1. Aqueous ammonia Article.pdf

WORD COUNT 4890
TIME SUBMITTED 22-JUL-2022 12:29PM
PAPER ID 88688345

ISSN: 04532198
Volume 62, Issue 03, April, 2020

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

Novia Novia*, Muhammad Said, Asyeni Miftahul Jannah, Pebriantoni Pebriantoni, Muhammad Bayu
Chemical Engineering Dept., Faculty of Engineering, Universitas Sriwijaya, Indonesia
*Corresponding author: E-mail: novia@ft.unsri.ac.id

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 °C. 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 °C. 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 °C to 100 °C 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 85ºC 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 121ºC 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 35ºC 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 ºC 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 ºC and agitated at 200 rpm for 24 h. Furthermore, its temperature was decreased to 30ºC. 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 ºC 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 ºC for 20 min. 1 mL of pre-cultur was added into erlenmeyer that contained slurry. It was stirred at temperature of 30 ºC 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 fibers 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.

ISSN: 04532198
Volume 62, Issue 03, April, 2020

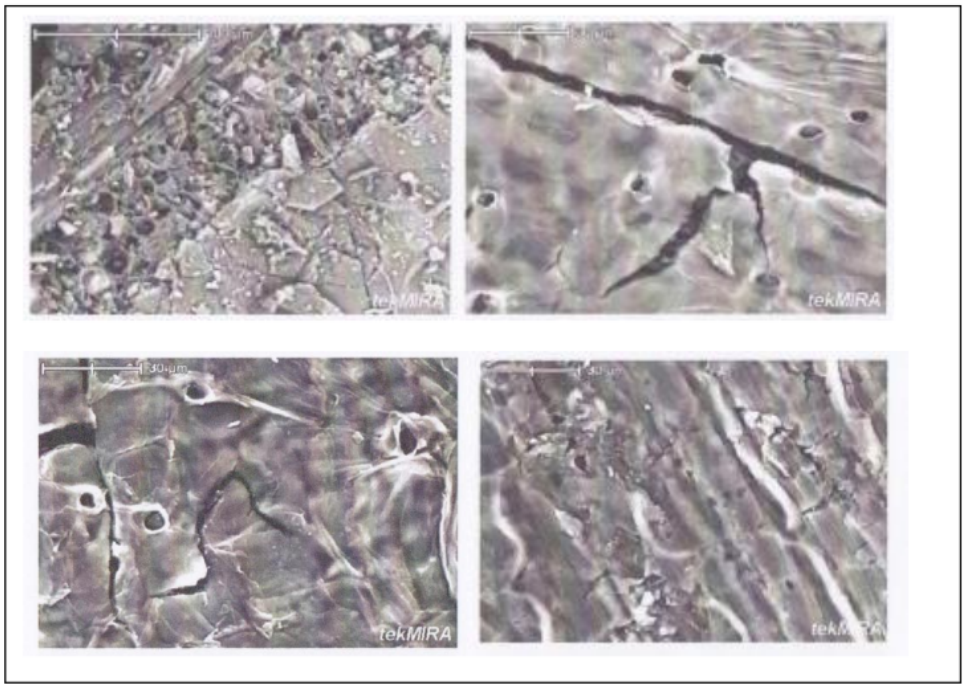

Fig. 1. Scanning Electronic Microscopy (SEM) pictures of various cellulosic materials: (a) Unpretreated rice hull (UTRH), (b) AAS D1 pretreated rice hull (AD1RH), (c) AAS D2 pretreated material (AD2RH), (d) AAS D3 pretreated material (AD3RH). Magnification of image provided in Figure 1a is 500 X, while, 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.

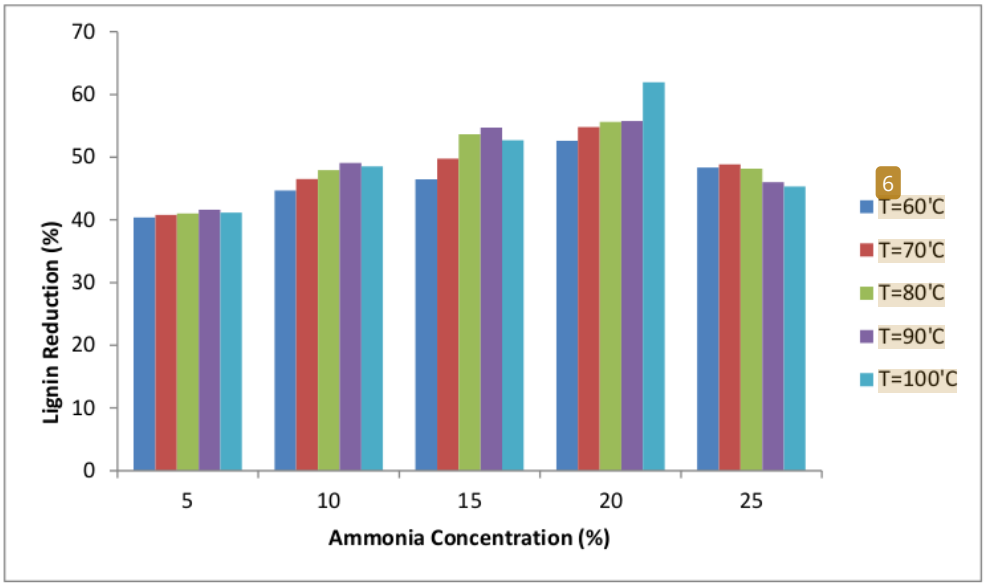

Fig. 2. Effect of ammonia concentrations and temperatures on percentage of lignin reduction during the AAS-Dilute Acid pretreatment of rice hull.

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 °C 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 °C 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].

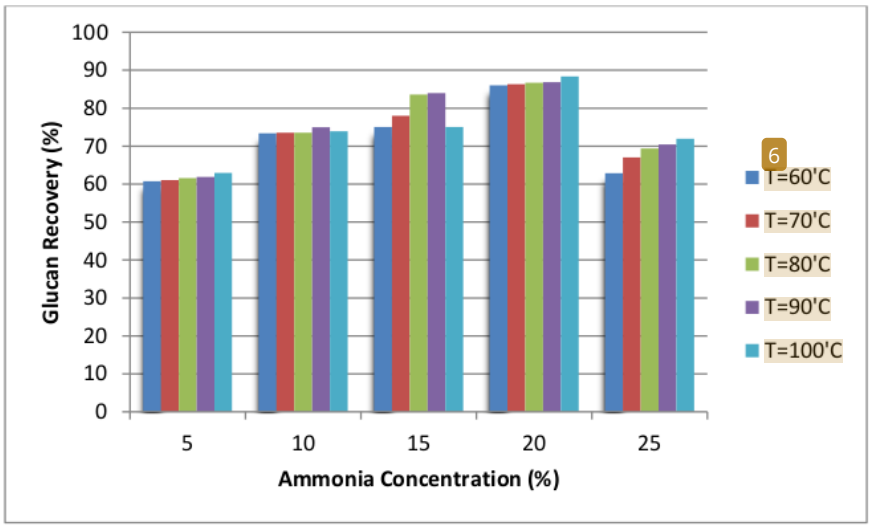

Fig. 3. Effect of ammonia concentrations and temperatures on glucan recovery during the AAS-Dilute Acid pretreatment of rice hull.

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 °C. 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 °C 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 a 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.

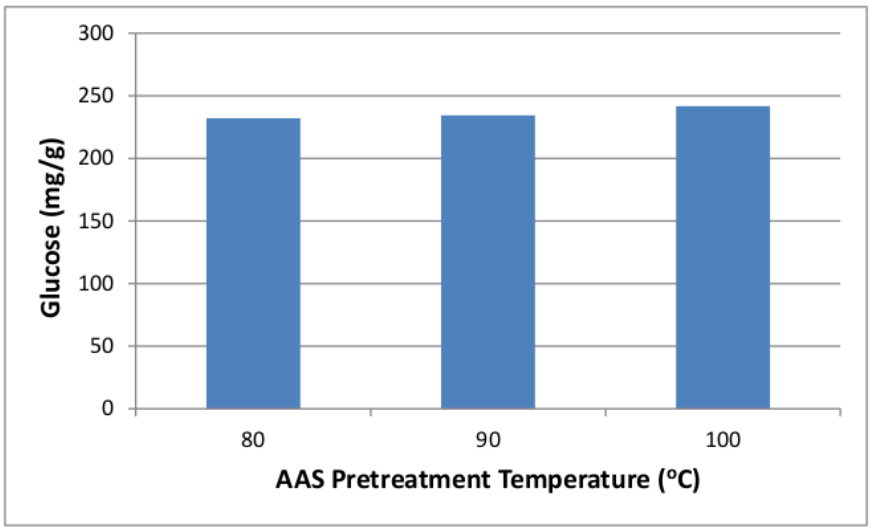

Fig. 4. Effect of pretreatment temperatures on glucose concentration during enzymatically hydrolysis.

3.5. Effect of pretreatment temperatures on ethanol yield during fermentation

Fig. 5 describes the ethanol yields produced from 80°C, 90°C, and 100°C 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 °C. The highest ethanol yield was about 5.86 % produced by AAS pretreatment temperature of 100 °C 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.

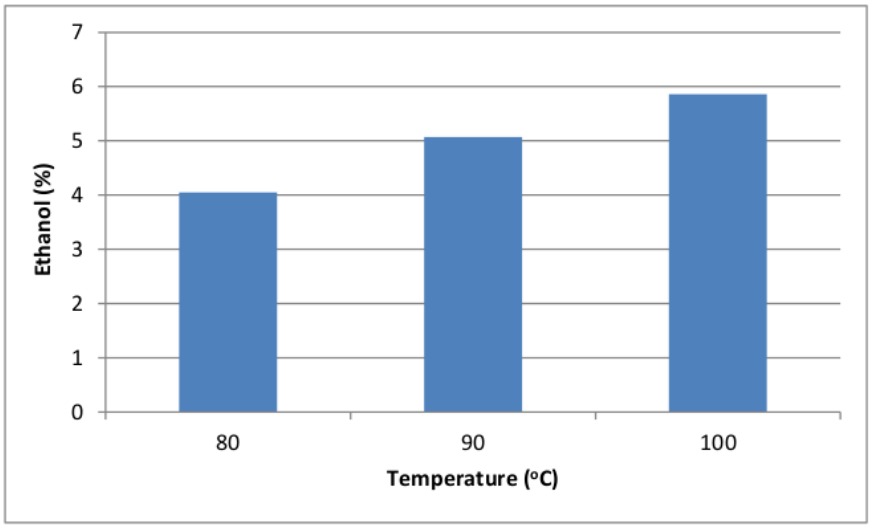

Fig. 5. Effect of pretreatment temperature on ethanol yield during fermentation.

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 °C 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 °C and ammonia concentration of 20% (v/v). The highest ethanol yield was about 5.86 %.

5. Acknowledgments

The authors gratefully acknowledge the financial supports provided by Universitas Sriwijaya for funding the research project through Anggaran DIPA Badan Layanan Umum No. SP DIPA-042.01.2.400953/2019, tanggal 05 Desember 2018 (as Research Contract: Unggulan Kompetitif Universitas Sriwijaya, No. 0015/UN9/SK.LP2M.PT/2019 Tanggal 21 Juni 2019).

6. References

[1] Novia, V. K. Pareek, and T. E. Agustina, "Bioethanol production from sodium hydroxide - Dilute sulfuric acid pretreatment of rice husk via simultaneous saccharification and fermentation," in *MATEC Web of Conferences*, 2017, vol. 101, pp. 1–5.

[2] Y. C. Park and J. S. Kim, "Comparison of various alkaline pretreatment methods of lignocellulosic biomass," *Energy*, vol. 47, no. 1, pp. 31–35, 2012.

[3] I. del Campo, I. Alegria, M. Zazpe, M. Echeverria, and I. Echeverria, "Diluted acid hydrolysis pretreatment of

agri-food wastes for bioethanol production," *Ind. Crops Prod.*, vol. 24, no. 3, pp. 214–221, 2006.
[4] C. Cara, E. Ruiz, I. Ballesteros, M. J. Negro, and E. Castro, "Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification," *Process Biochem.*, vol. 41, no. 2, pp. 423–429, 2006.
[5] W. H. Chen, B. L. Pen, C. T. Yu, and W. S. Hwang, "Pretreatment efficiency and structural characterization of rice straw by an integrated process of dilute-acid and steam explosion for bioethanol production," *Bioresour. Technol.*, vol. 102, no. 3, pp. 2916–2924, 2011.
[6] C. Fang, J. E. Schmidt, I. Cybulska, G. P. Brudecki, C. G. Frankaer, and M. H. Thomsen, "Hydrothermal pretreatment of date palm (Phoenix dactylifera L.) leaflets and rachis to enhance enzymatic digestibility and bioethanol potential," *Biomed Res. Int.*, vol. 2015, 2015.
[7] X. Li, T. H. Kim, and N. P. Nghiem, "Bioethanol production from corn stover using aqueous ammonia pretreatment and two-phase simultaneous saccharification and fermentation (TPSSF)," *Bioresour. Technol.*, vol. 101, no. 15, pp. 5910–5916, 2010.
[8] E. P. Dagnino, E. R. Chamorro, S. D. Romano, F. E. Felissia, and M. C. Area, "Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production," *Ind. Crops Prod.*, vol. 42, no. 1, pp. 363–368, 2013.
[9] G. Ralph Rolly and S. Kim, "Dark fermentative hydrogen production following the sequential dilute acid pretreatment and enzymatic saccharification of rice husk," *Int. J. Hydrogen Energy*, vol. 42, no. 45, pp. 27577–27583, 2017.
[10] J. M. Lee, H. Jameel, and R. A. Venditti, "A comparison of the autohydrolysis and ammonia fiber explosion (AFEX) pretreatments on the subsequent enzymatic hydrolysis of coastal Bermuda grass," *Bioresour. Technol.*, vol. 101, no. 14, pp. 5449–5458, 2010.
[11] Q. Shao *et al.*, "Enzymatic digestibility and ethanol fermentability of AFEX-treated starch-rich lignocellulosics such as corn silage and whole corn plant," *Biotechnol. Biofuels*, vol. 3, no. 1, pp. 1–10, 2010.
[12] D. A. Salvi, G. M. Aita, D. Robert, and V. Bazan, "Ethanol production from sorghum by a dilute ammonia pretreatment," *J Ind Microbiol Biotechnol*, vol. 37, pp. 27–34, 2010.
[13] G. Chaudhary, L. K. Singh, and S. Ghosh, "Alkaline pretreatment methods followed by acid hydrolysis of Saccharum spontaneum for bioethanol production," *Bioresour. Technol.*, vol. 124, pp. 111–118, 2012.
[14] A. Hedayatkhah, H. Motamedi, H. N. A. Jafzadeh, G. Ghezelbash, M. A. Bahnamiry, and K. Karimi, "Improvement of Hydrolysis and Fermentation of Sugarcane Bagasse by Soaking in Aqueous Ammonia and Methanolic Ammonia," vol. 77, no. (7), pp. 1379–83, 2013.
[15] Y. H. Jung *et al.*, "Ethanol production from oil palm trunks treated with aqueous ammonia and cellulase," *Bioresour. Technol.*, vol. 102, no. 15, pp. 7307–7312, 2011.
[16] J. K. Ko *et al.*, "Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes," *Bioresour. Technol.*, vol. 100, no. 19, pp. 4374–4380, 2009.
[17] T. H. Kim, F. Taylor, and K. B. Hicks, "Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment," *Bioresour. Technol.*, vol. 99, no. 13, pp. 5694–5702, 2008.
[18] J. W. Kim *et al.*, "Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid," *Bioresour. Technol.*, vol. 102, no. 19, pp. 8992–8999, 2011.
[19] H. Li, N. J. Kim, M. Jiang, J. W. Kang, and H. N. Chang, "Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid-acetone for bioethanol production," *Bioresour. Technol.*, vol. 100, no. 13, pp. 3245–3251, 2009.
[20] N. Mosier *et al.*, "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresour. Technol.*, vol. 96, no. 6, pp. 673–686, 2005.
[21] T. A. D. Nguyen *et al.*, "Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars," *Bioresour. Technol.*, vol. 101, no. 19, pp. 7432–7438, 2010.
[22] M. Balat, H. Balat, and C. Oz, "Progress in bioethanol processing," *Prog. Energy Combust. Sci.*, vol. 34, no. 5, pp. 551–573, 2008.
[23] L. P. Devendra and A. Pandey, "Hydrotropic pretreatment on rice straw for bioethanol production," *Renew. Energy*, pp. 1–7, 2016.

1. Aqueous ammonia Article.pdf

ORIGINALITY REPORT

15%
SIMILARITY INDEX

PRIMARY SOURCES

1	Phitsuwan, Paripok, Chutidet Permsriburasuk, Rattiya Waeonukul, Patthra Pason, Chakrit Tachaapaikoon, and Khanok Ratanakhanokchai. "Evaluation of fuel ethanol production from aqueous ammonia-treated rice straw via simultaneous saccharification and fermentation", Biomass and Bioenergy, 2016. Crossref	40 words — 1%
2	libprint.trisakti.ac.id Internet	40 words — 1%
3	coek.info Internet	37 words — 1%
4	Young Hoon Jung, In Jung Kim, Jae Jin Kim, Kyeong Keun Oh, Jong-In Han, In-Geol Choi, Kyoung Heon Kim. "Ethanol production from oil palm trunks treated with aqueous ammonia and cellulase", Bioresource Technology, 2011 Crossref	34 words — 1%
5	Praveen Kumar Keshav, Naseeruddin Shaik, Sravanthi Koti, Venkateswar Rao Linga. "Bioconversion of alkali delignified cotton stalk using two-stage dilute acid hydrolysis and fermentation of detoxified hydrolysate into ethanol", Industrial Crops and Products, 2016 Crossref	31 words — 1%

6	upcommons.upc.edu Internet	31 words — 1%
7	N Novia, L Cundari, A M Jannah, M K Haphsari, M Zulfani. "Hydrodynamic Analysis of Hydrolysis of the Rice Husk Cellulose by Using CFD Modeling", IOP Conference Series: Materials Science and Engineering, 2019 Crossref	30 words — 1%
8	Nguyen, T.A.D.. "Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars", Bioresource Technology, 201010 Crossref	30 words — 1%
9	pubs.rsc.org Internet	27 words — 1%
10	Abolghasem HEDAYATKHAH, Hossein MOTAMEDI, Hossein NAJAFZADEH VARZI, Gholamreza GHEZELBASH et al. "Improvement of Hydrolysis and Fermentation of Sugarcane Bagasse by Soaking in Aqueous Ammonia and Methanolic Ammonia", Bioscience, Biotechnology, and Biochemistry, 2014 Crossref	23 words — 1%
11	Swain, Manas R., and Chandraraj Krishnan. "Improved conversion of rice straw to ethanol and xylitol by combination of moderate temperature ammonia pretreatment and sequential fermentation using Candida tropicalis", Industrial Crops and Products, 2015. Crossref	19 words — < 1%
12	José María Cerveró, Pernille Anastasia Skovgaard, Claus Felby, Hanne Risbjerg Sørensen, Henning Jørgensen. "Enzymatic hydrolysis and fermentation of palm kernel press cake for production of bioethanol", Enzyme and	16 words — < 1%

Microbial Technology, 2010
Crossref

13 Shirkavand, Ehsan, Saeid Baroutian, Daniel J. Gapes, and Brent R. Young. "Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment – A review", Renewable and Sustainable Energy Reviews, 2016.
Crossref
16 words — < 1%

14 Arosha Loku Umagiliyage, Ruplal Choudhary, Yanna Liang, John Haddock, Dennis G. Watson. "Laboratory scale optimization of alkali pretreatment for improving enzymatic hydrolysis of sweet sorghum bagasse", Industrial Crops and Products, 2015
Crossref
15 words — < 1%

15 L Cundari, K F Sari, L Anggraini. "Batch Study, Kinetic and Equilibrium Isotherms Studies of Dye Adsorption of Jumputan Wastewater onto Betel Nuts Adsorbent", Journal of Physics: Conference Series, 2018
Crossref
15 words — < 1%

16 Jung, Y.H.. "Ethanol production from oil palm trunks treated with aqueous ammonia and cellulase", Bioresource Technology, 201108
Crossref
13 words — < 1%

17 cyberleninka.org
Internet
13 words — < 1%

18 lib.dr.iastate.edu
Internet
11 words — < 1%

19 link.springer.com
Internet
10 words — < 1%

www.tandfonline.com

20	Internet	10 words — < 1%
21	Chang Ho Choi, Jun Seok Kim, Kyeong Keun Oh. "Evaluation the efficacy of extrusion pretreatment via enzymatic digestibility and simultaneous saccharification & fermentation with rapeseed straw", Biomass and Bioenergy, 2013 Crossref	9 words — < 1%
22	Karagoz, P.. "Alkaline peroxide pretreatment of rapeseed straw for enhancing bioethanol production by Same Vessel Saccharification and Co-Fermentation", Bioresource Technology, 201201 Crossref	9 words — < 1%
23	Kim, Ilgook, and Jong-In Han. "Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology", Biomass and Bioenergy, 2012. Crossref	9 words — < 1%
24	Kim, J.W.. "Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid", Bioresource Technology, 201110 Crossref	9 words — < 1%
25	Li, C.. "Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification", Bioresource Technology, 201007 Crossref	9 words — < 1%
26	Pereira, Claudia Patricia Pabon. "Anaerobic Digestion in Sustainable Biomass Chains.", Wageningen University and Research, 2021 ProQuest	9 words — < 1%

27	bioresources.cnr.ncsu.edu Internet	9 words — < 1%
28	digitalcommons.lsu.edu Internet	9 words — < 1%
29	journal.unnes.ac.id Internet	9 words — < 1%
30	D. Mikulski, G. Kłosowski, A. Menka, B. Koim-Puchowska. "Microwave-assisted pretreatment of maize distillery stillage with the use of dilute sulfuric acid in the production of cellulosic ethanol", Bioresource Technology, 2019 Crossref	8 words — < 1%
31	Domanski, Jaroslaw, Sebastian Borowski, Olga Marchut-Mikolajczyk, and Przemyslaw Kubacki. "Pretreatment of rye straw with aqueous ammonia for conversion to fermentable sugars as a potential substrates in biotechnological processes", Biomass and Bioenergy, 2016. Crossref	8 words — < 1%
32	Junaid Akhtar, Ani Idris. "Oil palm empty fruit bunches a promising substrate for succinic acid production via simultaneous saccharification and fermentation", Renewable Energy, 2017 Crossref	8 words — < 1%
33	Kim, Sung Bong, Eunji Kim, Hah Young Yoo, Minsu Kang, Seong Woo Kang, Chulhwan Park, Jun Seok Kim, and Seung Wook Kim. "Reutilization of carbon sources through sugar recovery from waste rice straw", Renewable Energy, 2013. Crossref	8 words — < 1%
34	Kusmiyati, Duwi Maryanto, Ringga Sonifa, Sabda Aji Kurniawan, H. Hadiyanto. " Pretreatment of	8 words — < 1%

Starch-Free Sugar Palm Trunk ( ) to Enhance Saccharification in Bioethanol Production ", MATEC Web of Conferences, 2018
Crossref

35 Umagiliyage, Arosha Loku, Ruplal Choudhary, Yanna Liang, John Haddock, and Dennis G. Watson. "Laboratory scale optimization of alkali pretreatment for improving enzymatic hydrolysis of sweet sorghum bagasse", Industrial Crops and Products, 2015.
Crossref
8 words — < 1%

36 Yamashita, Y.. "Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo", Carbohydrate Polymers, 20100317
Crossref
8 words — < 1%

37 core.ac.uk
Internet
8 words — < 1%

38 eprints.soton.ac.uk
Internet
8 words — < 1%

39 hdl.handle.net
Internet
7 words — < 1%

40 Chaturvedi, Venkatesh, and Pradeep Verma. "An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products", 3 Biotech, 2013.
Crossref
6 words — < 1%

41 Susmita Sen Gupta, Krishna G. Bhattacharyya. "Adsorption of metal ions by clays and inorganic solids", RSC Adv., 2014
Crossref
6 words — < 1%

42 repositorio.unal.edu.co
Internet
6 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	OFF