but chopped, further confirming the importance of the preparative steps for pretreatment of the lignocellulosic feedstocks, along with the stringent preparative procedures required, illustrates the difficulty of using lignocellulosic feedstocks for ethanol production. The observed differences in the amount of reducing sugars produced from roots and other plant parts, further confirms this fact. Thus, the choice of feedstocks should encompass the time and effort required as well as the cost of preparation and hydrolysis, which has a bearing on the fermentation as well as the final product. The ability to produce sufficient amount of reducing sugars determines the importance of a particular feedstock for ethanol production. Feedstocks with ability to produce high amounts of glucose using simple hydrolysis procedures are important alternatives for biomass fuel production. All the factors mentioned above will in the end determine the final cost of the ethanol produced. The physiological state of the feedstock and the environmental conditions under which the feedstocks are grown, are also important factors to consider.

3.3. Fermentation of cassava peel using yeast isolated from durian

Yeast plays an essential role in fermentation process to convert carbohydrate into bioethanol. *S.cerevisiae* has well known as fermentation agent because of his ability to convert glucose into bioethanol. Yeast isolate used in this experiment was obtained from durian (*Durio zhibetinus*) fruit. Growth of yeast colonies when streaked onto YPDA agar media, it grew in small or large round, shiny, smooth and creamy morphology as shown in Figure 3. Utilization of yeast isolates from durian can explore indigenous yeast strain.

Figure 3. Colony of yeast isolated from Durian (*Durio zhibetinus*)

Fermentation of cassava peel hydrolysate was performed under room temperature condition with shaking 150 rpm. Bioethanol content generated by this fermentation was analyzed in the 2^{nd}, 4^{th}, 6^{th} and 8^{th} fermentation day time using Gas Chromatography. Based on the results obtained, 30 min hydrolysis with day 8 of fermentation showed the highest ethanol content in water which is 1.63 %, followed by 45 min hydrolysis in day 4 which is 1.42 %, then hydrolysis time at 60 min was 1.20% in day 8 . The lowest ethanol concentration in water with water was achieved at 30 min hydrolysis without shaking incubator time .

However this study shows lowest ethanol concentration in water at 30 minutes hydrolysis without shaking incubator time. This may be due to the disability of the yeast to fermentation at 0 day. There are other possibilities; the yeast that was used to conduct the experiment may be old. Old yeast will not carry out fermentation process efficiently compared to new yeast. This statement is not applicable for this study since at 30 minutes hydrolysis there is almost no ethanol production.

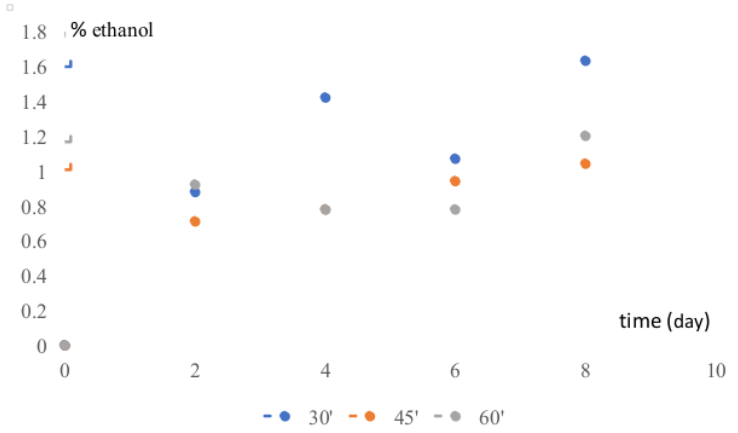

Figure 4. Bioethanol production from hydrolysate of cassava peel during fermentation, sample were analyzed in 2nd, 4th, 6th, and 8th day. Hydrolysis times were 30 min, 45 min, and 60 min.

Figure 4 shows three different hydrolysis times in affecting the bioethanol production. Overall, we can see that all of this have the same trend. They are on increasing trend. The production of bioethanol growed higher by days. However in the middle of timeline. In day 6 for 30 and 60 minute of the hydrolysis time, the production of ethanol seems to go down but go up again after that. But in the hydrolysis time 60 minute, in 4th day the production go down but go up again later. And all of productions in 3 conditions peak at the last days.

The high content of dry matter observed in the parts of cassava plants may be hydrolyzed into fermentable sugars. This has a bearing on the final yield of reducing sugars, since high contents of dry matter are desirable in ethanol production . The ethanol content between the different times of the hydrolysis and fermentation present study, 30 minutes hydrolysis in day 4th ,6th, 8th showing high levels of ethanol content compared to other 45 and 60 minutes hydrolysis, were notable since a significant relationship between ethanol properties and hydrolysis process in three different times existed. Long time hydrolysis content resulted in production of lower quantities of ethanol.

Fermentation efficiency depended on the ability of the yeast to utilize particular feedstocks based on their characteristics and compositional differences. The absence of differences in fermentation efficiency during the first 8th day was due to the yeast establishing itself in the fermenting solution, growing to a certain colony volume able to utilize the existing sugars. The variations in fermentation efficiency may be attributed to the type of sugars produced as well as substrate preferences by the fermenting organism, since different times of the hydrolysis produce different sugar types apart from. Although ethanol can be converted from glucose, however it reported that yeast cell growth and ethanol fermentation performance have no significantly difference from the cultures of glucose, corn stover hydrolysate liquid, and the pretreated corn stover solids as carbon sources, respectively [12]. Other study reported that increasing the yeast inoculum or cellulose concentration did not significant improve the ethanol yield or concentration [13].

The pretreatment process of feedstocks affect their hydrolysis, consequently affecting the type of reducing sugars produced and hence moderating the type of metabolism carried out by yeast under shaking in incubator. In particular, lignocellulosic materials in hydrolyzed peels may result in production of small molecular weight compounds such as furan derivatives, phenolic compounds, and amine-based compounds such as vanillin, all inhibiting fermentation. The low ethanol percentage obtained with progressive fermentation of sugar is most likely due to the fact which may inhibit its metabolism and hence reduce its efficiency.

4. Conclusion :

The use of cassava peel for bioethanol production as an alternative source of fuel provides a starting point for improvements in cultivation and adoption of cassava as well as improving food security.
This study concluded that the optimum concentration of sulphuric acid of the hydrolysis process was 30 minute, and duration time of fermentation process by shaking incubator was 8 days, while the concentration of bioethanol for the optimum of hydrolysis and fermentation process was 1.63 %. These indicated that it needs to do more experiment to improve the higher yield.

Aknowledgment

This project was financed by a grant from Scheme "Hibah Kompetitif UNSRI 2017".

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