**BIOETHANOL PRODUCTION FROM CASSAVA AND BAGASSE BY THERMAL HYDROLYSIS PROCESS AND LOCAL YEAST FERMENTATION**

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**ABSTRACT**

This study aims to utilize cassava and sugarcane bagasse to produce bioethanol as an environmentally-friendly alternative fuel. The raw materials were degraded by thermal hydrolysis at 145°C, 165°C, and 185°C, with the cassava to bagasse ratios at 1:2 and 1:3. The degraded material was then fermented using local yeast for 72 hours to produce bioethanol and finally, the resulting bioethanol was distilled at 85°C. The results showed that the process variables affect the yields and ethanol content produced. The best conditions were obtained in the sample with the cassava to bagasse ratio of 1:3, hydrolysis time of 120 minutes at a temperature of 185°C, and a fermentation time of 72 hours, from which the ethanol yield of 12.40% and ethanol content of 42.85% was obtained.

**Keywords:** Thermal Hydrolysis, Cassava, Bagasse, Fermentation, Local Yeast, Bioethanol.

**INTRODUCTION**

The growing demand for energy and the increasing atmospheric contamination by combustion gases have paved the way for new, safe, effective, and more accessible energy sources. In line with this, public understanding of domestic energy consumption and investigations of possible ways to produce energy from available resources, especially from renewable and environmentally friendly raw materials, are becoming increasingly necessary. Bioethanol is one of the alternative energies produced from sugar carbohydrates and biomass containing lignocellulose. Currently, bioethanol is mostly produced from cellulosic and lignocellulosic materials, especially from biomass waste which is very inexpensive and environmentally friendly. The raw materials for the biological production of bioethanol generally contain sucrose (e.g., sugar beet, sorghum, and sugar cane); starchy materials (e.g., wheat, corn, and barley), and lignocellulosic biomass (e.g., wood, straw, and grass). The main challenge in sustainable bioethanol production lies in the selection of raw materials and the right processing technology to produce clean energy without compromising food and water security. Changes in land use for the cultivation of energy crops will generally affect other sectors such as the environment and food sectors. Only when it is carried out in degraded areas or marginal lands will the energy crops cultivation have a positive impact on reducing GHG emissions. One option is to cultivate cassava plants that can grow in degraded grasslands or marginal lands so that they do not compete directly with food security. Apart from that, bioethanol produced from lignocellulosic biomass materials emits less carbon dioxide (CO₂) and poses fewer threats to food security than bioethanol produced from food crops or animal feed. One of the lignicolous biomass that has the potential to be used as raw material for bioethanol production is bagasse. The United Nations Committee on World Food Security (CFS) recommends the use of combined energy as a government policy to ensure that when biofuel production is increased, there is no risk to other resources, especially in the food security sector, such as land, biodiversity, water, and labour. For this reason, this study used a combination of raw materials derived from cassava and bagasse to produce bioethanol to minimize the risk of threats to food...
security. Bagasse (a complex polymer of cellulose, hemicellulose, and lignin) is a plentiful, renewable waste that can be used as a low-cost raw material in a variety of processes, including fermentation, biocatalysis, and chemo-catalysis, to produce value-added products such as biofuels, biopolymers, and other important and useful chemicals. In addition, bagasse contains sugar, one of the raw materials for biological bioethanol production. In such a production, sugar conversion is carried out through direct fermentation. Meanwhile, cassava has a high starch content, where the conversion of starch into ethanol is carried out through starch hydrolysis to form sugar as cassava contains 83.8% starch. Thus, the combination of cassava and bagasse is expected to increase the levels of bioethanol produced.

The conversion of starch and lignocellulosic materials into bioethanol involves three stages: pre-treatment, fermentation, and purification. Pre-treatment steps are required for the destruction of the rigid lignocellulosic structure and the hydrolysis of polysaccharides (i.e., cellulose and hemicellulose) into monosaccharides. Increasing the concentration of monosaccharides in the hydrolyzate is very important to obtain a high concentration of ethanol. Many pre-treatment methods have been developed to break down lignocellulosic biomass for bioethanol production including the use of banana peels with acid hydrolysis process, bagasse with enzymatic hydrolysis, and alkaline hydrolysis using NaOH. The utilization of these processes in the production of bioethanol is still not effective because it has a complicated process, uses chemicals that are not environmentally friendly, and requires high costs. Therefore, other methods are needed to degrade the lignocellulosic structure with a simpler process, avoid the formation of by-products that can inhibit further processes, and cost less. One of those methods is thermal hydrolysis. The thermal hydrolysis process is a process involving high pressure and temperature to break down organic compounds into low-molecular-weight organic acids. The advantage of this process is that the hydrolysis process by heat treatment does not require further steps such as pH adjustment or the use of a catalyst.

This study aims to utilize bagasse and cassava for bioethanol production by a fermentation process assisted by thermal hydrolysis. Fermentation was carried out using mixed culture local yeast containing microbes such as *Saccharomyces cerevisiae*, *Pichia sp*, *Kluveromyces sp*, *Candida sp*, *Sporotrichum sp*, *K. marxianus*, and moulds/filamentous fungi as microorganisms to convert glucose into alcohol and carbon dioxide. This mixed culture local yeast was chosen because this type of yeast is still rarely used in bioethanol production. Several studies in bioethanol production generally use a single culture such as *Saccharomyces cerevisiae* in the fermentation stage.

**EXPERIMENTAL**

The hydrolysis reactor was designed to be oval and uses anti-corrosion materials so it is safe to use at high temperatures. The reactor is equipped with a metal enclosure and can be operated up to 200°C. Samples in the form of cassava and bagasse were mashed and dried for 2-3 days to reduce the water content. Then the sample was mixed with 2000 mL of distilled water with cassava to bagasse ratio of 150:300g (1:2) and 150:450g (1:3). The samples were then hydrolyzed at temperatures of 145, 165, and 185°C for 60, 90, and 120 minutes to form a slurry. Then the tank was removed and put into a bucket filled with cold water to stop the reaction. The outcome of the hydrolysis was put into a fermentation container, to which local Aceh yeast with a concentration of 5% w/w was added. The sample was stirred until the yeast was evenly mixed, then fermented for 72 hours at room temperature under anaerobic conditions. Such fermentation usually forms three layers, with the bottom layer being a precipitate, and the top layer being water and ethanol. Water and ethanol were separated from the precipitate using a separatory funnel, after which further filtration was carried out on filter paper. Furthermore, purification was carried out using a distillation apparatus to separate water and ethanol at 85°C. Finally, chemical compounds or components in the bioethanol were identified using a Fourier Transform Infrared (FTIR) Spectrophotometer and Gas Chromatography (GC). Analysis of the physical characteristics of the bioethanol product was carried out by calculating the yield, density, viscosity, and pH.

**RESULTS AND DISCUSSION**

**Effects of Weight Ratio and Hydrolysis Time and Temperature on Bioethanol Yields**

The hydrolysis process aims to break lignin bonds, remove lignin and hemicellulose content, damage the cellulose crystal structure, and increase the porosity of the material being processed. Damage to the crystalline structure of cellulose will make it easier for cellulose to decompose and be converted into...
glucose. In addition, hemicellulose decomposes into simple sugar compounds such as glucose, galactose, mannose, hexose, pentose, xylose, and arabinose, among other things. Microorganisms will ferment these simple sugar compounds, resulting in the production of ethanol. Parameters of acid concentration, temperature, and time of hydrolysis are very important in the hydrolysis process to minimize the product in the hybrid which in turn increases the ethanol yield in the fermentation process. Figure-1 shows the effect of hydrolysis temperature on the yield of bioethanol produced. Hydrothermal pre-treatment at high temperature and pressure led to the disintegration and separation of the lignocellulosic matrix. Most of the hemicellulose and some of the lignin could be degraded during hydrothermal treatment. Therefore, an increase in temperature and hydrolysis time will increase the yield obtained, which is resulted from the increasing number of sugar monomers being broken down and their bonds degraded. Ethanol production increased because the lignin content of the solid residue decreased.

Figure-2 also shows the effect of hydrolysis time on bioethanol yield at various cassava to bagasse ratios. The bioethanol yield which was obtained at the ratio of 1:2 and 1:3 at the hydrolysis time of 60, 90, and 120 minutes and the hydrolysis temperature of 185°C were directly proportional. The highest bioethanol yield (12.40%) was obtained from the sample in a 1:3 ratio with a hydrolysis time of 120 minutes at 185°C. The result is higher than that in previous studies using bagasse with a thermal hydrolysis process, which resulted in a lower ethanol yield (11%).

Effects of Weight Ratio and Hydrolysis Time and Temperature on Bioethanol Content
In the hydrothermal process, there will be ionization of water which produces H⁺ ions in the subcritical region. The production of H⁺ ions which increases with increasing operating temperature can cut more glycosidic bonds in cassava and bagasse biomass. H⁺ ions will attack the glycosidic bonds in starch, causing instability due to the transfer of carbon-oxygen electrons to form monomers and oligomers from polysaccharides, thereby forming reducing sugars which get higher as the hydrothermal temperatures rise. In Fig.-3 it can be seen that the hydrolysis time and temperature greatly affected the bioethanol content produced. In the 1:3 sample, 60-minute hydrolysis at the temperatures of 145°C, 165°C, and 185°C, the ethanol content produced was 36.63; 44.19; and 46.03% consecutively. Then, at 90 minutes the ethanol content became 38.63; 47.07; and 46.94%. Meanwhile, at 120 minutes of hydrolysis, the ethanol content
rose to 38.56; 54.06; and 58.48%. In other words, as the hydrolysis time and temperature increase, the ethanol content produced also increases. The rise of the temperature of the water as a solvent to approach the critical point in the hydrolysis process can increase the production of hydronium ions (H$_3$O$^+$) and hydroxide (OH$^-$). This makes various reactions with water more likely and follows the Arrhenius law of reaction rate constants. The high ionization product indicates that there is a strong acid and a strong base at the same time. Due to the presence of these acids and bases, water used as a solvent in the hydrolysis process will act as a degradation agent, where lignin will be degraded so that starch, cellulose, and hemicellulose which are glucose in polymer form will be broken down into glucose in the form of monomers. Then the increasing hydrolysis temperature and time will cause more lignocellulosic bonds to be broken and separated so that more glucose will decompose. An increase in the decomposed glucose will have an impact on increasing ethanol content due to more sugar being converted through the fermentation process.

Figure 4 shows that the ethanol content increases along with the increase in the cassava to bagasse ratio. At 1:2 ratio and 185°C hydrolysis temperature and 60, 90, and 120 minute hydrolysis time, bioethanol content obtained was 20.28; 22.98; and 30.34% respectively while at 1:3 ratio and 185°C, the bioethanol content was 46.03; 46.94 and 58.48% for 60, 90, and 120-minute hydrolysis time respectively. In a previous study, in the bioethanol production process from sugarcane bagasse with the addition of hydrolyzate in the form of acid, the bioethanol yield was lower (2.51%), and when raw materials were combined, the ethanol content obtained would jump significantly to the range of 10.60%-42.85%. This proves that the combination of raw materials will produce higher ethanol content.

Characteristics of FTIR and GC
FTIR analyzes the chemical structure by identifying the functional groups present in each sample analyzed. The identification of each specific bond absorption of each functional group serves as the foundation for the interpretation of the infrared spectrum, which is a complex process. The results of the functional group analysis using FTIR are in the form of a spectrum so that the functional groups obtained can be analyzed.
FTIR results show that at wavenumber 3259.70 cm\(^{-1}\) there is strong and wide absorption, indicating that there is an interaction between electronegative O molecules with positive H which forms hydrogen bonds. The O-H bond in alcohol absorbs at a higher wavenumber than an acid, which is between 3230-3550 cm\(^{-1}\). Small or weak absorption in the wavenumber region of 2900-3000 indicates the presence of asymmetric and symmetrical C-H bonds of CH\(_2\). In the spectra, a stretch area is seen with a wavenumber of 1639.49 cm\(^{-1}\), where at a wavenumber of around 1600-1700 cm\(^{-1}\) there is absorption indicating the presence of O-H bending bonds, while absorption at a wavenumber of 1100 cm\(^{-1}\) indicates a stretching C-O bond. Accordingly, it can be identified that the sample has O-H, C-H, and C-O bonds. This indicates that the sample from the experimental results is ethanol and is following the spectra of the standard solution as shown in Figure-5. Energy from infrared radiation is absorbed at a specific frequency by each signal, resulting in bond vibrations within the molecule as a result of the energy absorption. A variety of signals are easily used to identify a particular type of bond in a molecule. The area to the right of the diagram (from 1500 to 500 cm\(^{-1}\)) usually contains very complex absorbent forms. This is because all types of molecules absorb in this region. Based on the data of ethanol standard area, further data on the retention time of the bioethanol and its concentrations were obtained and are presented in Table-2.

Table-2: Results of Bioethanol Analysis by GC

| Percent Bioethanol (%) | Retention Time (min) | Area (Counts*s) |
|------------------------|----------------------|-----------------|
| 10.60                  | 3.94                 | 78.201          |
| 42.85                  | 3.94                 | 329.998         |

The results show that the ethanol content of 10.60% and the retention time of 3.94 minutes have an area of 78.201, while the ethanol content of 42.85% has a retention time of 3.94 and an area of 329.998. These results indicate that in this sample there is only ethanol produced from distillation with the glucose fermentation process in cassava and bagasse. The process of glucose fermentation produces ethanol.

Test Results of Bioethanol Physico-Chemical Characteristics
Samples of bioethanol obtained at optimum conditions were taken, to which the testing of physical and chemical characteristics including density, viscosity, pH, and ethanol content was performed. The results based on the resulting wavenumber. Figure-5 shows the FTIR spectra of ethanol. Identification of the functional groups of bioethanol and ethanol is presented in Table-1.
were then compared with the standards in the SNI (the Indonesian National Standards). This was carried out to see whether the bioethanol produced is in accordance with SNI so that the product feasibility can be determined. Table-3 shows the physicochemical characteristics of bioethanol that this study produced.

| Parameters | Unit | Bioethanol in this study | SNI No. 7390: 2012 |
|------------|------|--------------------------|-------------------|
| Densities  | (g/mL) | 0.886 - 0.982 | Max. 0.7890 |
| Viscosities | mm²/s, cSt | 0.811 - 1.207 | 1.16 - 1.17 |
| pH         | - | 4.0 - 7.0 | 6.0 - 9.5 |
| Solubility | - | Soluble | Max. 0.7807 |
| Colour     | - | Clear (colourless) | Clear (colourless) |
| Bioethanol content | (%) | 8.96 - 42.853 | 94.0 - 99.5 |

Based on the physical and chemical characteristic tests carried out on the bioethanol sample from the study, several variables have not met the Indonesian National Standard 7390:2012 yet. The resulting bioethanol still has a high content of water as a result of a simple distillation process, in which the separation did not take place completely. In addition, since the mass of the sample used was not on a large scale, the conversion of glucose produced was very small so that during the fermentation process, the conversion of glucose into bioethanol produced a small amount of ethanol as well.

**CONCLUSION**

The combination of cassava and bagasse can be a promising raw material for bioethanol production. It is more suitable for the biorefinery approach due to its worldwide abundance, high carbohydrate content, and the fact that the use of these two raw materials does not compete with the available land for food and feed production. The results showed that the increase in temperature, time, and material ratio had a significant effect on the increase in the ethanol content produced. The highest ethanol concentration achieved in this study was 42.85% with a 12.40% yield in the sample with a weight ratio of 1:3 and at the hydrolysis temperature and time of 185°C and 120 minutes. The results of the physical characterization of the resulting bioethanol which included density, viscosity, and pH showed better values than those in the previous studies. The presence of special functional groups of ethanol such as O-H, C-H, and C-O groups was also identified in the FTIR analysis.

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