The conversion of lignocellulosic biomass to bioethanol: pretreatment technology comparison

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Abstract. The barrier to realising the potential of lignocellulosic bioethanol is the recalcitrance of cellulosic biomass. Overcoming this biomass recalcitrance is the key challenge to large scale production of lignocellulosic bioethanol. Pretreatment is an important and critical step that enables enzyme hydrolysis of lignocellulose conversion to ethanol. Finding a pretreatment method for reducing the high recalcitrance via cost-effective pretreatment methods would therefore be of great benefit. This study aims at investigating the effect of pretreatment on delignification process of sugarcane bagasse and oil palm trunk. Two methods of pretreatment were compared i.e. alkaline hydrogen peroxide pretreatment (1% and 5% H₂O₂) and subcritical water pretreatment (170°C, 2.2 MPa) for the effectiveness of reducing the lignin content. Scanning Electron Microscopy (SEM) analysis was also performed to investigate the effect of pretreatment on surface of lignocellulosic biomass. It was observed that alkaline hydrogen peroxide pretreatment gave better results than subcritical water pretreatment based on the lignin content for both lignocellulosic biomass. Pretreated sugarcane bagasse presented lower lignin content than pretreated oil palm trunk. Lignin was successfully removed until 56.99% under the best condition of 5% of alkaline hydrogen peroxide, at 28°C for 72 hours incubation. This study confirmed that alkaline pretreatment was found to have a better method for the conversion of lignocellulosic materials. Furthermore, sugarcane bagasse has a greater potential as basic raw materials used for the bioethanol production than oil palm trunk because it has lower content of lignin with higher content of cellulose.

1. Introduction

Lignocellulosic biomass is a low-value product from various types of industrial sectors, such as sugarcane bagasse and oil palm trunks. Both are found in Indonesia with abundant quantities, but they are not optimally utilised. Sugarcane bagasse contains 30% cellulose while oil palm trunk has 42.51% [1,2]. Their high cellulose content functions as raw materials for ethanol production. Lignocellulosic biomass has a recalcitrant substance, which causes industries difficult to convert biomass into bioethanol [3].

Pretreatment process is highly essential for bioethanol production because it facilitates hydrolysis by enzymes. It plays a vital role in reducing the levels of lignin bound with cellulose, by which cellulose levels increases. Another function of pretreatment is to reduce the physical size of the material, to ease the hydrolysis process, avoiding the degradation of sugars (mainly pentoses), minimising the formation of inhibitors for subsequent fermentation steps [4, 5]. There are various types of pretreatment methods, but each sample requires the right model for the optimal results at a
low cost. One of the most commonly-used methods is pretreatment with alkali peroxide due to the high availability of the ingredients and the lack of inhibitor formation [6, 7, 8]. Pretreatment with alkali peroxide uses a solution of hydrogen peroxide with a high pH, where hydrogen peroxide forms hydroxide anion radical, which breaks down lignin bonds [9]. Physicochemical pretreatment such as subcritical water reactor is also frequently used because it is environmentally friendly, and it does not require more costs for the use of chemicals [10]. During the process, subcritical water enters the lignocellulose structure and breaks the bonds of ester and ether within lignin and hemicellulose [11, 12].

The current study aimed to obtain the highest increase of cellulose and the highest reduction of lignin through alkali peroxide method and subcritical water pretreatment in two samples, sugarcane bagasse and oil palm trunk.

2. Materials and Method
2.1 Chemicals and materials
Hydrogen peroxide 30%, NaOH, distilled water, and sulfuric acid were purchased from Merck. The sugar cane bagasse was obtained from Kebon Agung Sugar Factory, Malang, while the oil palm trunk was obtained from PT. Sampoerna Agro Tbk., Palembang. The samples were sterilised and cut into approximately 1 cm in length.

2.2 Alkaline hydrogen peroxide pretreatment
A 3.3 mL of H₂O₂ 30% was added to 96.7 mL distilled water to produce a working solution of 100 ml H₂O₂ 1%. Besides, for the solution of 100 mL of H₂O₂ 5%, 16.6 mL of H₂O₂ 30% was added into 83.4 mL distilled water. The solution of H₂O₂ was then added NaOH to achieve the expected pH (11.5). Pretreatment was performed by adding 100 mL working solution and 5 g sample into a 250 ml Erlenmeyer. Pretreatment was conducted through the combination of incubation time (3 hours and 72 hours) and incubation temperature (80 °C and 28 °C). After pretreatment, the temperature of the sample mixture was frozen and justified into that of the room and filtered by vacuum filtration. The remaining solid residue was rinsed with distilled water to pH 7. Then, it was stored in an oven at 106°C until it achieved constant weight [13].

2.3 Subcritical water pretreatment
A 5 g sample, 55 mL distilled water, and magnetic stirrer were put into the reactor. The reactor was then heated, and N₂ was injected into it until it reached the desired pressure. Pretreatment was carried out at 170 °C, 320 psi, and 500 rpm for 20 minutes. The sample was allowed to cool then filtered with a vacuum filtration. The solid residue was put into the oven at 106°C until it reached a constant weight.

2.4 Chesson analysis
A 1 g of dry sample was put into 250 mL Erlenmeyer, and 150 mL of distilled water was added. The solution was then heated with reflux for 2 hours. The sample was then filtered and the solid residue was dried in an oven of 106°C to a constant weight. The dried sample was weighed (b), and put back into 250 mL Erlenmeyer 150 mL before H₂SO₄ 1 N was added. The sample was refluxed a second time for 2 hours, filtered, and dried in an oven 106°C until it reached a constant weight. The sample then was dried a second time and weighed (c). Then, 10 mL H₂SO₄ solution was added into the sample and incubated at room temperature for 4 hours. After incubation, 150 ml of H₂SO₄ solution was added and the sample was re-heated with reflux for 1.5 hours. It was then filtrated while the solid residue was dried in an oven of 106°C to a constant weight. The dried sample was again weighed (d). The dried sample then processed to analyse mineral content with a muffle furnace. Finally, dried sample was weighed (e). The level of hemicellulose, cellulose, and lignin was measured by Equation 1, 2 and 3 [14].
Hemicellulose (%) = \frac{\text{Weight b} - \text{Weight c} \times 100\%}{\text{Weight a}} \quad (1)

Cellulose (%) = \frac{\text{Weight c} - \text{Weight d} \times 100\%}{\text{Weight a}} \quad (2)

Lignin (%) = \frac{\text{Weight d} - \text{Weight e} \times 100\%}{\text{Weight a}} \quad (3)

2.5. SEM analysis

The surface of material was analysed by using Scanning Electron Microscopy (SEM) (Quanta FEG 650, FEI, United States) in 7.50 kV beam voltage.

3. Results and Discussion

3.1 Effect of pretreatment on the composition of solid material

The purpose of pretreatment was to break down lignocellulosic biomass into materials with much cellulose and little lignin [15]. The increase of cellulose in sugarcane bagasse samples reaches up to 52.66% with alkaline peroxide pretreatment with 1% hydrogen peroxide at 28°C for 72 hours. Besides, that of the oil palm trunk sample reaches up to 47.89% alkaline peroxide pretreatment with 5% hydrogen peroxide at 28°C for 72 hours (Table 1). It occurs due to the loss of lignin and hydrolysis of hemicellulose [13,16].

Table 1. The percentage of the increase of cellulose level after pretreatment

| Pretreatment                  | The increase of cellulose level (%) |
|-------------------------------|-------------------------------------|
|                               | Sugarcane Bagasse | Oil Palm Trunk |
| H₂O₂ 1% 80°C 3 hours          | 45.28               | 24.71          |
| H₂O₂ 5% 80°C 3 hours          | 32.56               | 35.23          |
| H₂O₂ 1% 28°C 72 hours         | 52.66               | 44.86          |
| H₂O₂ 5% 28°C 72 hours         | 52.01               | 47.89          |
| Subcritical water             | 40.50               | 34.01          |

Table 2. The percentage of decrease in hemicellulose level after pretreatment

| Pretreatment                  | The decrease of hemicellulose level (%) |
|-------------------------------|----------------------------------------|
|                               | Sugarcane Bagasse | Oil Palm Trunk |
| H₂O₂ 1% 80°C 3 hours          | 11.23               | 14.67          |
| H₂O₂ 5% 80°C 3 hours          | -                    | 5.76           |
| H₂O₂ 1% 28°C 72 hours         | 7.79                 | 20.16          |
| H₂O₂ 5% 28°C 72 hours         | 30.79                | 47.96          |
| Subcritical water             | 31.81                | 53.23          |

Notes: - symbol means no decrease of hemicellulose level
The highest decrease of hemicellulose level occurs in alkaline peroxyde pretreatment with hydrogen peroxyde 5%, 28°C for 72 hours for sugarcane bagasse and pretreatment with subcritical water to oil palm trunk (Table 2). The hemicellulose in the sample decreases because it has an amorphous structure which more easily hydrolysed in alkaline conditions (alkali peroxyde method) or in high-temperature and high-pressure (subcritical water) [17, 18].

Lignin reduces in the sample with alkali peroxyde pretreatment at 28°C for 72 hours, with the most optimal lignin reduction using 5% hydrogen peroxyde (Table 3). Lignin reduction may occur due to the decomposition of hydrogen peroxyde, which forms hydroxyl and superoxide anion radicals attacking the lignin structure. Lignin then dissolves into the solution phase and increases the digestibility of the solid phase of biomass [9, 19].

### Table 3. The percentage of decrease in lignin level after pretreatment

| Pretreatment          | The decrease of lignin level (%) |
|-----------------------|----------------------------------|
|                       | Sugarcane bagasse | Oil palm trunk |
| H₂O₂ 1% 80°C 3 hours  | 28.62              | -              |
| H₂O₂ 5% 80°C 3 hours  | -                  | -              |
| H₂O₂ 1% 28°C 72 hours | 51.67              | 2.79           |
| H₂O₂ 5% 28°C 72 hours | 58.49              | 16.89          |
| Subcritical water     | -                  | -              |

Notes: - symbol means no decrease of lignin level

The percentage shows that the best pretreatment occurs in alkali peroxyde method with 28°C incubation for 72 hours and 5% hydrogen peroxyde pH 11.5. Hydrogen peroxyde in an alkaline condition quickly decomposes and forms hydroxyl and superoxide anion radicals, which cause oxidation in the lignin structure, break some inter unit bonds, and dissolve lignin in the end [20]. The more lignin dissolves, the more cellulose releases. The pretreatment with subcritical water does not result in optimum lignin decrease due to the extreme pretreatment of subcritical water (temperatures above 300°C with 22.1 MPa pressure). Thus, the structure of lignocellulose is not damaged [21].

### 3.2 Surface analysis

SEM analysis was conducted on samples before pretreatment, after the pretreatment of alkali peroxyde method (incubation temperature 28 °C for 72 hours, hydrogen peroxyde 5%), and pretreatment with subcritical water.

![Figure 1. SEM images of untreated sugarcane bagasse](image1)

![Figure 2. SEM images of untreated oil palm trunk](image2)
Figure 1 shows that sugarcane bagasse before pretreatment has a closed surface, and it is more open after being treated with subcritical water (Figure 5) even if the structure of the sample is still intact. Figure 3 explains that alkali peroxide pretreatment methods cause damage to the formation of lignocellulose from sugarcane bagasse. The oil palm trunk samples, before pretreatment, contains a surface with layers such as clumps (Figure 2). After pretreatment with alkali peroxide method, the surface of the sample experiences erosion with some holes (Figure 4). The pretreatment with subcritical water (Figure 6), shows the same erosion, but it is less compared to that in the sample results of pretreatment with alkaline peroxide method.

4. Conclusions
This study aims to obtain the highest increase of cellulose and the highest reduction of lignin through alkali peroxide method and subcritical water pretreatment in two samples, sugarcane bagasse and oil palm trunk. The analysis of the Chesson method concludes that the best pretreatment method to decrease lignin and increase cellulose was an alkali peroxide pretreatment with 28°C incubation for 72 hours and 5% hydrogen peroxide. The decrease in lignin levels for the sugarcane bagasse was 58.49% and for the oil palm trunk was 16.89%. At the same time, it causes an increase of cellulose levels by 52.01% in sugarcane bagasse and by 47.89% in oil palm trunk. The best pretreatment of the current study was in line with SEM results, in which the damage to the structure and surface on both samples was more severe due to the alkali peroxide pretreatment method. In sum, sugarcane bagasse was the better material for bioethanol production because of the higher cellulose before and after pretreatment and more reduction in lignin.
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