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Article Reviews: Bioethanol Production from Biomass Waste Using Fermentation with The Assistance of Yeast

Wiwik Pratiwi[1], Ni Made Suaniti[2*]

[1]Master of Chemistry, University of Udayana, Indonesia

[2]Chemistry Department, University of Udayana, Indonesia

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Abstract. Bioethanol is a biomass-based ethanol compound containing starch, sugar, and cellulose. The most common production of bioethanol uses hydrolysis and fermentation methods. The following article aims to compare the levels of bioethanol produced from various types of biomass using multiple methods. The process can vary in the concentration of acids and bases used in hydrolysis, fermentation time, and yeast mass. The article review results found that the highest bioethanol content was obtained from bioethanol made from bagasse, with an ethanol content of 41% by adding 1% sodium hydroxide to dextrose agar (PDA) media.

Keywords: *Bioethanol, Fermentation, Glucose, Hydrolysis, Yeast.*

1. Introduction

Bioethanol is a biochemical liquid from a fermentation process that contains glucose, cellulose, and starch or carbohydrates with the help of microorganisms. Bioethanol is a renewable energy source with the properties of a clear, colorless liquid that can be biodegraded and is an environmentally friendly fuel [1]. Bioethanol can be used to reduce CO_2 (carbon dioxide) emissions and as a substitute for fossil fuels because it can be produced from renewable biomass. Bioethanol has a higher O_2 (oxygen) content than BBM, which is 35%. Bioethanol has an octane number of 118 and a lower CO (carbon monoxide) gas content ranging from 19 – 25% [2].

Bioethanol production from lignocellulosic generally goes through the stages of delignification, hydrolysis, fermentation, and distillation [3]. The delignification process is carried out to facilitate the breakdown of lignin, a cellulose fermentation inhibitor, into simple sugars [4]. This process can be done with the help of chemicals such as NaOH. The

* corresponding author: madesuaniti@unud.ac.id

microorganism used to produce bioethanol in this study was Saccharomyces cerevisiae [5]. This type of bacteria is a bacterium that is resistant to alcohol from a reasonably high fermentation yield (12 – 18% v/v), is resistant to high sugar levels, and remains active in carrying out fermentation influenced by fermentation temperature factors and fermentation time [6].

Raw materials that can become bioethanol come from corncob, cassava peel, bagasse, molasses, and organic waste [7]. Agricultural and organic waste require special handling to avoid harming the environment. Organic waste such as vegetables, fruits, household waste, and food waste can be converted into bioethanol. Retno & Nuri (2011) have also researched using banana peels to produce bioethanol. Bioethanol from bagasse was also proven by Restiawaty *et al.* in 2020. Waste paper, seaweed, rice straw & Dao skin have been studied and confirm that these materials can be used as a fuel in bioethanol [8].

Based on this background, problems related to environmental waste management can be addressed so that it can be reused. The author will develop this in the title "Article: Production of Bioethanol from Biomass Waste Using Fermentation with the Assistance of Yeast".

2. Methods

The most frequently used methods in making bioethanol are hydrolysis and anaerobic fermentation. Hydrolysis is the process of breaking the bonds of a molecule using water. Hydrolysis in making bioethanol aims to obtain glucose [9]. Compounds that can be used in hydrolysis are acidic compounds, one of which is HCl. H^+ ions in HCl during hydrolysis will change the fiber from plants so that they become free radical groups. Then the OH^- group of the H_2O molecule will bind to a free radical group to produce glucose. The high concentration of an acidic compound used during hydrolysis will affect the glucose levels produced [10]. However, the higher the acid concentration, the lower the water content in the hydrolysis solution.

Retno and Nuri (2011) produced bioethanol from banana peels using hydrolysis and fermentation. Banana peels, which will be processed into bioethanol, are pretreated first by grinding them to make banana peel starch. Banana peel starch was analyzed for water content and starch content. The following process was hydrolysis, which in this study used an acidic compound, namely 0.5 N sulfuric acid (0.5 N H_2SO_4), which was heated at 100 °C for 2.5 hours [11]. The hydrolysis results are added with nutrients for fermentation in the form of ammonium

sulfate and urea. Pasteurization was carried out at 120 °C for 15 minutes. The fermentation process is carried out with the help of the yeast Saccharomyces cerevisiae. The fermentation was anaerobically (not requiring oxygen) at 27 – 30 °C for 48, 96, 144, and 192 hours. Bioethanol formed after fermentation was analyzed for its levels [12].

Research conducted by Restiawaty et al (2020) entitled Bioethanol Production from Sugarcane Bagasse using Neurospora Intermedia in an Airlift Bioreactor used a chemical treatment method from bagasse, with the help of 1% NaOH. Bagasse that has gone through the alkaline treatment process is neutralized with water to a pH close to 7 and then dried at 60 °C for 48 hours [13]. The inoculum used, N. intermedia, was prepared first in dextrose agar (PDA) media in an incubator for 7 – 10 days at 30 °C. In an airlift bioreactor, media nutrient preparation was carried out for the Simultaneous Saccharification and Fermentation (SSF) process. The fermentation process in this study produced a 41% yield of bioethanol [14].

Zimife et al (2002) in a study of bioethanol production from paper waste began with a pretreatment process in the form of sulfuric acid with concentrations of 5, 10, and 50% as much as 40 grams, and allowed to stand for 6 hours. In addition, it also uses variations of pretreatment in the form of alkaline compounds using sodium hydroxide, which is the same treatment as acid pretreatment. Hydrolysate from paper waste pretreated with acidic and alkaline compounds can stand at room temperature for 5 – 7 days [15]. The substrate undergoing pretreatment is added with water and DNS (Determination of Reducing Sugar Concentration). The mixture was placed in a water bath at an increased temperature for 5 minutes. The mixture is added with Rochelle salt and left to cool. The solution was analyzed using a UV spectrophotometer at 540 nm. Bioethanol production is carried out with the help of yeast in the form of Saccharomyces cerevisiae [16]. The substrate and yeast were stirred at 800 rpm. The stirring is carried out for 1 hour daily for up to 3 days. After fermentation, the mixture is purified by distillation at 78 °C [17].

Kolo et al (2021) produce bioethanol from seaweed using acid hydrolysis (H_2SO_4) and alkaline hydrolysis (NaOH). The process of preparation and delignification of seaweed using sun drying. The powder produced after milling was analyzed for its morphology using SEM [18]. Delignification uses an alkaline compound, 2% NaOH, and is heated at 90 °C for 30 minutes. The delignified samples were hydrolyzed with an acid compound (2% H_2SO_4). The filtered delignified solid fraction was analyzed for morphology by SEM, while the searched fraction was analyzed for reducing sugar content by the DNS method using a UV-Vis

spectrophotometer. The hydrolysate was sterilized by autoclaving at 12 °C for 15 minutes before being used for the fermentation process [19]. The fermentation process uses the yeast Saccharomyces cerevisiae at 30 °C for 6 days with a pH of 4.5. The inoculum used was 10% (v/v). The fermented product is purified by distillation at a temperature of 75 – 80 °C for 1 – 2 hours or until the bioethanol no longer drips [20]. The resulting bioethanol was analyzed qualitatively using the oxidation of Potassium dichromate ($K_2Cr_2O_7$) [21]. Bioethanol is added with potassium dichromate and concentrated H_2SO_4. A change from orange to green indicates a positive sample containing bioethanol—quantitative analysis of bioethanol using a pycnometer and gas chromatography [22].

Baharuddin et al (2016) produced bioethanol from rice straw and Dao tree bark. The process involved cutting and delignification using 0.5 N NaOH at 105 °C for 24 hours. It was then crushed to produce cellulose powder. Inoculums were prepared from *Aspergillus niger* with liquid media in the form of glucose and nutrient broth [23]. The fluid medium was shaken for 48 hours at 130 rpm to produce an *Aspergillus niger* suspension. This process also uses cellulase enzymes from solid-liquid media. Rice straw is used and added with nutrients urea, magnesium sulfate heptahydrate, potassium hydrogen phosphate, and distilled water. The pH media was adjusted to pH 5 and then sterilized by autoclaving at 121 °C for 15 minutes. The media was incubated for 96 hours at 30 °C. The fermented product was extracted with distilled water, stirred for 1 hour at 150 rpm, and then filtered to obtain enzymes. Rice straw is used in the simultaneous saccharification and fermentation process (SSF) and is added with cellulase enzymes [24]. The mixture was shaken at 170 rpm for 24 hours, and *Saccharomyces cerevisiae* was added, and stirred at 150 rpm. The saccharification and fermentation process was carried out within 3, 5, 7, and 9 days. The solution is filtered and then distilled at 80 °C for 1.5 – 2 hours. The bioethanol content obtained was analyzed, and the glucose level was tested using the Nelson-Somogy method [25].

3. Result and Discussion

The results obtained from the journal review are the percent yield of bioethanol produced from various raw materials and the various methods used. Research conducted by Retno & Nuri (2011) carried out a banana peel pretreatment process to produce banana peel starch. The resulting banana peel starch was analyzed for its starch content, so the water and starch contents were 93.8610 and 3.68%, respectively. Banana peel starch is hydrolyzed using

sulfuric acid compounds to produce a glucose level of 3.13% [26]. The hydrolyzed filtrate was fermented with the help of 0.0624 grams of Saccharomyces cerevisiae yeast with variations in fermentation time, namely 48, 96, 144, and 192 hours. Variation in fermentation time certainly results in the amount of bioethanol and the percentage of bioethanol produced. The percentage of bioethanol produced from each fermentation time is presented in Table 1.

Table 1. Results of the Effect of Fermentation Time on the Percentage of Alcohol at a Temperature of ± 27 °C and a Yeast Weight of 0.0624 Grams

Fermentation Time	Alcohol	
(Hours)	Weight (Grams)	(%)
48	4.7502	3.8737
96	4.7205	7.7828
144	4.6796	13.5406
192	4.6794	13.4416

(Source: Retno and Nuri, 2011)

The data obtained related to the levels of bioethanol produced from various time variations is presented in graphical form to determine the effect of time variations on the levels of bioethanol produced. The data is contained in Figure 1.

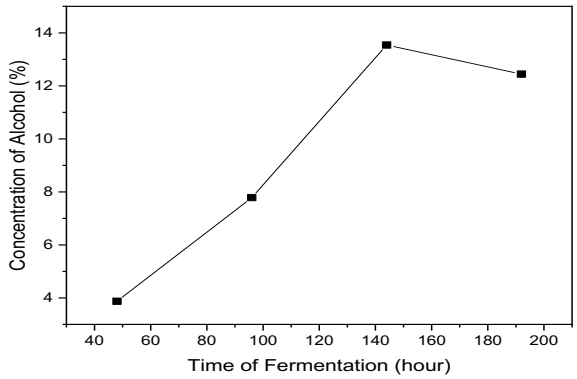

Figure 1. Graph of the Effect of Time Variation on Alcohol Levels

The alcohol content increased from 48 to 144 hours of fermentation. The increase in alcohol content was because during this time the yeast microorganisms used experienced good growth, and the yeast still received sufficient nutrition to grow and produce bioethanol [27]. However, at 192 hours, the alcohol content decreased; this was caused by the yeast having reached the maximum limit for breeding and producing bioethanol. This can also be affected by the nutrients used for fermentation that have run out, so that the yeast cannot produce bioethanol, so the yeast eats the bioethanol, and the formation of acetic acid occurs. In addition

to time variations, this study used variations in the weight of the yeast used. The weight of the yeast used was analyzed based on the influence of the yeast on the alcohol content produced. The optimum yeast weight used was 0.0624 grams to produce an alcohol content of 13.53%. The yeast mass is the optimum amount according to the nutrients used [28].

The research results by Restiawaty et al (2020) obtained different bioethanol levels for the Potato Dextrose Broth (PDB) concentration used. The highest bioethanol yield was produced from a PDB concentration of 13.25 g/L, which was 44%. This is because more *N. intermedia* biomass is formed. The amount of *N. intermedia* formed will undoubtedly affect the yield of the resulting bioethanol. This is consistent with the rate of ethanol production, which depends on the rate of glucose production from glucose degradation by cellulase enzyme activity.

Research conducted by Zimife et al (2002) suggested that the pretreatment process using acidic compounds was more effective in breaking down the lignocellulosic structure than alkaline compounds. The concentration of sulfuric acid used is 10%; this concentration has a mild nature so that it can avoid the formation of compounds that can inhibit the work of enzymes [29]. Pretreatment using alkaline compounds will produce relatively low sugar concentrations because alkaline compounds cannot break down the paper's structure, so that cellulose will be easily susceptible to enzyme action. The effect of acid concentration on ethanol content is presented in Figure 2, while the impact of base concentration on ethanol content is presented in Figure 3.

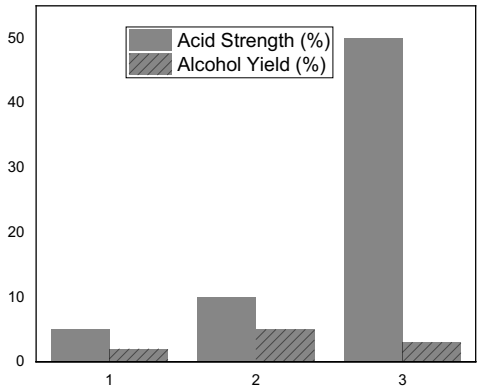

Figure 2. Effect of Acid Concentration on Ethanol Content.

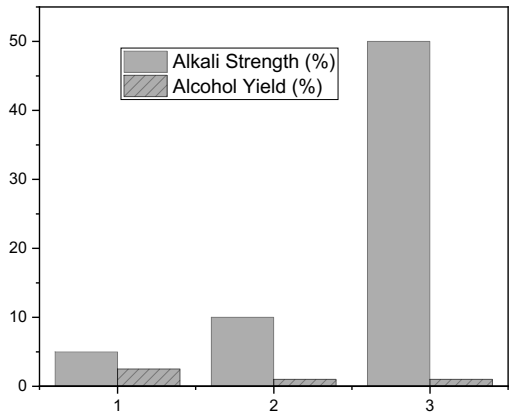

Figure 3. Effect of Alkali Concentration on Ethanol Content

Kolo et al. (2021) researched bioethanol production from seaweed using hydrolysis. Before being hydrolyzed, the seaweed powder that had gone through the pretreatment process was analyzed for its morphology using Scanning Electron Microscopy (SEM), so that the result was that before the hydrolysis and delignification process, the surface structure of the seaweed powder was still intact and compact. However, it differs from the results of the analysis of seaweed powder that has gone through the hydrolysis and delignification processes, namely, the surface texture of the seaweed powder is significantly damaged. This can happen because the delignification process uses the basic compound NaOH, which can damage the lignin structure of the seaweed powder, and is also caused by the hydrolysis process [30]. Hydrolysis using sulfuric acid can break the β-1,4-glycosidic bond in cellulose and break β-1,4-D-pyrasonyl in hemicellulose to become a simple sugar. The process uses hydrolysis with delignification and hydrolysis without delignification using different temperature variations, producing various levels of reducing sugars. The hydrolysis process without delignification produced a reducing sugar content of 23.7% at 150 °C, which is the optimum temperature that can be achieved to deliver the highest reducing sugar content. As for hydrolysis by delignification, a reducing sugar content of 27.3% was obtained at 150 °C. This result is the optimum sugar content that can be achieved in this study. In addition to the effect of temperature, this study also used time variations to reduce sugar levels. According to this study, the optimum time for hydrolysis without delignification was 50 minutes with a maximum reducing sugar content of 33.4 g/L. As for the hydrolysis process by delignification, the optimum time was 60 minutes with a reducing sugar content of 28.0 g/L. The hydrolysis process using a low acid concentration will obtain a glucose yield of 50% in a relatively short time.

The fermentation process was carried out after hydrolysis and delignification with the help of the yeast Saccharomyces cerevisiae. The fermentation results were tested qualitatively using the oxidation method with Potassium dichromate ($K_2Cr_2O_7$). The testing process uses pure ethanol as the reference standard. The fermented product is proven positive for containing ethanol when the color changes from orange to green. Quantitative analysis of the levels of bioethanol produced using a pycnometer and gas chromatography (GC) [31]. Tests using a pycnometer obtained the density of seaweed bioethanol at 0.9927; this result is higher than the absolute ethanol density of 0.789. The difference in results could be because the bioethanol from the research results still contains water, as it had not been purified by repeated distillation. The result of the conversion of specific gravity is only 1% of the resulting bioethanol. The results of the quantitative test by gas chromatography showed that the purity of the bioethanol obtained by this method was 5.02% with a retention time of 3.264 minutes. The difference in results between the qualitative test (pycnometer) and the quantitative test (gas chromatography) can be caused by the extended time gap between pycnometer analysis and GC analysis. Another thing that can affect it is the density pycnometer method used, namely, the density of a mixture of water and bioethanol, while in gas chromatography, only bioethanol is detected to measure the concentration of bioethanol.

Baharuddin et al (2016) produce bioethanol from rice straw and Dao trees, which produce bioethanol content of 0.24 and 0.97%, respectively. The delignification process on rice straw and Dao tree was 105 and 216 mg/L. The difference in glucose levels between rice straw and the Dao tree is due to lignocellulose in the Dao tree, which contains more cellulose than rice straw.

Using Benedict's reagent, qualitative tests can also be carried out to determine the presence of glucose from hydrolysis. It is said to be positive for glucose, seen from the color change to greenish yellow and an orange precipitate. Quantitative test of bioethanol resulting from rice straw fermentation obtained a maximum bioethanol content of 0.242% with an optimum fermentation time of 7 days, while in Dao trees it was 0.978% with a fermentation time of 7 days. The increase in bioethanol levels from day 3 to day 7 was caused by yeast and nutrients being able to develop optimally, so the bioethanol produced increased. However, after 7 days, the bioethanol content decreased; this could be because the substrate to be converted into bioethanol had run out, so the resulting bioethanol would turn into organic acids. The GC method is also used to analyze the levels of bioethanol produced in the fermentation process.

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In gas chromatography, the bioethanol content produced from rice straw was 0.24% with a retention time of 2.703 minutes, while in dao trees it was 0.97%. The research results have been summarized in Table 2.

Table 2. Results of Research on the Production of Bioethanol from Biomass

Raw Material	Method	Results	References
Banana Peel	Pretreatment,acid hydrolysis fermentation using the *Saccharomyces cereviseae*	-Glucose content hydrolyzed by 3.13% -The resulting bioethanol content is 13.540%	[2]
Sugarcane Bagasse	Pretreatment (physics and chemistry). Sugar and bioethanol were analyzed using High Performance Liquid Chromatography (HPLC)	Yield bioethanol is 41% and the optimum pH medium is 3 – 3,5. NaOH 1% added results in increased cellulose content from 25,3 to 54%	[3]
Paper Waste	Pretreatment using H_2SO_4 and NaOH. Fermentation using *Saccharomyces cereviseae* with bioethanol separation using the distillation method at 78 ^oC	The concentration of reducing sugar is obtained at a concentration of 0.8 ppm with an absorbance value of 0.41 nm (10% sulfuric acid). yield alcohol produced by 8% with the addition of sulfuric acid by 10%	[4]
Seaweed	Pretreatment using H_2SO_4 and NaOH. Inoculum media using *Saccharomyces cereviseae*	Seaweed powder was analyzed for morphology using the Scanning Electron Microscope (SEM) method. The results after the delignification and hydrolysis processes showed that the surface texture of the powder was damaged. The highest reducing sugar content at 50 minutes of 33.4 g/L for hydrolysis without delignification while for hydrolysis with delignification of 28 g/L within 60 minutes	[5]
Rice straw and dao tree bark	Using NaOH as delignification process. Hydrolysis using cellulase enzymes (saccharification). Fermentation using *Aspergillus niger* inoculum	Glucose and dao tree levels were 105 and 216 mg/L, respectively. The ethanol content of rice straw cellulose through the process of simultaneous saccharification and fermentation (SFS) is 0.24%. while in the dao tree of 0.97%.	[6]

4. Conclusion

Bioethanol can be produced from raw materials such as sugarcane bagasse, banana peels, seaweed, paper waste, rice straw, and dao trees. Methods that can be used to produce bioethanol are hydrolysis, delignification, and fermentation. The fermentation used is anaerobic; that is, it does not require air or oxygen. Yeast Saccharomyces cerevisiae was chosen

as an assistant in fermentation to produce bioethanol. One of the most significant levels of bioethanol produced is 41% from sugarcane bagasse with the addition of 1% NaOH.

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