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Alkaline pretreatment and enzymatic saccharification of oil palm empty fruit bunch fiber for ethanol production [1)]

Pengolahan awal dengan basa NaOH dan sakarifikasi enzimatis serat tandan kosong kelapa sawit (TKKS) untuk produksi etanol

Yanni SUDIYANI[*)], Kiky C SEMBIRING, Hendris HENDARSYAH & Syarifah ALAWIYAH

Research Center for Chemistry - Indonesian Institute of Sciences
Kawasan Puspiptek Serpong, 15314, Tangerang, Indonesia

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Abstract

Alkaline pretreatment of oil palm empty fruit bunch (EFB) fiber was conducted to improve enzymatic saccharification of EFB fiber for ethanol production. EFB as one of the major biomass wastes from palm oil industry is a complex lignocellulosic material consists of 41.3 – 46.5% of cellulose, 25.3 – 33.8% of hemicellulose and 27.6 – 32.5% of lignin. Alkali pretreatment of EFB using NaOH 1 N with temperature at 30 and 60^{0}C and reaction times of 30, 60, 90, 120 and 150 minutes were investigated. Furthermore, the enzymatic saccharification of pretreated EFB was examined. The pretreated substrate was subjected to an enzymatic saccharification using meicelase (10, 20 and 40 FPU/g substrate) at 40^{0}C, pH 4.5, 100 rpm for conversion of cellulose and hemicellulose in palm oil EFB to monomeric sugars. The alkali pretreatment of EFB using NaOH can significantly improve the enzymatic saccharification of EFB by removing more lignin and hemicellulose and increasing its accessibility to hydrolytic enzymes. The results showed that the optimum pretreatment condition was NaOH 1 N at 30^{0}C and 90 minutes with the optimum component loss of lignin and hemicellulose was 45.8 % and 35.6 % respectively. The saccharification of EFB pretreated by NaOH 1 N (at 30^{0}C and 90 minutes) for 45 hours and pH 4.5 resulted in optimum saccharification of 63.8 %.

[*Keywords*: Lignocellulosic, oil palm, EFB, saccharification ethanol, NaOH]

Abstrak

Pengolahan awal (pretreatment) serat tandan kosong kelapa sawit (TKKS) dengan basa NaOH telah dilakukan untuk meningkatkan sakarifikasi enzimatik TKKS menjadi etanol. TKKS merupakan bahan lignoselulosa yang terdiri dari selulosa 41,3– 46,%, hemicellulosa 25,3 – 33,8% dan lignin 27,6 – 32,5%. Pretreatment TKKS dilakukan dengan NaOH 1 N dengan variasi suhu 30^0 dan 60^{0}C dan variasi waktu 30, 60, 90, 120 dan 150 menit. Konversi selulosa dan hemiselulosa hasil pretreatment TKKS menjadi gula dilakukan dengan sakarifikasi enzimatik menggunakan enzim meiselase (10, 20 dan 40 FPU/g substrat) pada suhu 40^{0}C, pH 4,5 dengan shaker 100 rpm. Pretretament TKKS dengan basa NaOH dapat meningkatkan sakarifikasi enzimatik dengan berkurangnya lignin dan hemiselulosa secara signifikan dan memudahkan masuknya enzim hidrolitik. Hasil pretreatment dengan NaOH 1N pada suhu 30^{0}C dan 90 menit menunjukkan kondisi optimum untuk penghilangan lignin dan hemiselulosa berturut-turut sebesar 45,8 % and 35,6 %. Hasil sakarifikasi optimum yaitu 63,8 % dicapai setelah 45 jam sakarifisi pada pH 4,5.

[*Kata kunci* : *Lignoselulosa, kelapa sawit, TKKS, sakarifikasi, etanol, NaOH]*

Introduction

Lignocellulosic materials, such as crop residues, grasses, sawdust, woodchips etc., are inexpensive, abundant and renewable sources for ethanol production (Sun & Cheng, 2002). Utilization of lower-value substrates such as lignocellulose offers a great potential for reducing the raw material handling cost and increasing the use of ethanol as a fuel additives (Zhao *et al*., 2008).

Indonesia is the largest palm oil producer in the world. Indonesian palm oil industry generates approximately 15.2 x10^6 t of solid wastes consisting of oil palm empty fruit bunches, fiber and fruit shell every year. One of the major solid wastes from palm oil industry is empty fruit bunch (EFB) which is a complex lignocelllulosic material consists of 41.3 – 46.5% cellulose, 25.3–33.8% hemicelluloses and 27.6–32.5% lignin (Syafwina *et al*., 2002). This lignocellulosic material has a great potency as raw materials for the fermentative production of bioethanol, a promising alternative fuel to gasoline.

The major constraint to the development of successful bioconversion process of lignocellulosic materials is the physical protection of cellulose by lignin against cellulolytic enzymes (Havannavar & Geeta, 2007). Therefore, for the utilization of lignocellulosic materials in a bioconversion process involving enzymatic hydrolysis followed by fermentation, pretreatment is required in order to break down the complex structure of lignocellulose, to reduce the lignin content, cellulose crystallinity and to increase

*) Corresponding authour Email: sudiyani@ gmail.com Phone: +62-21-7560090

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the surface area for enzymatic reactions (Zhao *et al*., 2008; Mtui & Nakamura, 2005). Alkaline pretreatment is a widely used lignocellulosic materials pretreatment approach based on the chemical reaction between alkali and lignocellulosic materials. The decrease of hemicellulose and lignin because of its solubilisation in the NaOH aqueous solution. The increase of cellulose come from the solubilisation of other components in the NaOH aqueous solution. The alkali pretreatment of lignocellulosic materials could increase its enzymatic hydrolysis rate by removing more lignin and hemicellulose and increasing its accessibility to hydrolytic enzymes (Zhu *et al.,* 2006a; 2006b).

The objective of this research was to develop an efficient alkaline pretreatment method for bioconversion of EFB into ethanol and to study the effects of enzyme loadings on sugar obtained after enzymatic saccharification of pretreated EFB.

Materials and Methods

Raw material

Oil palm EFB was collected from an Oil Palm Plantation belongs to PT Perkebunan Nusantara VIII, in Pandeglang, Banten, Indonesia. The EFB was pretreated physically by drying and grinding to get particle sizes that passed 30 mesh sieve, then it was stored in sealed plastic bag at room temperature until be used for pretreatment.

Characterization of EFB

The EFB was measured for its moisture content by gravimetric method and oil content by Soxhlet extraction as well as its lignin, cellulose and hemicellulose contents (Goering & Van Soest, 1970).

Pretreatment

Alkaline pretreatment of EFB fiber was carried out in 200 mL Erlenmeyer flask. 100 mL of NaOH 1N was added to physically-treated EFB samples at 12.5% (w/v) solid loading. The samples were heated at 30^0 and 60^0C for 30, 60, 90, 120 and 150 minutes. The pretreatment conditions were selected based on previous research on hydrolysis of various lignocellulosic raw materials. After pretreatment reaction, the samples were filtered to separate the insoluble solid fiber from the soluble fraction. The insoluble solid fiber was washed with water until neutral pH, and dried at 105^0C until reached a constant weight. The fiber was then determined for its lignin, cellulose and hemicellulose contents (Goering & Van Soest, 1970) to determine the decreasing of lignin, cellulose, and hemicellulose contents after pretreatment. Lignin, cellulose and hemicellulose losses were also calculated.

Enzymatic saccharification

Enzymatic saccharification of pretreated EFB fiber was carried out using a commercial cellulase (Meicellase from Meiji Seika, Japan). Two grams of pretreated EFB fiber was added with 50 mL acetate buffer solution 20 mM, pH 4.5 and 5.0, respectively. Cellulase was added to the samples at 10, 20 and 40 FPU/g, then, the samples were incubated on shaker 100 rpm at 40^0C, for 48 hrs. Glucose and xylose concentration was determined by Nelson-Smogyi method.

Results and Discussion

Characterization of EFB

Characterization of EFB was carried out to determine major of principal components of EFB. Table 1 shows the chemical composition of initial oil palm EFB fiber (before pretreatment). From Table 1, it can be seen that the carbohydrate fraction (holocellulose fraction) of EFB was 56.49 % of the total biomass, which consist of 33.25 % alfa-cellulose and 23.24 % hemicellulose. The major component of EFB was alfa-cellulose (33.25 %), a polymer of glucose which is very potential as a sugar source for ethanol production. The lignin content of EFBs was 25.83%, comparable to the lignin content of hardwoods (18-25%) (Syafwina *et al*., 2002). This high lignin content is the main reason of alkali (sodium hydroxide) pretreatment which would be applied to oil palm EFB.

The main effect of alkali pretreatment on lignocellulosic biomass is delignification by breaking ester bonds cross-link lignin and xylan, thus increasing the porosity of the biomass (Silverstein *et al*., 2007).

The effect of NaOH pretreatment on lignocellulosic components

Alkaline pretreatment of lignocellulosic biomass is one of the most effective pretreatment methods which predominantly affect lignin content of biomass. The main effect of sodium hydroxide pretreatment on

Table 1. Chemical contents of oil palm EFB fiber.

Tabel 1. Kandungan kimia dari serat TKKS.

No	Components	Percentage (%)[a]
1	Water content	8.56
2	Oil content	0.98
3	Lignin	25.83
4	Holocellulose	56.49
5	Alfa-cellulose	33.25
6	Hemicellulose	23.24
7	Extractive, others component	4.19

[a] Composition percentages are on dry-weight basis

lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass (Silverstein *et al*., 2007). Sodium hydroxide pretreatment of lignocellulosic materials also causes swelling, leading to an increase in internal surface area and a decrease in the degree of polymerization and crystallinity (Sun & Cheng, 2002). It seems that sodium hydroxide pretreatment is more effective to enhance the enzymatic digestibility than acid pretreatment, since alkali has a stronger delignification ability. Therefore, sodium hydroxide were used to pretreat the EFB in order to improve the enzymatic saccharification and fermentation of EFB to ethanol. The degradation of lignin and hemicelluloses in the EFBs fiber after NaOH pretreatment are represented as the component loss of lignin and hemicelluloses, shown in Figures 1 and 2. The results showed that the optimum pretreatment condition was NaOH 1 N at 30^0C for 90 minutes with the optimum component loss of lignin and hemicellulose was 45.8 % and 35.6 % respectively. Figures 1 and 2 show that the increase of heating time only affected the loss of lignin and hemicellulose from 30 to 60 and 90 minutes and there was no significant effect of heating time after 90 minutes pretreatment on the loss of lignin and hemicelluloses. This indicated that the severity of the pretreatment did not show any significant improvement to lignin and hemicellulose degradation. There was also no significant effect of temperature increase from 30^0 C to 60^0 C on the loss of lignin and hemicellulose in the EFB fiber.

An effective pretreatment is characterized by several criteria. The loss of lignin in the pretreatment is one of the most important indicators of pretreatment effectiveness because the presence of lignin impedes enzymatic hydrolysis of the carbohydrates Mosier *et al*. (2005). Lignin interferes with hydrolysis by blocking the access of cellulases to cellulose and by irreversibly binding hydrolytic enzymes (Sun & Cheng, 2002). As shown in Figure 1, NaOH 1N-pretreatment at 30^0C for 90 minutes gave optimal condition for delignification of EFB with the loss of lignin reach 45.8 %.

The content of cellulose, hemicellulose and lignin EFB fiber before and after NaOH pretreatment in the optimum pretreatment condition (at 30^0C for 90 minutes) is shown in Table 2. As seen in Table 2, the pretreated EFB fiber contains 33.25 % cellulose, 23.24 % hemicellulose and 25.83 % lignin. Compared with the chemical components in the initial EFB fiber, it was clear that NaOH pretreatment increased cellulose by 18.6 %, and decreased hemicellulose by 35.6 % as well as lignin by 45.8 % respectively. The increase of cellulose content and the decrease of hemicellulose and lignin content can facilitate the process of enzymatic hydrolysis.

The effect of NaOH on enzymatic saccharification of EFB

The EFB fiber treated by NaOH 1 N at 30^0C for 90 minutes, was subjected to enzymatic saccharification. This condition was selected based on the highest component loss of lignin and hemicellulose content of EFB (45.8 % and 35.6 %, respectively) during the alkali pretreatment. The residual solid of alkali-pretreated EFB was treated with a commercial cellulase preparation, at enzyme loadings of 10, 20 and 40 FPU/g. Cellulase is a mixture of several

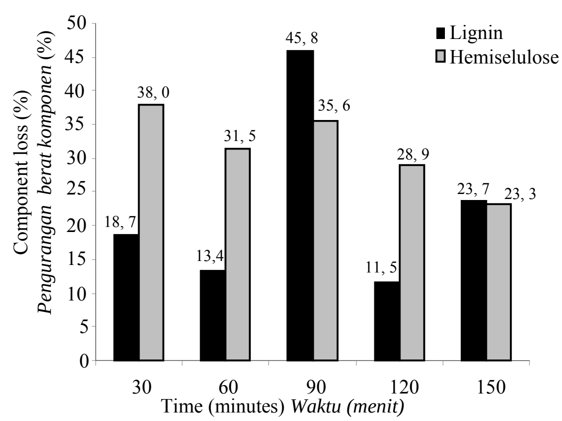

Figure 1. Loss of lignin and hemicellulose during alkali pretreatment of EFB fiber with NaOH 1N at 30^0 C for 30, 60, 90, 120 and 150 minutes.

Gambar 1. Pengurangan lignin dan hemiselulosa serat TKKS akibat pretreatment dengan NaOH 1N pada suhu 30^0 C selama 30, 60, 90, 120 dan 150 menit.

enzymes. There are at least three major groups of cellulases that involved in the hydrolysis process: 1) endoglucanase, which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; 2) exoglucanase or cellobiohydrolase, which degrades the molecule further by removing cellobiose units from the free chain-ends; and 3) β-glucosidase, which hydrolyzes cellobiose to produce glucose (Silverstein *et al.,* 2007). The effect of enzyme concentration on the saccharification of alkali-pretreated EFB is shown in Figure 3.

Results of this research show that the optimum saccharification obtained of alkali-pretreated EFB was 63.8 %, during 48 hours of saccharification. Figure 3 shows that the enzyme loading of 40 FPU/g after 45 hours saccharification, has not changed the saccharification significantly, from 63.5 to 63.8 %. From this result, it can be concluded that the optimum saccharification process of alkali-pretreated EFB took 45 hours.

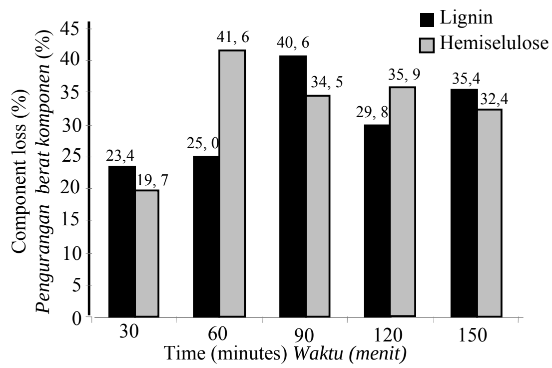

Figure 2. Loss of lignin and hemicellulose during alkali pretreatment of EFB fiber with NaOH 1N at 60^0 C for 30, 60, 90, 120 and 150 minutes.

Gambar 2. Kehilangan lignin dan hemiselulosa serat TKKS akibat pretreatment dengan NaOH 1N pada suhu 60^0 C selama 30, 60, 90, 120 dan 150 menit.

Table 2. The content of cellulose, hemicellulose and lignin EFB fiber before and after NaOH pretreatment at 30^0C for 90 minutes.

Tabel 2. Komposisi selulosa, hemiselulosa dan lignin serat TKKS sebelum dan sesudah pretreatment dengan NaOH pada suhu 30^0C selama 90 menit.

Pretreatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Before NaOH	33.25	23.24	25.83
After NaOH	48.82	18.52	17.32

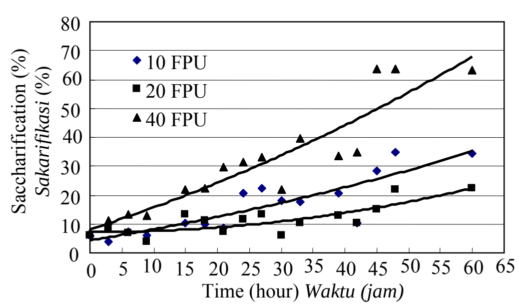

Figure 3. Effect of enzyme concentration on saccharification of EFB fiber pretreated by NaOH 1 N, 30^0C, 90 minutes, pH 4.5.

Gambar 3. Pengaruh konsentrasi enzim pada sakarifikasi perlakuan awal serat TKKS dengan NaOH 1N, 30^0C, 90 menit, pH 4,5.

Conclusion

As expected, higher enzyme concentration results in higher yield of sugar. In the present study, the highest saccharification was obtained at enzyme loading 40 FPU/g. Even though increase of cellulase loading in the process to a certain extent can enhance the yield and rate of the hydrolysis it, however would also significantly increase the cost of the process. Therefore, an appropriate amount of enzyme loading should be considered to obtain an optimum saccharification with a minimum cost.

Pretreatment with NaOH resulted in a high sugar yield of EFBs associated with the reduction in lignin and hemicelluloses content and the increase in cellulose content. Further research will be conducted to ferment sugar into ethanol using SSF (simultaneous saccharification and fermentation) method.

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