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Abstract :

MARDI developed the RiceFert formulation to assist in fertilizer recommendation rate for optimal paddy growth. The formulation followed an adjustable soil test–target yield equation (ST-TY) into dedicated software. The output from the RiceFert software includes a total rate of N, P2O5, and K2O fertilizer (kg/ha) and split application or straight fertilizer rate (kg/ha). Additionally, the output from RiceFert was integrated to GIS to produce maps according to the specific fertilizer recommendation rates. With interpolation techniques, thematic maps added more information, such as total area coverage according to individual classification classes. This paper discusses the overall process of fertilizer recommendation rate for paddy fields, starting with the RiceFert formulation, followed by map production using interpolation techniques according to the fertilizer recommendation output. The RiceFert strategy is expected to benefit local authorities because it offers vital information to farmers for optimal rice growth and output.

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Abstract :

Geothermal manifes-tations of hot springs are formed due to the flow of hot fluid that comes from rock fractures that are below the surface. The nature of various rocks and the heat produced from below the surface makes the characteristics of each geothermal system different. One of them has an impact on each chemical content of the fluid that appears on the surface varies. The purpose of this research was to find out more about the condition of existing hot springs as the object of study in determining the type of fluid and functional groups manifestation geothermal hot

springs of Tolok I village, Tompaso, Minahasa Regency. For determining the type of fluid laboratory tests were carried out and the results of these laboratory tests were conducted by Liquid Chemistry Plotting on the ternary plot diagram. To analyze fluid functional groups, water samples were tested using FTIR spectroscopy. The measurement of physical parameters was also carried out to support this research. The results showed that the type of fluid in the study area was chloride type with a percentage of 51% and the functional groups obtained were functional groups of amides, ketenes and carbonates.

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Title : Aqueous Ammonia Soaking-Dilute Acid Pretreatment to Produce Bioethanol from Rice Hull

Abstract :

The objective of this work is to investigate the effect of aqueous ammonia concentrations and pretreatment temperatures of rice hull to produce bioethanol. Aqueous Ammonia Soaking (AAS) and dilute acid were used to pretreat rice hull. The rice hull composition was analyzed by SEM and Chesson methods. AAS pretreatment was carried out at various aqueous ammonia concentrations of 5, 10, 15, 20, 25 % (v/v) and temperatures of 60, 70, 80 90, 100 oC. The result showed that the highest lignin reduction and glucan recovery were about 61.97%, 88.39%, respectively at ammonia concentration of 20% (v/v) and pretreated temperature of 100 oC. Furthermore, the result produced the highest glucose of about 241.8 mg g-1 and the bioethanol yield of 5.86%

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Aqueous Ammonia Soaking-Dilute Acid Pretreatment to Produce Bioethanol from Rice Hull

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Abstract— The objective of this work is to investigate the effect of aqueous ammonia concentrations and pretreatment temperatures of rice hull to produce bioethanol. Aqueous Ammonia Soaking (AAS) and dilute acid were used to pretreat rice hull. The rice hull composition was analyzed by SEM and Chesson methods. AAS pretreatment was carried out at various aqueous ammonia concentrations of 5, 10, 15, 20, 25 % (v/v) and temperatures of 60, 70, 80 90, 100 ℃. The result showed that the highest lignin reduction and glucan recovery were about 61.97%, 88.39%, respectively at ammonia concentration of 20% (v/v) and pretreated temperature of 100 ℃. Furthermore, the result produced the highest glucose of about 241.8 mg g^{-1} and the bioethanol yield of 5.86%.

Keywords— Aqueous Ammonia, Bioethanol, Dilute Acid, Fermentation, Enzymatic Hydrolysis.

1. Introduction

Lignocellulosic biomass is renewable natural resources. The world production of lignocellulosic material reached 200 Billion tons/year, of which over 90 % from lignocellulosic biomass residue (Saini et al., 2015). Rice hull is obtained from rice grain collection. Indonesia has a great potential of rice hull wastes to be utilized. Indonesia paddy production on 2018 was about 56.5 million ton dry grain milled. It is about 11 million ton of rice hull wastes was produced. Farmers commonly remove rice hull by fire before the further cultivation period. The firing of rice hull is a conventional agricultural tradition since it produces the pollutions to atmosphere. Therefore, rice hull removal requires to be recovered to decrease the effect on the environment and public physical condition. For defending of environment and enhancing of its value, rice hull has been utilized as a natural resource to produce bioethanol. According to previous study [1], the compositions of rice hull were 37.48% cellulose, 10.4% hemicellulose and lignin 16.71%.

Pretreatment has been investigated as a main stage for the succesfull uitilization of lignocellulosic raw material to produce bioethanol. A pretreatment stage is required to break down lignosellulosic material structure to make it more acceptable by enzyme during hydrolysis. The pretreatment requires the following prerequisites: (1) enhance the creation of sugars, (2) reduce the degradation of carbohydrate, (3) prevent the creation of inhibitor on the hydrolysis and fermentation, and (4) less cost.

Numerous researches have been performed to improve pretreatment methods. Pretreatment aims are to reduce cellulose crystallinity, enhance hemicellulose, destroy the lignin cover [2]. The perfect pretreatment comprises on preventing the demand for decreasing the material particles size, protects the hemicellulose parts, restricts creation of decomposition compounds that prevent microbes growth on fermentation, reduces energy needs and decreases costs [3]. The type of numerous pretreatments and their combinations have been investigated [4]–[7]. Pretreatment types of lignoselllosic biomass comprise dilute-acid [8], [9], ammonia fiber explosion (AFEX) [10], [11], aqueous ammonia soaking (AAS) [7], [12], and alkaline pretreatments [13].

Aqueous Ammonia Soaking (AAS) is one of the alkaline pretreatments and its main objective is to reduce lignin content on biomass. AAS is a promising technique for biomass pretreatment. Using Aqueous Ammonia Soaking (AAS) pretreatment, the lignin substance of the lignosellulosic material has been reduced to a preferred amount [14], [15]. Previous authors [14] researched AAS method to improve a yield of 95.9% for hydrolysis of sugarcane bagasse and of 92.45% for fermentation process. Other researchers [16] studied influence of AAS on rice straw and found a yield of 71% for sugar and of 83% for ethanol. Use of AAS method has not yet been investigated for rice hull. Contrasting to other alkalis, ammonia is highly effective for lignin removal [14]. Aqueous Ammonia Soaking (AAS) was selected because this pretreatment gives the benefits of a moderate temperature condition and high elimination of lignin [17]. It has also already been performed to several kind of lignosellulosic materials, such as barley hull, rice straw, sugarcane baggase, oil palm trunk, corn stover, and sorghum, rye straw [7], [14]–[17].

A lower content of hemicellulose and lignin from biomass is a key important step to produce ethanol from lignocellulosic materials. In addition, the succesful degradation of hemicellulose and lignin as well as lowest cellulose elimination and byproducts minimum creation has been complicated due to the dissimilar conditions of enzymatic hydrolysis for hemicellulose and lignin. Dilute acid pretreatment was applied by the further step which highly reduced of hemicellulose, partly destroyed the cellulose crystalline composition, and increased the cellulose enzymatic digestibility. It was investigated by previous study [18] that an aqueous ammonia followed by dilute acid pretreatment encountered the effective goal campare than one step pretreatment. They found that the enzymatic hydrolysis yield of pretreated sample was 96.9% at enzyme loading of 60 FPU g-1 of glucan.

In this work, rice hull was treated with Aqueous Ammonia Soaking (AAS) and followed by dilute acid pretreatment. The first step was performed by Aqueous Ammonia Soaking (AAS) to remove lignin content. Once dissolving the lignin at moderate temperature while sustaining cellulose and hemicellulose complete. The following step was conducted by a low concentration of sulfuric acid to solubilize hemicellulose with a low temperature condition. Effect of various ammonia concentrations and pretreatment temperatures was studied as to enzymatic hydrolysis and fermentation processes. The purpose of pretreatment developments was also considered by analysis of the configuration and morphology of rice hull with Scanning electron microscope (SEM).

2. Materials and Methods
Rice hull was gathered from a Pemulutan Village Ogan Ilir, South Sumatera, Indonesia. It was dehydrated by sunshine for 1 d. Furthermore, it was crushed and then screened to get particles of 0.85 mm. Initial components structure of rice hull was investigated by Chesson standard methods (Datta, 1981). The results of of these analyzes showed that the rice hull was comprised of 39.34% cellulose, 5.30% hemmicellulose, 37.22% lignin and 10.80% ash on a dry weight basis. Scanning electron microscope (SEM) micrographs of the sample was conducted by a SEM-EDS.

2.1. AAS-Dilute Acid Pretreatment
50 g of rice hull (dry weight) was prepared in 1000mL Erlenmeyer. Then various volume fraction of aqueous ammonia solutions were also fed into erlenmeyer of about 5% to 25% at solid-to-liquid ratio of 1:10. Pretreatment was carried out at various temperature of 60 ºC to 100 ºC during 5 h. There was no agitation during pretreatment. The slurry was filterred and separated with a vacuum fliter. The solid phase was neutralized until a pH of 7. Then AAS pretreated samples were investigated by a Chesson method (Datta, 1981). The micrograph pictures of the AAS pretreated samples were analyzed by a SEM-EDS.

25 g of AAS pretreated sample was inserted into 500 mL Erlenmeyer. 250 mL of H_2SO_4 solution with a volume fraction of 2% was placed into erlenmeyer. Slurry was agitated for 1 min. In addition, slurry was treated at temperature of 85°C for 75 min. Then slurry was filterred and separated with a vacuum fliter. The solid phase was neutralized until a pH of 7. Furthermore AAS-dilute acid pretreated samples were analysed by using a Chesson method (Datta, 1981). The micrograph pictures of the AAS-dilute acid pretreated samples were investigated by a SEM-EDS.

2.2. Preparation of Cellulase Enzyme from Aspergillus niger

The inoculum culture 100 mL was prepared, consisted of 12.5% of sucrose, 0.25% of $(NH_4)_2SO_4$ and 0.2% of KH_2PO_4. Solution pH was adjusted to pH of 3 by using HCl 1M. Then the end of the wire loop dipped in 96% ethanol and heated in a Bunsen flame until red colored. Aspergillus niger culture PDA media were taken by using a wire loop dipped in liquid media. This work was carried out in the aseptic room. Liquid medium was then covered with the cotton and incubated with temperature of ± 30 °C at 24 h.

20 g rice hull (dry weight) was inserted into 250 mL beaker glass and prepared by media comprising: 0.03 g urea, 0.005 g $MgSO_4.7H_2O$, and 0.0023 g KH_2PO_4. The aqueous 80 mL was inserted to a media and setted to pH of 5. Media was autoclaved at 121°C for 15 min. After cooling process, 10 mL of the suspended spores of Aspergillus niger was added into the media. It was incubated for 96 h at 35°C and shaken at 150 rpm. After the incubation, the contents of these flasks were aseptically centrifuged and were used for enzymatic hydrolysis.

2.3. Enzymatic hydrolysis

100 g of pretreated sample was placed in 1000 mL Erlenmeyer. Then a media consisted of 5 g L^{-1} yeast, 7.5 g L^{-1} $(NH_4)_2SO_4$, 3.5 g L^{-1}, K_2HPO_4, 0.75 g L^{-1}, $MgSO_4•7H_2O$ and 1 g L^{-1} $CaCl_2•2H_2O$, was inserted with a mass to volume ratio of 1:10 and pH of 5. The mixture was autoclavated at temperature of 121 °C for 60 min [19]. Then, slurry was cooled and then added enzyme with a volume to mass of 10%. The hydrolysis reaction was applied at temperature of 50 °C and agitated at 200 rpm for 24 h. Furthermore, its temperature was decreased to 30°C. The glucose concentration was determined by Spektrofotometric UV-VIS.

2.4. Preparation of Yeast Saccharomycess cerevisiae

This research used yeast Saccharomycess cerevisiae. Pre-cultur was innoculated by 1 loopfull yeast in 500 mL shake-flask bottles with 150 mL of YPD-medium. It was then put on infors HT Ecotron Rotary Shaker at temperature of 32 °C for 24 h. YPD-medium contained of 10 g yeast extract, 20 g pepton and 20 g glucose.

2.5. Fermentation conditions

All of laboratry equipment used for fermentation process was pasteurized in a autoclave at temperature of 120 °C for 20 min. 1 mL of pre-cultur was added into erlenmeyer that contained slurry. It was stirred at temperature of 30 °C homogenly. Then, erlenmeyer was closed by clog rubber which has two branch (for CO_2 gas and sampling). Furthermore, it was put on rotary shaker at 120 rpm for 5 d. After fermentation process, residue was separated by using centrifuge. Then, ethanol was separated from its mixture by distillation process. The ethanol concentration was analyzed by a gas chromatograph (model GC-2014: Shimadzu) with a flameionization detector (FID).

2.6. Analytical methods

The configuration of either untreated or AAS-Dilute Acid pretreated rice hull were investigated by using using a Chesson method.(Datta, 1981) The cellulose recovery (%, mass fraction) was defined by dividing the value of pretreated rice hull -cellulose by initial cellulose content of raw rice hull and multiplying by 100. The glucose concentration was determined by Spektrofotometric UV-VIS. The ethanol concentration was analyzed by a gas chromatograph (model GC-2014: Shimadzu) with a flameionization detector (FID) at 250 ∘C. A column temperature was kept isothermally at 150 ∘C and nitrogen was applied as carrier gas. The ethanol standards were provided by a commercial grade ethanol.

2.7. Scanning electron microscopy
Surface morphological characteristics of unpretreated rice hull and AAS-Dilute Acid pretreated rice hull were analyzed by a JEOL scanning electron microscope (SEM) model JED-2200 SERIES. The SEM images were take taken at magnifications of 500–1000 by utilizing accelerating voltage of 15–20 kV.

3. Results and discussion
The main parts of lignosellulosic raw materials are cellulose, lignin and hemicellulose. Commonly, the structures of the untreated rice hull (UTRH) consist of cellulose (39.34%), hemicellulose (5.30%), lignin (37.22%), ash (10.80%) and hot water soluble (7.33%).

3.1. Biomass analysis using Scanning electron microscopy
SEM images of untreated and AAS pretreated rice hull are ilustrated by Fig. 1. Unpretreated rice hull described rigid and hard fibril configurations (Fig. 1a) which enlarged next AAS pretreated (using 20% aqueous ammonia, temperature of 80ºC – 100ºC) due to lignin and hemicellulose fractionation (Fig. 1b-1d). AAS pretreatment produced in more fibres ruptures and coarser building of the fibres. In the AAS pretreated rice hull, the shell film was detached and the cell wall was changed, causing in contact of inner buidings (Fig. 1b-1d) which was reliable with observation study of sorghum sturcture by previous author [12]. They also reported a damage of crystal constructions of sorghum with ammonia pretreated. Changing of lignin structure of rice hull can also be seen from its particle physic form. Before pretreatment, the colour of particle was light yellow and rigid surface structure. However after AAS pretreated, its colour was dark yellow and more surface area due to lignin destructed. It can be seen from fig 1b to 1d that texture of biomass destroyed so leads more pore. This is due to lignin bond decomposed and broken down carbohydrate on biomass. The increasing of surface are of biomass led contact between enzym and cellulose easier, higher conversion of glucose. Minor fiber frames (most possibly lignin) persisted by the AAS pretreated material, signifying that mostly cellulose was enzymatically hydrolyzed to bioethanol. Other researcher [12], studied a devastation of sorghum configurations of dilute ammonia pretreatment. Their result showed that dilute ammonia pretreatment lead enlarging and swelling highly for sorghum, as contrasted with unpretreatment.

The high digestibility of pretreated lignosellulosic material is possibly due to the increasing of cellulose accessibility as a outcome of hemicellulose removal and lignin rearrangement (Harun et al., 2013). Furthermore, the analysis of the hydrolysis trends of AD1RH, AD2RH and AD3RH, the SEM analysis presented additional knowledge of the hydrolysis of the AD1RH, AD2RH and AD3RH substrates based on morphological alterations of the UTRH and AAS pretreated rice hull surface. SEM pcitures of UTRH illustrated that mostly shell and silica coats on the surface were previously cracked through the crushing which prominently assisted AD1RH term by means of the surface resistance was lower than uncrushed rice hull. When the rice hull was rigorously pretreated with the AD2RH condition, it degraded lignin structures. It produced more hydrolysis operation. The surface of AD3RH was less compact contrasted to AD2RH, describing the effect of high ammonia concentration on lignin content on pretreatment.
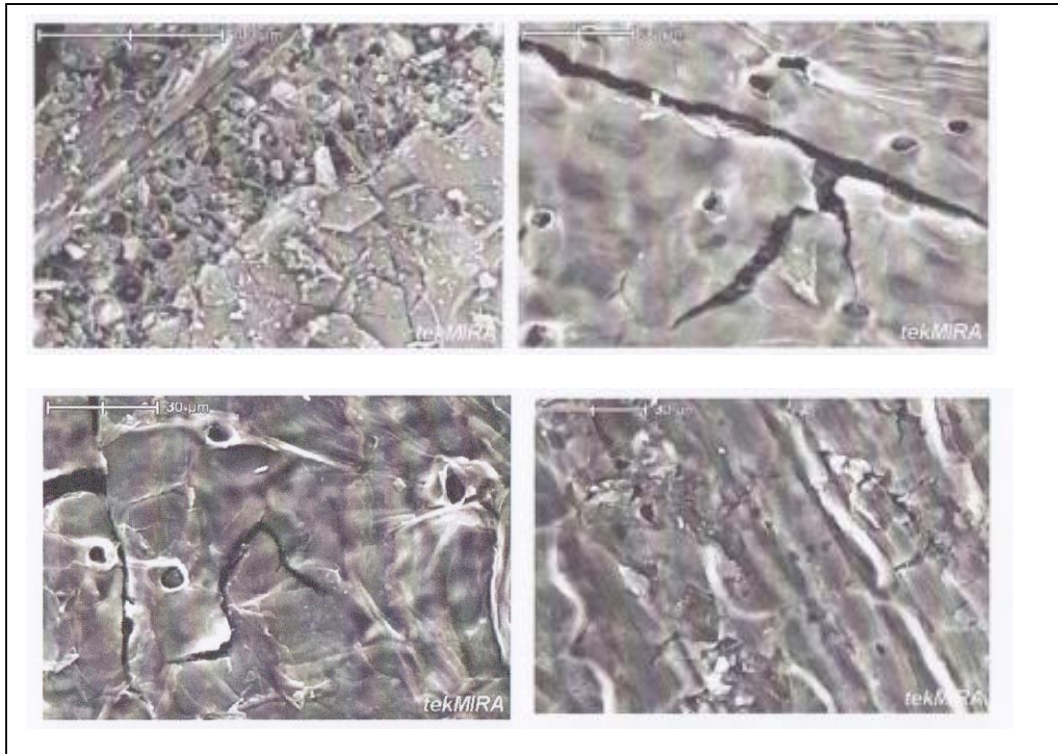
Fig. 1. Scanning Electronic Microscopy (SEM) pictures of various cellulosic materials: (a) Unpretreated rice hull (UTRH), (b) AAS D1 pretreated rice hull (AD1RH), (c) AAS D2 pretreated material (AD2RH), (d) AAS D3 pretreated material (AD3RH). Magnification of image provided in Figure 1a is 500 X, while, all other images are magnification at 1000X.

Figure 1 illustrates the SEM pictures of outer hull surface of unpretreated rice hull (UTRH – 1a) and after AAS pretreatment rice hull (AD1RH – 1b; AD2RH – 1c; AD3RH – 1c) samples. In AD1RH and AD2RH, several silica parts were uncovered on cellulose fibrils because of removal of epidermis layer by moderate AAS pretreatment (Figure 1 (1b, 1c)), since silica is accumulated as a film under a shell layer. When the cellulose structures was completed, some receptor structures were destroyed, illustrating a breakdown of several shell layers. AAS D1 and AAS D2 conditions (AD1RH and AD2RH) disposed few shell layers. However, it was not approriate enough to create the cellulose more reachable to the enzymes. SEM pictures describe that AD3RH had a quite spotless cuticle surface (Figure 1(1d)). Most of shell and silica films were reduced, and a great bonds together with lignin were distorted. The absence of shell and silica coats, clear cellulose fibers, enhanced the capability of the enzymes to acces cellulose. It produced highest concentration of bioethanol.

3.2 Effect of ammonia concentrations on percentage of lignin reduction during the AAS-Dilute Acid pretreatment of rice hull at various temperature.
The major component of raw rice hull was taken as 39.34% cellulose, 5.3% hemicellulose and 37.22% lignin. During biomass pretreatment, lignin prevents carbohydrates from changing to chemicals, thus it is the most key important issues influencing the effectiveness [20]. The effective factors of biomass pretreatment have been determined by highly recovery of cellulose and hemicellulose to produce sugars and less lignin concentration in pretreated material in order to enhance the cellulose acceesibility to

enzymatically hydrolysis [21]. Based on these factors, this research combined aqueous ammonia soaking (AAS) and diluce acid pretreatment.
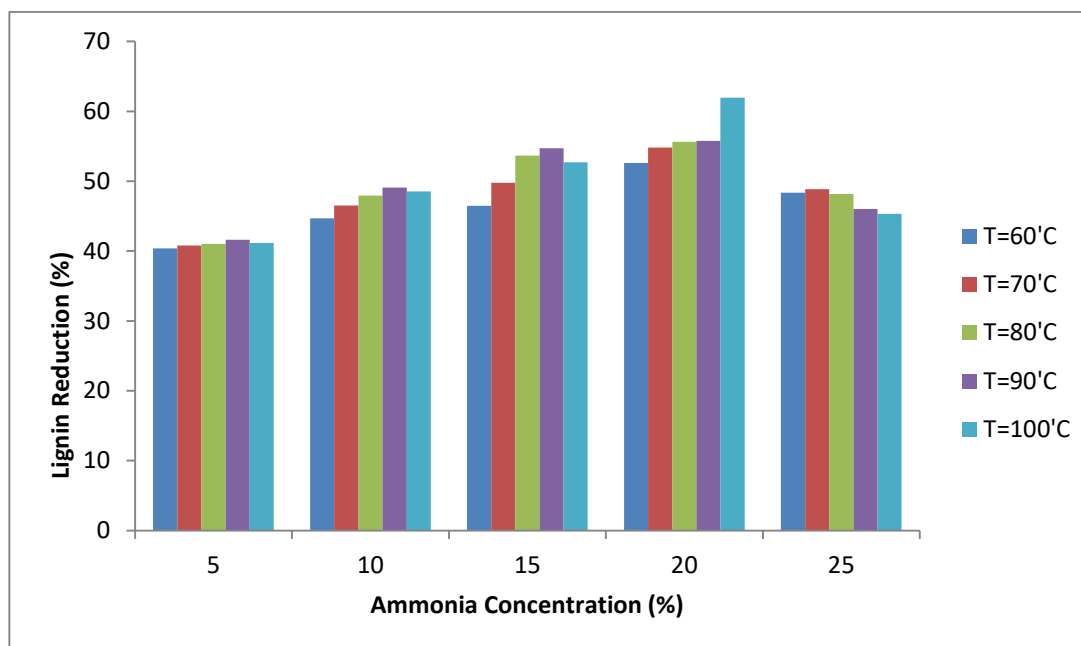

Fig. 2. Effect of ammonia concentrations and temperatures on percentage of lignin reduction during the AAS-Dilute Acid pretreatment of rice hull.

Lignin reduction is the major objective of pretreatment [22]. Fig. 2 describes the percentage of delignification of rice hull for 5 h of the AAS pretreatment time and 1:10 of solid-to-liquid ratios. The effect of five volume fractions of ammonia at 5, 10, 15, 20 and 25% and pretreatment temperature of 60, 70, 80, 90 and 100 ºC were studied. As described in Fig. 2, the decreasing of lignin between 40.39 to 61.97% of its of its early amount in rice hull. This significant amount of lignin reduction taking place in the AAS-Dilute Acid pretreatment of rice hull triggered the enhancing of enzymatic digestibility. When the ammonia concentration enhanced from 5 to 20 %, the percentage of lignin reduction raised from 40.39 to 61.97%. The highest percentage of lignin reduction, 61.97%, was produced at the ammonia concentration of 20%. Nevertheles, further enhances in the ammonia concentration to 25% gave a lower lignin reduction percentage of 15%. This is due to a critical pH of ammonia pretreatment has been attained at 20% concentration [15]. In addition, higher concentration af ammonia did not produce an enhance of enzymatic digestibility. Other researchers [16], [17] studied aqueous ammonia soaking to pretreat biomass, the optimum ammonia concentration between 15% to 21% at temperatures of 60 to 75 ºC. According to previous author [15], a higher ammonia concentrations did not effected in an enhance of enzymatic digestibility. It was shown that lignin reduction by 20% of ammonia was higher than other concentrations. In addition, an ammonia concentration of 20%, which provided a lignin reduction percentage of 61.97, was decided as the optimum concentration for AAS pretreatment of rice hull. Once an ammonia concentration higher than 20 % did not effectt in a considerable improve lignin removal percentage, an ammonia concentration of 20%, which produced a lignin reduction percentage of 61.97%, was chosen as the optimal ammonia concentration for further study. According to previous author [15], if the pH lignocellulose is the important effect in the AAS pretreatment, the optimum ammonia concentration was different from the other works employing different lignocellulose raw materials.

A lower temperature range was selected to alleviate the influence of autohydrolysis reaction occuring at a higher temperature which will cause loss of carbohydrates [23]. Throughout the AAS pretreatment, thermal energy disrupts the bonding structures of rice hull. The AAS pretreatments have been considered by obtainiing an effective capability of delignification without significantly influencing the other components structure [15]. In this study, 61.97% of the lignin content was eliminated after the AAS-dilute acid pretreatment (Fig. 2). This amount was similar to previous works [16], [17]. They conducted AAS (15% volume fraction aqueous ammonia solution) pretreatment of barley hulls at 70 ºC with two solid-to-liquid ratios: 1:6 and 1:10. It was obseved that the increase in aqueous ammonia solution concentrations significantly enhanced delignification. The lignin reduction of 20-60 % in a biomass is adequate to enhance the enzymatic digestibility [16].

3.3. Effect of ammonia concentrations and temperatures on glucan recovery during the AAS-Dilute Acid pretreatment of rice hull

Based on the results shown in Fig.2, 100 ºC and 20%, which produced a lignin reduction percentage of 36.62, was selected as a the optimum temperature and ammonia concentration for further enzymatical hydrolysis in this work, respectively. The glucan recovery of rice hull increased from 60.76% to 88.39% after the AAS-dilute acid pretreatments (Fig. 3). Therefore, the combination of AAS and dilute acid pretreatments were successful for the removal of lignin, producing in high cellulose contents in the solid phase that has been enzymatically hydrolyzed and fermented to ethanol subsequently. The highest recovery of glucan after pretreatment was about 88.39% which is considerable value with other works [15]–[17].
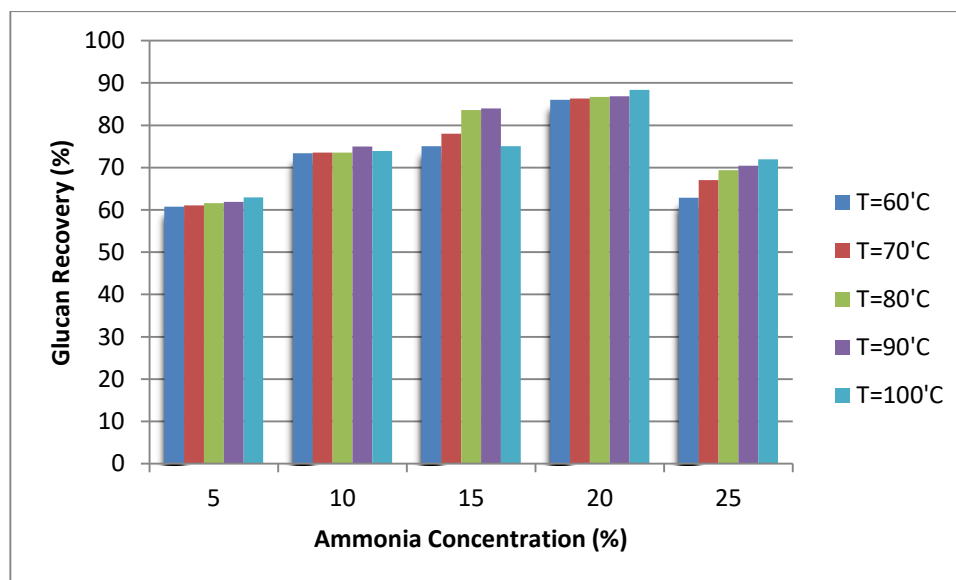

Fig. 3. Effect of ammonia concentrations and temperatures on glucan recovery during the AAS-Dilute Acid pretreatment of rice hull.

3.4. Effects of pretreatment temperatures on glucose concentration during enzymatically hydrolysis

Fig. 4 presents glucose concentration during enzymatically hydrolysis at various pretreatment temperatures. The hydrolysis of AAS-dilute acid pretreated rice hull was conducted by using cellulase enzyme from Aspergillus niger with concentration of 10% (v/w) at a range of pretreated temperature of 80-100 ºC. As shown in Fig. 4 that glucose concentration increased with pretreatment temperature. The glucose concentration of AAS-dilute acid pretreated rice hull were as low as 232.2 mg g^{-1}, 235.3 mg g^{-1}, and 241.8 mg g^{-1}, respectively, after 24 h enzymatic hydrolysis. The highest glucose concentration was about 241.8 mg

g^{-1} obtained at temperature of 100 ºC and ammonia concentration of 20%. The high glucose concentration in pretreated rice hull contributed to lignin reduction due to cutting of lignin-carbohydrate polymer complex by ammonia. This can cause the formation of pore and swelling on biomass polymer so the surfaec area enhanced and the accesibility of enzyme improved [20]. At the higher concentration, ammonia altered the crystalline configuration of cellulose and enhanced the glucose production. Meanwhile, lignin degradation also occured, however had no influence on the enzymatic hydrolysis. As shown in Fig. 2 and Fig.3 before, AAS-dilute acid pretreated rice hull at aqueous ammonia concentration of 20% had more glucan recovery and lignin reduction than others, which made cellulose more accesible to enzym and produced high cellulose digestibility. This result illustrates that the AAS-dilute acid preteatment leads succesful enzymatic hydrolysis of rice hull.
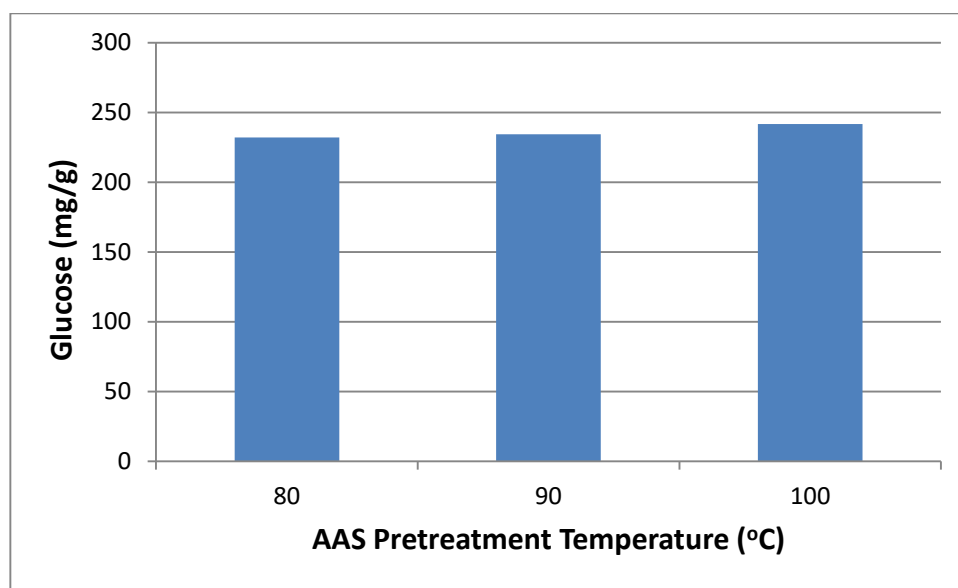

Fig. 4. Effect of pretreatment temperatures on glucose concentration during enzymatically hydrolysis.

3.5. Effect of pretreatment temperatures on ethanol yield during fermentation

Fig. 5 describes the ethanol yields produced from 80ºC, 90ºC, and 100ºC AAS-dilute acid pretreated ruce husk. The fermentation was carried out with 1 mL of yeast sacharomicess cerevisiae pre-cultur for 5 d at a range of pretreated temperature of 80-100 ºC. The highest ethanol yield was about 5.86 % produced by AAS pretreatment temperature of 100 ºC and ammonia concentration of 20% . As illustrated in Fig. 5 that ethanol concentration enhanced with pretreatment temperature. This trend is similar to glucose concentration profile. The ethanol yields of AAS-dilute acid pretreated rice hull were as low as 4.05%, 5.07%, and 5.86%, respectively, after 5 d fermentation.
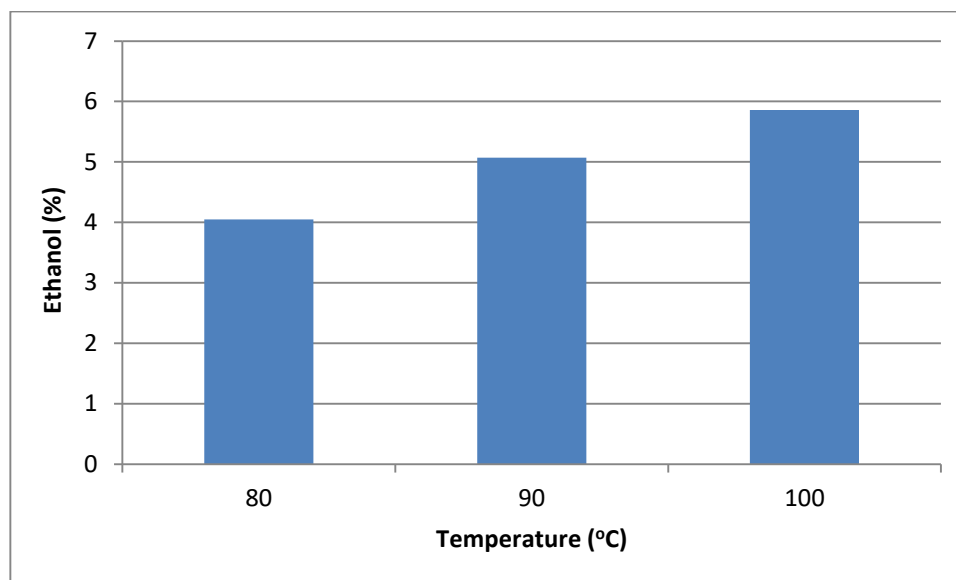
Fig. 5. Effect of pretreatment temperature on ethanol yield during fermentation.

4. Conclusion

Unpretreated rice hull had rigid and hard fibril structures which enlarged after AAS pretreatment due to lignin and hemicellulose fractionation. The increasing of surface are of biomass led contact between enzym and cellulose easier, higher conversion of glucose. The effects of five volume fraction of ammonia at 5, 10, 15, 20 and 25% and pretreatment temperature of 60, 70, 80, 90 and 100 ºC were studied. The reducing of lignin between 40.39% to 61.97% of its of its early amount in rice hull. The amount of lignin reduction taking place in the AAS-Dilute Acid pretreatment of rice hull triggered the enhancing of enzymatic digestibility. The highest percentage of lignin reduction, 61.97%, was produced when the ammonia concentration was 20%. The glucan recovery of rice hull increased from 60.76% to 88.39% after the AAS-dilute acid pretreatments. Therefore, the combination of AAS and dilute acid pretreatments were successful for the removal of lignin, producing in high cellulose contents in the solid phase that has been enzymatically hydrolyzed and fermented to ethanol subsequently. The highest recovery of glucan after pretreatment was about 88.39%. The highest glucose concentration was about 241.774 mg g^{-1} obtained at temperature of 100 ºC and ammonia concentration of 20% (v/v). The highest ethanol yield was about 5.86 %.

5. Acknowledgments

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