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Reuse black liquor of alkali pretreatment in bioethanol production

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Abstract

Over the past years, lack of energy fossil has become an important issue around the world, especially in developing countries such as Indonesia. Alternative energy is needed to replace utilization of fossil fuels. Bioethanol is providing one window of potential alternative energy. Lignocellulosic from oil palm empty fruit bunch (EFB) could be a promising bioethanol raw material because it will not interfere food supply. Pretreatment is a one of the steps in the bioconversion of lignocellulosic material. Pretreatment also contributed the largest cost in the bioethanol production. Black liquor as the waste of pretreatment provides significant environmental impacts. Black liquor recycling is expected to increase economic efficiency by reducing the cost of pretreatment and the amount of wastewater. This experiment used black liquor with NaOH addition as a pretreatment solution. The pretreatment process was conducted at 5 liter reactors at 150^{o}C and 160^{o}C. The result using black liquor was not significant different with using NaOH solution in the pretreatment process at 160^{o}C, delignification and cellulose content were 84.01% and 77.10%, respectively, but the results in pretreatment at 150^{o}C gave the lower value. The maximum ethanol concentration in variation of pretreatment solutions: NaOH solutions, black liquor with NaOH addition at 150^{o}C, and at 160^{o}C are 67.42 gl^{-1}, 61.14 gl^{-1} and 67.03 gl^{-1}, respectively, at a pretreated biomass loading of approximately 15% (w/v) with an enzyme dosage of 30 FPU g/dry biomass.

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Keywords: Black liquor; economic efficiency; ethanol; pretreatment; reuse

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1. Introduction

The future risks of global warming and shortage of petroleum, as well as the superior environmental characteristics of ethanol as an oxygenate for fuels, promote the production and usage of bioethanol. Ethanol from renewable resources as an alternative fuel or oxygenated additive to the current fossil fuels has been interesting issues in recent decades. Lignocellulosic materials, which are relatively cheap and plentiful, are considered the main source of feedstock for low-cost bioethanol production. Oil palm empty fruit bunch (EFB) is one of the abundant lignocellulosic waste materials in the world. In 2008, production of crude palm oil in Indonesia achieved more than 17.5 million ton and each ton of it generally produces 1.1 ton EFB 0. EFB material contains cellulose, hemicelluloses, lignin, ashes and the extractive. In terms of chemical composition, the EFB predominantly contains cellulose, hemicelluloses and lignin. Cellulose as the major fraction of lignocellulosic biomass can be hydrolyzed to glucose by cellulase enzymes and then glucose can be fermented to ethanol by *Saccharomyces cerevisiae*0.

Difficulties to degradation of lignocelluloses matters are caused by strength of compact structure made by their components. Lignin is further linked to both hemicellulose and cellulose forming a physical seal around the latter two components that are an impenetrable barrier preventing penetration of solution and enzymes. Because of the difficulty in dissolving the lignin without destroying it and some of its subunits, its exact chemical structure is difficult to ascertain. For this reason, delignification is processed that must be done before saccharification to an optimum result in bioconversion lignocelluloses to fermentable suga r0.

Over the years, a number of different pretreatment methods, including uncatalysed steam explosion, liquid hot water, dilute acid, flow-through acid pretreatment, lime, wet oxidation and ammonia fibre/freeze explosion, have been developed for the pretreatment of lignocellulosic biomass 0. Pretreatment is necessary to prepare the feedstock in order to improve conversion of sugar 0. In alkaline process the mechanism was saponification of ester linkages between cross-linked molecules and other molecules, such as lignin and hemicellulose. The loss of these crosslinking caused an increasing of material porosity 0. Dilute sodium hydroxide solution also caused swelling of the material, so that it increased internal surface area in the material, reduced degree of polymerization and crystallinity, caused scission of chains between lignin and carbohydrates, and degraded lignin structures. The goal of any pretreatment process is to alter or remove structural and compositional obstacles to hydrolysis, to improve the rate of enzyme digestibility, and to increase the yield of fermentable sugars from substrates 0. Therefore, to be efficient the selected pretreatment method also should have a number of features. It has to: require a low energy demand or be performed in a way so that the energy can be re-used in other process steps as secondary heat and require low capital and operational cost0.

Although the yields improved, higher environmental impact in second-generation ethanol scenarios are mainly related to high NaOH consumption for delignification prior to hydrolysis, being the most impacting parameter in global warming 0. Pretreatment process used a large amount of NaOH, this condition also increased the operational cost of ethanol production. According to this, technological improvements, such as NaOH recycling, are necessary in this process for the environmental sustainability of second generation ethanol production.

Han *et al.* 0 was used black liquor in *Miscanthus* pretreatment process, and for five times recycle gave the results the cellulose content were not much significant different. Rocha *et al.* 0 also reuse black liquor in the pretreatment process for sugarcane bagasse. The current study aimed to reuse the black liquor for EFB's pretreatment process to minimize the wastewater and reduce cost production of bioethanol. The ethanol production from pretreated EFB using recycled black liquor was also investigated.

2. Materials and methods

2.1. Material

Oil palm empty fruit bunch (EFB) used in this experiments was obtained from a Palm Oil Mill, Musi Banyuasin, South Sumatera, Indonesia. EFB was still big shape dried in the open air. The first step in using EFB for ethanol production was physical pretreatment through milling and sieved to a particle size below 5 mm and then stored in a plastic container at room temperature. The commercial enzymes Cellic® Ctec2 and Cellic® Htec2 from Novozyme were applied to the saccharification process. The vendor reported that the activity of CTec2 was 144 FPU (Filter

Paper Unit)/ml enzyme, whereas HTec2 was 240 CBU (cellobiose unit)/ml. The commercial dry yeast (*Saccharomyces cerevisiae*) used for the fermentation process. All reagents (except sodium hydroxide using industrial grade) used in this study were of analytical grade.

2.2. Chemical pretreatment

Chemical pretreatment was performed by heating 500 g of EFB with 2,500 ml alkali solution in stirred reactor. In this study, the pretreatment conducted using two kind of solution, which are 10% NaOH solution and black liquor with NaOH addition. Pressure was controlled at 4 bars, the temperature reactor was set at 150°C with reaction time 30 minutes and 160°C with reaction time 40 minutes. After pretreatment, solid fraction was washed and neutralized with tap water then dried at 50-60°C overnight, the dry pretreated EFB stored at room temperature prior to saccharification and fermentation process.

Characterization of EFB was measured before and after pretreatment. Cellulose, hemicellulose and lignin content were analyzed based on a method from National Renewable Energy Laboratory (NREL)0. Oil Palm EFB (300 mg, dry weight) was subjected to acid hydrolysis for lignin, cellulose and hemicellulose content analysis. After hydrolysis, acid insoluble lignin (AIL) was weighed using Sartorius BS224S and acid soluble lignin (ASL) was measured using Spectrophotometry UV/Vis Spectrophotometer Optizen 2120 UV at 205 nm. Total lignin was obtained from the sum of AIL and ASL. On the other hand, after hydrolysis, cellulose and hemicellulose were measured by HPLC Waters e2695.

2.3. Saccharification

Hydrolysis/saccharification process was conducted in 250 ml Erlenmeyer, 15% (w/v) or 15 g dry weight basis as a substrate, then added 80 ml of 0.05 M citrate buffer pH 4.8. Citrate buffer and substrate were sterilized at a temperature of 121°C for 15 minutes using an autoclave. The enzyme and buffer citrate addition was added in the sample until the total volume is 100 ml. Two kinds of enzymes, CTec2 and HTec2 were added with the ratio 5:1. The CTec2 loading was 30FPU/g pretreated EFB. The saccharification was conducted in a shaker incubator for 72 hr at temperature 50°C, and 150 rpm agitation. The temperature was adjusted based on optimum condition of enzyme.

2.4. Fermentation

One percent (1 g/100 ml) of commercial dry yeast (*S. cerevisiae*) was added in each flask after 72 hours saccharification process finished. Fermentation was conducted in a shaking incubator with 32°C during 72 hours. Velocity of agitation was set 150 rpm. Ethanol content was predicted using HPLC Waters e2695.

2.5. Analysis

The sample (1 ml) was withdrawn from saccharification and fermentation medium every 24 h, centrifuged and analyzed for glucose, xylose and ethanol. They were then analyzed by an HPLC Waters e2695. Glucose, xylose and ethanol were analyzed on an HPLC using Aminex HPX-87H column (Bio-Rad, Richmond, CA, USA) at 65°C with 0.6 ml min^{-1} eluent of 5 mM sulfuric acid along 25 min retention time. Prior to HPLC injection, all samples filtered through 0.2μm syringe filters.

3. Results and discussion

3.1. Pretreatment

The chemical composition of oil palm empty fruit bunch in this study was listed in Table 1. Before pretreatment total carbohydrate in the oil palm EFB was 61.57%, and total lignin fraction was 26.53% of the dry biomass. The lignin content was comparable to the lignin content of hardwoods 0.

Table 1. Chemical composition of untreated EFB

Composition	Dry matter (%)
Cellulose	36.60
Hemicellulose	24.97
Asid soluble Lignin	3.50
Acid insoluble lignin	23.03
Ash	1.79
Other	10.11

Table 2. Chemical composition of pretreated EFB using NaOH solution and black liquor

Composition	Temperature 150°C, time 30 minutes			Temperature 160°C, time 40 minutes		
	NaOH 10%	BL + 60 NaOH	BL + 125 NaOH	NaOH 10%	BL + 60 NaOH	BL + 125 NaOH
Cellulose	72.54	65.80	65.85	71.73	71.36	77.10
Hemicellulose	14.22	16.15	13.21	9.37	13.87	17.20
Lignin	6.17	11.47	10.79	3.47	4.88	4.23
Ash	2.12	0.95	1.92	1.66	1.88	1.88

BL + 60 NaOH: preteatment using black liquor with 60 g of NaOH addition

BL + 125 NaOH: preteatment using black liquor with 125 g of NaOH addition

The sugar content after pretreatment was increased both of using NaOH solution and black liquor. The composition of pretreated EFB with different pretreatment solution was listed in Table 2. The alkaline pretreatment was increasing cellulose content and decreasing hemicelluloses and lignin contents in all cases. Lignin content at both of pretreated EFB was lower than untreated EFB. Cellulose content in pretreated EFB was higher than untreated EFB. In NaOH solution, 250 g of NaOH was diluted in 2500 water (10% NaOH solution). After pretreatment, the NaOH content in black liquor is around 7.6% together with dissolving lignin. For the second pretreatment, we use black liquor with 60 g of NaOH addition, its mean the black liquor with $\pm$7.5% NaOH was added by $\pm$2.5% NaOH. In this process, we can save 190 g (76%) of NaOH utilization. In another process, we used black liquor with 125 g of NaOH addition; it means we can save 50% utilization of NaOH in the pretreatment process. From Table 2, it was observed that at temperature 150^{o}C and 30 minutes pretreatment conditions, after the initial pretreatment using NaOH solution, the cellulose content was 72.54%. But after use the recycle black liquor both of 60 g of NaOH addition and 125 g of NaOH addition, the cellulose content tended to lower than NaOH solution were 65.80% and 65.85%, respectively. This results in contrast with research by Han *et al.*0 they done recycle preteatment solution for Miscanthus up to five times with the result the cellulose content is not significant different than the use of NaOH solution.

Hemicellulose content also decreased after pretreatment because pretreatment with NaOH can also dissolve hemicellulose as has been done by Wan *et al.*0 on soybean straw. Besides influenced by the alkali solution, the pretreatment process was also influenced by time and temperature 0. Therefore, in this study was also carried out pretreatment process at a temperature of 160°C and 40 minutes. These pretreatment conditions resulted in better-pretreated biomass. Reuse of black liquor with the addition of NaOH resulted in chemical composition values are nearly same with the use of NaOH solution. The cellulose and delignification was decreased but no significant.

Pretreatment was changing the EFB composition. The aim of chemical pretreatment process is to reduce the lignin, the compound acetyl group, and slightly dissolves hemicellulose. The process of chemical pretreatment with NaOH can also cause the swelling in the cellulose structure, reduce cellulose crystallinity, and to increase the material porosity, so the cellulose can be easily hydrolyzed0. One of the purpose alkaline pretreatment is to reduce lignin content called delignification. Delignification can improve the ability of cellulase enzymes in the saccharification process. Millet *et al.*0 in Kang *et al.*0 it is reported that the performance of the enzyme increased by 14% to 55% on the hardwood when the lignin content was reduced from 24-55% to 20%. The delignification in variety of pretreatment condition was shown in Fig. 1. Reuse of black liquor in the pretreatment process, not yet produce the same pretreated biomass result with the use of NaOH solution. Delignification with NaOH solution reached 76.74%, while the use of black liquor only reached 59.30%. However, by increasing the temperature of the pretreatment process can improve the delignification to reach more than 80% both in the NaOH solution or reuse of black liquor.

Reduction of lignin, hemicellulose and other chemical compounds would cause a reduction in biomass produced. The effect of time pretreatment processes affects the biomass recovery. The longer time of the process would be less biomass recovery. Similar with reaction time, the temperature also gave the influence to the pretreatment process. The higher temperature in the pretreatment process will decrease the biomass recovery. This is caused by the increasing number of lignin and hemicellulose was dissolved in the black liquor. Fig. 1 showed the biomass recovery in all condition pretreatment process. The biomass recovery at 150°C and 30 minutes pretreatment process was higher than 160°C and 40 minutes process. The recovery biomass in 150°C (30 minutes) with NaOH solution, black liquor with 60 g of NaOH and 125 g of NaOH addition were 35.97%, 39.82%, and 37.90% respectively. Pretreatment process at 160°C (40 minutes) gave the recovery biomass values were 32.82%, 30.66% and 26.30% respectively. Very high recoveries were found for most of the treatments for carbohydrate, both for cellulose and hemicellulose. Cellulose was almost totally left in the solid phase. All the pretreatments yielded in fact recoveries higher than 60%.

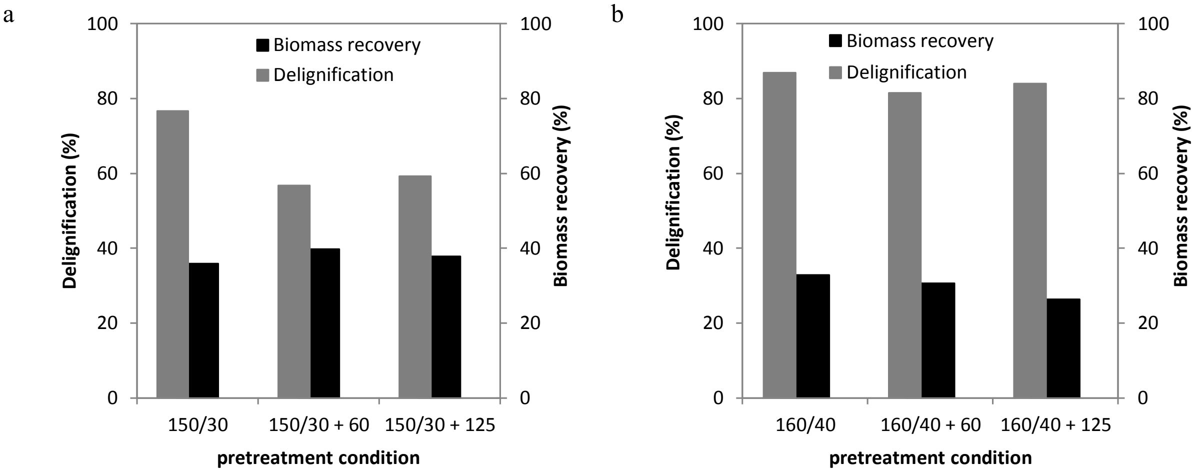


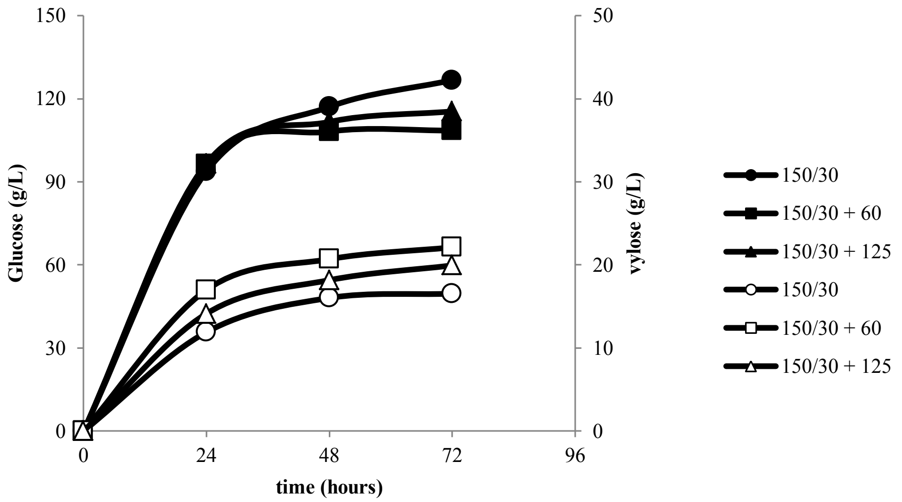
Fig. 1. Delignification and biomass recovery at pretreatment condition; (a) T 150°C, t 30 min; (b) T 160°C, t 40 min.

3.2. Saccharification

After alkaline pretreatment, 15% (w/v) pretreated EFB in variation of the pretreatment process then hydrolyzed using commercial enzymes. In this research, two enzymes were used, cellulase (CTec2) and hemicellulase (HTec2). The dose of enzyme in enzymatic hydrolysis was 30 FPU, this dose according to Kim *et al.*0 reported that an effective concentration of enzyme for cellulose hydrolysis has been determined to be 10 to 60 FPU per gram of dry cellulose or glucose.

The result of sugar from saccharification process could be seen in Fig. 2. This figure showed the glucose and xylose concentration obtained from HPLC analysis. Saccharification increased significantly with the increase of reaction time of saccharification. Alkaline explosion pretreatment of biomass increased the cellulose accessibility and its hydrolysis by enzymes. In the pretreated EFB using pretreatment at 150°C, the highest glucose content was reached 126.7 g l^{-1} by pretreated EFB using NaOH solution. The glucose content by recycle black liquor with 60 g of NaOH and 125 g of NaOH addition only reached 108.5 g l^{-1} and 115.4 g l^{-1} respectively. But in process pretreatment at 160°C, the glucose content is similar between preteated EFB using NaOH solution and using black liquor with 125 g of NaOH addition, reached 135.7 g l^{-1} and 137.0 g l^{-1}, respectively. The higher temperatures produce higher delignification and swelling structure in EFB, it's caused the pretreated EFB easier to hydrolysis by enzymes0. The process using black liquor with 60 g of NaOH addition gave the lower glucose content. Xylose content after 72 hours hydrolysis is approximately same in both of pretreatment process with range 16-22 g l^{-1}.

a



b

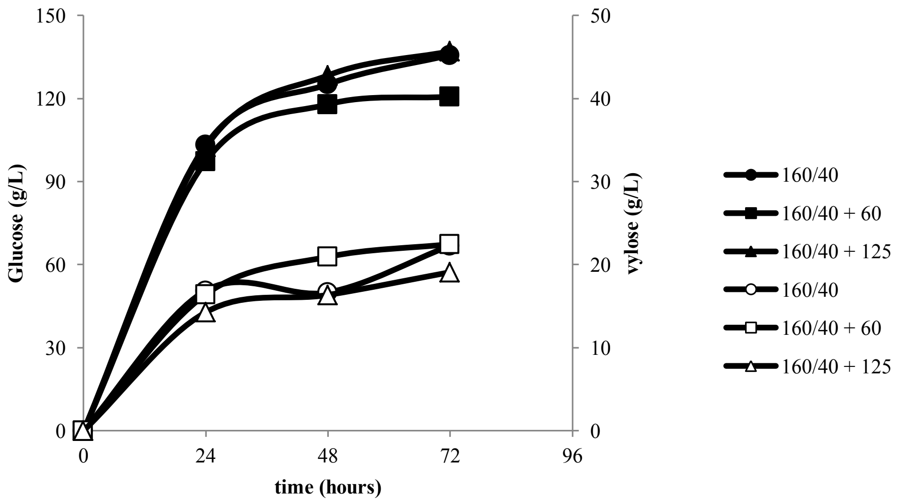


Fig. 2. Glucose (fill marker) and xylose (blank marker) at pretreatment condition; (a) T 150°C, t 30 min; (b) T 160°C, t 40 min.

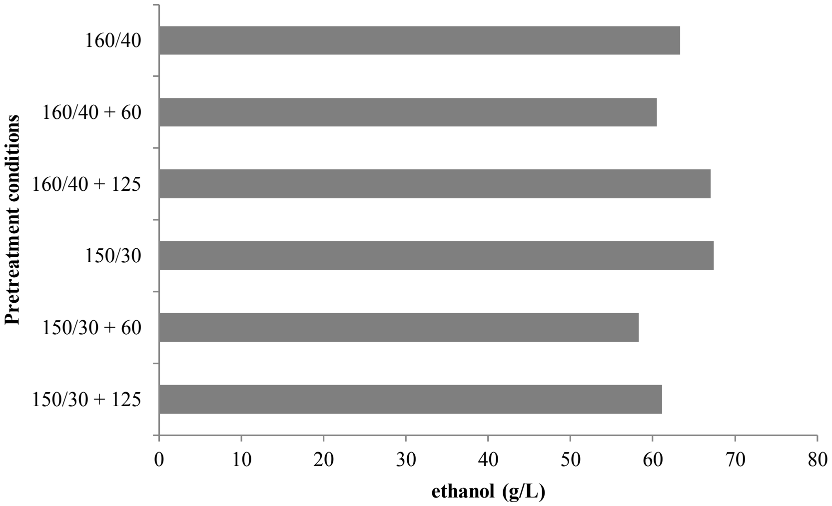


Fig. 3. Ethanol production.

S. cerevisiae converted glucose to ethanol by *Embden-Mayerhof pathway* or often referred as glycolysis. In glycolysis, one mole of glucose is converted to two moles of pyruvate. Pyruvate in *S. cerevisiae* can be converted to ethanol by *Pyruvate decarboxylase* (PDC) and *Alcohol dehydrogenase* (ADH) enzymes0. However, *S. cerevisiae* cannot convert xylose into ethanol; fermentation of xylose can be done by using *Pichia stipitis*0.

Fig. 3. shows ethanol produced using all pretreated EFB. The highest ethanol 67.03 g l^{-1} was produced by pretreated EFB that using reuse black liquor in the pretreatment process at 160°C, 40 minutes with 125 g of NaOH addition. This ethanol in accordance with the content of reducing sugar produced it higher than others doses. The maximum theoretical ethanol yield of glucose fermentation is 0.51 g ethanol/g glucose[2], so, its theoretical yield was 67.90 g l^{-1} by calculation (using pretreated substrate). It was almost the same as the experimental value; the ethanol yield reached 98.57%. Ethanol produced was higher than Muryanto *et al.*0 that use *Rhizopus oryzae* in EFB's fermentation, and also higher than Dahnum *et al.*0, that used palm oil frond fermentation with *S. cerevisiae*. Fermentation process in 72 hours was called well enough because almost of the glucose convert into ethanol.

4. Conclusion

This study has shown that black liquor could be used in the pretreatment process. The highest delignification in this research reached 86.92% using NaOH solution at 160°C, 40 minutes, but the result of delignification from reuse black liquor from the pretreatment process at 160°C, 40 minutes also reached 84.01 %. The highest glucose concentration from saccharification process was 137.90 g l^{-1} by using reuse black liquor at 160°C, 40 minutes, and 125 g of NaOH addition, with ethanol production reached 67.03 g l^{-1}. The reuse black liquor with NaOH addition for pretreatment solution gave the result as good as the NaOH solution in pretreatment process at 160°C. Thus, recycling the black liquor increases economic efficiency and reduces the cost of treatment by minimizing wastewater and radically reducing the amount of expensive alkali used in lignocellulosic biomass pretreatment.

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