Black Liquor Pretreatment of Concorb and Enzymatic Hydrolysis for Lignocellulosic Bioethanol

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Abstract. Lignocellulosic bioethanol from corncob could be a promising alternative fuel because of abundant resources, and it will not interfere with the food supply. Pretreatment is the first step in a lignocellulosic bioethanol process. One of the effective pretreatments is alkaline pretreatment because it gives the cellulose-rich material for enzymatic hydrolysis. However, it produces black liquor as wastewater that provides environmental impacts. The black liquor from Empty Fruit Bunch (EFB) pretreatment was collected and used for pretreatment of corncob to reduce wastewater from bioethanol production. The pretreatment process was conducted in a 5-liter reactor. The pretreated corncob was hydrolysis with cellulase enzyme and converted to ethanol by Saccharomyces cerevisiae. The delignification of corncob using EFB-black liquor was 45.82%. The black liquor pretreatment was increasing ethanol production 3.25 times than untreated substrat after saccharification and fermentation process by Saccharomyces cerevisiae (36.39 g/l).

1. Introduction

Corncob is one of the abundant lignocellulosic waste materials in Indonesia. Main components of corncob are cellulose, hemicellulose that can be converted to fermentable sugar for ethanol production. In 2012, corn plantation could produce 20 million tons and generate 1.94 million tons of corncob and estimation of ethanol production from corncob is around 0.38 million tons [1].

Ethanol production from lignocellulosic material consists of three main processes, which are pretreatment, hydrolysis, and fermentation. Pretreatment is needed to produce macroscopic and microscopic biomass structure used for hydrolysis and to break lignin and hemicellulose bonding and crystal structure [2]. Delignification must be done to destroy the lignin structure, reduce lignin content, and give an optimum result in bioconversion lignocelluloses to glucose [3].

The alkaline pretreatment process is widely used in ethanol lignocellulosic production. During alkaline pretreatment, high amount of black liquor is produced. Black liquor contains potentially toxic chlorinated compounds, suspended solids, phenolic, lignin, and spent alkaline [4]. Production 76.46 kg of bioethanol in a pilot plant will produce 3,000 liters of wastewater from a pretreatment process that is dominantly obtained from alkaline solution (sodium hydroxide) [4]. Black liquor wastewater releases black-colored effluent with high chemical oxygen demand (COD) and Total Suspended Solids (TSS) which makes it significantly toxic to the environment. Therefore, the black liquor must be treated before
discharge to the environment or can be minimized to utilize or reuse for another purpose. The high pH value in black liquor indicates rich in alkali content of spent alkaline. The high content of NaOH in black liquor has the potential to be used as a solvent in the pretreatment process.

Our previous research successfully used EFB based-black liquor as an alkaline pretreatment solvent for EFB [5,6] and sweet sorghum bagasse [7]. Therefore, in the present study, we use EFB based-black liquor as a solvent in corncob pretreatment to investigate its effect on enzymatic hydrolysis and ethanol production.

2. Material and methods

2.1. Materials

Corncob (Cc) used in this experiment was obtained from Indonesian Center for Agricultural Post Harvest R&D, Ministry of Agriculture. Physical pretreatment was conducted as the first step to reducing the size below 1 cm granule then stored in a plastic bag. The black liquor was collected from alkaline explosion pretreatment of EFB using 10% NaOH solution, 150°C, 4 bar and 30 minutes in Pilot Plant 2nd Generation Bioethanol, Research Center for Chemistry-LIPI. Commercial cellulase enzyme (Cellic® Ctec2 and Cellic® Htec2 supplied from NOVOzyme) were used in all the experiments as sole enzymatic complex. We have analyzed it by measuring its activity as FPU. The enzyme showed an activity of 132 FPU/ml. The organism used for the fermentation process in this study was commercial dry yeast of *Saccharomyces cerevisiae* (Fermipan, French).

2.2. Black Liquor Pretreatment

Black liquor pretreatment was conducted in the chemical explosion reactor [5,6]. 250 g of dry corncob (10 % moisture content) was mixed with 1,250 ml of EFB based-black liquor. The pressure was controlled at 4 bars; the temperature in the reactor was 130°C for 40 minutes. After pretreatment, the pretreated biomass was recovered by filtration, washed with water until neutral and dried in the oven at 50-60°C overnight.

2.3. Saccharification

The saccharification process was conducted in 250 ml Erlenmeyer with 15% (w/v) substrate, and then 70 ml of 0.05 M citrate buffer was added. The samples were closed and sterilized 15 min using an autoclave. After reaching the room temperature, enzyme and buffer citrate addition were added to the sample until the total volume is 100 ml. Two kinds of enzymes, CTeC2 and HTeC2, were combined with ratio 5:1. The CTeC2 loading was 30 FPU g-1 pretreated corncob. The saccharification was conducted in a shaker incubator for 72 hrs at temperature 40°C, and 150 rpm agitation.

2.4. Fermentation

The substrates after saccharification process were added with one percent dry yeast *Saccharomyces cerevisiae*. The fermentation process was conducted in shaking incubator at 32°C with 150 rpm agitation for 72 hours.

2.5. Analysis

Corncob composition was characterized before and after black liquor pretreatment based on a method from National Renewable Energy Laboratory/NREL [8]. Corncob (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 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).
The sample (1 ml) was withdrawn from enzymatic hydrolysis and fermentation medium every 24 h. Glucose, xylose, and ethanol were determined using high-performance liquid chromatography/HPLC (Waters e2695).

3. Results and discussion

3.1. Pretreatment

Characteristics, properties, and analysis of the components of untreated and pretreated corncob are shown in Table 1. The main components of untreated corncob are cellulose followed by the lignin and hemicellulose. The total carbohydrate of corncob is 56.08%, and total lignin fraction is 26.47% of the dry biomass. Corncob could potentially be used as raw materials for bioethanol production process due to the cellulose contains.

Pretreatment is the most critical process in converting lignocellulose. Lignin is amorphous heteropolymer which is insoluble in water [9]. Lignin is associated with cellulose and hemicellulose, and it can inhibit enzymatic hydrolysis process. The purpose of the pretreatment is to reduce the amount of lignin in biomass known as delignification. Alkaline pretreatment can improve the ability of cellulase enzyme in the enzymatic hydrolysis process. In this study, pretreatment is conducted using black liquor from alkaline pretreatment process of EFB. Based on Table 1, cellulose content in corncob increased after pretreatment process used black liquor. The cellulose content in pretreated corncob reached 48.24%, its mean 1.5 times higher than untreated corncob. Utilization of black liquor as a solvent in the pretreatment process can reduce the use of NaOH in the lignocellulosic bioethanol production process [6].

| Component        | Untreated | Pretreated |
|------------------|-----------|------------|
| Cellulose        | 31.69     | 48.24      |
| Hemicellulose    | 24.39     | 24.67      |
| Lignin           | 26.47     | 14.34      |
| Ash              | 1.05      | 0.18       |

Figure 1 shows the effect of EFB-based black liquor pretreatment against delignification, cellulose recovery, and biomass recovery. Alkaline pretreatment process can eliminate lignin, hemicellulose, and other compounds that cause a reduction in the mass of corncob produced. The pretreatment process with black liquor is included in the alkaline pretreatment. Alkaline pretreatment can reduce the lignin content in lignocellulose. Black liquor pretreatment process at 130°C/40 minutes can reduce lignin up to 45.82%. Reduction of lignin, hemicellulose, and compounds other chemicals will cause a reduction the Corncob mass collected. Biomass recovery generated from black liquor pretreatment reached 66.37%. In general, the lignocellulose that has been pretreated with alkaline pretreatment will lose almost half of the initial mass [5]. The temperature and process time of the pretreatment process significantly affect the biomass recovery in alkaline pretreatment because the process temperature has a more dominant role in dissolving the soluble compounds [10]. The alkaline pretreatment process will only dissolve lignin, whereas cellulose is not dissolved, it resulted in the biomass produced with a high cellulose content. Cellulose recovery in this study reached 90%, its mean black liquor pretreatment was useful for the corncob pretreatment process because the only small amount of cellulose was lost during the process.
The alkaline pretreatment process is suitable for bioconversion of lignocellulose into bioethanol because it gives the higher cellulose content in the biomass. Cellulose in lignocellulose will be converted into glucose and fermented into bioethanol. Hence, cellulose content is very important in the process of bioethanol lignocellulose. In black liquor, there is spent alkaline which can be used as a solvent of lignin. Besides to dissolve of the lignin, the black liquor pretreatment process also damages the cellulose structure. NaOH content in the black liquor caused swelling of biomass, and reduce cellulose crystallinity and polymerization [11]. Some of the cellulose will also dissolve and lost in this process. Cellulose recovery in corn cob reaches 90.95% its mean only the small amount of cellulose lost due to the black liquor pretreatment process.

Delignification is the main purpose of the pretreatment process. Reduced lignin content can increase the performance of cellulase enzymes in converting cellulose into glucose of the lignocellulose [12]. Sjöström [13] stated that several phases occur in dissolving lignin, namely initial delignification phase, intermediate delignification, and final delignification. The initial delignification that occurs at low temperatures is generally controlled by diffusion. Meanwhile, the next step is controlled by chemical reactions with the temperature above 140°C. In this phase, the reaction rate increases with the rising temperature. Delignification in the black liquor pretreatment process for corn cob at 130°C reached 66.37%. The results of our previous studies showed that black liquor cannot be used directly for the same lignocellulose pretreatment due to NaOH content is lower than initial concentration, and the make up NaOH is needed to reach the same result [5],[6]. Smaller NaOH content can be used for pretreatment of lignocellulose which has low lignin content. In the previous study, black liquor may be used for the pretreatment process of bagasse sorghum without the addition of NaOH and the delignification reached 60% [7].

3.2. Saccharification and Fermentation

Saccharification or enzymatic hydrolysis aims to hydrolyze cellulose into monomer sugar (glucose). Corn cob cellulose is converted into glucose using a cellulase enzyme. The results of the saccharification process are shown in Figure 2. The process of saccharification is carried out for 72 hours. The glucose content generated by saccharification increases along with the increasing processing time of saccharification. A dose of 30 FPU is an optimum enzyme dose based on our previous studies [14]. Corn cob untreated produces the smallest glucose content (24.96 g/l). Meanwhile, pretreated corn cob produces glucose with a value of 3.19 times greater than the untreated corn cob. The pretreated corn cob had higher cellulose and lower lignin content, that made enzyme easier to convert cellulose into glucose. Black liquor pretreatment may increase the concentration of glucose in the enzymatic hydrolysis process.
for the pretreatment increases the cellulose accessibility by enzymes. One purpose of the pretreatment is to increase the rate of enzyme digestibility [15].

Figure 2. Glucose concentration after enzymatic hydrolysis

![Glucose concentration after enzymatic hydrolysis](image)

**Figure 2.** Glucose concentration after enzymatic hydrolysis

Glucose generated by saccharification process is then converted into ethanol using *Saccharomyces cerevisiae* through a fermentation process. *S. cerevisiae* has been known as yeast that can transform hexose sugars (glucose) into ethanol [16]. Figure 3. shows the concentration of ethanol produced by *S. cerevisiae* after the fermentation process for 72 hours. *S. cerevisiae* can convert glucose either from a substrate that has been pretreated or from the untreated substrate. The ethanol concentration obtained from the pretreated substrate (pretreatment at 130°C/40 minutes) equal to 36.39 g/l. Similar to the saccharification process, in the fermentation process, the black liquor pretreatment process gave effect to the concentration of ethanol produced. In the untreated substrate, *S. cerevisiae* only produces ethanol by 11.21 g/l. This small ethanol concentration was due to less glucose contained in the untreated

![Ethanol concentration after fermentation](image)

**Figure 3.** Ethanol concentration after fermentation
substrate for the performance of cellulase enzymes in the saccharification process was hampered by the lignin [17]. It shows that black liquor can be used as a pretreatment process solvent of corncob with good results.

Table 2. Ethanol concentration and yield of ethanol production from corncob

| Var | max. ethanol concentration (g/l) | yield ethanol based on pret-CC (g/g pret-CC)a | yield ethanol based on untreated-CC (g/g untreated-CC)b |
|-----|---------------------------------|---------------------------------------------|-------------------------------------------------------|
| untreated | 11.21                          | 0.07                                         | 0.07                                                  |
| Cc 130/40 | 36.39                          | 0.24                                         | 0.16                                                  |

a yield ethanol based on pretreated corn cob = max ethanol/dry weight of pretreated corn cob (150 g/l)
b yield ethanol based on untreated corn cob = max ethanol x rec biomassa/dry weight of corn cob (150 g/l)

F = cellulose fraction in biomass

Table 2. shows the ethanol production from the process of saccharification and fermentation of pretreatment substrate with different conditions. The ethanol yield based on pretreated corn cob generated by *S. cerevisiae* is reached 0.24 g/g pretreated substrate, its higher than our previous study that used *Rizhupus oryzae* for corn cob fermentation [14]. This result showed that black liquor could be used as a pretreatment solvent. When viewed from the ethanol production to the initial substrate of corn cob before pretreatment, the ethanol production produced by black liquor pretreatment is higher than the untreated production reached 0.16 g/g untreated substrate (more than two times). This means that the ethanol produced by the black liquor pretreatment is more efficient than untreated process. Pretreatment with the use of black liquor has several advantages, saving the use of NaOH, reduce black liquor waste discharged into the environment and produce the same product with the use of alkaline pretreatment.

4. Conclusion
The use of black liquor as a pretreatment solvent can reduce lignin content in corn cob up to 45.82% under the process temperature conditions of 130°C for 40 minutes. The content of cellulose in pretreatment corn cob reaches 48.24%, with the results of saccharification using cellulase enzymes which produce glucose concentration of 79.62 g/l. Glucose generated by saccharification at the fermentation using *S. cerevisiae* produces ethanol reached to 36.39 g/l. The parameters generated by the black liquor pretreatment approach the results obtained using alkaline pretreatment. Therefore, the black liquor can potentially be used as a pretreatment solvent of the corn cob.

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