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To cite this article: A M Jannah *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **543** 012053

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# Biobutanol Production from Bagasse Using Ammonia Pre-treatment and Acid Hydrolysis Method

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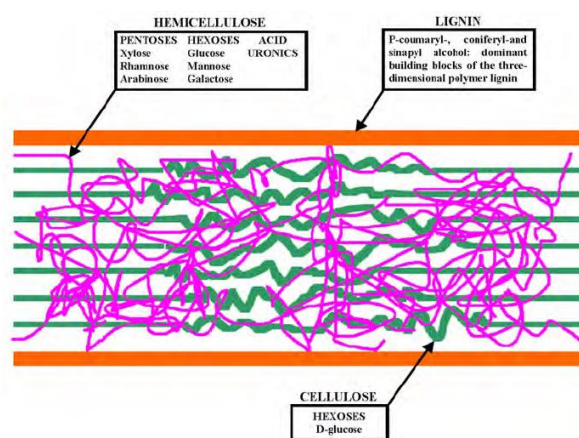
**Abstract.** Biobutanol is a type of alcohol that can be produced from biomass lignocellulose and used as renewable fuel. One of lignocellulose sources as raw material of making biobutanol is bagasse. Bagasse as agriculture waste was useless and did not have a potential benefit for farmers, but in this research it used as raw material because of the high cellulose contained. In this research for producing biobutanol, bagasse pre-treated by using ammonia with various concentrations (0 %, 1 %, 3 %, 5 %, 7%) and hydrolysis with 2 % sulphuric acid for 2 hours and 120 °C. Then the process continued with fermentation. In this step 10 mL *Clostridium acetobutylicum* bacteria was used and fermentation times were varied (2, 4 and 6 days). The aims of this research were analysed the content of cellulose, hemicellulose, lignin before and after pre-treatment processes and also biobutanol content after fermentation process. The results of this research showed that using 7 % ammonia was the highest lignocellulose content which had 69.68 % of cellulose, 14.39 % of hemicellulose and 9.15 % of lignin. The highest biobutanol content was 0.85 % that produced by using 5 % ammonia in the pre-treatment process and fermented for 6 days.

## 1. Introduction

As non-renewable, the quantity of fossil fuel decreased by the time. However, humans need the fuel every day. The decreasing of fuel quantity made many researchers were trying to find alternative fuel to substitute non-renewable energy and able to reduce the scarcity of fossil fuel. Alcohol has high potential to convert to be energy. Butanol is biofuel diversification that has high potential to be developed. Biobutanol have a great potential to partially or completely replace fossil fuels in the automotive industry due to their attractive characteristics [1]. At the first generation biobutanol used for fuel produced from organic compounds such as glucose (19.6 g/l ) and corn flour (15.8 g/l) [2]. But nowadays butanol also produced from organic waste called 2nd generation biofuel. Another agriculture waste has been researches. From Plant empty fruit bunches as raw material can be produced 0.84 g/L biobutanol [3]. Biobutanol also has been researched by using city organic wastes consist of fruits and vegetables waste from traditional market in Samarinda, Indonesia. In this research, organic waste also hydrolyzed by using 0.5 % - 1 % H<sub>2</sub>SO<sub>4</sub> with rasio 1:6 (g/v) at various temperature 100 – 130 °C. The result from that research produced 13.0 (g/L) biobutanol. The most glucose yield has been produced by sample with hydrolysed with 1 % H<sub>2</sub>SO<sub>4</sub> at 110 °C for 45 min [4]. In this study, biobutanol was produced from bagasse. Bagasse as a solid waste of sugar industry is very abundant in Indonesia. Statistics Indonesia released that Indonesia sugar cane (*Saccharum officinarum*) in 2016 has 482,239 hectares. One sugar cane can be obtained 30 % - 40 % of bagasse by volume. Because of this reason, Indonesia has huge potential to make bagasse as biofuel and produce sustainably. Biobutanol was produced from lignocellulose biomass with main constituent compounds are cellulose, hemicellulose and lignin. The main processes of biobutanol production were in hydrolysis process and fermentation. Bagasse as the raw material in this study was gotten from waste



of sugar industry. Bagasse can be used as a raw material to produce biobutanol because it contains 52 % cellulose [5]. Cellulose as a major compound of plant structure will be reacted with acid or alkaline solution and converted to become glucose. Then glucose will be fermented by using *Clostridium* bacteria in an anaerobe to produce biobutanol. In the plant structure compound, lignin covers cellulose and hemicellulose bonding. Lignin inhibits cellulose to react with catalyst and convert to be glucose. Because of this reason, lignin must be eliminated. Amount of lignin in raw material was quite influential in glucose production. The process to produce biobutanol was started by decrease the lignin component of biomass and continued to the hydrolysis process and fermented the bagasse by using *Clostridium acetobutylicum* bacteria. *Clostridium acetobutylicum* bacteria is a bacteria that can help in process of conversion sugar into the butanol organic. *Clostridium acetobutylicum* is a gram-positive bacillus [6]. This bacterium is working in anaerobic condition of fermentation. Some of the newest research has investigated alternative methods to produce the industrial solvent which *Clostridium acetobutylicum* has been used for the last century to produce. In particular, butanol has received particular attention as possible alternative fuel source for automobiles.



**Figure 1.** Lignocellulosic Materials: Composition of Major Compounds [7]

## 2. Processing Description

### 2.1. Delignification

Pre-treatment process for lignocellulose biomass aimed to degrade lignin content in the cell walls of plants. Bagasse as raw material in this research has 9.15 % by volume of lignin content. The amount of lignin has influence in the conversion process of biobutanol. Less lignin content in cell wall of bagasse was made the process of biobutanol production easier. Lignin is one of complex polymer with high weight molecule that composed of phenyl propane. This component fills the space between cellulose and hemicellulose in the cell walls [8]. The main process of biobutanol production was cellulose and hemicellulose conversion into glucose and the last product was alcohol. In this case, lignin will inhibit the process of glucose formation. Because of this reason lignin has to be delignified. The delignification process can be done by adding solvent and followed by heating. Delignification process can be done through chemical treatment, physic treatment, and chemical-physic treatment. Type of treatment used for raw material can be adjusted. Using only physic treatment for delignification was not effective to decrease lignin, therefore needed follow chemical treatment, so there will be chemical reaction to help lignin detached from the bond. Chemical delignification can be done with several treatments such as Liquid Hot Water, Acid Treatment, alkaline treatment, and Soaking in Aqueous Ammonia (SAA). Liquid Hot Water (LWH) was a biomass pre-treatment process using high temperature and high pressure water in to break the molecules. Other treatments were hydrothermolysis, hydrothermal, aqueous fractionation, solvolysis. While in Acid Treatment process was using acid in delignification. Both weak acid and strong acid can be used in this process. Using the acid in pre-treatment was effective to degrade the lignin. The

use of weak acid commonly followed by high temperature and continue flow process for 5 % - 10 % by weight for substrate concentrate of solid (low solid) and low temperature and batch process for 10 % - 40 % by weight for substrate concentrate of solid (high solid). The alkaline also can be used in degradation of lignin. Alkaline solution improved the affectivity of polysaccharide beside degraded the lignin. In this research, delignification process used ammonia solution called soaking in Aqueous Ammonia (SAA). The biomass soaked in ammonia solution in 28°C – 30°C with atmospheric pressure. This solvent was effectively to decrease lignin from biomass by hydrolysis ether bonds. The using of ammonia had several advantages. It has high selectivity to lignin, maintained carbohydrate in original form, very little interaction with hemicellulose and the ammonia itself can be regenerated. SAA pre-treatment was started by washing the bagasse and well dried under the sun light. Then the bagasse chopped and milled with grinder until 60 meshes. Took 50 g of bagasse was weighed and placed using Erlenmeyer for a sample and soaked with 500 mL of ammonia solution with several concentrations (0 %, 1 %, 3 %, 5 % and 7 %) by volume for 60 minutes with 121°C and 3.5 bar using autoclaved. After heating, the samples were filtered and washed until pH 7. Then the samples dried in the oven with 105°C until well dried (8 h drying). The dried samples were ready to be analysis for lignin, hemicellulose and cellulose content.



**Figure 2.** Dried bagasse as raw material

## 2.2. Acid Hydrolysis

Hydrolysis is a reaction involving water to break down a bond in a molecule using water. Breaking a bond of lignocellulose can be used water, alkaline solution, acid solution and enzyme. This treatment process generated inhibitory compounds, and the detoxification was required for removing these compounds found in the hydrolysate [9]. The success of hydrolysis process in term was depending on some factors. The main factor that influenced the hydrolysis process was the content of carbohydrate in the raw material. In hydrolysis process carbohydrate will be converted to glucose. The amount of carbohydrate content in raw material determined the amount of glucose. If in the raw material contented much carbohydrate, it decreased the molecular collisions between carbohydrate and water, so it decreased time of glucose form reaction. Bagasse as raw material in this research has 43.28 % cellulose, 16.01 % hemicellulose and 24.76 % lignin. Reaction between lignocellulose and water molecule was very slow, so needed the catalyst to make it faster. In hydrolysis cellulose and hemicellulose will be converted to become glucose by using acid solution as catalyst by activated the water, this process called acid hydrolysis. The main advantage of acid hydrolysis is that acids can penetrate lignin without any preliminary pre-treatment of biomass, thus breaking down the cellulose and hemicellulose polymers to form individual sugar molecules [10]. There were two types of acid hydrolysis, concentrated acid and dilute acid. Several types of acid, concentrated or diluted, can be used, such as sulphurous, sulphuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acid

[11], but commonly sulphuric and hydrochloric were used as catalyst for hydrolysis process [12]. The using of concentrated acid will be produced the high content of glucose (90 % glucose in theoretic). The high concentration of acid as catalyst in hydrolysis can be heated in low temperature but needed corrosion resistant equipment with high cost. It was the reasons the using concentrated acid catalyst did not common used in industry. Otherwise, the used of dilute acid was common used because this process did not need acid recovery, no acid lost in process [13] and low cost. In industry acid solutions that used were sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrochloric acid (HCl). In this research catalyst used was dilute sulphuric acid solution. This process was started by adding 500 mL sulphuric acid with 2 % concentration by volume to the samples that has been pre-treated. Then the samples heated 121°C for 120 min by using autoclaved. After heating, the samples were washed with pure water and filtered to separate the filtrate and sediment. The samples have to be washed to neutralize the bagasse.

### 2.3. Fermentation

Fermentation is a process of composition of organic compounds to produce energy accompanied by conversion of substrate to new product using microbes [14]. Fermentation has been done by scientists for a long time ago; it was one of the old technic in science. They used fermentation in process of making drinking of food. There were several factors that influenced the success of fermentation. The acidity or alkalinity solution in the term has important role in fermentation. Besides that the microbes itself has important influence in fermentation. Bacteria will grow in pH 4 – 5 [15]. The microbiology applied to fermented substrate has to have optimum temperature. All bacteria have their own optimum environmental surrounding and temperatures in which they thrive the most. In order to grow, bacteria must have an energy source. Energy sourced from carbon, other required nutrients, and a permissive range of physical condition such of oxygen concentration. In this research, to ferment bagasse used *Clostridium acetobutylicum*. *Clostridium acetobutylicum* is a commercially valuable bacterium belonging to genus *Clostridium*. *Clostridium acetobutylicum* naturally produces acetone, butanol and ethanol. *Clostridium acetobutylicum* thrived in pH 5. The samples adjusted with pH 5 and added with 5 mL *Clostridium acetobutylicum* starter. The sample fermented with varying time (2, 4 and 6 days) in anaerobe condition at 37°C to produce biobutanol. The last step, product of this research has to purify to separate pure biobutanol from aqua and others impurities using distillation.

## 3. Result and Analysis

### 3.1. Cellulose, hemicellulose and lignin Analysis

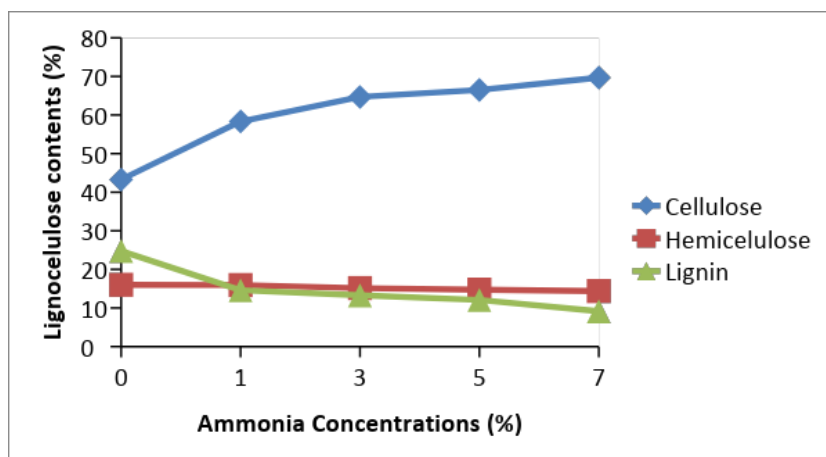
In this research, the delignification bagasse used dilute Ammonia solution. This process aimed to decrease lignin content in bagasse. Cellulose, hemicellulose and lignin content were analysed not only for samples after pre-treated but also applied to the raw material. Analysis samples to get the number of cellulose, hemicellulose and lignin content used Datta Method. One gram sample (a) was added 150 mL alcohol-benzene and refluxed at 100°C for an hour with water bath. Then the product was filtered and residue was washed with 300 mL hot water. The residue dried with oven with 100°C for 8 h until the weight constant (b). After drying the sample, 150 mL sulphuric acid 1 N was added and stirred until homogeny. This solution then refluxed with water bath at 100°C for 1 h. After an hour, the sample was filtered and washed with aqua until pH 7. The residue left at the filter was dried until the weight constant with oven at 100°C for 8 h (c). Dried residue was soaked with 100 mL of 72 % sulphuric acid by volume for 4 h. Then added 150 mL of 1 N sulphuric acid and refluxed at 100°C with water bath for 1 h. The residue was filtered and washed with H<sub>2</sub>O until the residue neutral. The neutral residue dried in the oven at 105°C until well dried (d). Then dried residue burned to ashes and weighed (e). Calculations of cellulose, hemicellulose and lignin content were used formulas below:

$$\text{hemicellulose content} = \frac{(b - c)}{\frac{a}{a}} \times 100 \% \quad (1)$$

$$\text{cellulose content} = \frac{(c - d)}{\frac{a}{a}} \times 100 \% \quad (2)$$

$$\text{lignin content} = \frac{(d - e)}{a} \times 100 \% \quad (3)$$

The result of ammonia pre-treatment showed in the graph below :



**Figure 3.** Cellulose, hemicellulose and lignin content after ammonia pretreatment

Pre-treatment process using ammonia aimed to break the bond between lignin and hemicellulose without this pre-treatment, hydrolysis of cellulose in biomass will be obstructed. From the figure 3, lignin content decreased significantly. The higher ammonia concentration used made more lignin depolymerized. The chemical structure of lignin can be changed naturally. Lignin will be agglomerated to be small particles and will be separated from cellulose [16]. The lignin content can be decreased until 63.05 % using 7 % of ammonia solution in pre-treatment Cellulose, hemicellulose and lignin content after ammonia pre-treatment. Bagasse pre-treatment used ammonia showed more lignin reduction compared to sodium hydroxide pre-treatment. When bagasse was soaked in 6 % of sodium hydroxide for 12 hours at room temperature can realize lignin up to 32.11 % [17], while the using of 5 % ammonia can reduce 51.21 % lignin. It showed the effectiveness of ammonia to break down lignin bond in pre-treatment process. The increasing cellulose content by increasing the concentration of ammonia solution in pre-treatment process was caused by degradation of other compounds. The other degradation compounds dissolved in black liquor decreased amount of other compounds in the substrate. Figure 3 showed amount of cellulose content increased by increasing ammonia concentrations. While hemicellulose content decreased by increasing ammonia concentration in pre-treatment. Hemicellulose and lignin were bound together by a variety of different chemical bond. Some of these attachments were easy to break but others were more difficult. When lignin dissolved in black liquor, so did amount of hemicellulose. It made hemicellulose content tended to decrease as well as lignin.

#### 4. Biobutanol Analysis

Biobutanol analysed using gas chromatograph. But for making sure the components of lignocellulose content, the samples must be analysed refractive index. Refractometer used to analyse the refractive index for identification of cellulose contents. The refractometer works to analyse the sample using finding the angle of reflection and correlated the numbers to refractive index. Based on the research, the results showed (Table 1) that higher ammonia concentration used in hydrolysis treatment and longer fermentation time spent made refractive index increase linearly.

**Table 1.** Refractive Index of Samples with Various Ammonia Concentrations and fermentation Times

| Ammonia | Fermentation (days) |
|---------|---------------------|
|---------|---------------------|

| (%) | 2       | 4       | 6       |
|-----|---------|---------|---------|
| 0   | 1.33007 | 1.33045 | 1.33107 |
| 1   | 1.33205 | 1.33207 | 1.33209 |
| 3   | 1.33305 | 1.33307 | 1.33405 |
| 5   | 1.34407 | 1.34507 | 1.34607 |
| 7   | 1.34645 | 1.34705 | 1.34707 |

After getting refractive index, samples analysed using gas chromatograph to get the biobutanol content. The principle of gas chromatography was injection sample solution into instrument entered a gas stream which transported the sample into a separation tube called “column”. Sample was injected to GC-2010 Plus Series with condition : column type RTX-1, column temperature was 90°C with 30 m length, inside diameter 0.25 mm, film thickness 0.25 µm with nitrogen for a carrier gas, column flow 1.13 mL/min, total flow : 60.8 mL/min, linear velocity : 30.4 cm/sec, purge flow : 3.0 mL/min, split ratio : 50.0, equilibrium time : 0.5 min, hold time : 2.4 min, injection temperature : 120°C, mode : split, detector type was FID (Flame Ionization Detector), detector temperature 120°C, sample speed : 40 msec. Sample was filtered using filter Whatman-42 before injected to the column. Then the sample analysed using internal method standard with 99 % purity of butanol. The computer will be analysed the combustion process delivered by detector and showed the result with graphic. Biobutanol content calculated with the formula below:

$$\text{Biobutanol content} = \frac{\text{area butanol sample}}{\text{area butanol standart}} \times 100 \% \quad (4)$$

In this research the highest biobutanol content has gotten in the sample treated with 7 % of ammonia and fermented for 6 days, was 0.85 %. The optimum fermentation time was in 6 days. It showed that more ammonia concentration used for pre-treatment in this research made the most biobutanol content as product.

## 5. Acknowledgement

The author are grateful to Universitas Sriwijaya for funding this research by Research of Technology Science and Art No. 1013/UN9.3.1/PP/2017.

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