DILUTE ACID PRETREATMENT AND ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS FOR BUTANOL PRODUCTION AS BIOFUEL

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ABSTRACT

Biobutanol is one of the promising biofuel for substituting gasoline. Biobutanol produced from biomass fermentation using solventogenic clostridia which are able to convert a wide range of carbon sources to fuels such as butanol. Therefore, lignocellulosic biomass has great potential as fermentation substrate for biobutanol production. Lignocellulosic biomass should be hydrolized before fermentation by a pretreatment process and enzymatic hydrolysis. The various lignocellulosic biomass pretreatment will influence in butanol production depending on fermentable sugars content. The objective of this research is to get potential lignocellulosic biomass using dilute acid pretreatment and enzymatic hydrolysis process for biobutanol production. Eight types of biomass from sugarcane bagasse, rice straw, rice husk, empty fruit bunch (EFB) of palm oil, corn cob, pulp waste, traditional market organic waste, and microalgae were used in this experiment. After hydrolysis, the high result of total fermentable sugars in corn cobs, bagasse, rice straw, and rice husk, shows good opportunity of these biomass to be used as fermentation feedstocks for biobutanol production. In addition, pulp waste, organic waste, and microalgae are prospective as raw material but require more appropriate treatment either for to break down the cellulose/hemicellulose or to enhance reducing sugar content. Fine milling and delignification have no significant effect on cellulosic biomass conversion into fermentable sugars. Therefore, the production cost can be reduced. In order to enhance the sugar content and reduce the formation of inhibitor product, it is necessary to examine dilute acid pretreatment variations and appropriate operating conditions of enzymatic hydrolysis process.

Keywords: biofuel, biobutanol, lignocellulosic biomass, dilute acid pretreatment, enzymatic hydrolysis

I. INTRODUCTION

Biofuels development for fossil fuels replacement has increased in recent years. One of the promising biofuel for substituting gasoline is biobutanol, a four carbon alcohol produced by biomass fermentation using anaerobic bacteria. Compared to ethanol, butanol has many superior properties as an alternative fuel. Butanol contains more energy, less hygroscopic, and easily mix with gasoline in any proportion. Furthermore the air-fuel ratio of butanol in engine combustion chamber is close to gasoline. Butanol can be used directly or blended with gasoline without any vehicle retrofit. In addition, butanol is also can be supplied through the existing gasoline pipes without any problems.

The most abundant sources of renewable biomass is lignocellulosic biomass obtained from energy crops, wood and agricultural residues. Using biomass to produce energy can possibly solve the problems that world faces because of excessive use of fossil fuels, and may significantly reduce greenhouse gas emissions, pollution and waste management problems. Indonesia has great natural resources and biomass to produce biofuels. Agricultural residues,
domestik organic waste and other non-edible are easily found in a large amount. According to Ministry of Energy and Mineral Resources Republic of Indonesia, the potency of Indonesian biomass for energy is 49.81 GW, while installed capacity is only about 1618 MW. It might be caused by a lack of biotechnology research for industrial-scale biofuels production.

Anaerobic bacteria such as solventogenic clostridia are able to convert a wide range of carbohydrates to biofuels and chemicals such as biobutanol. Therefore, the use of lignocellulosic biomass as a substrate is good approach for biobutanol fermentation. Butanol fermentation by different strains using various biomass substrates has been reported in recent years. These innovations may help reduce fermentation substrate costs. Biobutanol fermentation technology has changed rapidly in the last few years and a commercial scale process based on biomass materials is nearly achieved. The use of lignocellulosic substrates in combination with developed technology is expected to make the production of biobutanol economically viable.

Since microorganisms do not have enzymes to digest cellulose, it is essential to treat lignocellulosic materials before fermentation in order to break it down into simple sugar. The production of fermentable sugars from lignocellulosic biomass after initial mechanical process is usually carried out in two steps: a pretreatment process, and enzymatic cellulose hydrolysis. Pretreatment is a crucial process step and it has been recognized as one of the most expensive processing steps in cellulosic biomass to fermentable sugars conversion. The pretreatment process needed to liberate the cellulose from the lignin seal and its crystalline structure thus makes cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. One of the most effective pretreatment methods for lignocellulosic biomass is dilute acid pretreatment. Dilute acid hydrolysis has been extensively reviewed and is considered to be one of the treatment methods with greater potential for wide-scale application. For a given material, the best conditions for hemicellulososes removal and recovery in the hydrolysate do not always translate into the best enzymatic digestibility. Enzymatic hydrolysis is reaction using specific cellulase enzymes that brakes cellulose into glucose molecules. Obstacles in the pretreatment and hydrolysis processes include the insufficient conversion of cellulose to glucose, high lignin content which is recalcitrant fraction, high use of chemicals and/or energy, considerable waste production, and formation of by-products that inhibit fermentation. This research examine most of all Indonesian potential biomass such as sugarcane bagasse, rice straw, rice husk, empty fruit bunch of palm oil, corn cob, pulp waste, traditional market organic waste, and microalgae. The objective of this research is to get potential lignocellulosic biomass using combination of dilute acid pretreatment and enzymatic hydrolysis process for biobutanol production.

II. MATERIALS AND METHODS

A. Biomass Preparation

Eight prepared biomass from sugarcane bagasse, rice straw, rice husk, empty fruit bunch (EFB) of palm oil, corn cob, pulp waste, traditional market organic waste, and microalgae are used in this experiment. The sugarcane bagasse is obtained from sugar mill, whereas EFB and pulp waste are collected from palm oil mill and pulp industry respectively. Two types of pulp waste, i.e TR (rough) and TF (fine) are used in this experiment. The biomass used in this study are shown in Figure 1. All of the biomass used are based on % dry solid except for organic waste which is based on its % wet solid, because the structure and composition of carbohydrates may be degraded in the drying process and consequently affect reducing sugar yield.

B. Dilute Acid Pretreatment and Enzymatic Hydrolysis

Dilute acid pretreatment was conducted using 0.5 - 4% sulphuric acid with 1.5-12.5% w/v biomass. The process was carried out in an autoclave either at 121°C for 60 min or at 130°C for 30 min. The mixture was then cooled to room temperature and followed by neutralization with NaOH 10 M. The hydrolysate was finally separated from the solid fraction. The hydrolysate was then examined for reducing sugar content, while the solid fraction was subjected to hydrolysis process. The dilute acid pretreatment condition for each biomass is described in Table 1. In order to recognize the effect of lignin removal on reducing sugar enhancement, the delignification
A test was performed using pretreatment solid fraction from 3 biomass, i.e. bagasse, rice husk, and rice straw. The delignification process was conducted before enzymatic hydrolysis, using 1.5% NaOH at 100°C for 60 minutes, then neutralized with H₂SO₄ and washed with destillate water.

![Figure 1: Eight cellulosic biomass after mechanical preparation](image-url)
The solid fraction obtained from the previous process was then hydrolyzed using an enzyme mixture of Cellulase (Sigma, Cat. No. C1184) and Xylanase (Sigma, Cat. No. X2753). Each enzyme powder was dissolved 1 g/L in citrate buffer solution. Hydrolysis process was carried out in 500 ml Erlenmeyer flask containing 250 ml of enzyme solution in citrate buffer, incubated in a shaker incubator at 50°C and pH 4.8, with 120 rpm, for 72 hours. Finally, the flask was sterilized to denatured enzymes. Hydrolysate from the enzymatic hydrolysis process was sampled for reducing sugar analysis.

C. Analysis

The carbohydrate and reducing sugar were determined quantitatively using gravimetric method and Nelson-Somogyi method, respectively. Carbohydrate analysis was conducted in Centro Agro-Based Industry, Bogor and Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.

III. RESULTS AND DISCUSSION

A. Carbohydrate composition

The average content of cellulose, hemicelluloses, and lignin from each biomass are presented in Table 2. Analysis of biomass composition exhibits that the highest cellulose content found in pulp waste TF, followed by pulp waste TR, bagasse, and rice husk, respectively. The highest hemicellulose content found in organic waste, followed by corn cobs, and microalgae, respectively. The highest lignin content found in the EFB palm oil, followed by bagasse and organic waste respectively. EFB Palm oil which has highest lignin content is not appropriate for conventional butanol fermentation because of high energy consume for pretreatment and hydrolysis. According to Harmsen et al., biomass with high lignin is suitable for heat and

<table>
<thead>
<tr>
<th>No.</th>
<th>Biomass</th>
<th>Carbohydrate composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cellulose</td>
</tr>
<tr>
<td>1.</td>
<td>Rice straw</td>
<td>29.94</td>
</tr>
<tr>
<td>2.</td>
<td>Rice husk</td>
<td>33.85</td>
</tr>
<tr>
<td>3.</td>
<td>Bagasse</td>
<td>43.49</td>
</tr>
<tr>
<td>4.</td>
<td>Corn cob</td>
<td>23.29</td>
</tr>
<tr>
<td>5.</td>
<td>Microalgae</td>
<td>21.72</td>
</tr>
<tr>
<td>6.</td>
<td>Pulp waste TF</td>
<td>74.78</td>
</tr>
<tr>
<td>7.</td>
<td>Pulp waste TR</td>
<td>58.21</td>
</tr>
<tr>
<td>8.</td>
<td>EFB palm oil</td>
<td>25.24</td>
</tr>
<tr>
<td>9.</td>
<td>Organic waste</td>
<td>22.08</td>
</tr>
</tbody>
</table>
electricity production. Carbohydrate composition of lignocellulosic biomass vary greatly, depending on the type of plant, cultivation condition and the age of plant. Carvalho showed that there was different cellulose content between two types of bagasse which used in his research.

Based on the comparison of carbohydrate compositions (Figure 2), the highest fermentable carbohydrate from the total amount of cellulose and hemicellulose, is found in pulp waste TF, followed by organic waste and bagasse. The highest of cellulose and hemicellulose content in the biomass is expected to be superior in terms of highest butanol production yield.

B. Dilute Acid Pretreatment

The conversion process of carbohydrate into sugar has been commenced on dilute acid pretreatment, as indicated by the color changes of the solution (Figure 3).

The highest sugar content after pretreatment process is found in corn cob, followed by rice husk, bagasse, and organic waste, respectively. When compared between the percentages of reducing sugar per dry weight biomass, the organic waste give the highest result with 39.79% followed by corn cob and bagasse. Reducing sugar content of each biomass after pretreatment with dilute acid is shown in Table 3.

A comparison between the percentages of reducing sugar per weight biomass with hemicellulose content of each biomass is shown in Figure 4. It exhibit that the greater the hemicelluloses, the greater the sugar content obtained from the pretreatment process. The high difference between hemicelluloses and reducing sugar content on microalgae, pulp waste TF, and rice straw shows that optimal condition of dilute acid pretreatment process for these biomass has not been achieved.

The cellulose and hemicellulose are more easy to break down in small particles. However the examination of particle size effect on sugar content...
after hydrolysis process indicate that reducing the size of biomass is not followed by increasing sugar content. The increase of sugar content after milled only found in corn cobs (Figure 5). This means that only certain biomass undergoes increasing in sugar content when its particle size reduced. As a result, the elimination of fine milling process can reduce production costs. According to Carvalho\textsuperscript{13}, several types of high energy consuming grinding against some types of biomass, but it works much better with other biomass. However, grinding is an energy intensive process which is one of the most important limitations in the application of industry-wide scale.

C. Enzymatic Hydrolysis

The highest percentage of reducing sugar content after enzymatic hydrolysis is found in corn cobs, followed by rice husk, bagasse, and rice straw, respectively (Table 4). However, when compared with the biomass weight, microalgae has the highest value (78.74%) followed by corn cob (37.33%).
is assumed that the hydrolysis of carbohydrates in microalgae is complete. But the use of this biomass is not efficient if using low weight dry biomass. The microalgae biomass require more appropriate pretreatment to increase reducing sugar content.

The highest content of cellulose in the pulp waste does not generate the highest reducing sugar. This may be caused by the crystalline structure of cellulose which can inhibit the enzyme to access the biomass particles. Polymer solubility of cellulose intimately associated with the attainment of degrees of hydrolysis. Therefore, cellulose hydrolysis from pulp waste requires more specific study in order to achieved high fermentable sugars.

Reducing sugar content obtained via enzymatic hydrolysis process can be improved. The application of enzyme in the form of powder requires a preliminary test in order to determine the accurate activity of cellulase. The measurement cellulase activity will determine enzymes activity that can be used as efficiently as possible considering the expensive price of an enzyme. Anwar et al. stated that the greater the ratio of enzyme to the substrate, the greater the concentration of glucose is produced. However, the efficiency of the enzyme becomes smaller. The incomplete enzymatic hydrolysis may be caused by a number of factors including the presence of inhibitor for the enzymes. Furthermore knowledge about the physiology and genetics to make the enzymatic hydrolysis process becomes economically viable is required. The results from Qureshi et al. using strain C. beijerinckii showed that the use of enzymes for hydrolysis in butanol fermentation was not significantly different. It means that both glucose and xylose are utilized efficiently by the culture. Thus it is possible that low sugar content from biomass in this study can reach higher biobutanol production.

In the delignification test from bagasse, rice husk, and rice straw, the increasing reducing sugar content after enzymatic hydrolysis only found in rice straw. Whereas delignification in bagasse and rice husk decrease reducing sugar content (Figure 6). The delignification process does not affect the reducing sugar content in bagasse which contain more lignin than rice straw and rice husk. This phenomenon was described by Ishizawa et al. which found that nearly complete removal of lignin after dilute acid pretreatment have reduced access and conversion of cellulose. Rollin et al. stated that increasing cellulose accessibility was a more important pretreatment consideration than delignification for effectively releasing sugars from recalcitrant lignocellulose at high yield.

The total of reducing sugar content from hydrolyzates of dilute acid pretreatment and enzymatic hydrolysis shows high sugar yield from corn cobs, rice husk, bagasse, and rice straw, Table 4.

<table>
<thead>
<tr>
<th>No.</th>
<th>Biomass</th>
<th>% DS</th>
<th>% Reducing sugar</th>
<th>% Reducing sugar Biomass weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Corn cob</td>
<td>12.5</td>
<td>4.67</td>
<td>37.33</td>
</tr>
<tr>
<td>2.</td>
<td>Rice husk</td>
<td>18.75</td>
<td>3.97</td>
<td>21.17</td>
</tr>
<tr>
<td>3.</td>
<td>Bagasse</td>
<td>12.5</td>
<td>3.59</td>
<td>28.74</td>
</tr>
<tr>
<td>4.</td>
<td>Rice straw</td>
<td>12.5</td>
<td>3.28</td>
<td>26.27</td>
</tr>
<tr>
<td>5.</td>
<td>EFB Palm oil</td>
<td>12.5</td>
<td>2.95</td>
<td>23.56</td>
</tr>
<tr>
<td>6.</td>
<td>Pulp waste TR</td>
<td>12.5</td>
<td>2.47</td>
<td>19.76</td>
</tr>
<tr>
<td>7.</td>
<td>Organic waste</td>
<td>12.5</td>
<td>2.47</td>
<td>19.72</td>
</tr>
<tr>
<td>8.</td>
<td>Pulp waste TF</td>
<td>12.5</td>
<td>1.99</td>
<td>15.93</td>
</tr>
<tr>
<td>9.</td>
<td>Microalgae</td>
<td>1.875</td>
<td>1.48</td>
<td>78.74</td>
</tr>
</tbody>
</table>
respectively. The results of the total hydrolyzate of the dilute acid pretreatment and enzymatic hydrolysis are presented in Table 5.

**IV. CONCLUSIONS**

The current study demonstrates that some of biomass such as corn cobs, bagasse, rice straw and rice husk are good material for biobutanol production. Pulp waste and microalgae have a good chance. However, they still require more appropriate treatment to break down the cellulose/hemicellulose. Fine milling and delignification have no significant effect in cellulosic biomass conversion into fermentable sugars, therefore it can reduce

<table>
<thead>
<tr>
<th>No.</th>
<th>Biomass</th>
<th>% Reduction Sugar from Pretreatment</th>
<th>% Reduction Sugar from Hydrolysis</th>
<th>% Reduction Sugar from Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn cob</td>
<td>2.93</td>
<td>4.67</td>
<td>7.60</td>
</tr>
<tr>
<td>2</td>
<td>Rice husk</td>
<td>2.02</td>
<td>3.97</td>
<td>5.99</td>
</tr>
<tr>
<td>3</td>
<td>Bagasse</td>
<td>1.93</td>
<td>3.59</td>
<td>5.52</td>
</tr>
<tr>
<td>4</td>
<td>Rice straw</td>
<td>1.54</td>
<td>3.28</td>
<td>4.82</td>
</tr>
<tr>
<td>5</td>
<td>Organic waste</td>
<td>1.91</td>
<td>2.47</td>
<td>4.38</td>
</tr>
<tr>
<td>6</td>
<td>EFB palm oil</td>
<td>1.19</td>
<td>2.95</td>
<td>4.14</td>
</tr>
<tr>
<td>7</td>
<td>Pulp waste TR</td>
<td>0.59</td>
<td>2.47</td>
<td>3.06</td>
</tr>
<tr>
<td>8</td>
<td>Pulp waste TF</td>
<td>0.42</td>
<td>1.99</td>
<td>2.41</td>
</tr>
<tr>
<td>9</td>
<td>Microalgae</td>
<td>0.18</td>
<td>1.48</td>
<td>1.66</td>
</tr>
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</table>
production costs. In order to enhance the sugar content and reduce the formation of inhibitor product, it is necessary to examine the dilute acid pretreatment variations based on acid concentration, temperature, and retention time, and to search suitable operating conditions of enzymatic hydrolysis.

V. ACKNOWLEDGEMENT

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