POLBAN

Effect of Fermentation Time on the Production of Ambon Banana Weevil Waste Bioethanol

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ABSTRACT

The increase in population is directly proportional to the increase in the use of vehicles which results in an increase in fuel demand. The amount of fossil fuels continues to decrease and cannot be renewed, so alternative energy is needed, one of which is bioethanol. This study aims to make bioethanol from banana weevil waste, to determine the effect of fermentation time on the concentration of banana weevil bioethanol and to determine the concentration of bioethanol after going through the purification stage. The production of banana weevil bioethanol began with the process of cutting the banana weevil which was then mashed with the help of water and then squeezed and starch was obtained and then hydrolysed with the addition of glucoamylase and alpha-amylase enzymes for further anaerobic fermentation with the help of Saccharomyces cerevisiae with a concentration of 25%. In this study, variations of fermentation time were carried out for 5, 7, 9, and 10 days. The best fermentation time was obtained from 9 days of fermentation, where the conversion of glucose to bioethanol was 51%. The results of the analysis using a refractometer showed that the concentration of bioethanol obtained was 16.20% (v) which was obtained from fermentation for 9 days and purification using a rotary evaporator at a pressure of 360 mbar and a temperature of 50°C.

INTRODUCTION

The population in Indonesia is increasing every year, this has caused vehicle users to increase, as evidenced by data from the Central Bureau of Statistics in 2019 where the number of motor vehicle users was 133,617,012 and increased in 2020 to 136,137,451 [1]. This causes the community's need for fuel to increase, while the availability of petroleum is dwindling and non-renewable. Based on the press release of the Ministry of Energy and Mineral Resources no. 028.Pers/04/SJI/2021 oil reserves in Indonesia are only available for the next 9.5 years [2]. Based

on these data, alternative renewable energy is needed, one of which is ethanol. The uses of ethanol include as a solvent, antiseptic, mixing agent, chemical raw material, and as a fuel. Ethanol can be produced from plants that contain starch, where ethanol from these plants is referred to as bioethanol because it comes from plants that can be cultivated or in other words bioethanol is a renewable energy.

One of the plants that contain starch is the banana tree, where the part that has a high enough starch content is the weevil. The starch yield obtained from Ambon banana weevil is 11.63%, kepok banana weevil 12.56%, raja banana weevil 12.30%, Mahuli banana weevil 10.20%, and milk banana weevil 10.70% [3]. The banana tree is a tree that only bears fruit once in its growing season, so banana trees are usually cut down and thrown away after bear fruit [4]. This has the potential to produce organic waste, considering that Indonesia is an agricultural country where banana trees are planted quite a lot. According to data from the Central Statistics Agency of Indonesia, banana production in Indonesia reached 8.74 million tonnes in 2021. This amount experienced an increase of 6.85% compared to the previous year which amounted to 8.18 million tons [5]. Based on the description above, in order to increase the utilization of new and renewable energy and meet energy needs and efforts to utilize waste, this research was conducted.

Octavia et al. [6] using kepok banana stems and baker's yeast with a fermentation time of 7 days obtained a bioethanol concentration of 12.45%, while Bestari et al. [7] used kepok banana peels and baker's yeast with a fermentation time of 8 days, obtained a bioethanol concentration of 17.05%. Bioethanol concentration of 9.90% was obtained by Warsa et al. [8] from kepok banana weevil, using Saccharomyces cerevisiae and 7 days of fermentation time. Pranav et al. [9] obtained a bioethanol concentration of 4.80% using banana weevils with Saccharomyces cerevisiae and the fermentation time was only 5 days but the temperature was maintained at 35 oC and stirring at 150 rpm during the fermentation. Research conducted by Solikhin et al. [10] obtained a bioethanol concentration of 12.2% from the fermentation process using banana weevil with an acid hydrolysis process with a fermentation time of 5 days. Wastinah and Lukmaningsih [11] used sorghum flour and only obtained bioethanol with the highest concentration of 1.60% with a Saccharomyces cerevisiae concentration of 25% and a fermentation time of 72 hours. Meanwhile Pazmino-Hernandez et al. [12] used banana peduncle waste as a feedstock to produce bioethanol. The best result is from fermentation for 5 days of five times concentrated peduncle extract, containing 77.5 g/L total sugars, yielding 0.41 g ethanol/g sugars.

Based on previous research, the innovation of this research is to utilize banana weevils which have rarely been studied by previous researchers, namely Ambon banana weevils. Due to the different raw materials used, in this study the fermentation time for Ambon banana weevil into bioethanol was varied to obtain an optimal fermentation time.

LITERATURE REVIEW

The banana tree is native to Indonesia where this tree has fibrous roots with short internodes and grows up to 29 meters high [4]. Indonesia is a country that produces up to 6.20% of the world's

bananas [13]. In Indonesia, almost all parts of the banana can be utilized, starting from tubers, leaves, flowers, roots, tubers (shoots) and fruit. Banana weevil is the tuber stem of the banana plant. Banana weevil starch is like sago flour and tapioca. Starch is one of the polysaccharides found in all plants, especially corn, potatoes, maize, cassava, rice, and wheat [14]. Materials used as raw materials for bioethanol need to have a high starch content and high yield potential as well as flexibility in cultivation and harvest time [15]. Thus, the banana weevil has the potential to be used as a raw material for bioethanol production.

Bioethanol is the result of fermentation of raw materials by microorganisms through biological processes. Generally, biological raw materials that can be used as bioethanol are cellulosic materials (lignocellulosic), including bagasse, banana weevil, straw, and so on. Then, the sugary sap (sucrose) includes palm sap, coconut sap, palm sap, sugarcane sap, sweet sorghum sap, as well as ingredients containing starch including cassava, sweet potato, sago, arrowroot, canna, sorghum seeds, dahlia tubers [16]. Starch, which can be obtained from living plants, consists of two types of polysaccharides, both of which are polymers of glucose. Glucose polymers consist of α-D-glucose units connected in branch chains by α-1,4-glycosidic bonds and α-1,6-glycosidic bonds [17].

To obtain glucose from banana weevil starch, it is necessary to carry out the hydrolysis process. The hydrolysis carried out in this study was enzymatic hydrolysis, which consisted of dextrinization and saccharification. Dextrinization uses the enzyme alpha amylase where the enzyme breaks the α-(1,4) glycosidic bonds randomly on the inside of the substrate and produces reducing sugars and dextrins with a few glucose chains [18], [19]. The saccharification process uses the enzyme glucoamylase where the enzyme is able to hydrolyse α-1.4 bonds and a few α-1.6 bonds at the branching point. This enzyme will hydrolyse starch into oligosaccharides, mattotriose into maltose, and hydrolyse maltose into glucose [19], [20].

Hydrolysis is carried out to convert starch into glucose and followed by fermentation to convert glucose into ethanol with the help of microbes. The basic principle of fermentation is that certain microbial activities are activated to further produce good fermented products by changing the properties of the material. The following is the chemical reaction of the fermentation process:

$$C_6H_{12}O_6 \xrightarrow{\textit{Saccharomyces cerevisiae}} 2C_2H_5OH + 2CO_2$$
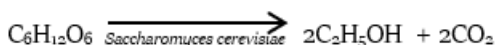
Factors that influence the fermentation process include types of microorganisms, media, temperature, pH, and fermentation time [21]. Bioethanol is obtained from sugar produced by the activity of yeast cell fermentation. Yeasts that can be used to produce bioethanol include Saccharomyces cerevisiae. Saccharomyces cerevisiae produces the zimase and invertase enzymes. The zimase enzyme breaks down sucrose into monosaccharides (glucose and fructose) and the invertase enzyme converts glucose into bioethanol [22]. In the fermentation process, optimal temperatures are needed, namely in the range of 25-30 0C and the degree of acidity (pH) adjusted for yeast growth, namely in the range of 4.0 - 5.0 [23].

In this study, the purification process was carried out using a rotary evaporator. Research on purification of bioethanol using a rotary evaporator, has been carried out by Sebayang et al. [24]. They carried out bioethanol purification at a temperature of 60 °C, a pressure of 175 mbar, and a stirring speed of 100 rpm with a bioethanol yield of 90.23%(v).

The main purpose of using a rotary evaporator is to lower the boiling point of the liquid and its relative volatility increases when the pressure is lowered. Boiling point can be defined as the temperature at which a liquid turns into vapor at atmospheric pressure or at a certain pressure. A decrease in pressure in the space of a liquid lowers the boiling point, but an increase in pressure at the surface of a liquid increases the boiling point [25]. Pure ethanol has a boiling point of 78 °C at atmospheric pressure. Because it is operated under vacuum pressure, it is necessary to lower the ethanol boiling point following the ethanol-water boiling point curve. The following figure (Figure 1) is the ethanol-water boiling point curve [26].
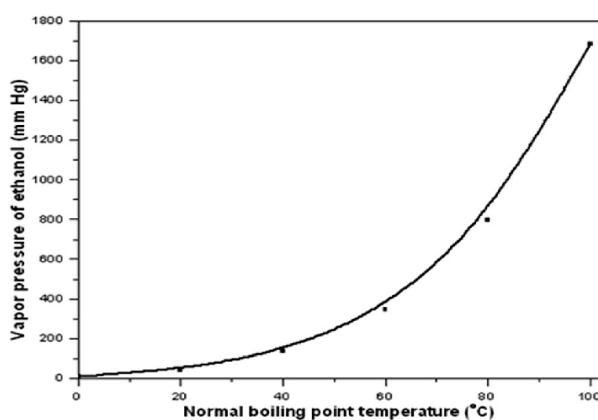

Figure 1. Ethanol-water Boiling Point Curve

RESEARCH METHOD

Obtaining Banana Weevil Starch

Banana weevil starch was obtained according to the stages as shown in Figure 2. One kg of banana weevil was cut into about 1-2 cm pieces and then washed using clean water, then pulverized using a blender while adding 1L of distilled water. The results were squeezed using a filter cloth to obtain the starch solution [6].
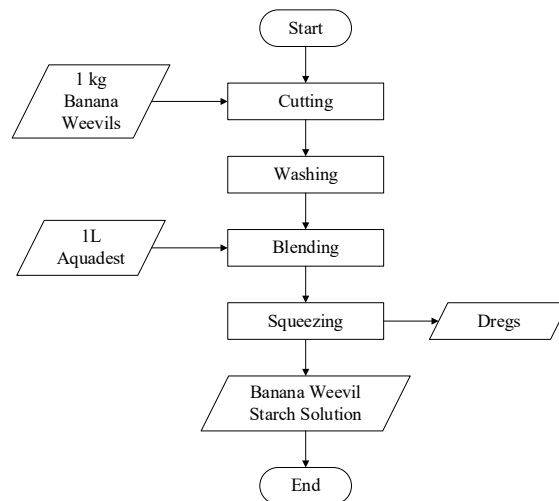
Figure 2. Stages to obtain Starch

Hydrolysis of Banana Weevil Starch

The stages of hydrolysis of banana weevil starch to obtain glucose are shown in Figure 3. The banana weevil starch obtained from the previous step needs to be maintained at pH of 6-6.5 before the dextrinization process. Then, 2 ml of alpha-amylase enzyme was added to the starch solution by heating at 90 ℃ for 4 hours. The pH after dextrinization was lowered in the range of 4-5. In the saccharification process, 2 ml of glucomylase enzyme was added by heating at 60 ℃ for 2 hours and then the pH was lowered again in the range of 3.5-5 to examine the glucose obtained [6].
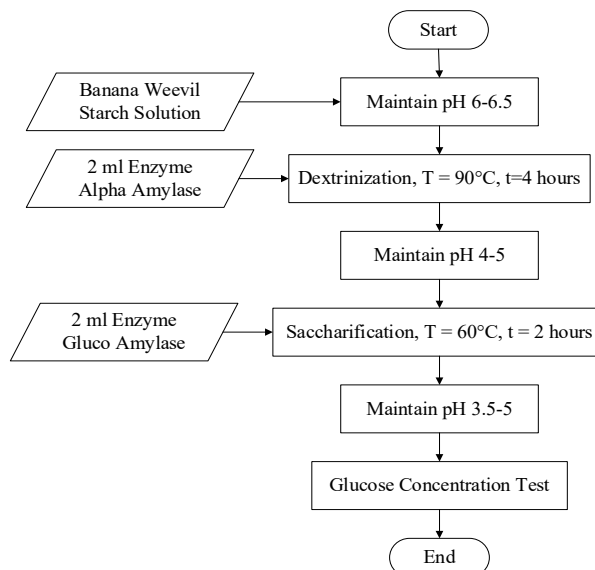

Figure 3. Starch Hydrolysis Stages

Preparation of Saccharomyces cereviseae Culture

Figure 4 shows the stages of preparation for Saccharomyces cerevisiae culture. The process of preparing Saccharomyces cereviseae culture began with growing Saccharomyces cereviseae microbes on sloped agar media for 24 hours. The microbes were then grown in an inoculum which contained nutrients for the microbes consisting of 2.5 g of yeast extract; 2.5 g of malt; 5 g glucose and 2.5 g peptone dissolved in 500 ml amidis. The inoculum media was incubated for 24 hours in a shaker incubator with a temperature of 30 °C and a rotation of 150 rpm.
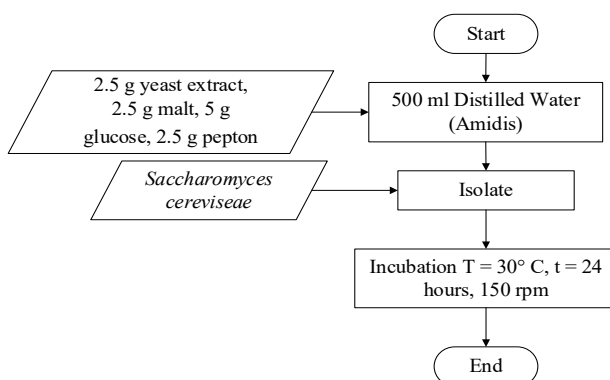
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                                    ┌─────────┐
                                    │  Start  │
                                    └─────────┘
                                         │
   ┌─────────────────────┐    ┌──────────────────────┐
   │ 2.5 g yeast extract, │    │ 500 ml Distilled Water│
   │ 2.5 g malt, 5 g      │───▶│      (Amidis)         │
   │ glucose, 2.5 g pepton│    └──────────────────────┘
   └─────────────────────┘               │
       ┌─────────────────┐    ┌──────────────────────┐
       │  Saccharomyces  │───▶│       Isolate         │
       │    cereviseae   │    └──────────────────────┘
       └─────────────────┘               │
                            ┌──────────────────────────┐
                            │ Incubation T = 30° C, t = 24│
                            │      hours, 150 rpm        │
                            └──────────────────────────┘
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                                    ┌─────────┐
                                    │   End   │
                                    └─────────┘
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Figure 4. Stages of Saccharomyces cerevisiae Culture Preparation

Fermentation and Purification

The fermentation process (Figure 5) begins with sterilizing the hydrolysed starch solution in a SELECTA autoclave at 121 °C for 15 minutes and then cooling the hydrolysed starch solution. In the starch solution, 0.25 g of urea and 0.5 g of NPK which had been previously mashed were added [6] and then added inoculum containing Saccharomyces cereviseae with a concentration of 25%(v) into each of 4 bottles [11]. The hydrolysed starch solution was then pour into each bottle with a hose connected to the bottle containing distilled water and placed in an incubator with a temperature of 30 °C. The purification process was carried out using a rotary evaporator at a temperature of 50 °C and a pressure of 360 mbar.
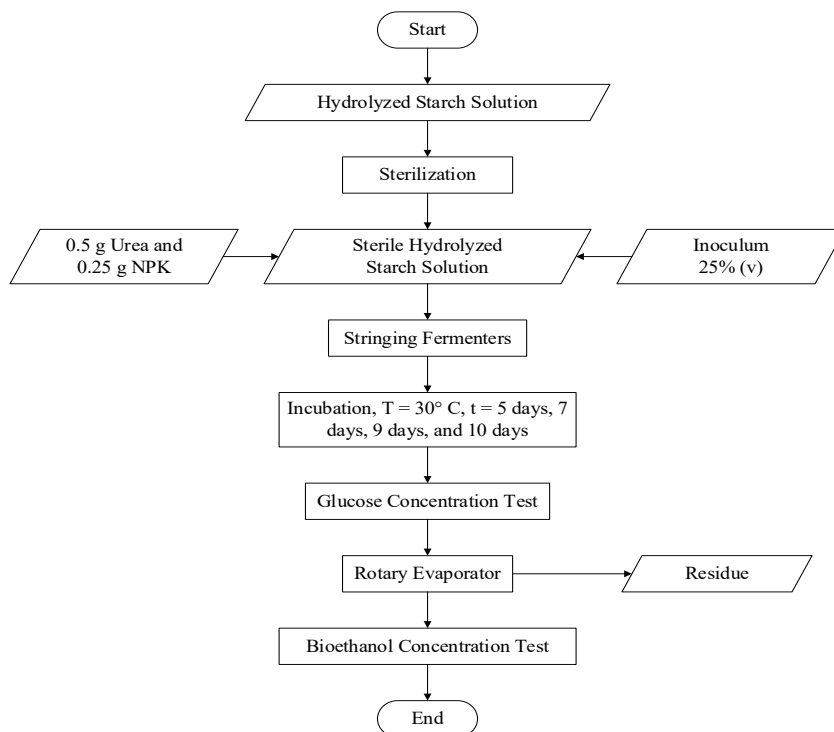
Figure 5. Stages of Fermentation and Purification

DISCUSSION

Hydrolysis-Fermentation

The starch solution obtained from 1 kg of banana weevil with the addition of 1L of distilled water was 1.682 L and the dregs obtained were 219.6 g. The pH of the starch solution during the hydrolysis process needs to be adjusted. The pH of starch solution was 6.49 before the dextrinization process. After the dextrinization process, a pH value of 6.24 was obtained. This pH was decreased using 0.25 ml of 96% H_2SO_4 until the pH decreased to 4.78. The hydrolysis process needs to be carried out at the optimum temperature and pH so that the enzymes can work optimally. If the hydrolysis process is carried out at too high temperature it will disrupt and damage the enzymes, while if it is carried out at too low temperature it will cause imperfect starch gelatinization [19]. After the saccharification process is complete, the pH of the starch solution needs to be adjusted to the optimum pH of 3.5-5 for the growth of Saccharomyces cereviseae [6]. In this study the banana weevil starch solution was fermented at an optimum pH of 4.78 to suits the optimum growth of Saccharomyces cereviseae utilized.

Banana weevil starch solution that has been hydrolysed needs to be tested for its glucose levels to determine the efficiency of the hydrolysis process, namely the conversion of starch to glucose. Testing of glucose levels based on %Brix using a refractometer. The standard curve was then made to determine the glucose levels. The following figure (Figure 6) is the standard curve of

%Brix vs glucose levels. From this standard curve, R2 = 0.9985 is obtained, which means that this curve can be used as a standard curve.
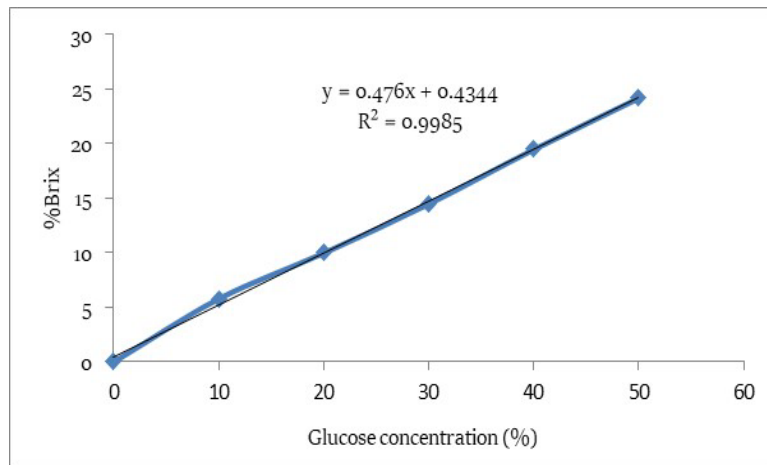

Figure 6. %Brix versus Glucose Concentration Calibration Curve

Variations in fermentation time in this study, namely 5, 7, 9, and 10 days were chosen based on previous research where researchers conducted fermentation for 5 days, 7 days, and 8 days to obtain optimal results. Therefore, this study added variations in the fermentation time of 9 and 10 days.

Based on the calibration curve above (Figure 6), the concentration of glucose resulting from fermentation can be obtained, that is based on the measurement of the % Brix in each fermentation time. Figure 7 shows the gain of glucose concentration for each variation of fermentation day, namely before fermentation or day 0, day 5, 7, 9, and day 10.
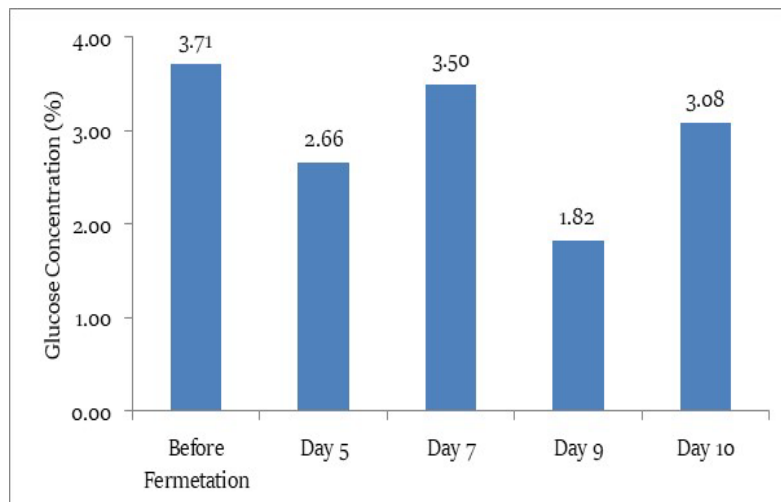

Figure 7. Glucose Concentration for each Variation

It can be seen that there is a change in glucose concentration before fermentation with glucose concentration after fermentation. This decrease in glucose concentration was due to the glucose content being converted by Saccharomyces cereviseae into bioethanol. According to Nasrun et al [23] the effect of fermentation time on the yield of bioethanol is as follows. The longer the fermentation time, the higher the yield of bioethanol is obtained, however, the longer the fermentation time causes the nutrients in the substrate to run out and the yeast Saccharomyces cerevisiae can no longer ferment the raw materials used. From Figure 7 it is found that the samples fermented for 9 days resulted in the highest conversion of % glucose to bioethanol compared to the other samples, or a yield obtained was 51%. This is because the variation of 9-day fermentation, the microbes experience an exponential phase or experience very fast growth so that the remaining glucose concentration is only 1.82%. This causes the most microbes to convert glucose in starch solution into bioethanol compared to other variations of fermentation time. Fermentation for 5 and 7 days did not give exponential growth which caused the conversion of glucose to bioethanol was not optimal but at 10 days of fermentation it may be caused by Saccharomyces cereviseae in the stationary or death phase so that the conversion of glucose to bioethanol was not as high as the results of fermentation for 9 days.

Effect of Fermentation Time on Bioethanol Concentration

Time is one of the factors that can affect the fermentation process. Fermentation was carried out under anaerobic conditions by placing the sample in a glass bottle and there was a hose connecting the sample bottle to the water bottle to reduce the oxygen content in the sample bottle. The use of Saccharomyces cereviseae starter at 25% (v) refers to research by Wastinah and Lukmanngsih [11]. After the completion of the fermentation process for 5, 7, 9, and 10 days, 2 layers were formed in the sample solution where the bottom layer was a precipitate containing protein and the top layer was a solution containing ethanol and H_2O [27].

Observation of ethanol concentrations was conducted based on the refractive index obtained, where the refractive index was measured using a refractometer. To obtain the ethanol concentration after fermentation according to variations in fermentation time, a calibration curve of ethanol concentration versus refractive index is needed. Based on this ethanol standard curve, an equation can be obtained which is then used to calculate the concentrations of bioethanol produced. The following figure is the refractive index calibration curve for ethanol (Figure 8). R2 on this curve = 0.9981 shows that this curve can be trusted as a standard curve.
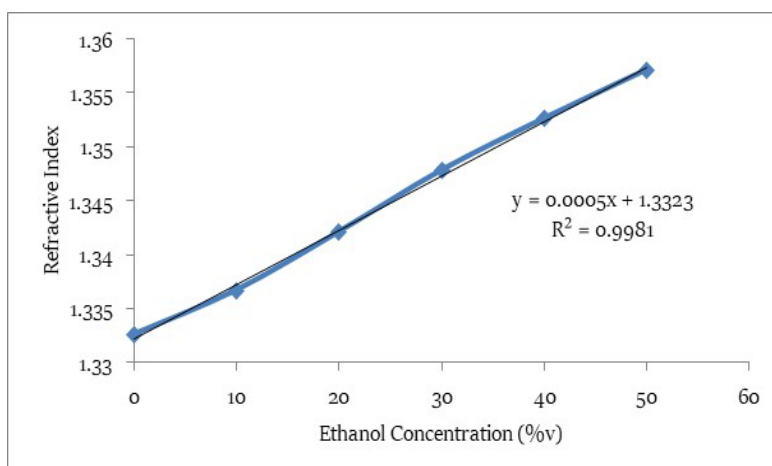
Figure 8. Refractive Index Calibration Curve for Ethanol Concentration

The purification process using a rotary evaporator was carried out on the 9th day variation sample because this sample was resulted from the highest decrease in glucose (Figure 7). The temperature and pressure settings of the rotary evaporator are based on the boiling point curve of ethanol-water where at a temperature of 50 °C, ethanol will evaporate at a pressure of 359.97 mbar or 360 mbar. The refractive index results obtained after distillation using a rotary evaporator were 1.3404 or a bioethanol concentration of 16.20% was obtained. The obtained bioethanol concentration from this study was higher than that of Solikhin et al [10] who obtained a bioethanol concentration of 12.2% using a starter concentration of 8% and an acid hydrolysis process. Research by Oktavia et al. [6] obtained a bioethanol concentration of 12.45% with the raw material being kepok banana stems and fermented using baker's yeast for 7 days without a purification process. Wastinah and Lukmaningsih's research [11] where the fermentation process was only carried out for 72 hours obtained the bioethanol concentration only 1.6%. Taro tubers, which compete with food ingredients, which are used as raw materials for bioethanol only provide a bioethanol concentration of 0.07% with a fermentation time of 8 days [28]. Thus, this study confirms that banana weevil waste can be used as raw material for bioethanol and at the same time helps to handle the utilization of one type of solid waste.

CONCLUSION

The best fermentation time obtained from this study was 9 days of fermentation. Bioethanol as a result of glucose conversion from banana weevil starch during 9 days of fermentation resulted in a yield of 51%. The concentration of bioethanol obtained from 9 days of fermentation was 16.20% (v) which was obtained from purification using a rotary evaporator at a pressure of 360 mbar and a temperature of 50 °C.

LIMITATION AND FUTURE RESEARCH

Based on the research that has been done, suggestions that can be followed up are examining the ethanol concentrations using Gas Chromatography (GC) to obtain more accurate quantitative data. Before using GC, the sample needs to be filtered or centrifuged so that it does not leave scale on the GC analyser.

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