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Characterization of Bioethanol from Tuber of Porang Waste Fermented with *Saccharomyces cerevisiae* Enzyme: Effect of Fermentation Time and Yeast Ratio

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ABSTRACT

The production of bioethanol from non-plant lignocellulosic materials has reached a commercial scale and is advocated as a possible solution for the decarbonization of the transport sector. Porang pulp tubers can be converted into bioethanol because they have abundant potential due to their high glucomannan content. The purpose of this study was to determine the effect of fermentation time and the ratio of yeast addition on bioethanol production. The methods used are hydrolysis, fermentation and distillation methods. The hydrolysis process used 5% (v/v) HCl catalyst, the fermentation process with 6 gr sample powder tuber of waste used *Saccharomyces cerevisiae* bacteria with varying ratios of 2.5, 4 and 6.5 g and variations in fermentation time for 2, 4 and 6 days at 38-40˚C. The results showed that the duration of fermentation had a significant effect on the yield of bioethanol, where microorganisms have the opportunity to break down more glucose to produce bioethanol. While the ratio of the addition of yeast added to the fermentation process, the greater the ratio of the addition of yeast, the greater the bioethanol produced. Where the addition of 6.5 grams of yeast and 6 days of fermentation time, resulted in a yield of 9.889%, a bioethanol concentration of 37.599%, a refractive index of 1.3642 and a density of 1.04 g/ml.

***Keywords*:** bioethanol, distillation, fermentation, hydrolysis, tuber of porang.

1. INTRODUCTION

Fossil energy is the main source which accounts for around 80% of global energy demand, but the drawback of using fossil energy is that it comes from non-renewable materials [1], erratic prices, resulting in global warming [2], ecosystem imbalance [3]. Therefore, an alternative that is environmentally friendly, abundant, safe and sustainable is needed [4,5] by developing renewable energy sources. Considerable attention has been focused on utilizing biomass as an alternative energy source not only to protect the environment but also to meet energy demands.

Interest in the production of biofuels (biomass-based fuels) has increased drastically to replace fossil-based fuels. Biofuels, such as bioethanol, biodiesel and biogas, has become a viable and economical solution. Bioethanol is a potential alternative fuel due to its properties of having a higher flash point, the heat of vaporization and octane number which are the main reasons to encourage its production [6] compared to gasoline. Bioethanol is by far the most widely used biofuel for transportation worldwide because it minimizes the use of fossil fuels and environmental pollution. The technology for bioethanol production from biomass feedstock consists of several stages and varies depending on the type of raw material used. Bioethanol is a liquid fuel derived from plants containing starch and cellulose

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components, through the process of glucose fermentation, distillation for the purification process [7].

Kafale, et al. [8] has conducted a study with coffee waste raw materials using saccharomyces bacteria at a fermentation temperature of 30℃ and pH 5, producing ethanol of 7.4 g/l, coffee skin waste produces bioethanol purity of 39.79% and using Zymomonas bacteria produces bioethanol of 38.78% [9]. Ginger fiber 43.4 g/l [10], tubers raw materials 50-80 g/l [11], Cassava Stem of 37.5 g/l [12], Corn Stover 41,9 g/l [5], fresh elephant ear plant weed 0,03 mg/ml [6] and from wild cassava crude starch 21,64 g/l [7].

However, until now no one has produced bioethanol from tuber of porang waste (Amorphophallus Muelleri), so researchers are taking advantage of the potential tuber of porang waste which has abundant potential sources due to its high glucomannan and sellulose content content [13]. Porang plants in Indonesia have production centres in East Java, NTT, Banten, Central Java, Kalimantan and Sumatra. Indonesia's porang tuber production in 2020 will reach 142,000 tons of which 89.65% is processed into porang chips for export purposes. In 2024 porang tuber production will reach 600,000 tons. Porang production which is quite large can be used as a raw material in bioethanol production [14]. Therefore, the purpose of this article is to determine the effect of bioethanol production on the time of fermentation using *Saccharomyces cerevisiae* bacteria and the optimal yield from the addition of yeast mass ratio. The results of this study are expected to produce bioethanol that is more optimal in terms of high concentrations compared to previous studies made from lignocellulosic raw materials.

2. METHODOLOGY

2.1. MATERIAL PREPARATION

The porang tubers used came from the city of Medan, Indonesia. The porang tubers were previously washed thoroughly using running water to remove the soil on the skin of the porang tubers. Next, peel the outer skin and chop it to a size of ± 5 cm, as shown in Figure 1 (b), then wash it with running water to remove the mucus that is attached to the flesh of the tuber.

Porang is a herbal plant that can grow up to 1.5 meters high with the Latin name Amorphophallus Muelleri. It is a potential source of glucomannan in Indonesia because of its high glucomannan content. Porang plants grow a lot around tropical forests and only grow under tree supports. Porang can survive on any type of soil at an altitude of 0 to 700 meters above sea level. The characteristics of the porang plant are that it has broad leaves and pointed leaf tips; smooth and yellowish bark; tubers are inedible and unpalatable as they contain high levels of calcium oxalate; on the surface of the tubers there are no nodules of fine fibrous tubers and are yellowish in colour [13,15].

This plant can be used as an alternative food ingredient since it has a starch content of 76.5%, 9.20% protein, 25% fiber, 0.20% lipid, and contains glucomannan compounds and oxalic acid crystals which are relatively high [16]. The dry porang tubers contains cellulose, hemicellulose and lignin respectively are 8.54%, 43.3% and 5.85%. The content of starch in the porang tubers is 71.25%, which strach is a polysaccharide compound which consists of amylose and amylopectin. Starch in the porang tubers is so high that the bulb can be converted into ethanol by using the enzyme amylase that will break the monosaccharide monomers on starch into glucose. Besides we can also use yeast S. cerevisiae to break down glucose into ethanol [17].

Porang tubers that have been chopped and washed are then mashed using a blender with a 1:1 ratio of water and tubers, then filtered to separate the starch and dregs from the porang tubers. The porang tuber pulp is then dried in the sun for 4-5 days, and then in the oven at 105˚C for 1 hour to dry. Porang tuber dregs that have been dried, then pulverized to a size of 80 mesh [18]. The preparation of raw as in flowchart Figure 2.

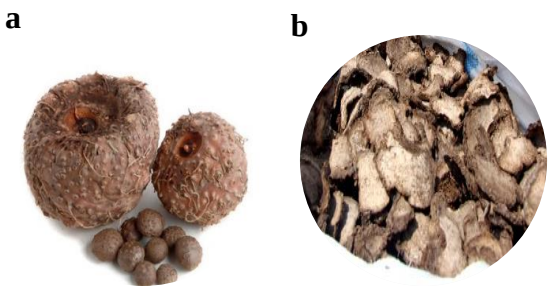

Figure 1. (a) tuber of porang (*Amorphophallus onchophyllus*) and (b) Skin tuber of porang.

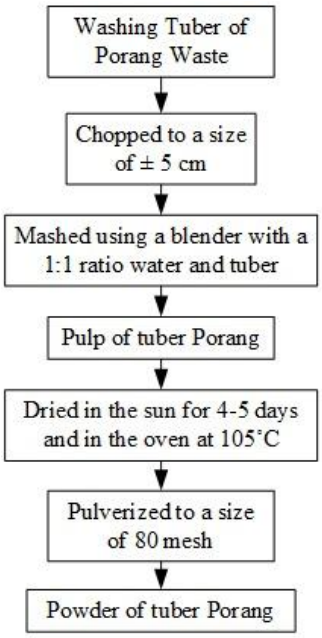

Figure 2. Flowchart preparation tuber of Porang waste.

2.2. HYDROLYSIS

The hydrolysis process is carried out in acidic conditions by adding a tuber of waste as much as 60 g using a 5% HCl catalyst (v / v) and inserted into a three-neck flask. Then the mixture is heated at a temperature of 110˚C for 3 hours (to prevent the loss of moisture, then the three-necked flask is equipped with reflux). Furthermore, the results of the hydrolysis solution are cooled at room temperature and filtered to separate residues and filtrates. Hydrolysis process as in flowchart Figure 3.

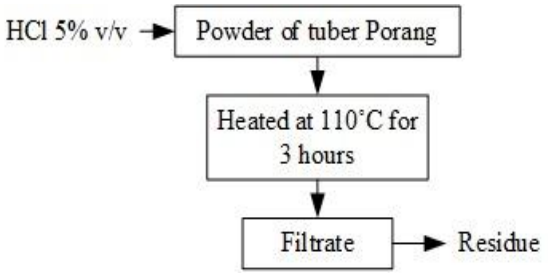

Figure 3. Flowchart hydrolysis process of Powder Porang waste.

2.3. FERMENTATION

The fermentation process begins by adding NaOH 1N solution to the filtrate solution resulting from hydrolysis until it reaches pH 5. After the filtrate solution reaches pH 5, *Saccharomyces cerevisiae* yeast is added with a ratio of 2.5, 4 and 6.5 grams, respectively, and nutrients are added in the form of a mixture of NPK and Urea. Then the filtrate solution is stirred for 5 minutes until homogeneous. The fermentation process is carried out anaerobically for 2, 4 and 6 days at a temperature of 38-40°C (ambient temperature). Previously, the fermentation container was purging using nitrogen gas to remove the air in the fermentation container.

2.4. DISTILLATION

The results of fermentation of porang tuber filtrate are then put into the distillation flask and refluxed at a temperature of 80 °C (estimated boiling point of the ethanol mixture is 80 °C). The distilled product is then put into a glass bottle and closed tightly, to minimize evaporation. Fermentation and distillation process as in flowchart Figure 4.

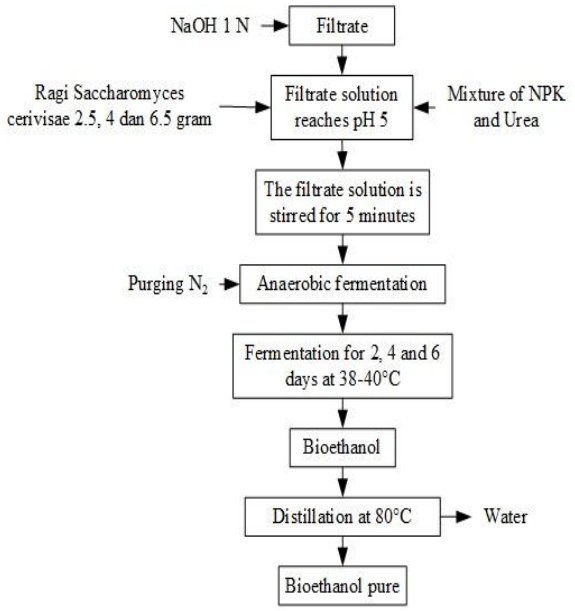

Figure 4. Flowchart fermentation and distillation process of filtrate Porang waste.

2.5. CHARACTERIZATION OF BIOETHANOL

The physical and thermophysical properties of bioethanol made from tubers of porang waste, a number of analyses were carried out including density, yield (amendment) analysis and Gas Chromatography Mass Spectrometry (GC-MS). Density measurements are carried out using a pycnometer is used ISO standard; ISO 1183-1:2004 and ASTM standard: ASTM D854. The chemical composition was analyzed using Gas Chromatography (Model 2010, Shimadzu, Japan) with FID detector and HP-FFAP column, 50 m × 0.20 mm ID. The temperature of the detector, oven and injector is set at 150, 200, and 120°C, respectively. The carrier gas used is Helium. A standard graph for ethanol concentrations produced by injecting variations in the concentration of a standard ethanol solution ranging from 0.1 to 5.0 v/v (%). The sample injected into the GC was 0.5 μL and ethanol retention time was 5.5 min. Refractive index was obtained using Automatic Refractometer, Koehler-Modell K-27550.

3. RESULTS AND DISCUSSION

The prepared samples are named based on the comparison of yeast mass and fermentation time used in this study, The naming in question can be shown in Table 1 below.

Table 1. Sample ID.

Sample ID	Description
SCC-1	*Saccharomyces cerevisiae* 2.5 gram and fermentation process for 2 days
SCC-2	*Saccharomyces cerevisiae* 4.0 gram and fermentation process for 2 days
SCC-3	*Saccharomyces cerevisiae* 6.5 gram and fermentation process for 2 days
SCC-4	*Saccharomyces cerevisiae* 2.5 gram and fermentation process for 4 days
SCC-5	*Saccharomyces cerevisiae* 4.0 gram and fermentation process for 4 days
SCC-6	*Saccharomyces cerevisiae* 6.5 gram and fermentation process for 4 days
SCC-7	*Saccharomyces cerevisiae* 2.5 gram and fermentation process for 6 days
SCC-8	*Saccharomyces cerevisiae* 4.0 gram and fermentation process for 6 days
SCC-9	*Saccharomyces cerevisiae* 6.5 gram and fermentation process for 6 days

3.1. BIOETHANOL YIELD

Figure 5. shows that the highest calculation result of bioethanol yield at variations in fermentation time and yeast period is found in SCC-9 with a fermentation time of 6 days and a yeast period of 6.5 g which is 9.889% (v/v) while the lowest yield is found in SCC-1 with a yeast period of 2.5 g and a fermentation time of 2 days which is 10.4% (v/v).

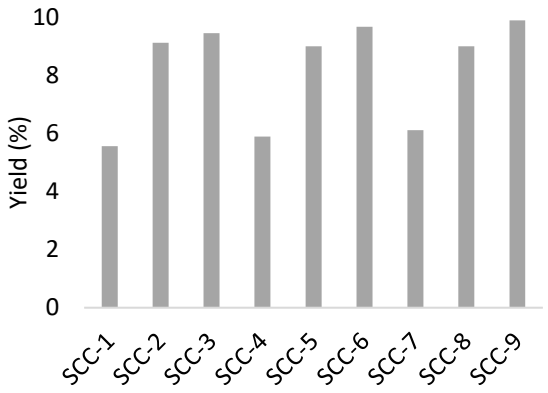

Figure 5. Correlation of yeast ratio and fermentation time to bioethanol yield.

The increase in the amount of bioethanol yield is influenced by the length of fermentation time, where microorganisms have the opportunity to break down more glucose to produce bioethanol. Fermentations that use less yeast result in less bioethanol yield compared to those that use yeast in larger quantities. This happens because the more *Saccharomyces cerevisiae* is given and the longer the fermentation time, the more bioethanol yield obtained, the same thing has also been reported by Aznury and Setiadi [19], fermentation process with the microbe Ralstonia eutropha JMP 134 has been carried out and utilizes volatile fatty acids (ALV) from the wastewater of the palm oil industry as a precursor, where the best yield obtained at the longest variation in fermentation time is at the 20th hour [20], obtained the best bioethanol product with a fermentation time of 96 hours from empty fruit bunches with the addition of 6 g/L yeast *Saccharomyces cerevisiae*. Awaliyah et al. [21] fermented seaweed waste with the microbe *Saccharomyces cerevisiae* obtaining optimum results at a fermentation time of 8 days with a yield of 16.67%. They also report that if there are too many microbes it will make the bioethanol formed decrease and when fermentation continues there is no more substrate, so the microbes will be in the premature death phase (the presence of secondary reactions).

The yield of bioethanol can continue to increase in line with the length of fermentation time. The results obtained as depicted in Figure 5 clearly show that the bioethanol yield increases as the yeast concentration increases to reach a maximum value of 9.89% (approached 10%) at a yeast concentration of 6.5 g, this is similar to the previously reported study by Egbosiuba et al. [22].

3.2. GAS CHROMATOGRAPHY (GC) ANALYSIS

The results obtained from the analysis using Gas Chromatography (Shimadzu, GC-2014) can be seen as tabulated and plotted in Figure 6.

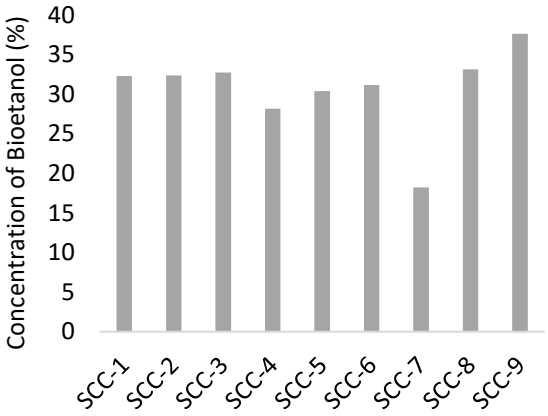

Figure 6. Correlation of fermentation time and percentage of bioethanol produced based on Gas Chromatographic results.

The results obtained from Figure 6 show that the retention time (Rt) is almost the same of all samples. This shows that the substances separated in the entire sample are the same, namely bioethanol. From the chart above, it can be seen that the concentration of bioethanol levels from the influence of fermentation time and yeast period is the highest in SCC-9, which is 37.599% with a peak retention time (Rt) of 2.995 minutes. As the fermentation time and yeast time concentration increase, the bioethanol produced increases. This happens because during the fermentation time bioethanol

bacteria have a longer chance of interacting to break down glucose into bioethanol. Similar findings have also been reported by Rebolledo-leiva et al. [23]. In addition, the more yeast periods with the same fermentation time, the amount of bioethanol concentration decreases. This difference in results is caused by the activity of bacteria that are reduced in nutrient sources (YSC, NPK and Urea), so that the longer the fermentation time is carried out, the impact on the level of bioethanol produced [5,24].

The concentration of bioethanol decreased very significantly and was the lowest in SCC-7 with a yeast period of 2.5 g against a fermentation time of 6 days, which was 18.210%. This is due to unstable temperatures so that the maximum concentration of bioethanol cannot be achieved. The most appropriate temperature to produce bioethanol is 30-35˚C [25]. GC chromatography for SCC-7 showed 2 almost identical peaks, namely 2,275 and 2,891 min. Results close to standard chromatography were at the second peak of 2,891 minutes. For the first peak that is at a retention time of 2,275 minutes it is possible that it is the peak of ethanol or other impurities that has a boiling point lower than ethanol.

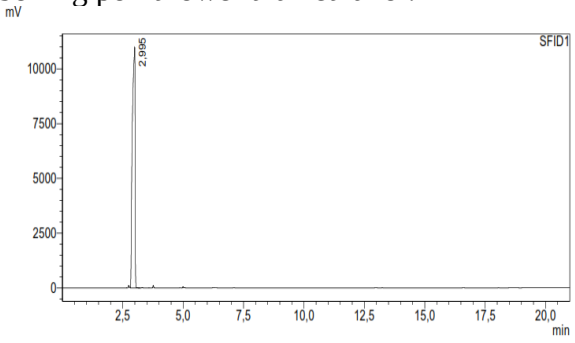

Figure 7. Chromatogram of GC analysis on SCC-9 samples.

Figure 7 shows the chromatogram results from the GC analysis with the highest bioethanol concentration of 37.6%. Zhang, et al. [26] has also produced bioethanol from sweet potato mash with *Saccharomyces cerevisiae* bacteria is 13.40%. Irmayadani, et al. [27] obtained 0.37 g/L of bioethanol from Dioscorea hispida tubers. From Hashem and Darwish [28], bioethanol obtained from potato starch is 5.52 g/L.

3.3. BIOETHANOL REFRACTIVE INDEX

Figure 8 shows the results of the analysis of the refractive index to the ratio of the amount of yeast and the time of fermentation. The greater the amount of yeast added, the resulting refractive index tends to increase, as does the fermentation time.

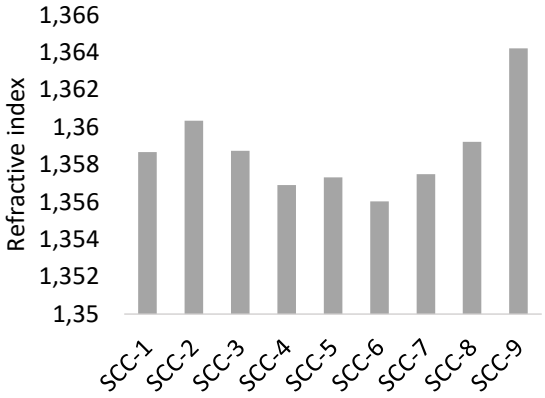

Figure 8. Correlation of fermentation time with the refractive index of bioethanol products to yeast.

The optimum result of the refractive index produced in this study was the SCC-9 with a bacterial mass of 6.5 g and a fermentation time of 6 days of 1.3642. While the lowest refractive index is SCC-6 with a bacterial mass of 6.5 g and a 4-day fermentation time of 1.3560. By knowing the refractive index value of bioethanol, it can be seen whether the quality is the same or not the standard ethanol being compared. The optimum refractive index value produced from porang tubers is slightly higher with the bioethanol standard of 1.3633 (SNI 7390:2008) [29].

The increased concentration of bioethanol causes the refractive index value to reject, so the relationship between ethanol levels and the refractive index is directly proportional. This has also been stated by Saka, et al. in his report on the production and characterization of bioethanol from sugarcane bagasse

obtaining a refractive index value of 1,356 [30]. Musa also reported bioethanol production from dika-nut shell of 1.40 [31]. The essence of measuring the refractive index of fuels is to verify the purity of the fuels, it can be deduced from the results obtained that the bioethanol produced is pure.

3.4. BIOETHANOL DENSITY

The results of the density analysis resulting from the acid hydrolysis stage with variations in yeast addition and fermentation time to the quality of bioethanol are shown in Figure 9.

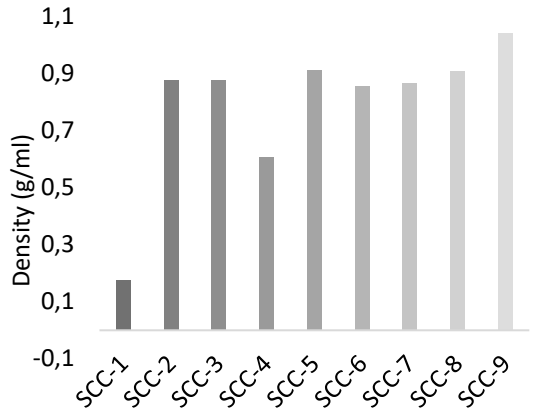

Figure 9. Correlation of fermentation time with purity of bioethanol products to yeast ratio.

Based on Figure 9, it can be explained that the highest bioethanol density value was obtained at SCC-1 with a bacterial mass of 2.5 g and a fermentation time of 2 days, giving a result of 0.814 g / ml and the lowest bioethanol density obtained at SCC-9 with a bacterial period of 6.5 g and a duration of 6 days fermentation time of 0.742 g/ml. The increase in bioethanol concentration causes the density value to decrease, so that the relationship between ethanol levels and density is inversely proportional, this has also been stated by Fadly et al. [32] in their report on the process of making bioethanol from pineapple skin waste. The density value closest to absolute bioethanol is 0.789 g/ml [24], which the researchers got its position in SCC-5 with a yeast period of 4 g and a fermentation time of 4 days, which is 0.788 g/ml. The results of this study show that fermentation time and yeast mass have an effect in producing optimal bioethanol.

Density is directly related to the concentration of bioethanol contained in the product. If the product's density gets closer to the bioethanol standard of 0.789 g/ml, the purer the bioethanol content in the product [29]. High density indicates high water content in the product because the density of the research results is closer to the density of water (1 g/ml).

4. CONCLUSION

The process of characterization bioethanol from porang waste tubes has been successfully developed by fermentation method using *Saccharomyces cerevisiae*. The addition of 6.5 grams of yeast with a fermentation time of 6 days showed ethanol productivity (yield) of 12.26%, this is also equivalent to 37.6% obtained from the GC analysis with a bioethanol density level of 0.844 g / ml and a resulting refractive index of 1.3642. In addition, another thing that increases the yield of bioethanol production is the longer the fermentation time and the ratio of yeast addition, so that the yield of bioethanol produced is increasing. The increase in bioethanol production can be caused by other things such as the length of fermentation time and the weight ratio of the addition of yeast.

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