Delignification As The Early Process Of Bioethanol Production From Paper Waste By Aspergillus Fumigatus

Amanda Frisilla, R. Ratnaningsih, Astri Rinanti

Abstract: This initial laboratory-scale research was carried out to test the potential of HVS paper waste as biomass containing lignocellulose to become bioethanol, an alternative energy source using the enzymatic ability of Aspergillus fumigatus fungi. Lignocellulosic biomass is a renewable resource of bioethanol raw material. This research was carried out by cultivating A. fumigatus fungi on Potato Dextrose Broth media and mechanically preparing the HVS paper waste into a paper pulp as the substrate. During the pretreatment process, A. fumigatus fungi were added to the container containing the substrate, in the ratios of 1:1, 1.5, and 1:10 respectively. The lignin level was determined using the gravimetric method with pretreatment conducted at each ratio using a contact time of 24, 72, and 120 hours. This was followed by hydrolysis to obtain sugar through fermentation, which was measured by the DNS method. The highest lignin removal efficiency at the pretreatment stage was 8.2%, while the hydrolysis stage produced as much as 1.288 g/L of sugar. Although the lignin removal efficiency was very low and the sugar produced was not much, this research provides some important information on the use of HVS paper waste as a bioethanol raw material and A. fumigatus fungi as a promising biocatalyst for future production.

Index Terms: Bioethanol, Paper, Pretreatment, Hydrolysis, Aspergillus fumigatus

1. INTRODUCTION

PAPER is one of the most common means of non-verbal communication in various sectors of society. Paper is the main medium for writing, printing and painting. Paper is also used as cleaning paper (tissue) that is used in everyday life. The main raw material in making paper is pulp. Pulp generally comes from wood, bamboo, rice and other plants that contain fiber, but generally the fibers commonly used as paper raw materials are wood fiber. In addition, pulp can also come from agricultural residues such as wheat straw or used paper. Plant fibers which are braid fibers that have been processed in such a way that they eventually form a sheet. Cellulose is a plant fiber that can be processed into paper raw materials [1]. The paper is in the form of thin sheets formed from compression of fibers originating from the pulp, which has undergone drying, and the addition of several additional materials that can stick together. Fiber used in making paper is usually in the form of natural fibers containing cellulose and hemicellulose. Currently, paper consumption in Indonesia and all over the world have continued to increase, thereby leading to a rise in papermaking industries [2]. It utilization all over the world is around 394 million tons and expected to increase to 490 million tons in 2020, while its domestic needs in 2011 were 5.2 million tons [3]. Furthermore, owing to its increase in production, its selling price has drastically reduced, thereby leaving the manufacturers with lots of pieces which are turned into waste. This waste is often thrown to the environment, piled up, and burned, thereby leading to environmental pollution with adverse health effects on human health. Currently, energy scarcity is also one of the major problems experienced by countries all over the world. Various parties need to strive to search for alternatives, one of which is renewable energy resources. Biomass therefore is one of such sources with great potential to be developed in Indonesia. It is used as a raw material in making bioethanol, which is an alternative fuel [4]. Lignocellulose biomass consists mainly of cellulose, hemicellulose, lignin and extract [5]. At present, three types of enzymes, namely, cellulase, hemicellulase and ligninase can be used to decompose lignocellulose biomass [6]. According to [7] lignocellulose biomass such as paper material is part of leafy plants or wood which basically consists of cellulose, hemicellulose and lignin compounds. The main structure of the plant cell wall is cellulose, which consists of a long chain of glucose which is connected by a bond (β-1, 4) glycosidic. The chains are arranged in bundles associated with hydrogen bonds which form the main components of paper products. Cellulose molecules are interrelated by other molecules, namely, hemicellulose which consists mainly of xylose. The strength of plant fibers is caused by the very complex molecular structure of lignin. According to [8] lignocellulose is an organic component that is abundant in nature. Lignocellulose consists of three types of polymers, namely cellulose, hemicellulose and lignin. The lignocellulose component is an important source of producing useful products such as sugar from the fermentation process. Lignin (C₇H₅O₂(OCH₃)ₙ) is a compiler of lignoselulose biomass of 10-25%. Lignin is the second most abundant natural polymer and is a constituent component of plants along with cellulose and other fiber ingredients. Lignin is insoluble in water and stable in nature and lignin acts as a connecting “glue” between cellulose and hemicellulose [9]. Ethanol (C₂H₅OH) is a liquid from the fermentation process of sugar originating from carbohydrate sources using microorganisms. Bioethanol can also be interpreted as a chemical produced by food containing starch, such as cassava, sweet potatoes, jangung, and sago. Bioethanol can be said as a fuel from vegetable oils that have properties such as premium oil. Ethanol is a product of the fermentation process derived from biological sources. Raw materials in the manufacture of ethanol can come from materials containing cellulose, polysaccharides and monosaccharides. In the process of making bioethanol the constraints refer to four major aspects, namely, raw materials,
conversion technology, hydrolysis processes and fermentation configurations [10]. According to [11] Ethanol can be produced from fermented sugars obtained from various biomass sources. These resources can be broadly classified into three types: sugar plants (sugar cane, sweet sorghum), plant starch (corn, wheat), and cellulose biomass (corn, switchgrass). Ethanol from sugar and starch raw materials is usually referred to as first generation biofuels and ethanol from cellulose biomass known as second generation ethanol. In addition to cellulose, the cellulose raw material mainly consists of hemicellulose and lignin, so it is often referred to as lignocellulose biomass. Cellulose biomass, such as agricultural residues, forestry waste, and energy crops, provides low-cost benefits, abundant availability, and most importantly, is a non-food source for biofuel production. Ethanol has economic value (economic value that can be used to have added value) containing high ethanol can be used as a solvent, fuel and so on [12]. Bioethanol has been considered a promising liquid fuel. Although the energy density of ethanol is about two thirds of gasoline, the amount of high octane ethanol mixture (106-110 vs 91-96 for gasoline) and 34.7% oxygen (zero in gasoline) provides several benefits when mixed with gasoline. In addition to high octane numbers, ethanol provides the advantages of higher evaporation enthalpies, higher laminar speeds and higher evaporation heat, which together make a very promising ethanol mixture of transportation fuels [13]. Delignification is an important step to break down carbohydrates or starch polymers into monomers such as glucose to produce bioethanol [14]. Pre-treatment of lignocellulosic biomass always results in changes in material properties, such as chemical composition, acetyl group, crystallinity, degree of polymerization, and accessibility [15]. According to [16] In the process of hydrolysis, cellulose present in the substrate is converted into ethanol using cellulase degrading organisms after the pre-treatment process. In a study conducted by [16] bacteria are used in the hydrolysis process compared to enzymes, because enzymes are expensive and can increase the cost of producing bioethanol. The hydrolysis process is carried out at neutral pH. Bioethanol production from enzymatic hydrolysis has greater potential than acid hydrolysis [17]. Enzymatic hydrolysis of cell walls uses less energy than chemical hydrolysis even though the number of selective enzymes used for effective saccharification is generally high [18].

Fig. 1. Ethanol Formation Mechanism

Paper waste is one of the sources of biomass rarely utilized. It contains lignocellulosic fibers which are converted into simple sugar using the hydrolysis process. Furthermore, the sugar is converted into ethanol through a fermentation process [4]. According to [23] lignocellulosic material is an abundant renewable resource for biofuel production obtained from the fermentation process by Saccharomyces cerevisiae microorganisms. Therefore, further research needs to be carried out regarding the use of paper waste in bioethanol production through the enzymatic process. This, thereby made it one of the alternatives to overcome the accumulation of paper waste in the environment, and to obtain ethanol as renewable energy. Figure 1 shows ethanol formation.

2 RESEARCH METHODOLOGY

2.1 Pretreatment of Paper Waste

HVS paper was obtained from household waste, cut into smaller pieces, soaked for one day, and mechanically crushed into a pulp, which was a substrate ready to be delignified by A. fumigatus at the pretreatment stage. Also, A. fumigatus fungi were obtained from the collection of the Laboratory of Environmental Biology/Microbiology, Trisakti University. The fungi were grown on potato dextrose agar (PDA) media for five days at 30°C till perfect sporulation was formed in the exponential phase. After the fifth day, the PDA containing fungi was stored at 4°C and used at the next stage [19].

2.2 Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted by A. fumigatus fungi, which were the catalyst in the pretreatment process. It was then put into a container containing potato dextrose broth (PDB) and sterilized using autoclave at 121°C, 15 PSI, for 15 minutes. The addition of Aspergillus fumigatus fungi to the substrate was set with a ratio of 1:1, 1:5, and 1:10 (w/w) respectively. The sampling and observations were carried out and filtered with its contents dried in an oven after the contact time of 24, 72, and 120 hours at 70°C.

2.3 Analytic Methods

The significant amount of lignin formed as a result of the delignification process was obtained using the gravimetric method. Its removal efficiency was calculated using equation 1.

\[
\text{% removal} = \frac{W_{(a)} - W_{(b)}}{W_{(a)}} \times 100
\]

Explanation:  
- \( W_{(a)} \): Lignin levels (%) before delignification  
- \( W_{(b)} \): Lignin levels (%) after delignification

The DNS method was used to determine and estimate the amount of sugar produced at the hydrolysis stage [20]. This was made by dissolving 1 gram of DNS, 50 mg of sodium sulfite and 1 gram of NaOH into 50 mL of distilled water, in a 50 mL colored (not clear) volumetric flask to avoid the oxidation process. The estimated process was carried out by adding 3 mL of DNS reagent into a test tube covered with aluminum foil. After that, it was heated at 90°C for 15 minutes/till a brownish-red color was formed. This was then followed by the addition of 1 ml Rochelle salt, and measured by a spectrophotometer at a wavelength of 575 nm. The Rochelle salt produced was carried out by dissolving 20 grams of Na-K Tartrat in a 50 mL volumetric flask.

The research needs to be continued by carrying out the fermentation and distillation process.
3 RESULT AND DISCUSSION
Facilitating the hydrolysis process was necessary to separate the lignin and carbohydrates. Table 1 shows the removal value of lignin content, while Figure 2 shows its removal pattern in the substrate.

### Table 1
LIGNIN REMOVAL EFFICIENCY (%) AT VARIOUS FUNGI TO SUBSTRATE RATIOS AND VARIOUS CONTACT TIMES

<table>
<thead>
<tr>
<th>Ratio Fungi : Substrat</th>
<th>Contact Time (hours)</th>
<th>Lignin Removal Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>24</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>6.5</td>
</tr>
<tr>
<td>1:5</td>
<td>24</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>6.3</td>
</tr>
<tr>
<td>1:10</td>
<td>24</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Table 1 shows the best delignification process that produced a lignin removal efficiency of 8.2%. This result occurred in the treatment with the ratio of fungi to the substrate of 1:1, at the contact time of 72 hours. Each variation of the contact time of 24, 72, and 120 hours and a ratio of 1:1 also had the highest efficiency, as shown in Figure 1. Therefore, the treatment with the ratio of 1:1 seems to be quite effective in converting the lignocellulose component found on the substrate by A. fumigatus fungi in this delignification process. The purpose of the pre-treatment process was to break down the lignin structure and disrupt the cellulose crystal structure for the acidic liquid or enzyme to easily access and hydrolyze the cellulose [21]. The use of A. fumigatus as the biological catalyst made this research eco-friendly and did not cause environmental pollution due to the use of a harmless catalyst. However, [22] states that Pre-treatment is the most expensive process in converting biomass to fuel, but has a great potential for increasing the efficiency and lowering costs through further research and development.

### Table 2
PRODUCTION AT THE HYDROLYSIS STAGE

<table>
<thead>
<tr>
<th>Ratio Fungi : Substrat</th>
<th>Contact Time (hours)</th>
<th>Sugar (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:01</td>
<td>24</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.103</td>
</tr>
<tr>
<td>1:05</td>
<td>24</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.141</td>
</tr>
<tr>
<td>1:10</td>
<td>24</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.132</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>1.288</td>
</tr>
</tbody>
</table>

4 CONCLUSION
This research proves that in the delignification and hydrolysis processes, A. fumigatus fungi plays a good role as a biocatalyst. Pre-treatment is an important part of converting biomass containing lignocellulose to biofuel. The ratio of A. fumigatus fungi to the substrate is 1:1 in the pretreatment process and is the most suitable for delignification because the highest lignin removal occurred at 8.2% at the contact time of 72 hours compared to other ratios. At a ratio of 1:10 with a contact time of 120 hours, the highest sugar content obtained was 1.288 g/L. The research needs to be continued by...
carrying out the fermentation and distillation process for further investigation to be conducted regarding the potential of HVS paper waste as a raw material in producing bioethanol.

REFERENCES