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, and 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^{o}C, 80^{o}C, and 100^{o}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^{o}C, 40^{o}C, 50^{o}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^{o}C which removed 14,53% of lignin and increased the cellulose content up to 50,23%. The best condition of the enzymatic hydrolysis was obtained at 50^{o}C and pH 4 which produced 7,57 gr/L reducing sugar.

***Keywords* :** coconut, enzyme, hydrolysis, lignocellulose

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 manufacturing industries are still largely focused on coconut meat manufacturing, while the manufacturing industry of its byproducts such as coconut water, coconut coir and coconut shell is still in small scale and traditional **[1]**. Whereas the potential of the byproduct is very large, especially the coconut coir.

Coconut coir is composed of cellulose, lignin, pectin, hemicellulose, and ash. Cellulose is a component of coconut coir that can be processed and converted into energy sources. Lignin is a component of coconut coir that is rigid and prevent the cellulose degradation **[2]**. 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 some kind of alkaline solutions such as NaOH and $Ca(OH)_2$ **[3]**.

The delignification can be carried out using NaOH solution that can attack the structure of lignin and disrupt the crystalline structure of cellulose **[3]**. In addition, the NaOH is relatively cheap, easily obtained and also more soluble in water than $Ca(OH)_2$ **[4]**.

This research studied the alkaline pretreatment using NaOH solution and the enzymatic hydrolysis of coconut coir. The hydrolysate as intended to be used as biohydrogen fermentation substrate.

2. Materials and Methods

2.1. Materials Preparation

The coconut coir was soaked for 24 hours, then washed with 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 3kW, 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^{o}C. The coconut coir loading was 15 grams in 200 ml solution of NaOH. The pretreatment process was conducted in a reactor with reflux for 60 minutes. After alkaline treatment, the solid 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 duplicated.

2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis was done using endoglucanase enzymes (Cellulase, *Trichoderma reesei* ATCC 26 921, Sigma Aldrich) and β-glucosidase (Cellubiase, *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 condition of hydrolysis process was maintained at temperature of 40^{o}C, 50^{o}C, 60^{o}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 then stopped by heating at 100^{o}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. Analysis

In this study, the analysis of cellulose and lignin content was conducted by Chesson method [5]. The reducing sugar content analysis performed by the method of DNS (Dinitrosalicylic Acid) [6].

3. Results and Discussion

The raw coconut coir contained 41.70% of cellulose and 30.54% of lignin. After delignification, it was observed that the cellulose content increased to 45.39%-50.23% while the lignin decreased to 26.10%-29.28%.

3.1. The Effect of the Delignification Temperature and NaOH Concentration on the 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.

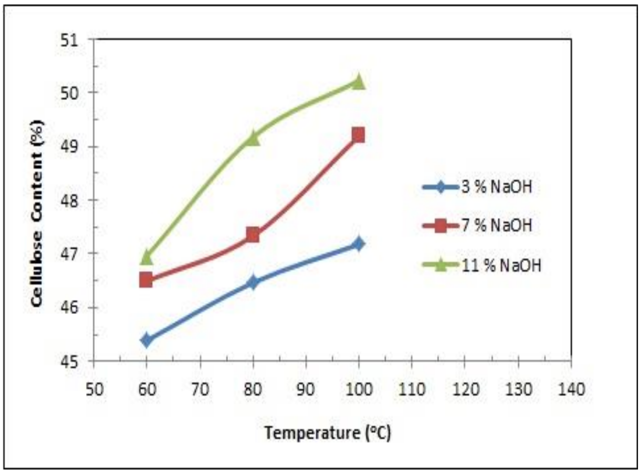

Figure 1. The Cellulose Content of The Coconut Coir after Delignification

The cellulose content of the coconut coir incresed with the delignification temperature. The delignification using 3% NaOH solution at 60^{o}C gave the lowest cellulose content than the delignification at 80^{o}C and 100^{o}C. The cellulose content increased by increasing the concentration of NaOH solution. As seen in figure 1, the delignification at 60^{o}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 happened during delignification.

3.2. The Effect of The Delignification Temperature and NaOH Concentration On The 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.

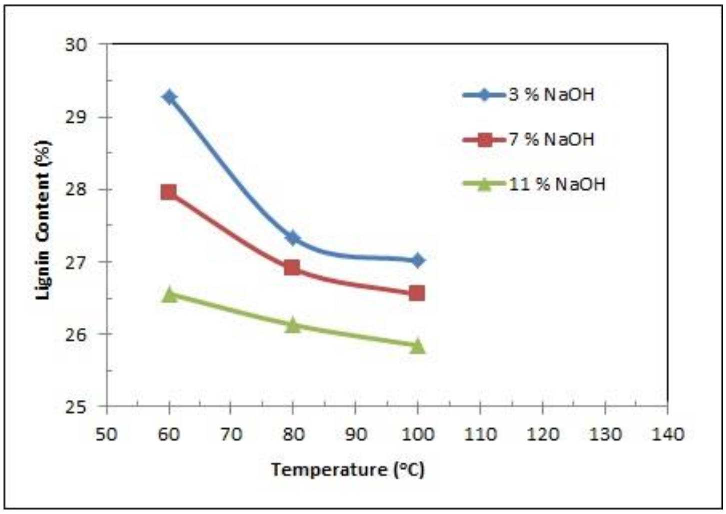

Figure 2. The Lignin Content of The Coconut Coir after Delignification

At concentrations of 3%, 7%, and 11% NaOH, the remaining lignin content at 60^{o}C was higher than 80^{o}C. The lowest remaining lignin content was observed at 100^{o}C for a particular 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 decreased the lignin content of the coconut coir.

The correlations of delignification temperature and NaOH solution concentration with the remaining lignin content of coconut followed the Arhenius theory as shown in equation 1 and 2.

$$k = k_o.e^{-(\frac{\varepsilon_o}{RT})} \quad (1)$$

$$-r_A = k.C \quad (2)$$

Where :

$-r_A$ = the rate of reaction
k = reaction rate constant
C = concentration
T = temperature

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.

3.3. The Reducing Sugar Content of the Hydrolysates

The raw coconut coir was hydrolyzed under the same condition as pretreated coconut coir, which was at 50^{o}C and pH 4. This hydrolysis resulted in 0.171 gr/L reducing sugar. The same step was given 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.

Table 1. The Reducing Sugar of The Pretreated Coconut Coir

Delignification Condition		Reducing Sugar (gr/L)
Concentration of NaOH (%)	Temperature (°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

As shown in Table 1, it could be concluded that the delignification could increase the reducing sugar content of coconut coir.

3.4. The Effect of NaOH Concentration on the Sugar Production

The reducing sugar of hydrolyzed coconut coir was affected by the concentration of NaOH solution. In the hydrolysis process at 50°C and pH 4, the result is presented in figure 3.

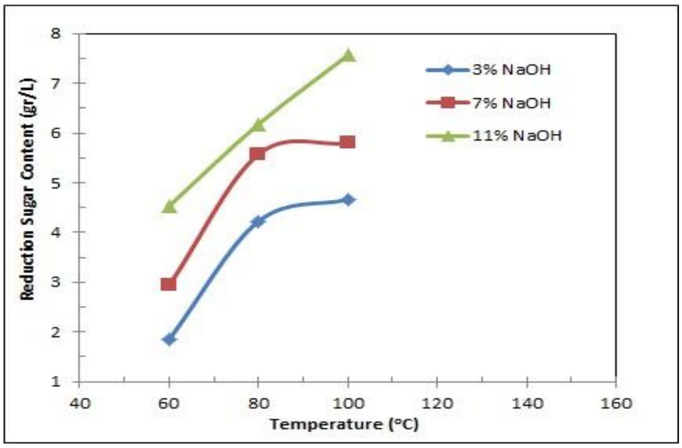

Figure 3. The Reducing Sugar Content at Various Concentration of NaOH Solution

The increased NaOH concentration enhanced the reducing sugar content of hydrolyzed coconut coir. At 60°C, the lowest hydrolysate reducing sugar concentration was obtained from enzymatic hydrolysis at 3% NaOH solution. This was because the higher concentration of NaOH solution removed more lignin during delignification, hence more available cellulose of coconut coir could be obtained. The more cellulose available obtained after delignification would enhance reducing sugar production in enzymatic hydrolysis.

3.5. The Effect of the Delignification Temperature on the Sugar Production

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

At 60°C with various concentration, the reducing sugar obtained was the lowest among the other conditions. The highest reducing sugar was obtained from the coir delignified at 100°C. The result showed that the higher the temperature of delignification the more effective the enzymatic hydrolysis would be. This was because 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.

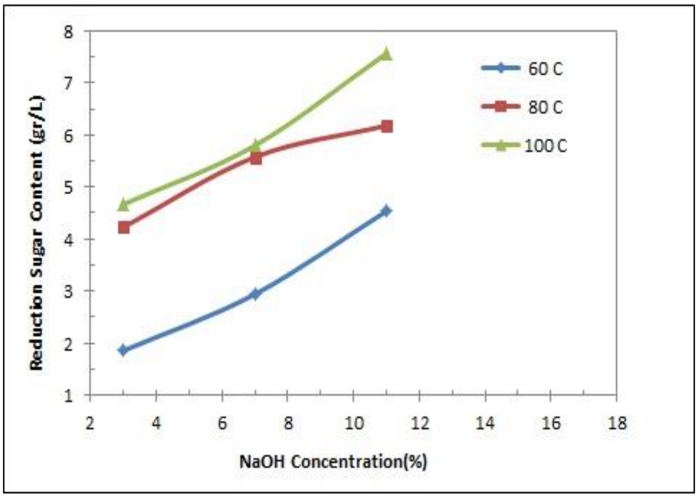

Figure 4. The Reducing Sugar Content at Various Temperature of Enzymatic Hydrolysis

3.6. The Effect of the Hydrolysis pH on the Reducing Sugar Production

After the best condition of delignification was obtained, then it was continued by enzymatic hydrolysis at 50^{o}C with variation of pH 3 and 5. The reducing sugar produced was shown in figure 5.

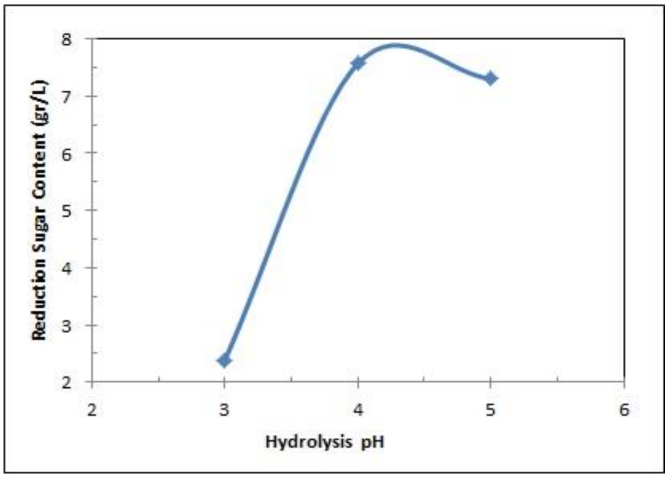

Figure 5. The Reducing Sugar Content at Various pH of Enzymatic Hydrolysis

The highest reducing sugar was obtained in the hydrolysis at pH 4. At pH 5 the reducing sugar produced was slightly lower than at pH 4, and the lowest one was obtained at pH 3. The optimum pH of hydrolysis in this research was observed at 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 **[7]**.

3.7. The Effect of Hydrolysis Temperature on the Sugar Production

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

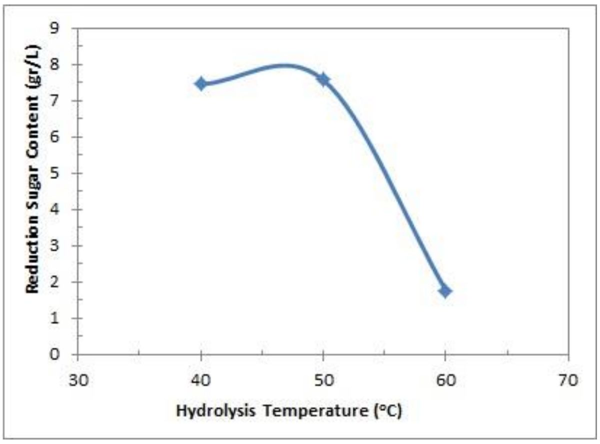

Figure 6. The Reducing Sugar Content at Various Temperature of Enzymatic Hydrolysis

The hydrolysis at pH 4 and 40°C, resulted in reducing sugar concentration slightly lower than that which carried out at 50°C. At 60°C, the reducing sugar concentration was much lower than those obtained at 50°C and 40°C. In other words, the highest reducing sugar was observed from hydrolysis at pH 4 and 50°C. The optimum hydrolysis temperature seemed to occur at temperature between 40-50°C. This could be concluded from the difference of reducing sugar produced from hydrolysis at 50°C and 40°C, where they were only slightly different. This behavior might be due to a temperature range between 40°C-50°C was very close to the optimum temperature of the enzyme, so that the enzyme was almost reached its optimal activity and the reducing sugar produced did not differ significantly or nearly constant. This followed the biochemical theory which stated that the thermal denaturation of the enzyme as a type of protein could occur at temperature of 45°C-50°C. Above 50°C enzyme gradually became inactive due to denatured protein [8].

4. Conclusion

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 was observed at 11% NaOH solution and 100°C which removed 14.53% of lignin content and increased the cellulose content up to 50.23%. The best condition of the enzymatic hydrolysis was obtained at 50°C and pH 4 which produced 7.57 gr/L reducing sugar. This was the maximum reducing sugar concentraion that could be achieved.

Acknowledgements

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