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Research Article

Application of Ionic Liquid [DMIM]DMP Pretreatment in the Hydrolysis of Sugarcane Bagasse for Biofuel Production

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Abstract

Sugarcane bagasse is one of lignocellulose materials that can be converted to biofuel. This work was aimed to develop new pretreatment combination methods to process sugarcane bagasse lignocellulose into biofuel (bio-hydrogen). Pretreatment of sugarcane bagasse using NaOH solution in combination with ionic liquid [DMIM]DMP enhanced the enzymatic hydrolysis significantly. After the pretreatment, the content of cellulose and hemicellulose increased by 29.31% compared to the untreated one. Cellulose and hemicelluloses were used as raw materials to produce reducing sugars, that can be converted to bio-hydrogen via fermentation. After being subjected to combined pretreatment processes, the crystalline index of sugarcane bagasse decreased significantly compared to solely NaOH pretratment. This indicates a more amorphous structure of the sugarcane bagasse, which makes it is easier to be hydrolyzed into reducing sugars. The recovery of cellulose + hemicellulose after pretreatment for 20 min and 120 °C was 92%, and the yield obtained was 0.556 g sugars/g (cellulose + hemicellulose) after 12 h and the bio-hydrogen yield was 0.46 mol H_2/mol sugars consumed after 48 h fermentation. The use of recycled of ionic liquid showed similar performance compared to the use of fresh ionic liquid.

Keywords: sugarcane bagasse; ionic liquid; [DMIM]DMP pretreatment; NaOH pretreatment; Hydrolysis; Reducing Sugar

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1. Introduction

Fossil fuels are actually the main sources of primary energy supply in the world, which contribute more than 80 % of the total energy production [1]. But recently, their resources facing many obstacles such as steady increase in price and depletion of raw material. In addition to the increase of atmospheric carbon dioxide due to the burning fossil fuels for energy, a gradual shift from fossil fuels to renewable energy sources seems to be serious.

Cellulose and hemicellulose are abundantly available in most developing countries, and are highly potential to bed converted into renewable energy or biofuels [2]. Recently, the efficient conversion of lignocellulosic materials into biofuel has become one of energy research priorities. The pretreatment of lignocelluloses is known to be the key step to the rapid enzymatic hydrolysis of cellulose [3].

Lignin could inhibit the hydrolysis process

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because it resides on the outer side of a lignocelluloses structure. It may restrict enzymes accesses in converting cellulose and hemicellulose into reducing sugar. So, a delignification process as a pretreatment is necessary. Some of well-developed delignification methods was using alkaline and acid, where alkaline method has more advantages. The alkaline method can degrade lignin significantly, does not produce side products, more environmentally friendly and can be carried out at a lower temperature (60-100 °C) [4].

Beside the presence of lignin on the outer side of lignocellulosic structure, the existence of intermolecular and intramolecular hydrogen bonds in cellulose, also inhibit enzymatic hydrolysis process. This is because the hydrogen bond forms crystalline structure of cellulose [5]. This kind of cellulose is difficult to dissolve in conventional solvents and also hard to be hydrolysed into reducing sugars [6]. Therefore, another pretreatment is an important step to disrupt the arrangement of crystalline cellulose fibrils. The easiest methods to reduce cellulose crystalline is using solvent. The solvent can be divided into derivative and non-derivative solvents. Derivative solvent is a solvent that can dissolve cellulose by convert it into temporary cellulose derivatives, whereas the solvent that can dissolve cellulose by disrupting the formation of hydrogen bonds without derivated cellulose is non-derivative solvent [7]. A number of non-derivative solvents was used to cellulose pretreated, such as N-methyl-morpholine-N-oxide (NMO), concentrated phosphoric acid, and the ionic liquid [8]. Among these solvents, ionic liquids have recently been developed as a promising non-derivative solvent for dissolving lignocellulose [9].

Ionic Liquid (IL) as a new type of solvent, considered as a potential solvent for dissolving cellulose because it is easily recycled, has a lower toxicity, better thermal stability and almost non-volatile [10]. Yang *et al*. [11] reported that among six alkyl-phosphate ionic liquids ([DMIM]DMP, [MEIM]DMP, [MAIM]DMP, [EMIM]DEP, [EEIM]DEP, [EAIM]DEP), [DMIM]DMP is the most effective ionic liquid to dissolve cellulose and giving favorable biocompatibility for enzymatic in situ saccharification process. Zhi *et al*. [12] reported that among three ionic liquids ([DMIM]DMP, [EMIM]DEP, [BMIM]DBP), [DMIM]DMP has the lowest viscosity, the highest polarity and the lowest ability to inhibit enzyme. Those are the reason why [DMIM]DMP was used in this work, in combination with NaOH pretreatment. In our work, sugarcane bagasse was pretreated using a combination pretreatment. They were alkaline pretreatment (NaOH 1%) to reduce the amount of lignin and ionic liquid [DMIM]DMP pretreatment to disrupt crystalline structure of cellulose pretreatment. Alkaline NaOH 1% (w/v) was an effective alkaline to reduce the amount of lignin on sugarcane bagasse and NaOH 1% pretreated was the best one compared to the other concentrations of NaOH [13].

This work focussed on the effect of those combination pretreatments. Determination of cellulose, hemicellulose, and lignin from sugarcane bagasse were analysed using Chesson method [14]. The Crystalline Index of sugarcane bagasse were analysed using XRD method. The hydrolysis product was measured using DNS method. And, the fermentation product, bio-hydrogen was measure using GC method.

2. Experimental

2.1. Materials

Sugarcane bagasse was used as a model of lignocellulosic material. It was collected in around Sepuluh Nopember Institute of Technology (Surabaya, Indonesia). It was ground and sieves until its particle were able to pass through a 60-mesh (0.3 mm) sieve. Then it dried for 24 hour in a 60 °C oven. IL 1,3-dimethylimidazolium diethylphosphate [DMIM]DMP was synthesized as the reported preparation procedures [14]. All other chemicals were from commercial source.

2.2. NaOH 1 % (w/v) pretreatment

One litre of NaOH 1% (w/v) and 50 grams of sugarcane sugarcane bagasse was added in 2 litres round bottom flask. The mixture was heated using oil bath for 16 hours at 80 °C. Then, the mixture was cooled and filtered. The solid was washed with 70 °C distilled water until pH 7 and dried at 60 °C for 24 hours in an oven. This dried sample was called NaOH treated sample.

2.3. Ionic liquid [DMIM]DMP synthesize

IL 1-methyl-3-methylimidazolium diethylphosphate [DMIM][DMP] was synthesized as the reported preparation procedures [15] by reacting *N*-methylimidazole and the corresponding trialkyl phosphate at 150 °C for 15 h. The resulting of ionic liquid, was washed three times with diethylether after cooling to room temperature, and then stirred and heated for 24 hours using oil bath at 80 °C to remove all volatile residues. Then, IL was heated again for

24 hours 80 °C before each used [11]. Reaction between N-methylimidazole and trimethylphosphate becomes ionic liquid [DMIM]DMP was shown in Figure 1.

2.4. Ionic liquid [DMIM]DMP Pretreatment

3.75 g of NaOH treated sugarcane bagasse was combining with 100 ml ionic liquid [DMIM]DMP in a 250 ml round bottom flask. The solution was prepared under nitrogen atmosphere to prevent uptake water from the air. It was heated in an oil bath at 100-120 °C for 10-900 min. To regenerate sugarcane bagasse from the solution, methanol were use as anti-solvent. The precipitated bulky material was filtered using Whatmann filter paper and washed with distilled water. And then, the regenerated sugarcane bagasse that have been washed, was dried at 60 °C for 48 h in an oven for the use of Chesson analysis and enzymatic hydrolysis.

2.5. Recovery of Ionic Liquid [DMIM][DMP]

A certain amount [DMIM][DMP] which has been used for sugarcane bagasse pretreatment was distilled using an oil bath at a temperature of 100 °C to separate the methanol from [DMIM][DMP]. So, [DMIM]DMP can be used again as [DMIM]DMP recycle.

2.6. Pretreatment using Ionic Liquid [DMIM]DMP Recycle

The method of pretreatment using [DMIM]DMP recycle, was same as Ionic Liquid [DMIM]DMP pretreatment. The differences only lies in the type of ionic liquid. Ionic liquid that was used in this process was ionic liquid recovered from previous pretreatment.

2.7. Sugarcane Bagasse Hydrolysis

1.5 grams of sugarcane bagasse from each variable pretreatment was added in 100 ml rockered flask. Then 27.9 U cellulase and 27.9 U Xylanase were added to that solid sugarcane bagasse, then buffer sitrat pH 3 also added to the mixture until the volume become 45 ml and then incubated at 60 °C for 12 hours. Furthermore, within a certain time interval, 1.5 ml of sugarcane bagasse hydrolyzate was centrifuged to separate the solid and liquid (supernatant), then total reducing sugars in the supernatant was measured by DNS method to know the increasing of sugars in this hydrolysis process each time.

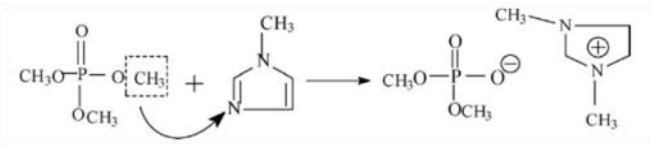

Figure 1. Reaction between N-methylimidazole and trimethylphosphate become ionic liquid [DMIM]DMP

2.8. Method of Analysis

Analysis of lignin, cellulose and hemicellulose from sugarcane bagasse was conducted by Chesson method [14]. Activity of the enzyme was done using DNS method, 0.2 ml of enzyme xylanase/cellulase and 1.8 ml of xylan/CMC put into a test tube and incubated for 10 min at 35 °C. Then DNS solution was added and mixed using vortex and it was put in boiling water for 10 minutes. Correction solution is made by 0.2 ml of enzyme solution was incubated in another test tube for 10 min at 35 °C, then DNS solution was added and mixed using vortex, then it was put in boiling water for 2 minutes. Then 1.8 ml xylan/CMC was added to the correction solution tube and heated again in boiling water for 10 minutes. Then, both of them was cooled in ice water 10 minutes. After the solution temperature become room temperature, the solutions absorbance were measured by spectrophotometer (CECIL CE 1011 1000) series at 540 nm wavelength.

2.9. Method of Calculation and Statistics

Crystalline index, reducing sugar yield and others was calculated using the following Equation (1) [16, 17]:

$$Cr.I = \frac{A_{cryst}}{A_{total}} \quad (1)$$

$$\text{Total Reducing Sugar} = \frac{\text{Sugars, g}}{(\text{cellulose} + \text{Hemicellulose}), \text{g}} \quad (2)$$

$$\text{Hydrogen Yield} = \frac{\text{Hydrogen, mol}}{\text{Total reducing Sugar Consumed, mol}} \quad (3)$$

$$\text{SCB recovery} = \frac{\text{Regenerated SCB, g}}{\text{Initial SCB, g}} \times 100\% \quad (4)$$

$$\text{Cellulose + hemicellulose recovery} = \frac{\text{Regenerated SCB (Cellulose + hemicellulose), g}}{\text{Initial SCB (Cellulose + hemicellulose), g}} \times 100\% \quad (5)$$

3. Result and Discussion

3.1. Mechanical Pretreatment of Sugarcane Bagasse

The first pretreatment process was mechanical pretreatment sugarcane bagasse. The purpose of this step was enlarging surface area of sugarcane bagasse. The result shown in Table 1 revealed that SCB in <60 mesh size after pretreated with 1% NaOH resulted in higher amount of cellulose and hemicellulose compared to 100-200 mesh. This is because SCB in <60 mesh gave higher remaining total solid after NaOH pretreatment. It means to produce the same amount of cellulose and hemicellulose with NaOH pretreatment, more sugarcane bagasse from 100-120 mesh sugarcane bagasse was needed than that from < 60 mesh sugarcane. Therefore, < 60 mesh sugarcane bagasse in the next process was used.

3.2. Effect of NaOH 1% w/v pretreatment

Lignin in sugarcane bagasse was reduced after NaOH Pretreatment. Lignin structure

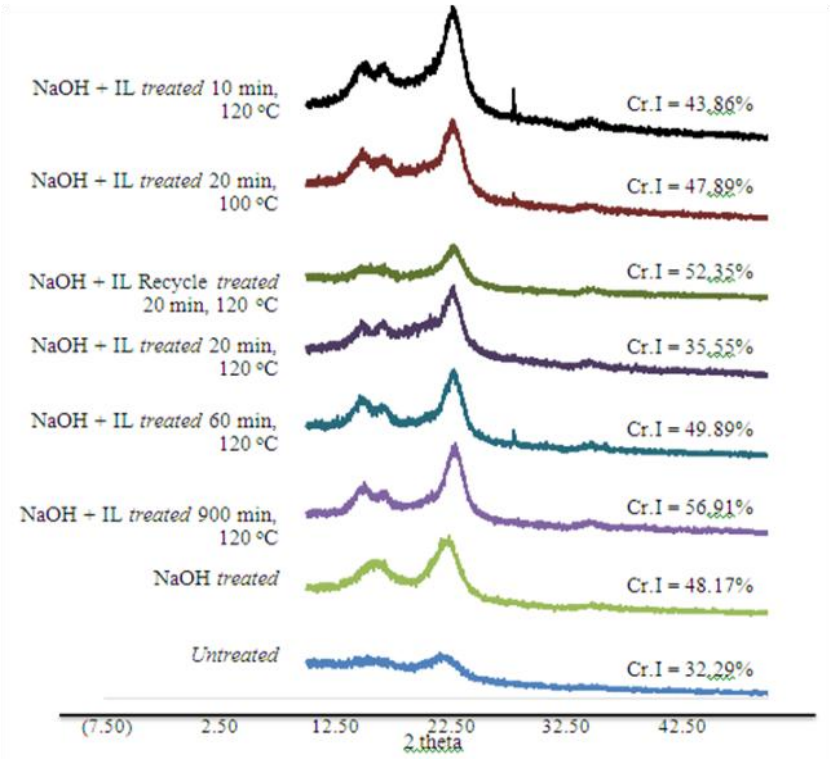

Figure 2. The XRD pattern of the regenerate sugarcane bagasse (untreated, NaOH treated and NaOH+IL [DMIM]DMP at 100 and 120 °C, for 10-900 min)

Table 1. Effect of Mechanical and NaOH pretreatment of sugarcane bagasse

Pretreatment type and size	Composition in solid residue (%)			TSR (%)	CHR (%)
	Cellulose	Hemicellulose	Lignin		
Untreated	32.87	26.30	24.81	-	-
NaOH treated (from < 60 mesh, SCB feed)	67.19	17.53	9.84	57.91	68.60
NaOH treated (from 100-120 mesh, SCB feed)	63.47	18.17	9.83	36.00	61.00

Note: TSR (%): Total solid after pretreatment (g) / Total solid before pretreatment (g) (%)
CHR (%): (cellulose+hemicellulose) after pretreatment (g)/(cellulose+hemicellulose) before pretreatment (g) (%)

around the sugarcane bagasse will block enzyme in the hydrolysis process. So, reducing lignin content was a useful treatment. As shown in Table 1, content of sugarcane bagasse after NaOH 1% w/v has smaller amount of lignin, and higher amount of cellulose and hemicellulose. It was compared with untreated sugarcane bagasse. The X-ray diffraction patterns of the sugarcane bagasse are shown in Figure 2. The lowest Crystalline Index of sugarcane bagasse was in untreated sample. It is undoubtedly due to the high presence of lignin that still remain in the outer space of cellulose structure. After NaOH pretreatment, the crystalline index increased. It was due to some of lignin was dissolved in NaOH [18].

3.3. Effect of ionic liquid [DMIM]DMP pretreatment time and temperature

Ionic liquid [DMIM]DMP can dissolve lignin, cellulose and hemicellulose in sugarcane bagasse. But, cellulose could be regenerated in the regeneration process. Therefore, the right condition was investigated where cellulose could be dissolved and regenerated well and hemicellulose was not too much dissolved. This work investigated two conditions, pretreatment time and temperature. Pretreatment time was varied from 10 min to 900 min, and the temperature varied 100 and 120 °C. During the pretreatment, the suspension turned a darker brown colour, presumably as a result of lignin extraction. The result of this process was shown in Table 2, the highest content of hemicellulose was detected in 20 min at 120 °C pretreatment variable, and the highest content of cellulose was detected in 10 min at 120 °C of pretreatment variable. This cellulose and hemicellulose was useful to produce reducing sugar, so it was needed to know the right pretreatment time and temperature to get as much as possible amount of cellulose and hemicellulose of regenerate sugarcane bagasse. The result of X-ray diffraction patterns of sugarcane bagasse are shown in Figure 2. The lowest Crystalline Index of sugarcane bagasse was on untreated sample. It is undoubtedly due to the high presence of lignin that still remain in the outer space of cellulose structure [18]. But, after ionic liquid [DMIM]DMP pretreatment, the lowest crystalline index was on NaOH+IL 20 min, 120 °C. It showed that after sugarcane bagasse treated by NaOH, the best condition for ionic liquid [DMIM]DMP pretreatment was at 120 °C for 20 min. Because, in that condition the sample showed the lowest crystalline index. This low crystalline index mean that cellulose in sugarcane bagasse has become more amorphous than others. This amorphous cellulose was accessed easier by enzyme in enzymatic hydrolysis process.

3.4. Regeneration of Celluose in Sugarcane Bagasse

The next process was regenerate sugarcane bagasse that dissolved to ionic liquid [DMIM]DMP. Methanol was used as antisolvent regenerate cellulose of sugarcane bagasse. Xiao *et al*. [19] reported the effect of deionized water, methanol and ethanol as antisolvent to regenerate cellulose from cellulose/IL solution, show that H_2O is the most beneficial for enzymatic hydrolysis. The order is: H_2O>Methanol>Ethanol. Methanol still give high TRS yield of total reducing sugar even though it was not as good as H_2O. That was the

Table 2. Effect of ionic liquid [DMIM]DMP pretreatment time and temperature to NaOH treated sugarcane bagasse

Pretreatment Time and Temperature	Content in solid residue (%)			SCB Recovery (%)	Cellulose + Hemicellulose Recovery (%)
	Cellulose	Hemicellulose	Lignin		
10 min 120 °C	65.23	12.64	7.85	94	90
20 min 120 °C	58.49	18.02	6.34	98	92
60 min 120 °C	55.39	8.67	6.10	82	65
900 min 120 °C	60.66	13.45	7.80	82	75
20 min 100 °C	64.15	14.83	9.14	97	93
20 min 120 °C (Using Recycle IL)	59.11	6.53	9.51	95	76

reason of using methanol as anti-solvent. The other reason was it could be reclaimed easily because of its low boiling point.

Methanol was added to the sugarcane bagasse/ionic liquid [DMIM]DMP mixture and precipitate immediately formed. Then sample was briefly stirred for an hour, then it filtered using Whatmann filter paper. Residual ionic liquid may cause certain degree of cellulose inactivation [20]. Therefore, the regenerated sugarcane bagasse required washing with aquadest to remove [DMIM]DMP from the solid. After regeneration process 82.4% - 97.8% of dry sugarcane bagasse was recovered.

The highest concentration of ionic liquid that still can be tolerate by enzyme was 10 %, because cellulose was not inactivated greatly in the medium that containing 10% [DMIM]DMP [12]. Amount of ionic liquid that still left in regenerate sugarcane bagasse was investigated by observing recovery of ionic liquid [DMIM]DMP. After ionic liquid [DMIM]DMP pretreatment process, solution that contain methanol and ionic liquid [DMIM]DMP could be separate using distillation method. Ionic liquid [DMIM]DMP that was obtained from distillation process was about 96-99%, which proved that ionic liquid left in regenerate sugarcane bagasse was lower than 10%.

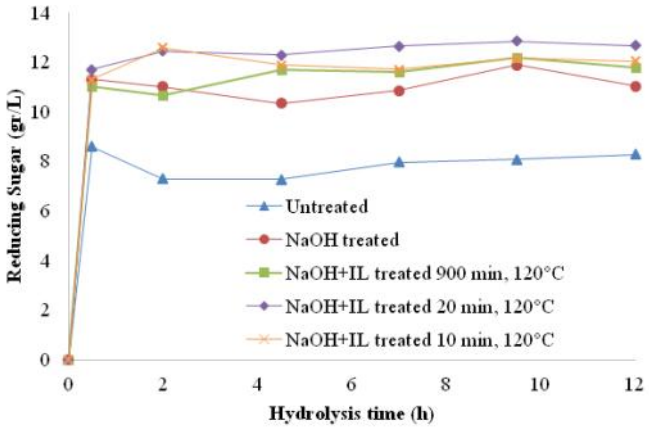

Figure 3. Effect of different IL pretreatment time on the enzymatic hydrolysis of the NaOH-pretreated sugarcane bagasse. The following reaction under this condition: 60 °C; 125 rpm, and 12 h

3.5. Profiles of Enzymatic Hydrolysis of Regenerated Sugarcane Bagasse

The next process, we evaluated the enzymatic hydrolysis of sugarcane bagasse that has pretreated in vary condition. The result showed that total reducing sugar that produce from regenerated each sample, increase rapidly with time, indicating that the combined pretreated sugarcane bagasse was hydrolysis rapidly by cellulase and xylanase enzyme. Moreover, total reducing sugar from sugarcane bagasse NaOH-pretreated in combination with [DMIM]DMP pretreated at 120 ^{0}C for 20 min was the highest, as shown in Figure 3-5. This result showed that this condition is the optimum condition of ionic liquid [DMIM]DMP pretreatment process among other condition that have been investigated in this work. This condition was the optimum condition because, cellulose can dissolve and regenerated well there, but there was not too much lost hemicellulose. It was relevant with the result shown in Table 2 sugarcane bagasse NaOH-pretreated in combination with [DMIM]DMP pretreated at 120 ^{0}C for 20 min-show the highest hemicelluloses content and

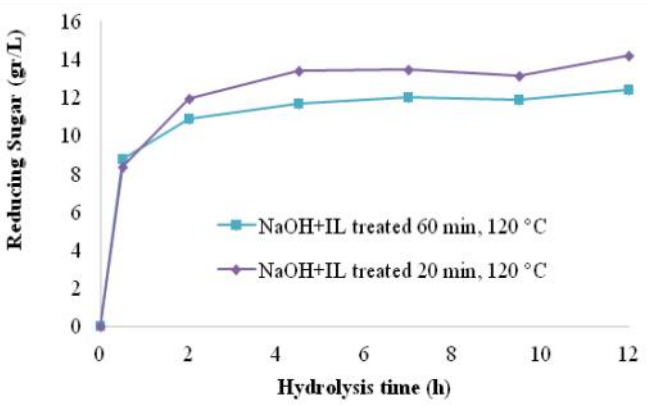

Figure 4. Effect of different IL pretreatment time on the enzymatic hydrolysis of the NaOH-pretreated sugarcane bagasse. The following reaction under this condition: 60 °C; 125 rpm, and 12 h

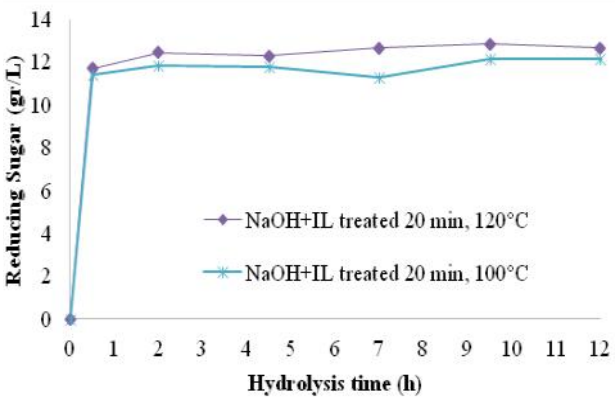

Figure 5. Effect ofdifferent IL pretreatment temperature on the enzymatic hydrolysis of the NaOH-pretreated sugarcane bagasse. The following reaction under this condition: 60 °C; 125 rpm, and 12 h

gave a high enough amount of cellulose+hemicellulose recovery. It also relevant with the result shown in the Figure 2, sugarcane bagasse NaOH-pretreated in combination with [DMIM]DMP pretreated at 120 ^{0}C for 20 min shows the most amorphous structure among other bagasse-NaOH+IL pretreated. Result of all variable hydrolysis process was shown in Figures 3-5. Reducing sugar yield-from sugarcane bagasse NaOH-pretreated in combination with [DMIM]DMP pretreated at 120 ^{0}C for 20 min was 0.556 g/g (sugar/cellulose + hemicelluloses). The yield of biohydrogen from this hydrolyzate was 0.46308 mol/mol (hydrogen/reducing sugar consumed). The reuse of ionic liquid [DMIM]DMP recycle show the similar performance compared to the use of new ionic liquid [DMIM]DMP. The use of individual [DMIM]DMP shows that combining pretreatment (NaOH+ionic liquid [DMIM]DMP) gave better result (data not shown).

The combination solvent (NaOH and ionic liquid [DMIM]DMP) gave better impact on enzymatic hydrolysis process, compare to the use of other solvents such as acid (H_2SO_4), individual alkaline (NaOH) and individual [DMIM]DMP. Ramadhan and Hendrawan [13] reported that pretreatment sugarcane bagasse using NaOH gave better effect then using H_2SO_4 [13]. Alkaline (NaOH) pretreatment is better than acid (H_2SO_4) pretreatment, because lignin in sugarcane bagasse could dissolve better in NaOH than H_2SO_4-. The lower amount of lignin in a substrat was better because presence of lignin in the outer space of sugarcane bagasse, make it difficult to be hydrolyzed by enzyme into reducing sugar. Combination of NaOH and ionic liquid pretreatment was better than individual NaOH pretreatment because ionic liquid pretreatment could convert crystalline cellulose in sugarcane bagasse become more amorphous (low crystalline cellulose). Low crystalline cellulose could be hydrolyzed easier than crystalline cellulose because it has more active site than high crystalline cellulose. Individual [DMIM]DMP was not better than NaOH+[DMIM]DMP because without NaOH pretreatment, much of lignin were still remain in the outer space of sugarcane bagasse. It also inhibit ionic liquid [DMIM]DMP pretreatment process.

This work showed that application of NaOH+ionic liquid [DMIM]DMP pretreatment in hydrolysis of sugarcane bagasse for biofuel production was a promising method. But the cost of ionic liquid appears to be the major barrier for the implementation. Sen *et al.* [21] reported the cost of ionic liquid was 93% of total operating cost of biomass to sugars using ionic liquid. At break event point, the MSP (minimum selling price) of fermented sugar is significantly higher than the current market price of sugars. However, this method still can compete with fossil fuels if lower ionic liquid consumption is applied, which can be conducted by lowering ionic liquid concentration and/or developing separation strategies that would allow high recycle of ionic liquids. Separation strategy has been used in this work. It resulted, ionic liquid recycle show the similar performance compared to the use of new ionic liquid. The best condition of lower ionic liquid concentration on the performance of producing biofuel should be investigated in the next research. Fu and Mazza [22] reported that optimum processing condition for fermentable sugars recovery was determined to 158 ^{o}C, 3.6 h and an ionic liquid concentration of 49.5% (w/w), the ionic liquid and substrate were [EMIM]Ac and wheat straw. The report indicated that low concentration of ionic liquid was allowed, to provides a potentially cost-effective way for biofuel production to compete with fossil fuels cost.

4. Conclusion

The experimental results show that the cellulose and hemicelluloce content in solid residue of NaOH pretreated sugarcane bagasse increases as most of lignin dissolves in NaOH solution. Ionic liquid [DMIM]DMP pretreatment of sugarcane bagasse under the best condition (at 120 ^{o}C for 20 min) has changed the crystallinity of sugarcane bagasse particles to be more amorphous, which facilitates easier them to undergo hydrolysis. The research presented a promising combined method of sugarcane bagasse pretreatment using ionic liquid [DMIM]DMP and 1% NaOH solution. The combined pretreatment yielded in 0.556 g/g of reducing sugar and 0.46308 mol/mol of biohydrogen. This value was higher than those obtained from individual NaOH pretreatment and un-treated sugarcane bagasse.

Acknowledgments

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References

[1] Climent-Font, A., Perlado, M. (2013). Sustainable energy for everyone; a challenge for the future. Foreword to the Workshop Proceedings. *Energy Procedia* 41: 1-3

[2] Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T., Vandenberghe, L.P.S., Mohan, R. (2000). Biotechnological potential of agro-industrial residues. II: cassava bagasse. *Bioresource Technology*, 74: 81-88.

[3] Zhao, H., Jones C.L., Baker, G.A.., Xia, S., Olubajo, O., Person, V.N. (2009). Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. *Journal of Biotechnology* 139: 47-54.

[4] Rocha, G.J.M., Martin, C., Silva, V.F.N.d., Gómez, E.O., Gonçalves, A.R. (2012). Mass Balance of Pilot-Scale Pretreatment of sugarcane bagasse by Steam Explosion Followed by Alkaline Delignification. *Bioresource Technology*, 111: 447-452.

[5] Azubuike, C.P., Rodríguez, H., Okhamafe, A.O., Rogers, R.D. (2011). Physicochemical properties of maize cob cellulose powders reconstituted from ionic liquid solution. *Cellulose*, 19: 425-433.

[6] Arantes, V., Saddler, J.N. (2011). Cellulose accessibility limits the effectiveness of minimum cellulase loading on the efficient hydrolysis of pretreated lignocellulosic substrates. *Biotechnology for Biofuels*, 4(3):1-16

[7] Liebert, T.F. (2010). Cellulose solvents- remarkable history, bright future. In T. F. Liebert, T. J. Heinze, & K.J. Edgar (Eds.), Cellulose solvents: for analysis, shaping and chemicalmodification (pp. 3–54). New York: Oxford University Press.

[8] Kuo, C.H. & Lee, C.K. (2009). Enhancement of enzymaticsaccharification of celluloseby cellulose dissolution pretreatments. *Carbohydr. Polym*. 77 (1): 41-46.

[9] Bian, J., Peng, F., Peng, X.P., Xiao, X., Peng, P., Xu, F., Sun, RC. (2013). Effect of [Emim]Ac pretreatment on the structure and enzymatic hydrolysis of sugarcane bagasse cellulose. *Carbohydrate Polymer*. 100: 211-217.

[10] Chaumont, A., Wipff, G. (2007). Solvation of big spherical solutes in room temperature ionic liquids and at their aqueous interface: A molecular dynamics simulation study. *Journal of Molecular Liquids*, 131-132: 36-47.

[11] Yang, F., Li, L., Li, Q., Tan, W., Liu, W., Xian, M. (2010). Enhancement of enzymatic in situ saccharification of cellulose in aqueous-ionic liquid media by ultrasonic intensification. *Carbohydrate Polymers* 81: 311-316.

[12] Zhi, S., Liu, Y., Yu, X., Wang, X., Lu, X. (2012). Enzymatic Hydrolysis of Cellulose after Pretreated by Ionic Liquids: Focus on One-Spot Process. *Energy Procedia* 14: 1741-1747.

[13] Ramadhan, A.G.G., Hendrawan, A.D. (2013). Perbandingan Pretreatment Degradasi Enzimatik Bagas Tebu Untuk Produksi Hidrogen. Teknik Kimia ITS. Surabaya.

[14] Datta (1981), Acidogenic Fermentation of Lignocellulose-Acid Yield and Conversion of Components, *Biotechnology and Bioengineering*, 23: 2167-2170 .

[15] Nie, Y., Li, C., Sun, A., Meng, H., Wang, Z. (2006). Extractive desulfurization of gasoline using imidazolium-based phosphoric ionic liquids. *Energy & Fuels*, 20: 2083-2087.

[16] Poletto, M., Zatera, A.J., Forte, M.M.C., Santana, R.M.C. (2012). Thermal Decomposition of Wood Components and Cellulose Crystallite Size. *Bioresource Technology* 109: 148-153.

[17] Popescu, M.C., Popescu, C.M., Lisa, G., Sakata, Y. (2011). Evaluation of morphological and chemical aspects of different wood species by spectroscopy and thermal methods. *J. Mol. Struct*. 988: 65-72.

[18] Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R.K., Pandey, A. (2012). Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renewable Energy* 37(1): 109-116

[19] Xiao, W., Yin, W., Xia, S., Ma, P. (2012). The study of factors affecting the enxymatic hydrolysis of cellulose after ionic liquid pretreatment. *Carbohydrate Polymers* 87: 2019-2023.

[20] Li, Q., He, Y.C., Xian, M., Jun, G., Xu, X., Yang, J.M., Li, L.Z. (2009). Improving enzymatic hydrolysis of wheat straw using ionic liquid 1-ethyl-3-methyl imidazolium diethyl phosphate pretreatment. *Bioresource Technology* 100: 3570-3575.

[21] Sen, S.M., Binder, J.B., Raines, R.T., Maravelias, C.T. (2012). Conversion of Biomass to Sugars via ionic liquid hydrolysis: process synthesis and economic evaluation. *Biofuels, Bioprod. Bioref*. 6: 444-452.

[22] Fu, D., Mazza, G. (2011). Optimization of processing conditions for the pretreatment of wheat straw using aqueous ionic liquid. *Bioresource Technology* 102: 8003-8010.