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PREFACE

BULLETIN OF CHEMICAL REACTION ENGINEERING & CATALYSIS (ISSN 1978-2993) is an electronic international journal. The journal is a media for communicating all research activities in chemical reaction engineering and catalysis fields, and disseminating the novel technology and news related to chemical reaction engineering, catalyst engineering and science, bioreactor engineering, membrane reactor, and catalytic reactor engineering.

This issue (BCREC, Volume 8, Issue 1, Year 2013) has published two review articles including mitigation of current environmental concerns from methanol synthesis, and heterogeneous acid-base catalysts for fragrances synthesis. Some original research articles focused on enzymatic catalysis have also been reported in this issue, including: enzymatic hydrolysis of alkaline pretreated coconut coir, production of sorbitol and mannitol from sugars catalyzed by Ni nanoparticles supported on alumunium hydroxide, and improved stabilities of immobilized glucoamylase on functionalized mesoporous silica. Some other articles focused on material studies have also been published in this issue, they are preparation and characterization of zeolite membrane for bioethanol purification, synthesis and characterization of high alumunium zeolite x, as well as preparation and characterization of acids and alkali treated kaolin clay. Studies about catalyst for oxidation and dehydrogenation processes have also been focused in this issue, they are Pt-Sn/Al_2O_3 catalyst for propane dehydrogenation, and Ag/$Ce_{1-x}Mn_xO_{2-\delta}$ catalyst for oxidation process.

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Research Article

Enzymatic Hydrolysis of Alkaline Pretreated Coconut Coir

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Abstract

The purpose of this research is to study the effect of concentration and temperature on the cellulose and lignin content, as well as the reducing sugars produced in the enzymatic hydrolysis of coconut coir. In this research, the coconut coir is pretreated using 3%, 7%, and 11% NaOH solution at 60 °C, 80 °C, and 100 °C. The pretreated coir were assayed by measuring the amount of cellulose and lignin and then hydrolysed using Celluclast and Novozyme 188 under various temperature (30 °C, 40 °C, 50 °C) and pH (3, 4, 5). The hydrolysis results were assayed for the reducing sugar content. The results showed that the alkaline delignification was effective to reduce lignin and to increase the cellulose content of the coir. The best delignification condition was observed at 11% NaOH solution and 100 °C which removed 14.53% of lignin and increased the cellulose content up to 50.23%. © 2013 BCREC UNDIP. All rights reserved. (*Selected Paper from International Conference on Chemical and Material Engineering (ICCME) 2012*)

Keywords: coconut; enzyme; hydrolysis; lignocellulose

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1. Introduction

The lignocellulosic biomass is represented by the high-level crop, hard wood and soft wood with cellulose, hemicellulose and lignin as the main component. One of the lignocellulosic biomass available in Indonesia is coconut coir. Coconut productivity in Indonesia is very high, reaching 15.5 billion coconuts per year, which is equivalent to 3.02 million tons of copra, 3.75 million tons of water, 0.75 million tons of shell, 1.8 million tons of coir fiber, and 3.3 million tons of coir dust. The coconut processing industries are still largely focused on coconut meat processing, while the process industry of its byproducts such as coconut water, coconut coir and coconut shell is still in small scale and traditional [1]. However, the potential of the byproduct is very large, especially the coconut coir.

Coconut coir is composed of cellulose, lignin, pectin, hemicellulose, and ash. Cellulose can be processed and converted into alternative energy sources such as ethanol, and biohydrogen via fermentation. In order to utilize the cellulose for fermentation substrate, it must be hydrolyzed either chemically or enzymatically to produce sugar.

Cellulose is the b-1,4-polyacetal of cellobiose which is a polymer of glucose. This cellulose mole-

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cule is a long straight chain and hydrogen bonds are formed between cellulose molecules. This makes the formation of a compound that is comprised of several parallel chains attached to each other called microfibrils which in turn are united into fibres. There are crystalline and non-crystalline region in the cellulose microfibrils. Lignin is an amorphous three dimensional polymer with polyphenols units as the predominant monomers. The most commonly found are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The bond between cellulose, hemicellulose and lignin includes ether, ester and hydrogen bonds [2]. Lignin is a component of coconut coir that is rigid and prevent the cellulose degradation [3].

Pretreatment is an important process in attempt to degrade cellulose to produce sugar. This prosess is intended to alter the cellulose structure so that it will be accessible for hydrolytic enzymes. The lignin degradation is necessary to increase the rate of the hydrolysis of lignocellulosic materials. This can be done by physical, chemical, and biological method. One method of chemical delignification is pretreatment using several kinds of alkaline solutions such as NaOH and $Ca(OH)_2$ [2]. The delignification can be carried out using NaOH solution that can attack the structure of lignin and disrupt the crystalline structure of cellulose [2]. The delignification of coconut coir using acidic condition has been investigated [4]. This research studied the alkaline pretreatment using NaOH solution and the enzymatic hydrolysis of coconut coir. NaOH is relatively cheap, easily obtained and also more soluble in water than $Ca(OH)_2$ [5].

2. Materials and Method

2.1. Materials and Instrumentations

The coconut coir was soaked for 24 hours, then washed using flowing water for 1 hour and dried. The dried coconut coir was cut (± 5 x 5 cm), and then milled using a disc mill (FFC type 23 A, with a speed of 5800 rpm, power 3 kW, Shandong Ji Mu Disk Mill Machinery). Finally, it was sieved (Retsch 5657 test sieve, no. mesh 40, a hole the size of 0.425 mm, stainless steel, W Haan, Germany) to obtain the particle size of 40 mesh.

2.2. Alkaline Pretreatment

In this study, the delignification was performed using various concentration of NaOH (3%, 7% and 11%) and temperature of 60°C, 80°C and 100°C. The coconut coir loading was 15 grams in 200 ml solution of NaOH. The pretreatment process was conducted in a reactor equipped with reflux for 60 minutes. After alkaline treatment, the slurry was filtered and then neutralized using distilled water and dried. Subsequently, the dried solid was tested for the content of lignin and cellulose. The experiment was done in duplicate.

2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis was done using endoglucanase enzymes (Cellulase, *Trichoderma reesei* ATCC 26 921, Sigma Aldrich) and β-glucosidase (Cellobiase, *Aspergillus niger*, Sigma Aldrich). The cellulase enzyme loading used was 15 FPU/g cellulose while the cellulase to cellobiase ratio was 2 FPU/CBU. The operating condition of hydrolysis process was maintained at temperature of 40°C, 50°C, 60°C and pH of 3, 4, 5 using 0.05 M citrate buffer. The hydrolysis was carried out for 2 grams of solids in 50 ml of citrate buffer solution in an incubator shaker (GFL brands 3032 models) at a speed of 90 rpm for 72 hours.

This enzymatic reaction was stopped by heating at 100 °C in a waterbath for 5 minutes. Filtration was then performed using filter paper and the reducing sugar content of the filtrate was analyzed.

2.4. Chemical Analysis

In this study, the analysis of cellulose and lignin content was conducted by Chesson method [6]. The reducing sugar content analysis performed by the method of DNS (Dinitrosalicylic Acid) [7].

3. Results and Discussion

The raw of coconut coir contained 41.70% of cellulose and 30.54% of lignin. After delignification, it was observed that the cellulose content increased to the range of 45.39% - 50.23%, while the lignin decreased to the range of 26.10%-29.28%. This experimental works was repeated twice and the chemical analysis of each experiment was also repeated twice. The maximum error was 3.27%.

3.1. Effect of Delignification Temperature and NaOH Concentration on Cellulose Content of Coconut Coir

Many factors could affect the cellulose content of the pretreated coconut coir. The two of them were temperature and concentration of NaOH solution which could be seen in Figure 1.

The cellulose content of the coconut coir increased with the delignification temperature. The delignification using 3% NaOH solution at 60 °C gave the lowest cellulose content. The cellulose content increased by increasing concentration of NaOH solution. As seen in Figure 1, the delignifi-

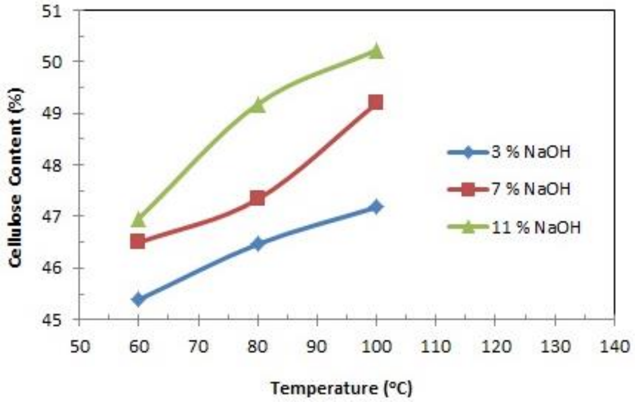

Figure 1. The Cellulose content of the coconut coir after delignification

cation at 60°C resulted in the highest cellulose content at 11% NaOH solution.

Figure 1 shows that the pretreatment using alkaline solution at various temperature and concentration could affect the cellulose content in coconut coir. This was caused by various reactions occurred during delignification.

3.2. Effect of Delignification Temperature and NaOH Concentration On Lignin Content of Coconut Coir

The lignin content of pretreated coconut coir was also affected by temperature and concentration of NaOH solution, as shown in Figure 2.
At NaOH concentrations of 3%, 7%, and 11%, the remaining lignin content at delignification temperature of 60°C was higher than that at 80°C. The lowest remaining lignin content was observed at delignification temperature of 100°C for a all concentration of NaOH. The remaining lignin content in the coconut coir decreased with temperature. The variation of concentration also gave the same effect to lignin content of the coconut coir. The higher concentration of NaOH the lower the lignin content of the coconut coir.

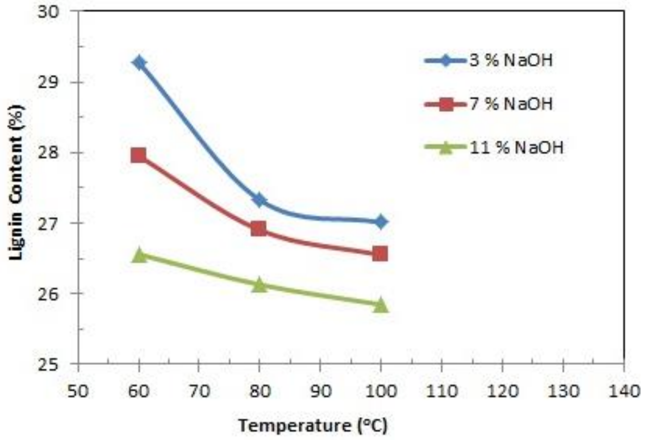

Figure 2. Lignin content of the coconut coir after delignification

As seen in Figure 1 and Figure 2, effect of NaOH concentration and temperature on the lignin concentration was in the contrary with that on the cellulose concentration. The presence of alkaline (NaOH) solution could degrade the lignin content of coconut coir. This degradation (delignification) reaction could be enhanced by increasing alkaline concentration and reaction temperature. Therefore, the lignin concentration decreased while the cellulose concentration increased with alkaline concentration and reaction temperature.

The correlations of delignification temperature and with the remaining lignin content of coconut coir can be explained using the Arrhenius theory as shown in Equation 1 and the reaction rate equation in Equation 2.

$$k = k_o . e^{-\frac{Ea}{RT}} \tag{1}$$

$$-r_A = k . C^n \tag{2}$$

where: $-r_A$: the rate of reaction; k : reaction rate constant; C : concentration; T : temperature; and n : reaction order.

According to the above equation, the higher the temperature, the greater the value of the reaction rate constant (k) would be. Therefore, at the same concentration (C), the rate of reaction ($-r_A$) would increase. Similarly, at the same temperature (T), if the concentration (C) increased, the rate of reaction would also rise. The increased of the reaction rate would speed up the process of lignin removal, and hence more lignin would be removed and more cellulose would be obtained.

Silverstein *et al.* reported that the dilute NaOH pretreatment has removed at 7.28%-14.301% lignin content from cotton stalk at 30 min reaction and temperature of 90°C using NaOH concentration of 0.5-2% [8]. In this research, the lignin content removal ranged from 4.096% to 14.55%.

3.3. Reducing Sugar Content of the Hydrolysate

The raw coconut coir was hydrolyzed under the same condition as pretreated coconut coir, which was at temperature of 50°C and pH 4. This hydrolysis resulted in 0.171 g/L reducing sugar. The same step was carried out to the pretreated coconut coir. The reducing sugar content obtained were varied depending on the variation of temperature and concentration of NaOH solution, as shown in Table 1. As shown in Table 1, it could be concluded that the delignification could increase the reducing sugar content of coconut coir.

3.4. Effect of NaOH Concentration on the Sugar Production

The reducing sugar of coconut coir hydrolysate was affected by the concentration of NaOH solution. In the hydrolysis process at temperature of 50 ^{o}C and pH 4, the result is presented in Figure 3.

Table 1. Reducing sugar of the pretreated coconut coir

Delignification Condition		**Reducing Sugar (gr/L)**
Concentration of NaOH (%)	**Temperature (^{o}C)**	
3	60	1.85
	80	4.23
	100	4.67
7	60	2.94
	80	5.58
	100	5.80
11	60	4.53
	80	6.18
	100	7.57

3.5. Effect of Delignification Temperature on the Sugar Production

Beside the concentration of NaOH solution, the temperature of delignification also affected the production reducing sugar in the hydrolysis, as shown in Figure 4.

At delignification temperature of 60^{o}C with various NaOH concentration, the reducing sugar obtained was the lowest among the other conditions. The highest reducing sugar was obtained from the coir delignified at temperature of 100^{o}C. The result showed that the higher the temperature of delignification the more effective the enzymatic hydrolysis would be. This phenomena was due to the higher the temperature of delignification, the higher the levels of available cellulose produced, and hence the more available substrate for enzyme would be obtained. This would resulted in higher concentration of reducing sugar produced.

3.6. Effect of Hydrolysis pH on the Reducing Sugar Production

After the best condition of delignification process was obtained, the experiment was continued to enzymatic hydrolysis at temperature of 50^{o}C with variation of pH 3 and 5. The reducing sugar produced is shown in Figure 5.

The highest reducing sugar was obtained in the hydrolysis at pH 4. At pH 5, the reducing sugar produced was slightly lower than that at pH 4, and the lowest one was obtained at pH 3. The optimum pH of hydrolysis in this research was observed at

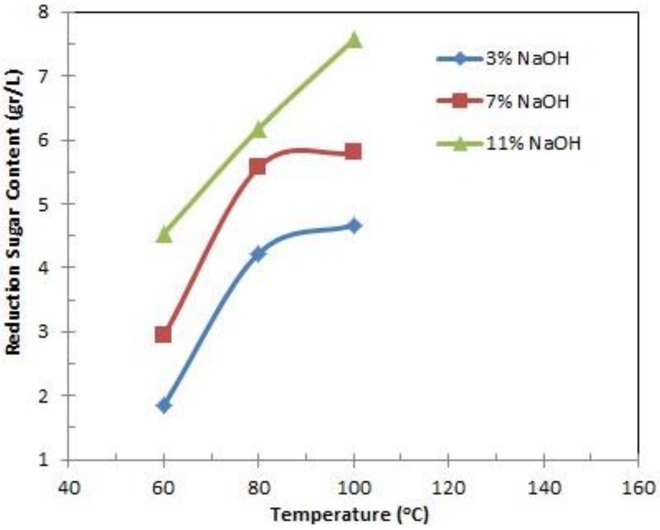

Figure 3. The reducing sugar content at various concentration of NaOH solution

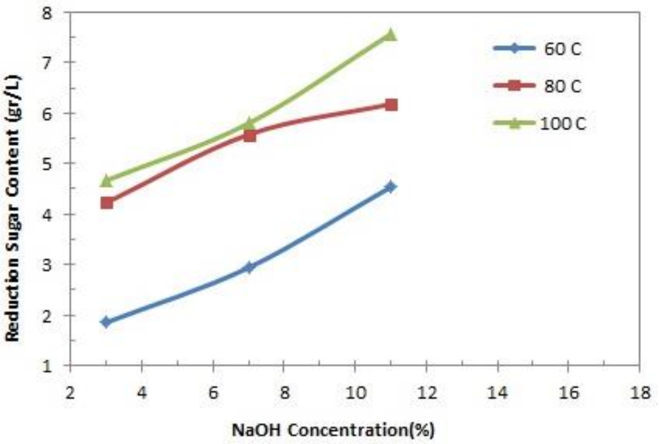

Figure 4. The reducing sugar content at various temperature of enzymatic hydrolysis

about 4 and 5.

The change of pH could affect the enzyme activity by changing the structure or the charge of the active site of enzyme. At the optimum pH, there was charge suitability between the enzyme and the substrate, so the substrate was exactly placed in the active site of enzyme. Those enzyme and substrate then formed enzyme-substrate complex and produced products. At pH lower than the optimum, the substrate lost its negative charge. The enzyme could not bind the substrate at higher pH because it was ionized and lost its positive charge [9].

3.7. Effect of Hydrolysis Temperature on the Sugar Production

After the best pH of hydrolysis was observed (pH 4), the hydrolysis process was continued at various temperature (40^{o}C and 60^{o}C). The reducing sugar obtained at this operating condition was shown in Figure 6.

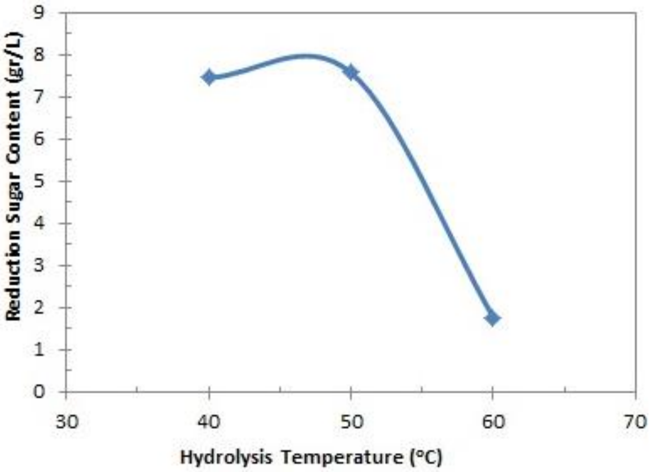

Figure 6. The Reducing Sugar Content at Various Temperature of Enzymatic Hydrolysis

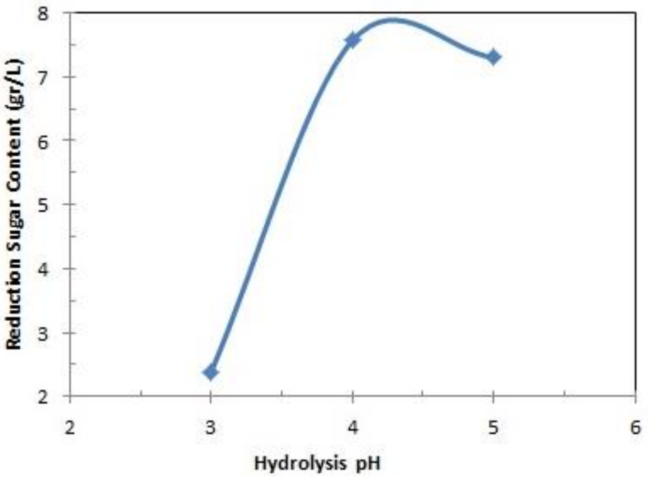

Figure 5. The Reducing Sugar Content at Various pH of Enzymatic Hydrolysis

The hydrolysis at pH 4 and temperature of 40^{o}C resulted in the reducing sugar concentration slightly lower than that which carried out at temperature of 50^{o}C. At temperature of 60^{o}C, the reducing sugar concentration was lower significantly than those obtained at temperature of 50^{o}C and 40^{o}C. In other words, the highest reducing sugar was observed from hydrolysis at pH 4 and temperature of 50^{o}C. The optimum hydrolysis temperature seemed to occur at temperature between 40-50^{o}C. This could be concluded from the difference of reducing sugar produced from hydrolysis at 50^{o}C and 40^{o}C, where they were only slightly different. This behavior might be due to a hydrolysis temperature range between 40-50^{o}C which was close to the optimum temperature of the enzyme. The enzyme was almost reached its optimal activity and the reducing sugar produced did not differ significantly or nearly constant. This followed by the biochemical theory which stated that the thermal denaturation of the enzyme as a type of protein could occur at temperature of 45-50^{o}C. At above temperature of 50^{o}C the enzyme gradually became inactive due to denatured protein [10].

The influence of delignification conditions on this research result is depicted in Figures 7 to 9. In those figures the experimental data (shown in asteriks) were presented along with the result from surface models. It can be concluded from those figures that the best condition for coconut coir delignification condition is at 11% NaOH concentration and temperature of 100^{o}C.

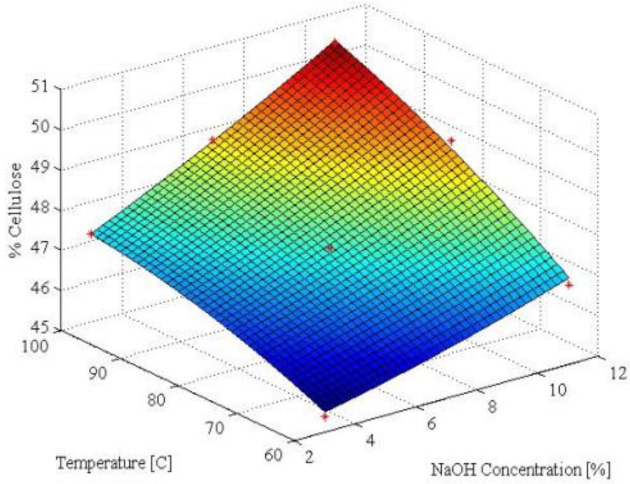

Figure 7. The effect of delignification condition on the cellulose content of coconut coir

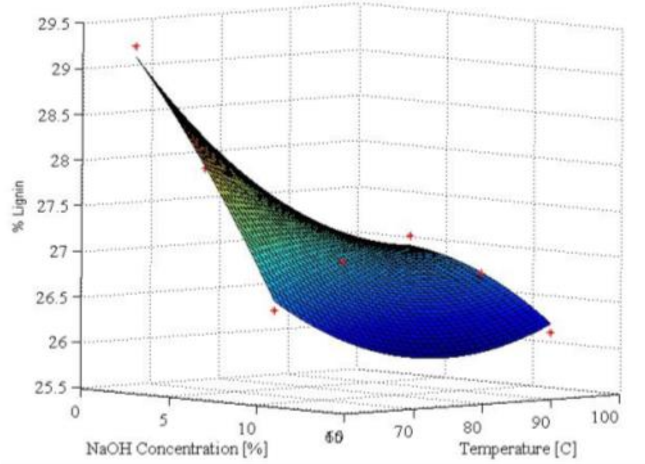

Figure 8. The effect of delignification condition on the lignin content of coconut coir

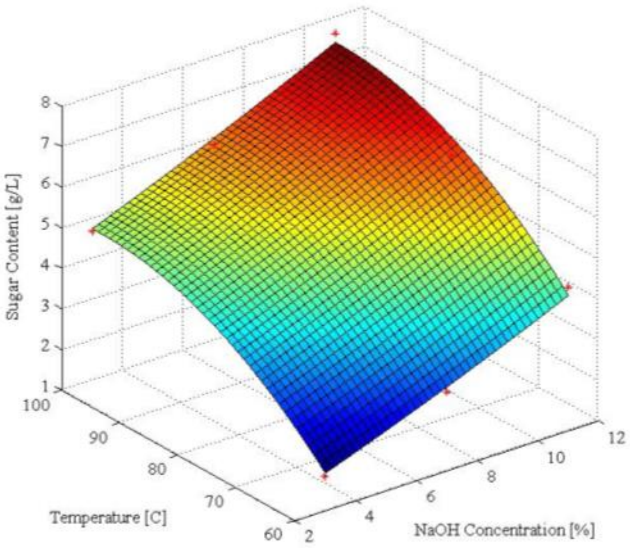

Figure 9. The effect of delignification condition on the sugar content of the hydrolysate

4. Conclusions

The delignification of coconut coir using dilute NaOH solution was effective to reduce lignin content and increase cellulose content of the coconut coir. The increasing temperature and concentration of NaOH would increase the amount of lignin removed, and hence as well as reducing sugars produced. The best condition of the delignification process was observed at 11% NaOH solution and delignification temperature of 100°C which removed 14.53% of lignin content and increased the cellulose content up to 50.23%. The maximum reducing sugar concentration that could be achieved was 7.57 g/L.

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