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Effect of Fermentation Time and Weight of Bread Yeast on Bioethanol Content from Glucose Hydrolysis of Cellulose Empty Bunches Palm Oil (*Elaesis guineensis Jacq.*) with HCl 30%

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Abstract. Research about the effect of fermentation time and weight of yeast bread on bioethanol concentration from the fermentation of the glucose from hydrolysis of cellulose oil palm empty fruit bunches (*Elaeis guineensis Jack*) with HCl 30% has been done. From the research, it found that oil palm empty fruit bunches contained cellulose of 24.1298 %. The cellulose was isolated from oil palm empty fruit bunches. It was hydrolyzed by HCl 30% to yield glucose and was analyzed by Nelson-Somogyi Method and the rate of the glucose was 17,1051 %. The fermentation of glucose used various periods of fermentation were 2 days, 4 days, and 6 days and various added baker yeast were 2 g, 4 g, and 6 g. The percentage of bioethanol was analyzed by using potassium dichromate titrations of the oxidation volumetric method. The result of the analysis shows that the highest percentage was 7.3922% with a period of fermentation was 6 days and baker yeast of 6 g.

Keywords: Empty Bunches Palm Oil, Fermentation, Yeast, Bioethanol

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1 Introduction

Oil palm is a plant with high economic value because it is one of the vegetable oil-producing plants. For Indonesia, palm oil has an important meaning because it can create job opportunities for the community and as a source of foreign exchange earnings for the country. Until now, Indonesia is one of the main producers of palm oil in the world besides Malaysia and Nigeria. One of the solid wastes of the palm oil industry is oil palm empty fruit bunches (TKKS). Solid waste has a characteristic in its composition. The largest component is cellulose, in addition to other although smaller components such as ash, hemicellulose, and lignin (Fauzi, 2003).

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Cellulose materials are agricultural waste that has not been utilized optimally and the amount is quite abundant. In addition, this material does not conflict with food needs. Among the cellulose materials that have the potential to be developed as raw materials for bioethanol are oil palm empty fruit bunches. It is available quite abundantly and so far it has not been used optimally. Besides that, the cellulose content is quite high (45%).

The processing of OPEFB into bioethanol is in principle the same as the process made from cassava, namely through the stages of hydrolysis, fermentation, and distillation. However, OPEFB requires additional treatment in the form of pretreatment.

To be able to remove lignin which can interfere with the hydrolysis of cellulose. Then proceed with hydrolysis using cellulase enzymes and glucose liquid is produced. Glucose liquid was fermented using yeast *Saccharomyces cerevisiae* with facultative anaerobic conditions, temperature 30ºC, pH 4.0 – 4.5, and sugar content 10 -18% for 30 to 72 hours and produced bioethanol. Bioethanol is then distilled to achieve a purity of 95 to 98%. Bioethanol is ready to be used as fuel in motor vehicles. Its use can be mixed with gasoline but can also be 100% bioethanol if the motor vehicle engine is specifically designed for bioethanol fuel (Hidayat, R. 2005).

Ethanol is a fermentation product that can be made from substrates containing carbohydrates (sugar, starch, or cellulose). Ethanol is a colorless liquid that has a characteristic odor, its specific gravity at 15^{o}C is 0.7937 and its boiling point is 78.3oC at a pressure of 76 mmHg. The most used ethanol is as a solvent (Judoamidjojo, M. 1992).

The background of this research is based on research on Making Bioethanol from Cassava by Fermentation Using Yeast Tape by Heppy Rikana and Risky Adam (2000). Where in this study, tape yeast can be directly used for the fermentation process without isolating the microbes present in the tape yeast first and other research, namely research on saccharification and fermentation of bagasse into ethanol using cellulase enzymes and cellobiase enzymes by Gozan, et al (2007). The results obtained the highest ethanol content in the hydrolysis process using cellulase and cellobiose enzymes as well as the addition of HCl and starting with the weathering process first, which was 13.72%. However, the use of enzymes will make production costs higher and the stages quite long.

In another study entitled Utilization of Cellulose of Oil Palm Empty Fruit Bunches in Making Bioethanol Using Yeast Tape by Nurfadillah (2011), where the ethanol content produced was 0.99%. In this study, the microbes used were isolated from tape yeast, and to hydrolyze cellulose 3% H_2SO_4 was used and the results of research conducted by Ideris (2007) entitled Acid Hydrolysis of Pretreated Palm Oil Lignocellulosic Wastes, hydrolysis using 30% HCl gave the highest glucose levels compared to using 10% and 20% HCl.

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Based on the description above, the authors are interested in researching the utilization of oil palm empty fruit bunches in the manufacture of bioethanol. Where the first stage is the isolation of cellulose from the pulp which is then hydrolyzed with 30% HCl which is then fermented using baker's yeast without isolation of *Saccharomyces cerevisiae* first.

2 Materials and Methods

2.1 Equipment

The tools used in the form of glass tools commonly used in the laboratory, autoclave, analytical balance, thermometer, oven, water bath, desiccator, electromantle, and distillation apparatus.

2.2 Materials

Oil palm empty fruit bunches, aquadest, yeast, $CuSO_4.5H_2O$, ethanol 99.9%, $Fe(NH_4)_2(SO_4)_2.6H_2O$, $FeSO_4.7H_2O$, glucose, $H_2SO_{4(c)}$, KH_2PO_4, $K_2Cr_2O_7$, $MgSO_4.7H_2O$, NaOH, Na_2SO_4, $HNO_{3(c)}$, $HCl_{(c)}$, $NaNO_3$, ferroin indicator, sodium citrate, sodium hypochlorite, potassium sodium tartrate, $Na_2HAsO_4.7H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$, $NaHCO_3$, 1,10-O-phenanthroline.

2.3 Isolation of α-Cellulose from Oil Palm Empty Fruit Bunches

75 g of oil palm empty fruit bunches that have been refined are put in a beaker glass. Then 1000 mL of 3.5% HNO_3 and 10 mg of $NaNO_2$ were added. Then it was heated using a thermostat for 2 hours at 80^{o}C. Then the residue was filtered and washed with distilled water until pH = 7. The residue was added to 375 mL of 2% NaOH and 375 mL of 2% Na_2SO_3. Heated using a thermostat for 1 hour at a temperature of 50^{o}C. Then the residue was filtered and washed with distilled water until pH = 7. The residue was added with 500 mL of 1.75% Na-Hypochlorite. Then it was heated using a thermostat for 30 minutes at a temperature of 100^{o}C which was then filtered and washed with distilled water until the residue was pH = 7. The residue was added with 500 mL of 17.5% NaOH. Then it was heated using a thermostat for 30 minutes at 80^{o}C. Then the residue was filtered and washed with distilled water until pH = 7. The residue was added with 500 mL of 1.75% sodium hypochlorite. Then heated for 5 minutes at a temperature of 100 ^{o}C. Then filtered and washed the residue with distilled water until pH = 7. After that, the residue was dried in an oven at a temperature of 60 ^{o}C. Then it was cooled and put into a desiccator. Enough cellulose is added to the drop plate and then dripped with 0.1 iodine solution which will show a positive test for cellulose if there is no color change.

2.4 Hydrolysis of Cellulose into Glucose

Add 0.502 g of OPEFB cellulose into a 250 mL Erlenmeyer glass. Added 5 mL of distilled water. Added with 8 mL of 30% HCl. Covered with cotton and aluminum foil. Heated in a thermostat at 80 ^{o}C for one hour. Cool to room temperature. Added 10% NaOH until pH = 4 –

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4.5 then filtered. Pipette 1 mL of the filtrate into a test tube. Add 5 mL of Benedict's solution. Heated in a thermostat until a brick red precipitate formed.

2.5 Measurement of Maximum Wavelength of Standard Glucose Solution

Weighed 500 mg of anhydrous glucose and dissolved with distilled water to a volume of 500 mL (1 mg/mL anhydrous glucose solution). Pipette 5 mL of 1 mg/mL glucose mother liquor and put it into a 100 mL (0.05 mg/mL) volumetric flask. Then it is diluted with distilled water to the limit and homogenized. Put 1 mL of 0.05 mg/mL glucose solution into a test tube, then add 1 mL of Nelson's reagent and cover it with cotton. Then heated to boiling for 20 minutes and then cooled. Add 1 mL of arsenomolybdate solution and shake until all precipitate is dissolved. Then added 7 mL of distilled water and shaken until homogeneous. Next, the wavelength absorption was measured at 600-800 nm (the maximum wavelength was obtained).

2.6 Preparation of Glucose Standard Curve

Prepared standard glucose solution in several test tubes with graded concentrations from 0.02 to 0.1 mg/mL. Added 1 mL of Nelson's solution then heated to boiling for 20 minutes and cooled. Then 1 mL of arsenomolybdate solution was added and then shaken until all the precipitate dissolved, then 7 mL of distilled water was added and then shaken until homogeneous. The absorption was measured at a wavelength of 760 nm. Then a standard curve was made showing the relationship between standard sugar concentration and absorbance.

2.7 Analysis of Glucose Content

Pipette 1 mL of the filtrate from the hydrolysis of OPEFB cellulose then diluted in a 100 mL volumetric flask and 1 mL is taken for analysis. Added 1 mL of Nelson's solution then heated to boiling for 20 minutes and cooled. 1 mL of arsenomolybdate solution was added and then shaken until all the precipitate was dissolved. Added 7 mL of distilled water and then shaken until homogeneous. The absorption was measured at a wavelength of 760 nm so that the reducing sugar content could be calculated.

2.8 Fermentation of Glucose into Bioethanol

Entered 100 mL of glucose solution into a 250 mL Erlenmeyer glass. Added 0.1502 g $MgSO_4.7H_2O$; 0.1306 g KH_2PO_4 and 1.2021 g $(NH_4)_2SO_4$. Sterilized using an autoclave at 121^oC for one hour and then cooled. Added bread yeast as much as 2 grams. Fermented for 2, 4, and 6 days. Then the fermented solution was distilled at 78°C to separate the ethanol from the other components. The same treatment was carried out for variations in yeast weight of 4 and 6 grams.

2.9 Analysis of Bioethanol Content

Prepared standard solution of ethanol with a concentration of 0.2; 0.4; 0.6; 0.8; 1.0; 1.2; 1.4; 1.6; 1.8 and 2.0%. 5 mL of each prepared ethanol solution was pipetted and then diluted into a

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100 mL volumetric flask. Then 1 mL of the diluted ethanol solution was pipetted and then put into an Erlenmeyer glass. Added 5 mL of $K_2Cr_2O_7$ 0.689 N and 3 drops of ferroin indicator which were then titrated with $Fe(NH_4)_2(SO_4)2.6H_2O$ 0.393 N until the solution turned reddish brown

3 RESULT AND DISCUSSION

3.1 Isolation of α-Cellulose From Oil Palm Empty Fruit Bunches

Cellulose isolation from empty oil palm fruit bunches in this study was carried out based on the cellulose isolation method by Ohwoavworhua (2005). To determine the cellulose content obtained, the ashing of the dried cellulose isolation was carried out. Where the weight lost in the ashing process is calculated so that the cellulose content obtained from the isolation of oil palm empty fruit bunches is 24.1298%.

3.2 Hydrolysis of Cellulose into Glucose

In this study, hydrolysis was carried out using acid, namely 30% HCl. So that the cellulose will be broken down into glucose and then after hydrolysis is done, it is necessary to qualitatively test the presence or absence of glucose in the sample using Benedict's reagent. From the qualitative test of glucose carried out on glucose hydrolyzed by cellulose from oil palm empty fruit bunches, a brick red precipitate of Cu_2O was formed after heating, so the sample contained reducing sugar or glucose.

Glucose content in this study was analyzed quantitatively using the Nelson-Somogyi method and using a visible spectrophotometer instrument. A spectrophotometer was used to determine the absorbance of the solution compared to a standard glucose solution. Where at a wavelength of 760 nm the glucose content obtained from the hydrolysis of empty fruit bunches of oil palm is 17.1051%.

3.3 Effect of Variation in Fermentation Time on Bioethanol Content

In the fermentation process, the number of microbes is influenced by the length of fermentation, namely the longer the fermentation, the greater the number of microbes and the higher the ethanol production. This process will stop if the ethanol content has increased until it can no longer be tolerated by the yeast cells. The high content of ethanol will inhibit the growth of yeast and only microbes that are tolerant to alcohol can grow.

From the results of research conducted, it can be seen that with increasing fermentation time, the bioethanol content produced will be higher which can be seen from the table in figure 1 we can compare the levels of bioethanol produced from fermentation carried out with the addition of 2 grams of yeast and variations in fermentation time 2, 4 and 6 days.

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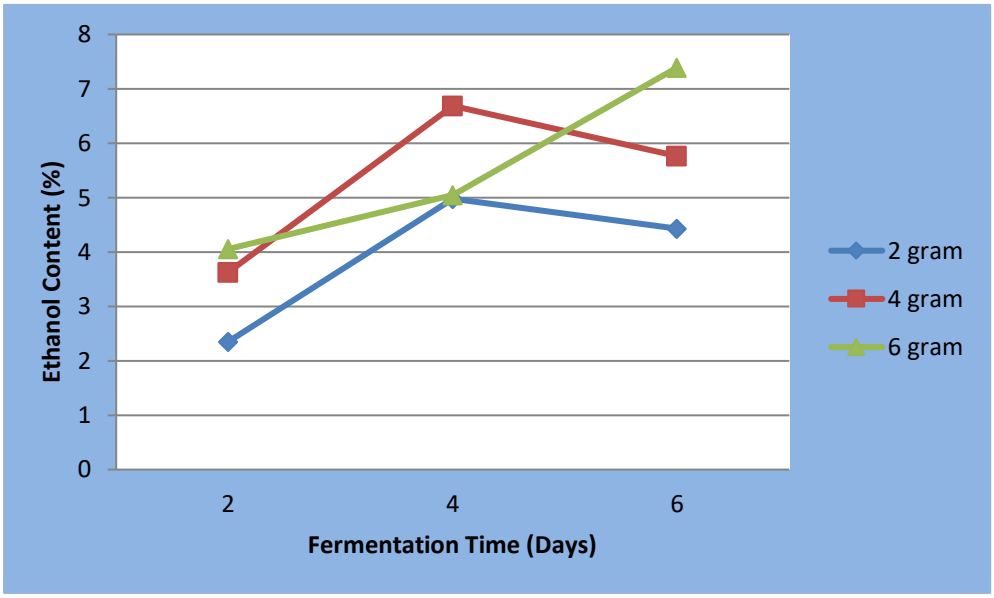

Figure 1. Bioethanol content curve with the variation of addition of bread yeast

Table 1. Data on titration volume of $FeSO_4(NH_4)_2SO_4$ 0.393 N and bioethanol content

Weight of yeast (g)	Fermentation time (day)	Volume of titration (mL)			Average titration volume (mL)	Ethanol content (%)
		I	II	III		
2	2	7,2	6,9	7,3	7,13	2,3532
	4	3,5	3,4	3,4	3,43	4,9792
	6	4,2	4,3	4,5	4,36	4,3404
4	2	5,4	5,3,	5,4	5,37	3,6307
	4	1,1	1,1	1,1	1,10	6,6860
	6	2,4	2,5	2,3	2,40	5,7599
6	2	4,7	4,8	4,9	4,80	4,0506
	4	3,3	3,4	3,5	3,40	5,0501
	6	0.1	0,1	0,1	0,10	7,3922

Where the smallest bioethanol content occurred at 2 days of fermentation with the addition of 2 grams of baker's yeast, namely 2.2532%. This is because the microbes are still in the adaptation phase and the microbial activity is also not optimal to decompose glucose into bioethanol. Meanwhile, the fermentation time of 4 days resulted in increasing levels of bioethanol, namely 4.9792%. On the fourth day, the microbes are in the exponential phase, and the most optimum time for microbes is to be able to decompose glucose into bioethanol. However, if the fermentation time is 6 days, the level of bioethanol produced decreases, namely 4.3404%. On the sixth day, the microbes entered the death phase due to a decrease in the number of nutrients so that the microbes were unable to convert glucose substrates into bioethanol as a result the levels of bioethanol produced decreased. For more details, see the graph in table 1, where the highest bioethanol content was obtained during 6 days of fermentation with the addition of 6 grams of baker's yeast, and the resulting bioethanol content was 7.3922%.

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3.4 Effect of Variations In Addition of Bread Yeast to Bioethanol Content

In the fermentation process, the number of microbes is very important in the formation of ethanol. The higher the amount of baker's yeast, the more yeast (*Saccharomyces cerevisiae*) that can break down glucose into alcohol. If the number of microbes is large, the production of ethanol will also increase until it can no longer be tolerated by yeast cells.

From the results of the research conducted, it can be seen that the more the amount of baker's yeast added, the higher the bioethanol content produced. This can be seen in Table 4.1 where the levels of bioethanol in fermentation for 2 and 6 days with variations in the addition of 2, 4, and 6 grams of baker's yeast increased in levels and the highest bioethanol content was obtained with the addition of 6 grams of baker's yeast with 6 days of fermentation, namely 7, 3922 %. This is due to optimal microbial activity in converting glucose into bioethanol. The more microbes there are, the more alcohol is formed.

However, there were different conditions in the 4-day fermentation with variations in the addition of 2, 4, and 6 g of baker's yeast. With the addition of 2 and 4 grams of yeast, the bioethanol content increased but decreased with the addition of 6 grams of baker's yeast. This is because the number of microbes that convert glucose into bioethanol is too much and is still in the growth phase so the microbes have not been able to break down glucose optimally within 4 days so the level of bioethanol produced is still low. More details can be seen in Table 1 and figure 1.

4 Conclusion

The cellulose content obtained from the isolation of cellulose from oil palm empty fruit bunches was 24.1298% and the glucose content obtained from the hydrolysis of cellulose from oil palm empty fruit bunches with 30% HCl was 17.1051% which was analyzed using the Nelson Somogyi method. Bread yeast can be used to ferment glucose resulting from the hydrolysis of cellulose from empty oil palm fruit bunches into bioethanol without going through the isolation of *Saccharomyces cerevisiae* first. The highest bioethanol content was obtained at 6 days of fermentation and with the addition of 6 grams of yeast, which was 7.3922%.

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