Bioethanol Production from Lindur Fruit (Burguiera Gymnorrhiza) Strach with Variation of Inoculum Volume of Zymomonas Mobilis

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Abstract: Bioethanol is the fermentation product of hydrolyzed carbohydrates by using acids or enzymes. Commonly, bioethanol uses fermented microbes, one of them is bacterium Zymomonas mobilis. Lindur (Burguiera gymnorrhiza) is the fruit of one kind mangrove plant which are not fully utilized. One of the chemical content of lindur Fruit is carbohydrate 23.53 %, it can be used for the production of bioethanol which uses fermentation of carbohydrates. Isolation of starch which is one kind of carbohydrates from lindur fruit by precipitation the starch using water. Pati will be hydrolyzed using HCl 20 % to obtain a solution of glucose around 7.49 %. after that, fermentation carried out using a different variation of the number of inoculums (5, 10 and 15 % (v/v)). Bioethanol obtained from the fermentation process will be measured using Gas Chromatography (GC), density, acidity, and evaporation residue also tested. The result shows the highest content of bioethanol is 43.75 %.

1 INTRODUCTION

Lindur fruit or Burguiera gymnorrhiza is the fruit of one kind mangrove plant. This mangrove plant grows a lot in tropical regions, especially Indonesia. These plants grow in the coastal area, it aims to prevent surface erosion by sea waves (abrasion)

Lindur fruit which is not fully utilized by many people. This fruit has a carbohydrate content of around 23.53 % (De, 2005). Carbohydrates are natural polymers that are abundant in nature, one type of carbohydrate is starch. Starch is a glucose homopolymer with α-glycosidic bonds. Lindur starch has an amylose content of about 31.56 % and an amylopectin content of about 26.17 % (Jacoeb *et al.*, 2014). Starches consisting of glucose can be used for bioethanol production.

Bioethanol can be produced using microbial help. Zymomonas mobilis is a bacterium that can ferment glucose and fructose (Gunasekaran and Chandra Raj, 1999) (Geeta, 2007). Bioethanol productivity obtained from zymomoas mobilis is higher when using the Entner-Duodoroff pathway (Obire, 2005) (Tripetchkul S.Z.D Hilary, 1998). Zymomonas mobilis is not harmful to humans and is often used as a natural inoculum to make traditional alcoholic drinks. Most of Zymomonas strains (90 %) can grow at pH 3.5. But it does not grow at pH 3.05 or lower. Because Zymomonas is rather thermolabile, the best condition for Zymomonas mobilis growth is at temperatures between 25 ^{o}C and 30 ^{o}C; 74 % of strains grow at 38 ^{o}C, but the growth will rarely occur at 40 ^{o}C.

Zymomonas mobilis has a tolerance to high substrate and product concentrations. Some types of Zymomonas can tolerate up to 30-40 % glucose and 13% (weight/volume) ethanol. This bacteria has a high tolerance to ethanol among the other bacteria, only the majority of bacterial growth is inhibited by ethanol concentrations of 1-2 % (weight/volume). As an explanation, the main protective function comes from hopanoids, which are pentacyclic triterpenoids, which are widely present in the Zymomonas mobilis cell membrane. Most likely, amphiphilic substances, such as sterols, stabilize Zymomonas mobilis cell membranes against dissolving with ethanol (Yanase, 2014).

This research aimed to determine the content of bioethanol produced from zymomonas mobilis fermentation in starch from acid hydrolyzed.

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2 METHODOLOGY

2.1 Preparation Lindur Fruit

The fruit obtained is then peeled and cleaned with clear water, then cut into cubes and then put into a bucket and soaked with 0.2 % $Na_2S_2O_5$ for 12 hours. Then blend until smooth by adding aquadest 1:5 (weight/volume) then let stand for 24 hours. Furthermore, starch is separated from the solution and roasted at a temperature of 45 ^oC for 24 hours.

2.2 Fourier Transforms Infrared Spectroscopy

The starch was prepared into pulp. Pulp slurry was examined in a thin film placed between flat plates of salt. The test was carried out by pinning the resultant film on the sample container. Then the film was placed on a plate in the direction of infrared light. The result will be recorded periodic paper in the form of a flow curve of 4000-200 cm^{-1} waves with intensity.

2.3 Starch Hydrolysis

10 g of starch obtained was then put into a 250 ml glass beaker, and then added with 100 ml 25% HCl and covered with alumanium foil. Then it was heated at 80 ^oC while stirring for 30 minutes. After that, it was cools down, the hydrolyzate was adjusted to neutral pH using 30 % NaOH.

2.4 Preparation YEPD Media (Yeast Extract Pepton Dextrose)

The preparation of YEPD media is by dissolving 4 g Yeast extract, 2 g $KH_2PO_{4\ (s)}$, 3 g $(NH_4)_2SO_{4\ (s)}$, 1 g $MgSO_4.7H_2O_{\ (s)}$, 3.6 g Pepton and 2% Bacto agar with 1000 ml Aquadest. Then heated on a hotplate until it was clear yellow.

2.5 Zymomonas Mobilis Bacteria Culture

Z. Mobilis bacterial culture is carried out in a sterile place near or around a burning Bunsen fire so that there are no contaminants that inhibit the growth of Z. Mobilis bacteria. Culture was carried out by inserting YEPD media into a petri dish, then 1 ose was taken from isolate Z. mobilis and then etched on a petri dish containing YEPD media. When the petri dish was closed and wrapped in plastic wrap, it was then incubated at 30 ^oC for 24 hours.

2.6 Hydrolyzed Fermentation using Inoculum Z. Mobilis

Fermentation using inoculum Z. Mobilis was done by dissolving 4 g Yeast extract, 2 g $KH_2PO_{4\ (s)}$, 3 g $(NH_4)_2SO_{4\ (s)}$, 1 g $MgSO_4.7H_2O_{\ (s)}$, 3.6 g Pepton and 2% agar Bacto with 1000 ml of hydrolyzate. Then sterilized using an autoclave for 2 hours at 121 ^oC. After the hydrolyzate was cooled, 5 %, 10 % and 15 % inoculum Z. Mobilis was added. Then it was tightly closed using alumanium foil and plastic wrap and put in a shaker incubator for 21 hours at 30 ^oC with 100 rpm speed.

2.7 Separation of Bioethanol from Fermentation Solutions

500 ml fermentation solution was put into a 1000 ml rotary evaporator flask then CaO was added to the fermentation solution at a ratio of 1: 2 (g / ml) and then in the rotary evaporator at 78^oC for 1 hour. Then the distillate obtained was test for ethanol qualitative.

Figure 1: Lindur Fruit.

2.8 Bioethanol Density Test

Bioethanol density test was carried out using a pycnometer, where the dry, clean and empty pycnometers weighed, then filled with water/ aquadest, and then it was dried and cleaned. Furthermore, weighed with an analytical balance to a constant weight. The same step was done by using a distillate (bioethanol).

$$\text{Density (g/ml)} = (a-w)/(b-w)$$

a = weight of empty pycnometer + sample
b = weight of empty picnometer + water
w = weight of the empty pycnometer

2.9 Determination of Bioethanol Content

Determination of obtained bioethanol content by using bioethanol density conversion tables with bioethanol content.

3 RESULTS AND DISCUSSIONS

3.1 Starch Isolation Results from Lindur Fruit

Starch isolation from lindur fruit was carried out by precipitating starch in water overnight, so that starch from lindur fruit has a brownish white color. Starch is a type of carbohydrate which is a glucose homopolymer with α-glycosidic bonds and there are many in all plants, one of which is fruit. Starch is in the cortical tissue in the fruit which is located in the xylem surrounded by phloem (Seknun, 2012) so that by destroying the fruit will damage the cortical tissue so that the starch of the fruit can be removed. Where the physical properties of starch that can not dissolve in aquadest it will precipitate starch at the bottom of the solution because the molecular mass of starch is heavier than the water molecules in the fruit juice.

3.2 Characterization using Fourier Transforms Infrared Spectroscopy

In the functional group analysis using FTIR for both the spectrum of the fruit starch and commercial starch showed that there was no significant difference between the starch band of the fruit and the commercial starch. It happened because both are starches. From FTIR spectra, there are widening bands in the absorption regions 3398 and 3286 cm^{-1} which show the existence of OH stretch vibrations from alcohol in the starch molecule, followed by the CH stretch vibrations of the alkane chains in the absorption area 2931 cm^{-1}.(Estrada-León *et al.*, 2016) In addition, the vibrational peak was also seen in the absorption area of 1145 cm^{-1} which showed the presence of C-O-C strain in the starch ring (Estrada-León *et al.*, 2016). Whereas the absorption area of 1338 and 1350 cm^{-1} indicates the presence of C-H groups (Wijaya *et al.*, 2019).

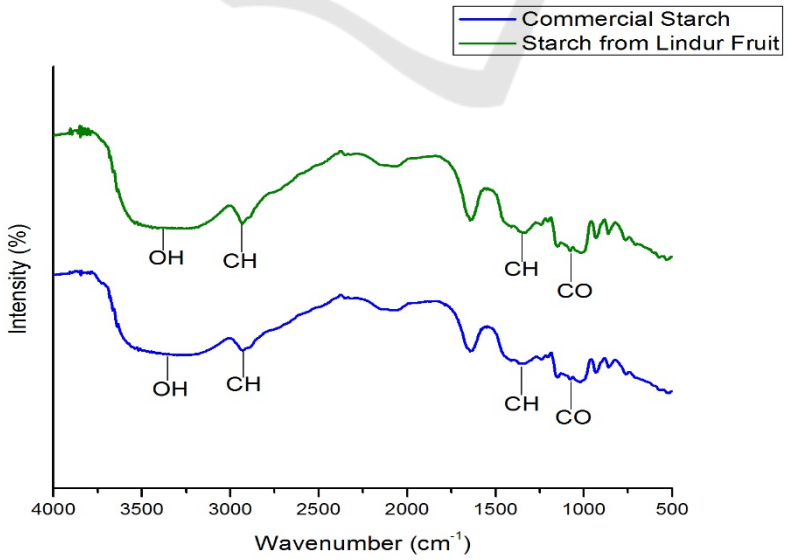

Figure 2: Spectrum FT-IR of Commercial Starch and Starch from Lindur Fruit.

Table 1: Results of Starch Fermentation from Hydrolyzed Lindur Fruit.

Treatment		Yield (%)	Bioethanol content (%)	Productivity (g / L. Hours)
Starch (%)	Inoculum Z. Mobilis (%)			
10	5	8.8	30.53	1.01
10	10	8.6	37.38	1.21
10	15	9.0	43.75	1.48

3.3 Hydrolysis Starch by Acid

Hydrolysis of lindur fruit starch using 25 % HCl solution and neutralization using 30 % NaOH solution to obtain a glucose solution of about 7.5 %. The hydrolysis of starch from the fruit yields 7.5 % glucose from 10% starch content. The added acid can hydrolyze because it can form hydroxonium ions (H_3O^+) which are electrophilic so that they attack the oxygen atom in the glycosidic α-1,4 bond and hydrolyze the glucosidic bond. Then the electrons in one of the carbon-oxygen bonds move to the oxygen atom and produce an unstable high-energy carbocation intermediary. Furthermore, intermediate carbocation reacts with water, which leads to the regeneration of hydroxyl groups (Hoover, 2000).

3.4 Fermented Hydrolyzed Starch

Results of fermentation of hydrolysis solution of starch fruit will be distilled using a rotarievaporator to separate the bioethanol obtained by boiling point differences. The bioethanol content obtained will be determined using gas chromatography (GC).

Zymomonas mobilis is widely used as a fermentation bacterium, which converts glucose, sucrose, and fructose into ethanol. Like Z. mobilis, Saccharomyces cerevisiae naturally consumes hexose sugar (for example, glucose, fructose).

The metabolism in Zymomonas is different from the metabolism of Saccharomyces in which glucose becomes pyruvate through the Enbden-Meyerhof-Parnas (EMP) pathway; ethanol is then formed from pyruvate. Instead, Zymomonas ferments sugar through the ED pathway, forming pyruvate from gluconate. As in Saccharomyces, the released pyruvate is decarboxylation, producing acetaldehyde and CO_2, after which acetaldehyde is reduced to produce ethanol.

When the formation of ATP through the EMP and ED pathways was compared, it was found that EMP produced 2 moles of ATP per mole of glucose, whereas the ED pathway produced 1 mole of ATP per mole of glucose. Thus, ATP cells result in less glucose in Zymomonas metabolism than in yeast. The equation describing molar fermentation is as follows:

$$1\,\text{mol}\,C_6H_{12}O_6 \longrightarrow 1{,}93\,\text{mol}\,C_2H_5OH + 1{,}8\,\text{mol}\,CO_2 + 0{,}053\,\text{mol}\,CH_3CHOHCOOH$$

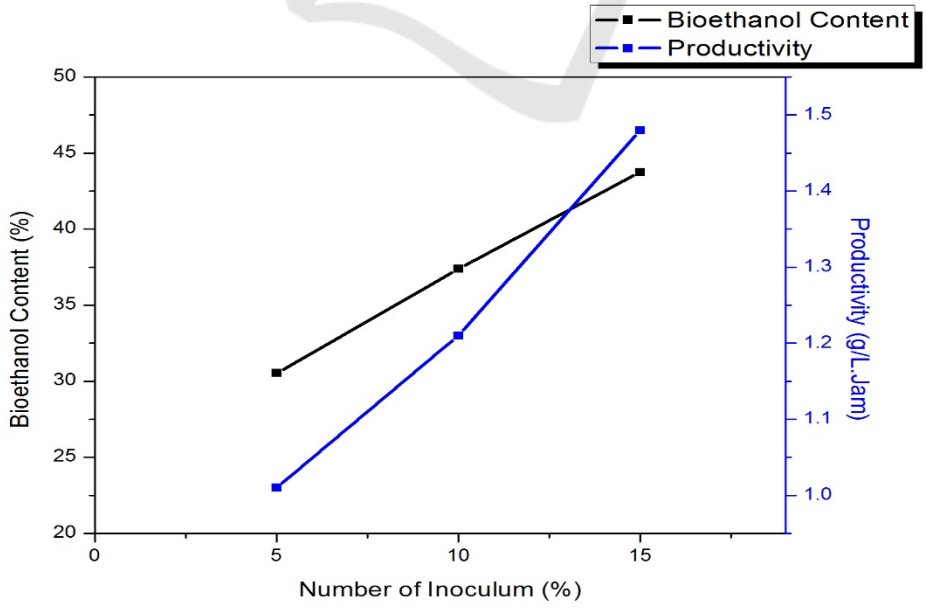

Figure 3: Fermentation Results Chart.

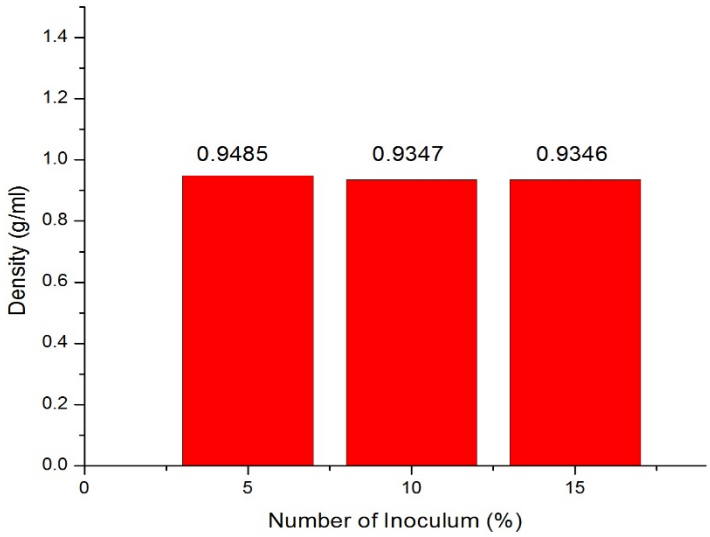

Figure 4: Bioethanol Density Graph.

In table 1 above, it can be seen that the % yield obtained from the distillation of fermentation solutions using 10 % starch content ranges from ± 9 % with bioethanol content ranging from 30-40 %.

Whereas in Figure 3 can be seen in the variation of the number of Zymomonas mobilis bacteria inoculums there is an increase in the productivity of bioethanol where in the starch variation of 10 % the highest productivity is 1.48 g / L. Hours. This is in accordance with (Fajrin, Amraini and Muria, 2008) , which with an increase in the number of inoculums will increase the productivity or bioethanol content produced, according to (Kusumaningati, Nurhatika and Muhibuddin, 2013) with an increase in the number of inoculums, it will increase its bioethanol levels due to more microorganisms that can utilize reducing sugars. According to (Prescott, 1981) there are two factors that influence the occurrence of increased bioethanol content, namely the number of substrates (sugar) and the number of microbes.

3.5 Density Test

In Fig. 4 above it can be seen that the highest density value on the variation of starch is 10 % and the amount of inoculum is 5 % with a density of 0.9485 g / ml while the lowest density with an amount of inoculum of 15 % is 0.9346 g / ml. This is due to the imperfect distillation process so that bioethanol is still mixed with water where the pure bioethanol content has a density of 0.798 g / ml but the density value obtained exceeds the density of pure bioethanol. So it can be concluded that the density value has decreased with the addition of the number of inoculum.

This decrease in density is due to the amount of inoculum which can increase bioethanol levels or productivity. High bioethanol content will have a low density value. So it can be explained that the density value of bioethanol is inversely proportional to the bioethanol contentwhere the higher the bioethanol content, the lower the density value.

4 CONCLUSIONS

Lindur fruit can produce starch that has a brownish white color. Lindur fruit starch hydrolyzed using acid produces a glucose solution of about 7.5 % with 10 % starch content. Fermentation of hydrolysis solution of lindur fruit starch showed productivity of 1.48 g / L.Hours

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