

The Effect of Alkaline Concentration on Coconut Husk Crystallinity and the Yield of Sugars Released

H F Sangian¹ and A Widjaja^{2,*}

¹Jurusan Fisika, Universitas Sam Ratulangi Manado, Sulawesi Utara, Indonesia 95115

²Jurusan Teknik Kimia, Institut Teknologi Sepuluh Nopember Surabaya, Jawa Timur, Indonesia 60111

*arief_w@chem-eng.its.ac.id

Abstract: This work was to analyze the effect of alkaline concentration on coconut coir husk crystallinity and sugar liberated enzymatically. The data showed that the employing of alkaline on lignocellulose transformed the crystallinity. The XRD peaks increased highly which indicated that cellulose was more opened and exposed. After pretreatment, the chemical compositions (cellulose, hemicellulose, and lignin) were changed significantly. The employing 1% alkaline, the cellulosic content inclined if compared to that of non-pretreatment. When the alkaline concentration was added to 4%, the cellulose was decreased slightly which indicated that a part of cellulose and hemicellulose was dissolved into solution. It was found the alkaline pretreatment influenced by the biochemical reaction of treated substrates in producing the reducing sugars. The amounts of sugar liberated enzymatically of coconut husk treated by 1% and 4% alkaline increased to 0.26, and 0.24 g sugar/g (cellulose+hemicellulose), respectively, compared to that of native solid recorded at 0.18 g sugar/g (cellulose+hemicellulose).

1. Introduction

The lignocellulose, a renewable- and a recycled carbon material account of 10-90% cellulose which has a linear polysaccharide 1,4- β -D-glucose. The cellulosic structure has a strong connection called a glycosidic linkage β (1 \rightarrow 4) and an H bond at the hydroxyl OH serves as a scutum of simple monomers [1]. Prior to the hydrolysis of lignocellulosic materials into sugars, lignin must firstly be degraded and the cellulose structure is changed from crystalline- into amorphous structures so that enzymes can attack easily the cellulose [2, 3].

The initial process to treat the lignocellulosic material prior to hydrolysis is called a pretreatment which is the most crucial step for producing of sugar and ethanol [4]. The objectives of pretreatment (including lignin degradation) were to modify the crystal structure of cellulose for increasing the digestibility of the enzyme work on the hydrolytic process [5]. The weakness of sub- and super-critical methods is difficult to scale up because of cost and safety considerations [6].

However, from the perspectives of sustainable technology and desirable cost, the conventional pretreatment must be improved the waste management. The preparation of sugar derived from lignocellulosic materials requires more environmentally friendly and more efficient method that utilizes a less heat, operates in mild condition, and uses the cheap solvents. The ionic liquid pretreatment is a new method and has many advantages in operation such as low vapor pressure, high thermal stability, easy to dissolve many compounds, and it can be recycled [7]. The lignocellulosic materials have been converted into sugars by ionic liquid pretreatment by delignification [8]. The



delignification process is to reduce barriers, mostly caused by lignin, in the enzymatic hydrolysis process. The low lignin lignocellulose, ionic liquid interacts well with cellulose through electrostatic interaction mechanism [9].

The usage of ionic liquids, however, is costly since their prices are very expensive [10]. It is necessary to manufacture the fermentable sugars from lignocellulose treated by the solvent in which it does not harm the environment nor was expensive. This work was to manufacture of reducing sugar hydrolyzed from coconut husk pretreated by alkaline whose concentrations were varied at 1 and 4% and biocatalysts used were the pure cellulase and xylanase.

2. Experimental Setup

2.1. Material preparation

The raw material, coconut husk, was kindly given by the owner of the coconut husk factory in South Minahasa, North Sulawesi Indonesia. Prior to pretreatment, coconut husk was prepared by the procedures as follows: the raw material was heated under sunlight radiation for days and milled and then filtered to the size of 120 mesh using screener apparatus (Retsch GmbH Rheinische strade 362/2781, Haan Germany).

One and four percent alkaline were employed to carry out pretreatment on coconut husk in which method was adapted from previous work [5]. Six grams' lignocellulose was mixed into one percent and four percent alkaline (250mL, 80°C) for 16 h and then the solid was washed by hot water passing through Whatman paper via reduced pressure. An XRD (*Philips X'Pert X-Ray Diffractometer*) was employed to analyze the structure of treated- and untreated substrates of coconut husk as a previously referred study [11]. The chemical composition of coconut husk treated by alkaline was analyzed using the Chesson technique [12].

2.2. Enzymatic hydrolysis

The enzymatic hydrolysis of treated- or untreated- coconut husk and sugar analysis were conducted in the same procedure as previously reported work [13]. Suspension solution was prepared by adding the sodium acetate buffer (pH 3, 30 mL, 0.1M) into one-gram substrates inside Erlenmeyer reactor. A suspension was controlled and incubated in an oil bath at 60°C under slow stirring for 48 h. The enzymatic reaction was begun by adding 0.2 ml of pure cellulase or mixture of cellulase + xylanase from A. Niger. The pH was controlled at 3.0 by adding sodium citrate, or citric acid. Two 0.2mL samples were removed periodically and diluted with 1.8mL pure water and 3 mL DNS solution and then the amount of sugar was measured using spectrophotometer (CECIL 1001). The mixture was isolated in the tube and put in boiling water for ten minutes. The sample tube was moved to an ice-water bath and then was rotated in centrifuge equipment (Hermle Labortechnik GmbH-Z 326 K, Wehingen Germany) to settle the solid and pulp. The measurement of sugars fraction (glucose, xylose and lactose) used HPLC technique (Waters 1515 Isochoric HPLC Pump, 2414 RI and Aminex HPX87P (Bio-Rad, CA) using standard concentrations: as follows glucose: 50.040, 25.020, 12.510; Xylose: 40.070, 20.037, 10.019; and galactose: 20.044, 10.022, and 5.011.

3. Results and Discussion

3.1. The crystallinity analysis

One percent alkaline solution dissolved lignin and hemicellulose so that cellulose I crystal was more exposed. The properties of cellulose I am near to those of pure cellulose, whose crystallinity is high and peaks are vivid at the angles 17.00°, 23.00° and 35.00° in which their Miller indices were (101), (002) and (040), respectively, as previously described works [11]. The XRD curve of 4%NaOH-pretreated substrate, the peak of (002) plane declined slightly whereby a part of cellulose was dissolved as shown in Fig. 1a.

The graph shows the peak symmetry of treated crystal was shifted at 0.5° appertaining to the dilatation of space of the substrate. Some authors reported lignocellulose that has been treated, could

increase the digestibility of enzymatic hydrolysis as previously published work [14]. An alkaline solution dissolved lignin and hemicellulose so that cellulose crystal was porous.

3.2. Analysis of chemical compositions

The average chemical compositions of treated- and untreated-coconut husk are shown in Fig. 1b. Chemical compositions of original coconut husk were 26.60% (cellulose), 17.74% (hemicellulose) and 41.18% (lignin), respectively. While cellulosic compositions of 1%NaOH- and 4% NaOH-pretreated substrates were 43.18 percent and 38.30 percent, which is higher lignin removal compared to that of control. The data shows 1%NaOH pretreatment caused the cellulose and hemicellulose improved significantly. When the alkaline concentration was added to 4% for pretreatment the cellulose composition declined because of dissolution of cellulose into solution. The origin, type, and maturity of coconut husk could affect to their compositions [15].

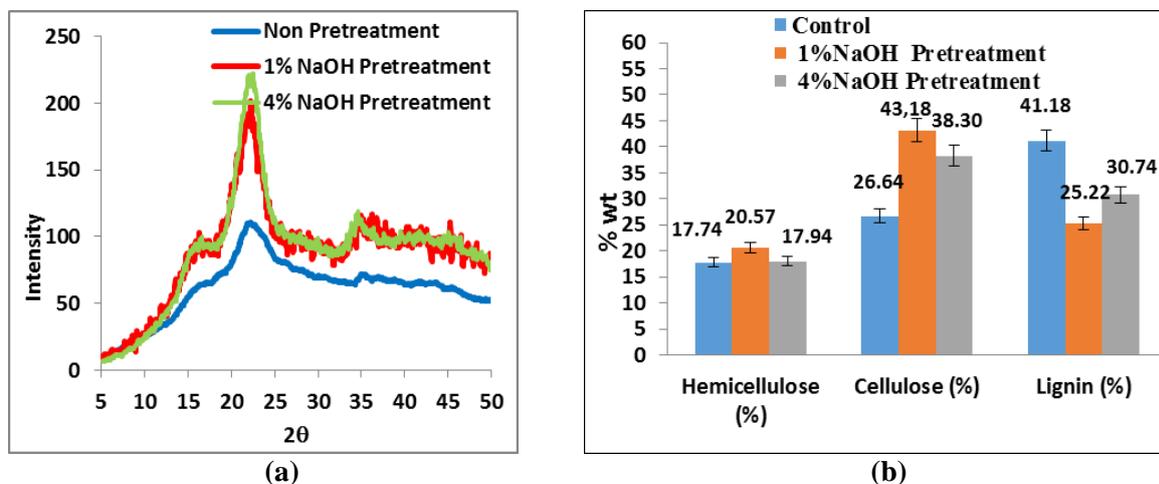


Figure 1. The XRD patterns (a) and chemical compositions (b) of pretreated coconut husk pretreated by 1 & 4% NaOH (80°C,16h) and compared with non-pretreatment as a control.

3.3. Enzymatic conversion

Preparation of enzyme solution and standard graphs of sugars was adapted from procedures that have been used since 1959 [16]. Fig. 2a shows sugar concentrations of enzymatic hydrolysis of coconut husk pretreated with 1%NaOH, 4%NaOH and original solid as control with using a single pure cellulase.

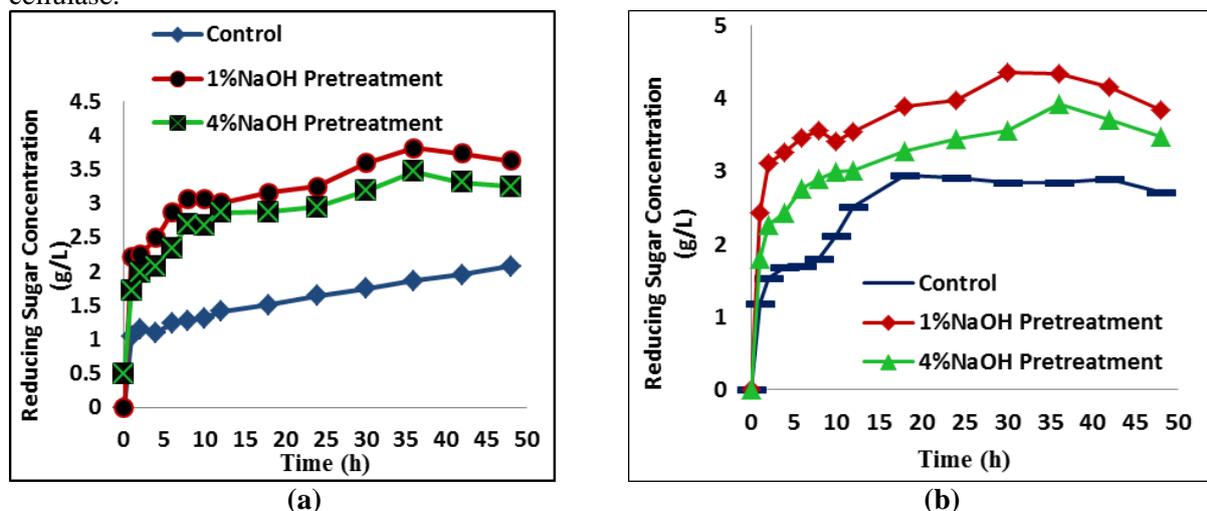


Figure 2. Time course of enzymatic hydrolysis (60°C for 48 h) using pure cellulase (a) and mixture cellulase+xylanase (b) of coconut husk pretreated with 1%NaOH, 4%NaOH (80°C,16 h) and original coconut husk as a control.

When coconut solid was pretreated by 1%NaOH and 4%NaOH, the maximum sugar concentrations obtained were of 3.814 g/L and 3.464 g/L, respectively. The optimum sugar hydrolyzed from the original substrate as control was 2.074 g/L, which was the least sugar concentration. These results attributed to the chemical compositions and crystalline structure of substrates before and after pretreatment. The higher of lignin content of substrate was the lower of sugar liberated from the substrate. It was found that lignin factor was a major barrier of catalyst or cellulase enzyme to attack cellulose and hemicellulose to liberate sugars as a previously reported study [17, 18]. The second step was the utilization of cellulase+xylanase to enzymatic hydrolysis of pretreated- and unpretreated-substrates. When substrate pretreated by 1%NaOH and 4%NaOH, sugar concentrations were 4.966g/L, 3.928g/L, respectively as shown in Fig. 2b.

Table 1. Comparison of reducing sugars concentration hydrolyzed from pretreated substrates using cellulase and cellulase+xylanase for 48 h.

Pretreatment	Sugar concentration (g/L)		Increase (%)
	Cellulase	Cellulase+xylanase	
1%NaOH	3.629	3.834	9.88
4%NaOH	3.239	3.475	7.30

Table 1 shows the fermentable sugar concentrations hydrolyzed from substrates treated by one and four percent alkaline using cellulase and then compared to those of employing cellulase+xylanase for 48 h. After employing cellulase+xylanase into one percent alkaline-treated substrate, the sugar concentration increased by 9.88% to 3.834g/L compared to 3.629g/L recorded with using single cellulase. While the increase of sugar concentration converted from four percent alkaline-treated substrate, was 7.30 percent to 3.475 g/L with employing cellulase+xylanase from 3.239g/L recorded by applying only cellulase enzyme.

Generally, the amount of sugar liberated from lignocellulose using a mixture of cellulase+xylanase increased only slightly from to those of using single cellulase. Two yield calculations employed as follows: the first yield was to compare an obtained gram of total reducing sugar (TRS) and a gram of dried coconut husk; the second yield was the ratio of a gram of TRS and gram of theoretical total reducing sugar (T TRS). The gram of total reducing sugar is determined by multiplying the TRS concentration by the amount of suspension volume (mL). The theoretical total reducing sugars obtained from the chemical composition (cellulose+hemicellulose) of original coconut husk. For control, T TRS is 0.267 multiplied by the gram of the dried lignocellulose plus 0.177 multiplied by the gram of dried lignocellulose.

Table 2. Yield comparison of reducing sugar hydrolyzed from pretreated substrates using cellulase and mixture of cellulase+xylanase enzymes.

Pretreatment	Yield (%)			
	Cellulase		Cellulase+xylanase	
	Yield (1)	Yield (2)	Yield (1)	Yield (2)
Control	0.06	0.14	0.08	0.18
1%NaOH	0.11	0.25	0.12	0.26
4%NaOH	0.10	0.22	0.10	0.24

When cellulase+xylanase added into one 1% and 4% alkaline-treated substrate, the yield of sugar was 0.26, 0.24 g sugar/g cellulose+hemicellulose which was higher than that of non-pretreatment recorded at 0.18 g sugar/g (cellulose+hemicellulose) as displayed in Table 2. The increase of sugar yield was due to the synergy of cellulase and xylanase to attack cellulose and hemicellulose. This result revealed that the yield of sugar which used the mixture of cellulase and xylanase enzymes was generally larger than that used a single cellulase. The utilization of cellulase+xylanase on enzymatic hydrolysis aimed to improve the sugar yield liberated from substrates since there was a synergy of those enzymes. Cellulase enzyme chopped cellulose chain into glucose and xylanase enzyme cut off

hemicellulose (xylan) into [19]. It was expected that yields would improve using cellulase+xylanase compared to those of introducing only cellulase itself.

However, it was found that no significant differences of sugar yields converted from substrates using cellulase and those employing the mixture of cellulase plus xylanase. This result was typical for enzymatic hydrolysis of high-lignin lignocellulose, as coconut husk and due to the low content of hemicellulose in coconut husk as previously described above. The HPLC measurement was performed to verify and to conform the sugar results recorded from DNS method and to analyze the type of reducing sugar which liberated from coconut husk as described in Table 3. It was found the kinds of fermentable sugars measured were dominated by glucose, xylose, and galactose. Galactose concentration obtained was at 2.3142 g/L for four percent alkaline pretreatment.

Table 3. Total reducing sugar concentration measured by HPLC with pretreated substrates using cellulase+xylanase for 48 h hydrolysis.

Pretreatment	HPLC			
	Glucose	Xylose	Galactose	Total
Control	2.1558	0.1141	1.6832	3.9531
1%NaOH	1.6097	0.2723	1.2351	3.1171
4%NaOH	2.1196	0.0863	2.3142	4.5200

HPLC measurement showed and confirmed that amounts of sugar obtained using DNS method are realistic since those figures are close each other.

It was found that the alkaline concentration influenced significantly on the crystallinity and the yield of sugar obtained. One percent (W/V) of alkaline can be decreasing the biomass crystallinity but increasing the yield of sugar released from treated substrate. When the alkaline concentration increased to 4%, the sugars declined slightly from 1% treated solid since a part of cellulose and hemicellulose started dissolved into solution.

4. Conclusion

The alkaline pretreatment was considerable for the preparation of reducing sugars hydrolyzed from lignocellulose-based materials. After pretreatment, the cellulosic structure transformed in which their crystallinity increased attributing to the dissolution of lignin and hemicellulose. By employing an enzymatic reaction, the sugar liberated from 1%NaOH-treated substrate was of 0.26g sugar/g (cellulose+hemicellulose). If alkaline concentration added to 4%, the total sugar obtained was of 0.24g sugar/g (cellulose+hemicellulose) by applying pure cellulase+xylanase). The results showed that alkaline pretreatment influenced on substrate structure and also affected of the yield of sugar obtained via biocatalyst reaction.

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References

- [1] Zhao X, Zhang L, and Liu D 2012 Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose *Biofuels Bioprod Bioref* **6** 465-482
- [2] Sangian H F and Widjaja A 2017 Effect of pretreatment method on structural changes of coconut coir dust *BioRes.* **12** 4 8030-8046
- [3] Widjaja A, Agnesty S Y, Sangian H F and Gunawan S 2015 Application of Ionic Liquid [DMIM]DMP Pretreatment in the Hydrolysis of Sugarcane Bagasse for Biofuel Production *Bull. Chem. React. Eng. Catal.* **10** 70-77
- [4] Cardona C A, Quintero J A and Paz I C 2010 Production of bioethanol from sugarcane bagasse: Status and perspectives *Bioresource Technology* **101** 4754-4766

- [5] Asgher M, Ahmad Z and Iqbal H M N 2013 Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production *Industrial Crops and Products* **44** 488–495
- [6] Sangian H F, Ranggina D, Ginting G M, Purba A A, Gunawan S and Widjaja A 2015 Study of the preparation of sugar from high-lignin lignocellulose applying subcritical water and enzymatic hydrolysis: Synthesis and consumable cost evaluation *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry* **16** 1 13–27
- [7] Ha S H, Mai N L, An G and Koo Y M 2013 Microwave-assisted pretreatment of cellulose in ionic liquid for accelerated enzymatic hydrolysis *Bioresource Technology* **102** 1214–1219
- [8] Qiu Z and Aita G A 2013 Pretreatment of energy cane bagasse with recycled ionic liquid for enzymatic hydrolysis *Bioresource Technology* **129** 532–537
- [9] Feng L and Chen Z 2008 Research progress on dissolution and functional modification of cellulose in ionic liquids *Journal of Molecular Liquids* **142** 1–5
- [10] Sangian H F, Kristian J, Rahma S, Dewi H K, Puspasari D A, Agnesty S Y, Gunawan S, and Widjaja A. 2015 Preparation of Reducing Sugar Hydrolyzed from High-Lignin Coconut Coir Dust Pretreated by the Recycled Ionic Liquid [mmim][dmp] and Combination with Alkaline *Bull. Chem. React. Eng. Catal.* **10** 8-22
- [11] Park S, Baker J O, Himmel M E, Parilla P A, Johnson D K 2010 Cellulose Crystallinity Index: Measurement Techniques and Their Impact on Interpreting Cellulase Performance *Biotechnology for Biofuels* **3** 10 1-10
- [12] Datta and Rathin 1981 Acidogenic Fermentation of Lignocellulose- Acid Yield and Conversion of Components *Biotechnology and Bioengineering* **23** 2167-2170
- [13] Zhao D, Li H, Zhang J, Fua L, Liu M, Fua J and Ren P 2012 Dissolution of cellulose in phosphate-based ionic liquids *Carbohydrate Polymer*. **87** 1490-1494
- [14] Yuan T Q, Wang W, Xu F and Sun R C 2013 Synergistic benefits of ionic liquid and alkaline pretreatments of poplar wood. part 1: effect of integrated pretreatment on enzymatic hydrolysis *Bio resource Technology* **144** 429–434
- [15] Van Dam J E G Martien J A, Van den Oever, Keijsers E R P, Van der Putten J C, Anayron C, Josol F and Peralta A. 2006 Process for production of high density/high performance binderless boards from whole coconut husk Part 2: Coconut Husk Morphology, Composition and Properties *Industrial Crops and Products* **24** 96–104
- [16] Miller G L 1959 Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar *Analytical Chemistr.* **31** 3 426-428
- [17] Moniruzzaman M and Ono T 2012 Ionic liquid assisted enzymatic delignification of wood biomass: A new ‘green’ and efficient approach for isolating of cellulose fibers *Biochemical Engineering Journal* **60** 156–160
- [18] Muhammad N, Man Z, Bustam M A, Mutalib M I A, Wilfred C D, Rafiq S 2011 Dissolution and delignification of bamboo biomass using amino acid-based ionic liquid *Application Biochemical Biotechnology* **165** 998–1009
- [19] Sangian H F, Kristian J, Rahma S, Agnesty S Y, Gunawan S, Widjaja A. 2015 Comparative study of the preparation of reducing sugars hydrolyzed from high-lignin lignocellulose pretreated with ionic liquid, alkaline solution and their combination *J. Eng. Technol. Sci.* **47** 2 137-148