Effect of Time Fermentation and \textit{Saccharomyces Cerevisiae} Concentration for Bioethanol Production from Empty Fruit Bunch

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Abstract. Bioethanol is new renewable energy using the fermentation process from a substrate containing glucose. One of the materials which can convert to bioethanol is empty fruit bunch, which is in large amount in Indonesia. The plantation of palm oil has empty fruit bunch waste as much 23,988,293 tons in a year. The conversion from an empty fruit bunch to bioethanol also helps to reuse the waste which is not useful. There are a few steps to get bioethanol from an empty fruit bunch that is delignification of empty fruit bunch pollen using KOH solution which makes from empty fruit bunch ash getting from an incinerator. And then purification of pollen using H$_2$O$_2$ 3% solution, hydrolysis cellulose becomes glucose solution using H$_2$SO$_4$ 1% in process condition 100 $^\circ$C and 60 minutes. Fermentation using temperature condition 30 $^\circ$C, and stirring in 250 rpm. The variation of yeast \textit{Saccharomyces cerevisiae} concentration which is 4 g/L, 6 g/L, and 8 g/L. And time variation in 0, 24, 48, 72, 96, and 120 hours. The research result is shown that maximum glucose from hydrolysis is 112.44 g/L. And the highest bioethanol concentration from the process is 5.5% (v/v) or 41.411 g/L in yeast concentration 6 g/L and 96 hours.

Keywords: Bioethanol, Fermentation, Empty Fruit Bunch, \textit{Saccharomyces Cerevisiae}

1. Introduction
Recently, the depletion of fossil fuel stock has been occurred and has been so many interests to produce renewable energy from biomass. Biomass is organic material that is from herbs, this material can convert into bioethanol as a new source to use as fuel. Bioconversion of lignocellulosic waste material to biofuel is an interest in low cost and widespread. Indonesia is the largest palm oil producer, its because every year Indonesia produce 83.9 millions ton of fresh fruit bunch (FFB) in 2016. The process of FFB into crude palm oil (CPO) result in biomass waste in large amounts of empty fruit bunch (EFB). This EFB which is waste is not useful because sometimes burn in incinerator the ash spread in the field as fertilizer for the plant, but in the process of burning it can also create air pollution. The former research resulted reported that EFB containing 41,3-45% cellulose, 25,3-33,8% hemicellulose, and 27,6-32,5 lignin [1]. The type of main carbohydrates of EFB of palm oil are Glucan, xylan, and arabinan, each is 31,0: 17,3: and 0,5% [2]. Significantly high of lignocellulose content as material source bioethanol from EFB can degrade into sugar compounds by the fermentation process. Due to its high cellulose content, EFB of palm oil is the potential to be converted for the fermentative production of bioethanol, a promising alternative fuel to gasoline. Cellulose is a crystalline polymer that cannot easily be hydrolyzed to its monomer [3].
bioconversion of lignocellulosic waste of EFB to bioethanol consists of pretreatment for reducing the crystallinity of cellulosic material and removing of lignin, reducing hemicellulose also increasing of pore size of biomass, and the bleaching process to wash lignin that already removed, cellulosic hydrolysis or saccharification using acid or alkaline solution for fermentable sugar production, And fermentation of produce fermentable sugar to bioethanol. In this study pretreatment using KOH solution from were get from EFB ash to determine to reduce lignin content. This research will develop separated hydrolysis and fermentation (SHF) using the hydrolysis process to convert cellulose into glucose. The fermentation process using yeast *S. cerevisiae* to convert glucose into bioethanol.

2. Method

The material used in this study were empty fruit bunch, sulfuric acid 1%, hydrogen peroxide 3%, EFB ash solution as KOH, yeast *S. cerevisiae*, aqua dest. The main tool used in this research is Erlenmeyer using as the reactor. Also, the tool used in this research is a hot plate which serves for heating samples in hydrolysis and delignification process, mesh filter to get EFB pollen, as well as a hot plate, glass chemical, scales, thermometer, and magnetic stirrer used for the preparation of each process. The preparation of bioethanol production is the reduction of EFB into 1-5 mm. Reference [4] reported that pretreatment of lignocellulose rich material caused; fragmentation into small pieces, reducing lignin and hemicellulose, removed the crystalline structure, and increased the porosity of the materials. Then added extract EFB ash for delignification first process with EFB and extract comparison is 1:10, condition 100°C, 60 min, delignification second process call cooking with comparison 1:5 with same condition process. The solution washed with hot water. Bleaching process using H$_2$O$_2$ 3% and EFB pollen 10:1, in 90°C and 60 min. Hydrolysis process using H$_2$SO$_4$ 1% and EFB pollen with comparison 10:1 in 100°C and 60 min [5]. Made inoculum 200 ml volume with added nutrition KH$_2$PO$_4$ 1 g/l, MgSO$_4$. 7H$_2$O 0,05 g/l, (NH$_4$)$_2$SO$_4$ 2 g/l, inoculation time in 24 hours, temperature 30°C and 230 rpm. Finally, Fermentation used glucose from hydrolysis with 2 L fermentation volume, 30 °C temperature operation, magnetic stirrer used in 250 rpm. The variation of the fermentation process is time 0, 24, 48, 76, 96 and 120 hours, and yeast *S. cerevisiae* weight in 4 g/l, 6 g/l, and 8 g/l. Bioethanol got after separation used rotary evaporator from the sample that took every 24 hours from the fermentation process, then analyzed refractometer Brix alcohol.

3. Result and Discussion

3.1. Hydrolysis Cellulose

Hydrolysis is a process to cut the polymer chain of EFB to become monomers that more simple used of sulfuric acid in low concentration. The analyst of glucose concentration used a UV-Vis spectrophotometer. The analyst is shown in Tabel 3.1.

| Treatment | Glucose Concentration (g/L) |
|-----------|-----------------------------|
| Treatment 1 | 112.44                      |
| Treatment 2 | 108.78                      |
| Treatment 3 | 108.08                      |

Table 3.1 shown that all samples have not much different, and result in glucose high concentration. sulfuric acid resulted in more high glucose concentration the used chloride acid. More seriously of the cell structure damaged broaden accessible are of materials on the successive fermentation process. This condition is desirable because the saccharification and fermentation process increased yield of glucose [6].
3.2. Effect of glucose concentration against dry weight cell
Glucose as the result from the hydrolysis process used as fermentation substrate by yeast *S. Cerevisiae* to double cell and created bioethanol. The reduce of glucose by yeast is shown in Figure 3.1.

![Figure 3.1](image)

**Figure 3.1** Effect of Glucose Concentration for Against Dry Weight Cell (a) 4 g/l, (b) 6 g/l, (c) 8 g/l

Figure 3.1 The decreation of glucose mostly in 0-24 hours in every variation, and the increased of *S. Cerevisiae* mostly in 0-24 hours, but after that time glucose still available so the process continues with the small reduce of glucose and also small increased of *S. Cerevisiae*. After that, there will be competition in fellow *S. Cerevisiae* because food is not enough and will face of death phase [7]

3.3. Effect of Glucose Concentration Against Bioethanol
In every treatment, glucose was reduced because it has consumed by *S. Cerevisiae* and produce bioethanol. The reduce of glucose compared to bioethanol increased as shown in Figure 3.2.
Figure 3.2. The Effect of Glucose Concentration Against the Produce of Bioethanol, in Yeast Concentration (a) 4 g/l, (b) in 6 g/l and (c) 8 g/l.

Figure 3.2 Shown that the increased of Bioethanol mostly at time 0-24 hours and reduce of glucose also in that time very significant. That figure also has shown that glucose reduces in every number of times with a different number of reduced. The glucose, not all reduce become bioethanol but also there is another reaction that produces pyruvate acid as a secondary product. In time 96 to 120 hours bioethanol also reduce because of that secondary process.

3.4. Effect of *Saccharomyces Cerevisiae* Against Bioethanol

*S. Cerevisiae* is a microbes that used to convert glucose into bioethanol in this process, the effect of *S. Cerevisiae* is shown in Figure 3.3.

Figure 3.3 Effect of *Saccharomyces Cerevisiae* for Increased Bioethanol.

Figure 3.3 Shown that highest result is in variation 6 g/l *S. Cerevisiae* concentration which is 5.5% (v/v) in 96 times of fermentation. More and more time in fermentation was make more bioethanol, as long there still substrate the need to consume by *S. Cerevisiae*. Reference [8] reported If the microbes are too much will make bioethanol be formed and in the next time fermentation there no more substrate so the microbes will be in the death phase to early, the bioethanol will be decreased because of the secondary reaction.

4. Conclusion

Bioethanol production from an empty fruit bunch has successfully done by separated hydrolysis and fermentation (SHF) method. The highest result was obtained in 6 g/l *Saccharomyces Cerevisiae* concentration at 96 hours time fermentation which is 5.5% (v/v) equal to 41,411 g/l.
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