Production of Second Generation Bioethanol from Palm Fruit Fiber Biomass using *Saccharomyces cerevisiae*

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**Abstract.** Indonesia was the largest producer and exporter of palm oil in the world. As the plantation area expands, the increasing number of palm oil process industries results in large amounts of waste product. The biggest waste was of palm fruit fiber (fiber cake). It was known that 1 ton of fresh fruit bunches (FFB) of palm oil will produced 13% or 130 kg of fiber cake. By look at the potential that can be generated from the fruit fiber of palm oil (fiber cake) coming from the CPO process industry has a value that to use as the main ingredients in the making of alternative bioethanol fuel. The purposes of this study were to synthesize bioethanol from the raw material of palm fruit fibers, to determine the influence of the amount of *Saccharomyces cerevisiae* weight on bioethanol, and to determine the optimum time of bioethanol production from the raw material of palm fruit fibers by separation hydrolysis and fermentation (SHF) method. The stages of this study were delignification used KOH solution obtained from the extract of Palm Empty Cluster Ash, then the purification process using 3% H2O2 solution. Then the hydrolysis process using 2 M H2SO4 for 3 hours at 100°C. The last process was fermentation. In the fermentation process, variations of *Saccharomyces cerevisiae* concentration were 4, 6, 8 and 10 gr/L and fermentation time were 24, 48, 72, 96, and 120 hours. In acid hydrolysis, a maximum sugar concentration of 131 gr/L was produced. The best *Saccharomyces cerevisiae* concentration in this study was 4 gr/L at the best fermentation time of 96 hours with the obtained bioethanol content of 7% or 55.25 g/L.

**Keywords:** Bioethanol, Fermentation, Hydrolysis, Palm fruit fiber, *Saccharomyces cerevisiae*

1. **Introduction**

Tropical countries like Indonesia generally have abundant biomass. About 250 billion tons per year is produced from forest biomass and agricultural waste. Agricultural waste generally comes from oil palm, sugar cane, coconut plantations and the rest of the harvest and others which reach 40 billion tons per year. Palm oil is one of the plantation commodities which has an important role in economic activities in Indonesia. Indonesia is the largest producer and exporter of palm oil in the world. Based on BPS data from Indonesian palm oil in 2015 Indonesia's palm oil production in 2015 reached 31.28 million tons. Riau Province is the largest palm oil producing province in Indonesia with a total production of 7.33 million tons. Indonesia's palm oil production in 2015 increased 6.85% or 2 million tons compared to the previous year. The vast area of oil palm plantations will produce a lot of palm solid waste. The palm oil solid waste produced can be in the form of shells, stems, empty bunches, midribs, etc. which are the rest of the palm oil industry that has not been used optimally [1].
The largest amount of waste in the palm oil processing industry is the waste of palm oil fruit fibers (fiber cake). It is known that 1 ton of fresh fruit bunches (FFB) of oil palm will be able to produce 13% fiber or 130 kg fiber \(^{[2]}\). By looking at the potential that can be produced from fiber fruits of palm oil (fiber cake) originating from the CPO processing industry has a use value that can be used as the raw material in making bioethanol alternative fuels (2nd generation Bioethanol). The chemical components that make up palm fruit fiber can be seen in Table 1 as follows.

**Table 1 Chemical Components of Palm Fruit Fiber\(^{[3]}\)**

| Chemical Components | Composition (%) |
|---------------------|-----------------|
| Cellulose           | 29.02           |
| Hemicellulose       | 27.49           |
| Lignin              | 31.13           |

Bioethanol is an alternative fuel that is processed from biological sources, namely plants, which has the advantage of a relatively high octane number of 129 and can reduce CO\(_2\) emissions by 19-25% lower when compared to fossil fuels. Addition of bioethanol by 3% in gasoline can reduce CO\(_2\) emissions by 1.3%. In general, the synthesis of bioethanol derived from biomass consists of two main stages, namely hydrolysis and fermentation. The method consists of two main stages, namely hydrolysis and fermentation. In the previous method the process of hydrolysis and fermentation was carried out separately or Separated Hydrolysis and Fermentation (SHF) and the latest was the Simultaneous Saccharification and Fermentation (SSF) process or Simultaneous Saccharification (SFS). SHF is a bioethanol production method where the substrate hydrolysis process and fermentation process take place separately. The advantage of SHF is that hydrolysis by cellulase enzymes and fermentation by microorganisms can be carried out at each optimum condition \(^{[4]}\). Development of bioethanol with raw materials from foodstuffs will actually disrupt national food security. To overcome the energy crisis, it should not cause new problems that interfere with food security which is also no less important than the energy crisis. Therefore, the development of agricultural waste for bioethanol production will not disrupt national food security due to the increasing consumption of non-fossil fuels. In the previous study \(^{[5]}\) said that Europe and America suspect that the conversion of food / feed ingredients into bioethanol is one of the causes of rising prices of food and animal feed in the world.

In producing bioethanol the fermentation process is influenced by the activity of microorganisms in the substrate remodel into certain products which are influenced by several factors: fermentation media, temperature, nutrition, pH, starter volume, sugar concentration, *Saccharomyces cerevisiae* content, and fermentation time. Therefore this study is aimed at developing bioethanol with raw materials from crude palm oil (CPO) industrial waste, namely from palm fruit fibers as an alternative energy source to produce bioethanol using the method of Separated Hydrolysis and Fermentation (SHF), and to determine the effect of yeast concentration *Saccharomyces cerevisiae* and fermentation time for bioethanol produced.

2. **Research Methods**

2.1. **Raw materials Source**

The main raw materials used in this study were palm fruit fibers and palm empty fruit bunches ash used by cooking solution (KOH) obtained from PTPN Sei Galuh, Kampar, Riau.

2.2. **Experimental Procedure**

The implementation of bioethanol production from palm fruit fibers with separate hydrolysis and fermentation (SHF) method was carried out with two variables: weight concentration of *Saccharomyces cerevisiae* and fermentation time. In general, the stage of this research consists of four stages, namely the preparation stage of the raw material, the hydrolysis stage, the fermentation stage and the results analysis stage. These stages are shown in Figure 1 below.
Extract Abu Empty Fruit Bunch Oil
- Ash TKS 40 mesh: Aquades (1:4)
- stir for 15 minutes, let stand for 48 hours, then filter

Pretreatment of palm fruit fibers
- Fiber is reduced in size by blender then sieved with a size of 40 mesh

Delignification of palm fruit fiber powder,
- Fiber: palm empty fruit bunches ash extract (1:10), at T = 100 °C, for 60 minutes
- After the process is finished, the filtrate is removed and the residue/pulp is washed with distilled water
- The fiber pulp in the oven to a constant

Bleaching of fiber powder
- Fiber powder: H₂O₂ 3% (1:5), at T = 90 °C, for 60 minutes
- After the process is finished, the filtrate is removed and the residue/pulp is washed with distilled water
- Fiber in the oven

Saccharomyces cerevisiae inoculation
- Inoculation using an incubator for 24 hours at 30°C with an inoculum volume of 200 ml and added nutrients.
- Sterilization of samples using autoclave
- Variation of yeast concentration of S. cerevisiae (4 g/L, 6 g/L, 8 g/L, and 10 g/L)

Hydrolysis
- Fiber powder (80 mesh): H₂SO₄ 2 M (1:20) with T=100°C, for 3 hours
- The results of hydrolysis are filtered, and the filtrate is taken
- the filtrate is neutralized with 50% NaOH to pH 4.5

Fermentation by Saccharomyces cerevisiae
- Fermentation volume 2 L
- Fermentation time (24, 48, 72, 96 and 120 hours) with room temperature and stirring 100 rpm

Bioethanol dan residual glucose

Purification

Bioethanol

Analysis
Measurement of bioethanol content using alcoholmeter and GC analysis, and sugar content with UV-Vis spectro analysis

Figure 1 Research Stages
2.3. Results Analysis

In this study the parameters analyzed were bioethanol concentration and substrate sugar concentration. Substrate sugar concentration in the form of initial sugar and final sugar content was analyzed by the antrone method. For the measurement of bioethanol levels, the bioethanol mixture in the fermented substrate is separated from *Saccharomyces cerevisiae* microorganisms, nutrients and residual sugar solutions, by evaporating the mixture of bioethanol and water using a rotary evaporator, then bioethanol contained in a mixture of bioethanol and water, will be measured by using an alcohol meter and GC analysis (gas chromatography).

3. Results and discussion

3.1. Chemical Hydrolysis Process

Hydrolysis is the process of chemical decomposition using aqueous solvents to break the chemical bonds of the substance[6]. Chemical hydrolysis reactions can be done using dilute acids or concentrated acids. According to previous research[7] hydrolysis using sulfuric acid produced a higher yield than hydrolysis using hydrochloric acid. Therefore, in this study, cellulose was hydrolyzed using sulfuric acid solution with a concentration of 2 M and a temperature of 100°C for 3 hours[8]. The concentration of the initial sugar solution in each of the results of hydrolysis of palm fruit fibers can be seen in Table 2 below.

| Substrate | Glucose concentration (gr/L) |
|-----------|------------------------------|
| Substrate 1 | 129.62                      |
| Substrate 2 | 132.72                      |
| Substrate 3 | 130.66                      |
| Substrate 4 | 131.00                      |

Table 2 Concentration of Initial Sugar Solution of Acid Hydrolysis Results

palm fruit fiber which will be used as a fermentation medium. The results of the sugar concentration obtained varied because in the initial process of pretreatment no pure cellulose was obtained. In addition, the reduction of palm fiber with a size of 80 mesh is not uniform, affecting the concentration of glucose obtained, where the smaller the size of the fiber, the faster the material is hydrolyzed to glucose. According to previous research[9], the size of the raw material will affect porosity so that it can maximize contact between the material and acid to increase the hydrolysis of hemicellulose. The smaller the size of the raw material used will facilitate the degradation of lignin so that cellulose and hemicellulose will hydrolyze optimally. Other components such as hemicellulose and lignin which are still present in the cellulose fraction are also hydrolyzed to form non-reducing sugars. According to research[10], acids are non-specific and randomly cut glycosidic bonds to produce non-uniform sugars (disaccharides and monosaccharides). Cellulose will produce glucose while hemicellulose will produce xylose, manose, galactose and glucose.

3.2. Analyze the Dry Weight of Cells against Glucose Concentration

Analysis of cell dry weight is used to measure cell concentration during the fermentation process. By measuring the dry weight of cells we can see microbial growth. The variable changed in this study is the concentration of *Saccharomyces cerevisiae* used for fermentation processes with concentrations of 4 gr/L, 6 gr/L, 8 gr/L, and 10 gr/L. Data from measurements of *Saccharomyces cerevisiae* cell dry weight during the process of palm fruit fiber fermentation can be seen in Figure 2 below.
Figure 2 Comparison Graph between *Saccharomyces cerevisiae* Dry Yeast Cell Weight on Glucose Concentration (a) 4 gr/L yeast concentration (b) 6 gr/L yeast concentration (c) 8 gr/L yeast concentration (d) 10 gr/L yeast concentration

Based on Figure 2 above, with increasing fermentation time, dry cell tends to increase. The increase in dry weight of this cell is inversely proportional to the concentration of glucose in the substrate during the fermentation process. A decrease in sugar concentration shows that microorganisms consume sugar in the substrate, which is used by microorganisms to multiply cells. At 24 hours fermentation, *Saccharomyces cerevisiae* dry weight is still relatively low, this is because, at the initial stage, the cell is still adapting or adjusting itself to the fermentation medium, so the number of cells produced is still not maximal. At 48 hours, the cell dry weight produced was more than the 24-hour fermentation time, this was because *Saccharomyces cerevisiae* had passed the adaptation phase so that the cells divide and multiply faster. At 72 hours and 96 hours of fermentation, the resulting dry weight of the cells was not very significant, it only slightly increased compared to the dry weight of the cells at 48 hours of fermentation. This indicates that at 72 and 96 hours, the cell enters the stationary phase, which is the phase where the growth of microorganisms reaches their maximum state and active and dead microorganisms are relatively balanced because food (nutrition) is relatively small. When the 120 hour fermentation decreases the dry weight of the cell, this indicates that the cell has experienced a slowing growth phase or death phase.

In the study[11] entitled The Effect of Inoculum Concentration and Fermentation Time in Bioethanol Production from Eucheuma Cottonii Seaweed Using Microbes Association, obtained results of 10% inoculum concentration and 15% showed a graph of increased bioethanol production from day 4 to day 7 where microbial growth occurs exponentially so that an increase in enzymatic secretion by microbes can eventually hydrolyze carbohydrates to glucose and then converted into bioethanol. The decrease in the concentration of ethanol production on day 8 was caused by the degradation process of both enzymatic carbohydrates into glucose and then into ethanol by microbes which had depleted all the nutrients available in the process. This problem coupled with microbes has entered a period of accelerated death, so that the process of enzymatic secretion decreases which has an impact on ethanol production.
3.3. Relationship of Glucose Concentration to Bioethanol Concentration

Glucose solution used as a medium in the fermentation process is consumed by *Saccharomyces cerevisiae* microorganisms. The decrease in sugar concentration shows that microorganisms consume sugar in the substrate, which is used by microorganisms to multiply cells and produce bioethanol. The relationship of sugar concentration to the level of bioethanol produced from the fermentation process can be seen in Figure 3 below.

![Figure 3](image)

**Figure 3** Relationship of Sugar Concentration to Bioethanol Level (a) 4 gr/L *Saccharomyces cerevisiae* concentration (b) *Saccharomyces cerevisiae* concentration of 6 gr/L (c) *Saccharomyces cerevisiae* concentration of 8 gr/L (d) *Saccharomyces cerevisiae* concentration of 10 gr/L

Based on Figure 3 above, shows the relationship between sugar concentration and the level of bioethanol formed. Overall, with reduced sugar concentration, bioethanol levels will increase. This shows that the sugar consumed by microbes will be converted to bioethanol. But when viewed from a decrease in glucose concentration, not all glucose consumed by microbes is converted to bioethanol. Based on previous research [12] said that sugar in the fermentation process is not only converted into bioethanol but also used for cell formation and also for the formation of secondary metabolites such as pyruvic acid. In general, the concentration of yeast is 4 gr/L, 6 gr/L, 8 gr/L, and 10 gr/L decreases the sugar concentration along with the increase in bioethanol levels. It can be considered that with more and more fermented sugar, more bioethanol is formed. At 10 gr/L yeast concentration shows the lowest sugar concentration, this is caused by the many concentrations of yeast/microbes contained in the substrate. Glucose in the fermentation process is not only converted into bioethanol but is also used for cell formation and also for the formation of secondary metabolites such as pyruvic acid so that little bioethanol is produced.
3.4. Effect of Saccharomyces cerevisiae concentration and fermentation time on Bioethanol

Bioethanol is the final product to be obtained in this study. Bioethanol is obtained from microbial metabolic processes, in which bioethanol is a product of primary metabolites. Bioethanol fermentation occurs in anaerobic conditions. Microbes used in this study are *Saccharomyces cerevisiae* derived from packaging yeast. Before being used to ferment substrates, these microbes need to be inoculated. The purpose of the process of inoculation of *Saccharomyces cerevisiae* inoculum is to adapt microbial cells and to obtain optimum cell numbers. So that when the fermentation process takes place, the adaptation phase in the fermentation process can occur briefly or not even undergo an adaptation phase at all because the microbes have adapted to the fermentation substrate during the inoculation process. The relationship between *Saccharomyces cerevisiae* concentration to bioethanol levels obtained can be seen in Figure 4 below.

![Figure 4 Relation of *Saccharomyces cerevisiae* concentration to bioethanol produced](image)

Based on Figure 4 above obtained maximum bioethanol results in the addition of *Saccharomyces cerevisiae* as much as 4 gr/L at 96 hours which is 7% v/v. The more *Saccharomyces cerevisiae* is added, the more bacteria that break down glucose into ethanol. However, in the use of *Saccharomyces cerevisiae* 6 gr/L, 8 gr/L, and 10 gr/L the percentage of the results obtained decreases. According to the study[13], the large number of *Saccharomyces cerevisiae* added to the substrate that still causes a tight competition in life so that the metabolism of glucose to alcohol is less optimal because of the large number of dead *Saccharomyces cerevisiae*. So, in these conditions cannibalism occurs so that the number of cells that live less and less the activity of *Saccharomyces cerevisiae* to convert glucose to ethanol decreases. In addition, this is because the amount of nutrients available is not comparable to the number of *Saccharomyces cerevisiae*, so that *Saccharomyces cerevisiae* lacks food which resulted in decreased *Saccharomyces cerevisiae* performance and decreased yield of bioethanol[14].

Figure 4 above also shows the relationship between fermentation time to the concentration of bioethanol obtained at each variation of yeast concentration. In each variation of the concentration of yeast with a fermentation time of 24 hours to 96 hours experienced an increase in the yield of bioethanol produced, this is because the growth of *Saccharomyces cerevisiae* microorganisms has decreased so that the substrate is only used as metabolism and produce bioethanol. The highest level of bioethanol concentration was obtained at 4 gr/L yeast variation of 7% (v/v) at 96 hours fermentation. The optimum fermentation time for each yeast concentration variation is at the 96th hour. This explains that *Saccharomyces cerevisiae* is in the stationary phase at this hour. The activity of microorganisms decreased after 96 hours at 4 gr/L yeast concentration, 6 gr/L, 8 gr/L, and 10 gr/L which showed that *Saccharomyces cerevisiae* experienced a phase of death and did not work optimally. The longer the fermentation time, the concentration of bioethanol produced is also increasing. However, after optimum conditions are reached, the concentration of bioethanol obtained tends to decrease, because the nutrients that are available as microbial foods also decrease. In addition, the decreased bioethanol concentration is influenced by the decreasing concentration of sugar, so that the yeast runs out of nutrients to survive and experience the phase of death. Furthermore, the
bioethanol produced is converted to other organic acids due to bioethanol oxidation reactions [15]. This is shown by the reaction below:

\[
\text{C}_2\text{H}_5\text{OH} + \text{O}_2 \rightarrow \text{CH}_3\text{COOH} + \text{H}_2\text{O}
\]

Bioethanol  Oxygen  Acetate Acid  Water

In this study, the highest bioethanol content was obtained in the concentration of *Saccharomyces cerevisiae* yeast 4 gr/L which had previously been measured using an alcohol meter. So that the samples in the best conditions were analyzed using gas chromatography in the lab. Integrated Research and Testing UGM, Yogyakarta. The results of bioethanol analysis using GC analysis can be seen in Table 3 below.

| No. | Sample (Hours) | Bioethanol Result (%) |
|-----|----------------|-----------------------|
| 1.  | 24             | 0.64                  |
| 2.  | 48             | 1.22                  |
| 3.  | 72             | 1.76                  |
| 4.  | 96             | 2.38                  |

Table 3 above shows the results of GC analysis to find out more accurate bioethanol levels on hydrolyzates which were previously carried out by acid pretreatment of 0.64 (% v/v), 1.22% (v/v), 1.76% (v/v) and 2.38% (v/v). From the results of the GC test it can be said that the level of bioethanol produced is small, this is caused by the oxidation of bioethanol to acetaldehyde and then oxidized to acetic acid. This condition will cause the fermentation media to become more acidic (there is a change in pH) so that the bioethanol level becomes small. In addition, things that can cause small amounts of ethanol to be obtained are due to the volatile nature of bioethanol itself which can occur when packing samples and sending samples [16]. The following is a comparison of bioethanol produced from several studies that have been carried out shown in Table 4 below.

| Research  | Siburian (2015) | Faizal et al, (2016) | Ni’mah et al, (2015) | This research |
|-----------|-----------------|----------------------|----------------------|---------------|
| Raw material | Empty fruit bunch palm oil | Empty fruit bunch palm oil | Fiber palm oil | Fiber palm oil |
| Process   | SHF             | SHF                  | SHF                  | SHF           |
| Mikroorganism | *S. cerevisiae* | *S. cerevisiae* | *S. cerevisiae* | *S. cerevisiae* |
| Optimum concentration yeast | 8 gr/L | 3 gr | 0,4 gr | 4 gr/L |
| Bioethanol concentration | 3% (v/v) | 5,42% (v/v) | 2,39% (v/v) | 7%(v/v) |

Based on Table 4.4 above, we can see the effect of yeast concentration on bioethanol produced. In some studies using *Saccharomyces cerevisiae* concentrations that were greater than other studies, the highest levels of bioethanol obtained were 7%. This can be caused by several factors, namely due to differences in the types of raw materials used so that it will affect the glucose levels produced. In addition, it is not comparable between the amount of yeast and nutrients available on the substrate so that it affects the bioethanol obtained.

4. Conclusion
The conclusion of this study is that in this study using raw materials for palm fruit fiber which will be fermented to produce bioethanol. Before fermentation, the fiber is pretreated first, then hydrolyzed using sulfuric acid to produce glucose. Glucose levels obtained were ± 131 gr/L. The best concentration of *Saccharomyces cerevisiae* yeast is 4 gr/L with the highest bioethanol content of 7% v/v (on measurements using alcohol meter) or 2.38% (GC analysis). At 4 gr/L *Saccharomyces*
cerevisiae concentration, 6 gr/L, 8 gr/L, and 10 gr/L produced the highest bioethanol content at 96 hours best time.

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