Bioethanol Production from Alkali Steam Explosion of Oil Palm of Empty Fruit Bunch Fiber

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Abstract. Bioethanol is the most promising biofuels and can be generated from lignocellulosic biomasses. The study of bioethanol production from oil palm empty fruit bunch (OPEFB) pretreated by alkali steam explosion was carried out employing separate enzymatic hydrolysis (SHF) and Simultaneous Saccharification and Fermentation (SSF) approaches. Alkaline extraction after steam explosion pretreatment of OPEFB led to the partial removal of lignin, hemicellulose and other degradation products from fiber surface. The pretreatment process is of great importance to ethanol yield. In the present study, the pretreatment was processed using a steam explosion reactor at 150°C, 4 bars for 30 minute with alkaline concentrations of NaOH 10% (kg/L). After pretreatment, the OPEFB substrates 10% and 15% (w/v) was saccharification using 30 FPU of Ctec2 and Htec2 enzyme and fermentation using yeast of Saccharomyces cerevisiae for 72 hours. The glucose obtained after 72 hours was 137.80 g/L (substrate 15%) and 97.90 g/L (substrate 10%). Bioethanol production in SSF mode faster and slightly more efficient process than SHF.

1. Introduction

Sustainability and environmental safety are major necessities in the current world that requires the utilization of renewable and eco-friendly alternative resources. Lignocellulosic biomass is one of the most promising alternative resources to be exploited since its utilization will not disturb food supply [1]. Lignocellulose can be obtained from many sources such as crops, forest materials, agricultural wastes, and aquatic plants [2] [3]. They are primarily composed of three primary bio-polymers: cellulose, hemicellulose, and lignin. These bio-polymers can be converted into high-value products such as biofuels, energy sources, and chemicals [4] [5]. One of the most widely practiced lignocellulose utilization is bioethanol production. Renewable and eco-friendly; bioethanol can be used to replace fossil fuels as primary energy source for transportation, industrial, and domestic uses. However, lignocellulose is relatively more recalcitrant to chemical process than sugar and starch due to many factors such as low available surface area, crystalline structure, protection of cellulose and hemicellulose by lignin, and heterogeneous nature of the biomass [6]. Therefore, bioethanol production from lignocellulosic materials requires three distinct processes: pretreatment of the biomass, hydrolysis/saccharification, and fermentation of monomeric sugars [2]. Proper understanding of these complex processes is necessary in order to break the lignocellulosic matrix and effectively convert the...
bio-polymers into fermentable monomeric sugars without producing excessive amount of side products or inhibitors that could disrupt the fermentation.

Pretreatment of lignocellulosic biomass can be performed through physical, chemical, physico-chemical, and biological methods. One of the most widely applied pretreatment methods is steam explosion due to its low environmental impact and modest cost [7].

Hydrolysis and fermentation is the next step conducted after pretreatment process for producing bioethanol. Separated Hydrolysis and Fermentation (SHF) was the conventional method that hydrolysis was carried out in the period and fermentation process after then. This process allowed hydrolysis process worked first to produce monosaccharide sugar, so sugar is ready when the fermentation begins. Another method of hydrolysis and fermentation was Simultaneous Saccharification and Fermentation (SSF). In SSF, hydrolysis and fermentation located in a single reactor, enzyme and yeast put together, so glucose is rapidly converted into ethanol [8]. Wyman et al. reported that SSF process gives higher yield of ethanol than SHF because low residual sugar relieves inhibition on the cellulase enzyme [9][10].

The objective of this study was to investigate the effect of alkaline (sodium hydroxide, NaOH) pretreatment of oil palm of empty fruit bunch (OPEFB) with provide highest cellulose to glucose conversion during enzymatic hydrolysis for ethanol production using two methods of SHF and SSF.

2. Materials and Methods

2.1. Raw Materials

OPEFB fiber used in this study was obtained from palm oil plantation in Palembang, South Sumatera Indonesia. It was dried (final moisture content 8.60%, measured by Moisture Analyzer OHAUS MB 45) and chopped (average particle size 6.5 mm). Sodium hydroxide (NaOH) pellets used in the NaOH pretreatment with steam explosion was industrial grade. Cellic® CTec2 and Cellic® HTec2 enzymes were procured from Novozymes, Denmark. The activity of both enzymes were 144 FPU/mL.

2.2. Pretreatment (Base-Catalyzed Steam Explosion)

Pretreatment process of oil palm OPEFB was carried out in a steam explosion reactor CHEMEX method using dilute sodium hydroxide solution. Concentrations of sodium hydroxide (solution which used in this study was 10% (kg/L), a solid-liquid ratio of 1:5 then steam exploded at 150°C and 0.4 MPa for 30 minutes. The pretreated biomass was filtered with hydraulic press filter, then washed with water to neutral pH. The washed biomass was then dried in the oven at 50°C until the moisture content is below 10%, then used for as substrate in the enzymatic hydrolysis.

2.3. Separate Hydrolysis and Fermentation (SHF)

This procedure was performed on 10, and 15% (w/v) of pretreated EFB fiber in 0.05 M citrate buffer with a pH of 4.8. The flasks containing the substrate in citrate buffer were autoclaved at 121°C for 20 minutes to ensure sterile condition. After cooling down, 30 FPU/g of Cellic® CTec2 and 6 FPU/g of Cellic® HTec2 enzymes were procured from Novozymes, Denmark. The activity of both enzymes were 144 FPU/mL.

Glucose and xylose were measured as a product in this hydrolysis. Data of glucose form HPLC was required to get the percentage of yield. The next process, fermentation, was conducted 72 hours after hydrolysis process. The temperature of shaking incubator was changed into 32°C. In the constant temperature, one percent (g/ml) of dry yeast, Saccharomyces cereviceae, was put in the each flask. Ethanol content, glucose, and xylose were monitored every 24 hour. The calculation percentage of yield in fermentation is as same as those in saccharification process i.e by comparing measured ethanol weight with the theoretical weight of ethanol. The anhydro correction is 0.51 (for glucose to ethanol)[11]. All process was conducted triplo.
2.4. Simultaneous Saccharification & Fermentation (SSF)
The procedure of SSF was similar with SHF, however 1% (w/v) of baker’s yeast *S. cerevisiae* yeast was added along with the enzymes. The substrate loading of the process was 10 and 15 % (w/v); and the Cellic® CTec2 enzyme loading was 30 FPU/g while Cellic® HTec2 was added 20% (v/v) of Cellic® CTec2. The process was conducted in a shaking incubator under temperature condition 32°C with velocity agitation 150 rpm for 72 hours. Samples were taken every 24 hours for analysis of ethanol by HPLC.

2.5. Products Analysis
Analytical procedure for the measurement of carbohydrate (glucose and xylose) and lignin component on the raw material and pretreated biomass, was provided by National Renewable Energy Labotatory (NREL) [26]. Glucose, xylose, and ethanol concentrations was measured by High Performance Liquid Chromatography (HPLC) Waters, USA. Bio-Rad Aminex HPX-87H column was used as the stationary phase while the mobile phase is 5 mM H2SO4 at 0.6 mL/min. The detector is Waters 2414 refractive index (RI) detector. The oven temperature was maintained at 40°C in the column input and 65°C in the output.

2.6. Calculations
Glucose yields from enzymatic hydrolysis were calculated according to Eqs. (1) based on the glucose content (as cellulose) of the steam-exploded OPEFB fiber, while xylose yields were calculated according to Eqs. (2) based on the xylose content (as hemicellulose) of the raw OPEFB fiber.

Glucose yield (% w/w) = \( \frac{\text{Glucose in hydrolyzate (g)}}{\text{Cellulose in substrate (g)} \times 1.11^* \times 100\%} \)  

Xylose yield (% w/w) = \( \frac{\text{Xylose in hydrolyzate (g)}}{\text{Hemicellulose in substrate (g)} \times 1.14^* \times 100\%} \)  

*1.11 and 1.14 are correction factors to compensate the addition of water molecule after the breakage of cellulose into glucose monomers and hemicellulose into xylose monomers, respectively [7].

Ethanol yields from SSF were calculated according to Eqs. (3) based on the theoretical mass of produced ethanol assuming that all of the cellulose in steam-exploded OPEFB fiber was converted into glucose, while all of the produced glucose was converted into ethanol with maximum efficiency.

Ethanol yield (% w/w) = \( \frac{\text{Ethanol in hydrolyzate (g)}}{\text{Cellulose in substrate (g)} \times 1.11 \times 0.51^{**} \times 100\%} \)  

**0.51 is a correction factor to represent the maximum possible ethanol yield from glucose based on the stoichiometric ratio between ethanol and glucose in glucose fermentation [13].

3. Result and Discussion

3.1. Composition of EFB after Steam Explosion
The composition change of the OPEFB reflect the efficiency of pretreatment. Table 1 depicts the changes in the composition of untreated and pretreated OPEFB. The composition of the untreated was 36.59% cellulose, 24.97% hemicellulose, 26.53% lignin and 1.79% ash. When the OPEFB was pretreated by alkali steam explosion, the composition of carbohydrate (cellulose and hemicellulose) increased to 70%. The OPEFB is more carbohydrate enriched because the percent composition of lignin decreased. The percentage of lignin decreased due to an effect of alkaline pretreatment. Over than 70% lignin removed and as a consequent, the cellulose content increased. Lignin was considered as an inhibitor because they could restrict the hydrolysis enzyme to attack cellulose. Furthermore, using sodium hydroxide would induce swelling leading on the increase of surface area [12]. In the present study, alkaline pretreatment OPEFB was aimed to alter the structure of cellulosic biomass by removing...
lignin and hemicelluloses, so that the cellulose became more accessible to the enzymes that convert carbohydrate polymers into fermentable sugars.

Table 1. Composition of EFB before and after steam explosion

| No | Component    | Before pretreatment (%) | After pretreatment (%) |
|----|--------------|-------------------------|------------------------|
| 1  | Lignin       | 26.53                   | 7.45                   |
| 2  | Cellulose    | 36.59                   | 78.02                  |
| 3  | Hemicellulose| 24.97                   | 12.64                  |
| 4  | Ash          | 1.79                    | 1.86                   |

3.2. Enzymatic saccharification

The result of glucose and xylose during saccharification process obtained from HPLC analysis was shown in Figure 1. This analysis predicted the sugar released by enzyme performance. Cellulose and hemicellulose were converted into glucose and xylose respectively using combined enzyme. As shown in Figure 1 glucose concentration reached the highest concentration after 72 h were 137.80 g/L and 97.90 g/L for pretreated OPEFB substrate 15% and 10% concentration respectively. As expected, higher glucose concentration results in higher yield of hydrolysis. As like as glucose concentration, xylose also increased during saccharification. The combination of two enzymes not only converted cellulose to glucose, but it also changed hemicellulose to xylose. However, the xylose concentration was lower compared with glucose concentration. The discussion will be focused on the cellulose hydrolysis since *S. cerevisiae* only metabolizes hexose sugars to bioethanol and cannot metabolize xylose or any other pentose sugar. The glucose concentrations and yields from enzymatic hydrolysis of the steam-exploded OPEFB are shown on Figure 1.

![Figure 1. Sugar yield obtained after 24, 48 and 72 h of enzymatic hydrolysis of pretreated OPEFB (a) substrate 10%; (b) substrate 15%](image)

3.3. Fermentation ; SHF versus SSF

Steam exploded alkali of OPEB was subjected to bioethanol fermentation by using *S. cerevisiae* yeast. Two different method were studied: a separate hydrolysis followed by fermentation (SHF) and a simultaneous saccharification and fermentation (SSF) process, in order to compare their efficiency in term, bioethanol concentration fermentation productivity, yield, and the overall time of the fermentation.
3.3.1. **Separate hydrolysis and fermentation process (SHF)**

SHF is one method of saccharification and fermentation process that involves two sequential steps: Enzymatic hydrolysis, often called saccharification and followed by fermentation process. First the cellulose in substrate was enzymatically hydrolyzed to glucose. Second, the glucose in the hydrolyzed was subsequently fermented to ethanol. For SHF study the previous saccharification was carried out for 72 h at 50°C, according to the condition described at section 2.3. After 72 h of enzymatic hydrolysis, process was continued by fermentation, thus the total time processes were 144 h. The fermentation step was performed using baker’s yeast *S. cerevisiae* at 32°C giving satisfactory results. The fermentation profiles of sugar consumption and ethanol production obtained presented in the Figure 2. For SHF study, the concentration of glucose at the beginning of fermentation step was 138 g/l after 72 h fermentation ethanol produced reach in the concentration 69.84 g/l and there is no glucose after 72 fermentation. In theory, maximum ethanol yield is 0.51 g ethanol/g glucose. In this study ethanol results from the process is 69.84 g/l, by calculation the yield ethanol is 94.29% (Table 2). The ethanol concentration obtained in this study 69.84 g/l was higher than 47.4 g/l [8]. OPEFB pretreated by steam explosion under similar conditions. Xylose concentration remained practically constant along the fermentation as expected, since native *S.cerevisiae* cannot metabolize xylose.

![Figure 2](image.png)

**Figure 2.** Ethanol concentration during fermentation of OPEB in the SHF process, substrate 15%.

3.4. **Ethanol Concentration & Yield from SSF**

SSF process allowed enzymes and yeast put together while working process was conducted at lower temperature. In this study, the used temperature was same with fermentation i.e., 32°C. More saving energy is one of the advantages of the SSF process. Time producing ethanol becomes shorter because the hydrolysis and fermentation were carried out at the same time. The enzymatic hydrolysis was accompanied by fermentation, almost from the beginning of this simultaneous process. At 24 h fermentation, glucose was completely exhausted and ethanol reached the concentration of 62.68 g/l for 15% substrate, however for 10% substrate 36.75% only. When enzyme produced glucose, the yeast, *S. cereviceae* changed to ethanol directly. It is why the concentration of glucose always zero in the 24, 48, 72 hour fermentation. The ethanol concentrations and yields from SSF are shown on Figure 3.
Figure 1. Producing Ethanol from OPEFB by SSF Process. Substrate (a) 15% and (b) 10%

Looking to the results obtained after 72 h of SSF of OPEB, the maximum ethanol yield was 84.29% (Table 2). The results from this study show that in the end of the assay the ethanol concentration obtained by SHF using 15% substrate concentration. The process of SHF for 144 h gave the highest yield of ethanol. However, it was not suitable time according to economic aspect. SSF method was considered as a better process than SHF due to rapidly ethanol production and the high concentration of produced ethanol.

Table 2. Yield of glucose and ethanol

|                      | SHF 10% | SHF 15% | SSF 10%  | SSF 15% |
|----------------------|---------|---------|----------|---------|
| Glucose yield        | 97.89   | 94.88   | -        | -       |
| Xylose yield         |         |         | Not calculated |   |
| Ethanol yield        | 40.74   | 94.29   | 66.84    | 84.62   |

4. Conclusion
Bioethanol production using pretreated of alkali steam explosion of Oil Palm Empty Fruit Bunch (OPEFB) was conducted in the area of hydrolysis and fermentation. Enzymatic hydrolysis and fermentation were studied in the two variation methods, SSF and SHF. The glucose obtained after 72 hours was 137.80 g/L (substrate 15%) and 97.90 g/L (substrate 10%). Using 15% of concentration substrate, it could be produce 69.84 g/l of ethanol in 72 hour fermentation by SHF process and 62.68 g/l of ethanol in 24 hour by SSF process. From this study, the SSF method was considered as a better process than SHF due to rapidly ethanol production and the high concentration of produced ethanol.

Acknowledgements
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