The effect of *Saccharomyces cerevisiae* concentrations on second generation bioethanol production from oil palm frond

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**Abstract.** Oil palm is one of the plantation commodities that have important role in economic activity in Indonesia. With a total area of 11.30 million hectares and 75,517,083 tons of oil palm waste per year and it has a calorific value of 3350 kcal/kg, it has the potential to contribute alternative energy from the biomass. In addition to being utilized as an environmentally friendly renewable energy source, conversion of oil palm to bioethanol also helps to reduce untapped waste. Producing bioethanol from oil palm frond can be done through fermentation process. One of factors that affect the fermentation process is the number of cells of microorganisms. The microorganism used in this study is *Saccharomyces cerevisiae*. The purpose of this study was to determine the effect of *Saccharomyces cerevisiae* concentration on fermentation process on bioethanol produced and determine the best time of fermentation to bioethanol production from oil palm frond. The production of bioethanol from oil palm frond includes stages of delignification of oil palm frond using KOH solution obtained from empty fruit bunches ash extract, purification of oil palm using 3% H\(_2\)O\(_2\) solution, hydrolysis using 1% H\(_2\)SO\(_4\) with 100°C for 60 minutes, and fermentation using *Saccharomyces cerevisiae* with variation of *Saccharomyces cerevisiae* concentration 4 g/L, 6 g/L, 8 g/L, and 10 g/L. The maximum sugar concentration produced by the hydrolysis process was 117.55 g/L. The best bioethanol concentration was obtained at 3.29% (v/v) or 25.97 g/L at *Saccharomyces cerevisiae* 8 g/L concentration and 96 hours fermentation time.

1. Introduction

Oil palm is one of the commodities which have an important role in economic activities in Indonesia. Indonesia is one of the largest palm oil producing and exporting countries in the world. With a plantation area of 11.30 million hectares in 2015 [1] and each tree producing 40-50 fronds/year [2], oil palm has the potential to contribute alternative energy from the biomass. Oil palm frond contains cellulose, hemicellulose and lignin, so it suitable for use to producing biofuel. The components of the oil palm fronds as show in Table 1 below.

**Table 1. Chemical components of oil palm frond [3]**

| Chemical component     | Composition (%) |
|------------------------|-----------------|
| Cellulose              | 35.88           |
| Hemicellulose          | 26.47           |
| Lignin                 | 18.90           |
| Extractive substances  | 9.05            |
| Water                  | 9.70            |
Cellulose is a main component of wood, about 40-50% of dry wood. Cellulose is a homopoly-saccharide composed of β-D-glucopyranose units that are bound to each other by β-(1,4)-glycoside bonds. As a fibrous structure and strong hydrogen bonds, cellulose has high tensile strength and is insoluble in most solvents. Cellulose dissolves in high concentrations of mineral acid (due to hydrolysis), and if the hydrolysis has not gone too far the cellulose can be precipitated to form smaller molecular weight solid polymer fragments through dilution of the solution in the strong acid and water [4].

In addition, cellulose has a high crystalline structure that is difficult to breakdown, so pretreatment is needed before the hydrolysis process. The purpose of pretreatment is to breakdown the cellulose structure on the cell wall of biomass and make cellulose easier to take when hydrolyzed (in the process of hydrolysis, cellulose is broken down into simpler sugars) [5]. Cellulose hydrolysis is the process of breaking the bonds of β-1,4-glycosides in cellulose. The hydrolysis process is an important process to produce ethanol which can be made from the fermentation of glucose obtained from cellulose hydrolysis. The process of cellulose hydrolysis can be done by the method of hydrolysis of acids, bases, and enzymes [6].

Alcohol fermentation is a process of alcohol production by utilizing yeast activity. The fermentation process is anaerobic, which converts glucose to alcohol without oxygen, but in the inoculating it takes an aerobic where oxygen is needed for cell culture [7]. The factors that influence fermentation include acidity (pH), temperature, oxygens, fermentation time, microorganisms, and nutrients.

Bioethanol is an ethanol produced from materials containing carbohydrates that have the formula (CH₂O)₅₆. The raw materials used to make bioethanol can be grouped into three main groups, there are biomass containing sugar, biomass containing flour, and biomass containing lignocellulose [8].

Every process in fermentation affect on the bioethanol produced. The number of microorganisms will also affect the yield of bioethanol. The different weight of Saccharomyces cerevisiae will produce different numbers of microorganism cells. Thus, the concentration of Saccharomyces cerevisiae in the optimum inoculum will produce maximum bioethanol as well.

The purposes of the research is to produce bioethanol from oil palm fronds using separate hydrolysis and fermentation (SHF) method, which is synthesizing bioethanol from raw oil palm fronds with SHF method, determining the effect of Saccharomyces cerevisiae concentration on bioethanol produced, and determining optimum time of bioethanol production from raw material of oil palm fronds using SHF method.

2. Research Methods

2.1. Raw Material Source
The main raw material used in this research is oil palm fronds obtained from the Agribusiness Incubator Universitas Riau. Other materials used are the ash of empty palm fruit bunches of palm oil mill incinerator PTPN V Sei Galuh.

2.2. Procedure
The implementation of bioethanol production from oil palm fronds using separate hydrolysis and fermentation (SHF) method was carried out with two variables: Saccharomyces cerevisiae concentrations (4 g/L, 6 g/L, 8 g/L and 10 g/L) and fermentation times (24 hours, 48, hours, 72 hours, 96 hours and 120 hours). Other process conditions are 2 liter fermentation volume [9], 24 hours inoculation time [10], 30°C fermentation temperature [11], fermentation pH 4.5 [12], stirring speed 250 rpm [9], and 10% inoculum volume (v/v) [13]. The results to be obtained from this study are bioethanol concentration. In general, this research consists of four stages: the material preparation stage, the hydrolysis stage, the fermentation stage and the results analysis stage. This research procedure is as shown in Figure 1 below.
2.3. Results Analysis

In this study, the parameters analyzed were bioethanol concentration, substrate sugar concentration, and number of cells. The initial and final glucose concentration was analyzed by the anthrone method using a UV-Vis Spectrophotometer. The bioethanol concentration will be analyzed using alcoholmeter and gas chromatography. Number of cell analyzed using dry cell weight method.
3. Results and Discussion

3.1. Cellulose Hydrolysis of Oil Palm Fronds

Cellulose is hydrolyzed using a sulfuric acid solution with a concentration of 1% with a temperature of 100°C for 60 minutes [12]. These sugar solutions used as a substrate in the fermentation process. The reduction glucose analyzed using the antrone method. Table 2 below is part of the hydrolysis process.

| S. cerevisiae concentration | Initial sugar concentration (g/L) |
|-----------------------------|----------------------------------|
| 4 g/L                       | 116.34                           |
| 6 g/L                       | 114.10                           |
| 8 g/L                       | 117.55                           |
| 10 g/L                      | 115.48                            |

The process of cellulose hydrolysis which contained oil palm frond in this study used dilute sulfuric acid solution. Hydrolysis using sulfuric acid gives higher results than hydrolysis using chloride acid [14]. In addition, the results of research on variations of acid to hydrolyze sago waste using chloride acid and sulfuric acid found that sulfuric acid produced higher sugar concentration compared to chloride acid [15].

3.2. Effect of Sugar Concentration on Number of Cells

Sugar solution from the hydrolysis process in this study was used as a fermentation substrate by Saccharomyces cerevisiae to multiply cells and produce bioethanol. Decrease of sugar concentration by microbes as shown in Figure 2 below.

![Figure 2](image-url)

*Figure 2.* Relationship between decreases of sugar concentration, number of cells and bioethanol concentration on Saccharomyces cerevisiae concentrations (a) 4 g/L, (b) 6 g/L, (c) 8 g/L, and (d) 10 g/L.
Based on Figure 2, the sugar concentration in the substrate decreases all the time during the fermentation process. This shows that microorganism activity occurs during fermentation to convert glucose to bioethanol [16]. In the variation of *Saccharomyces cerevisiae* concentration of 4 g/L and 6 g/L, it is seen that the decrease in sugar concentration is inversely proportional to the number of cells that increase all the time. This is because in the variation of 4 g/L and 6 g/L, the number of cells contained in the fermentation medium still uses the substrate to multiply itself. In this phase, the number of microbes reproduces rapidly. The optimal working time of *Saccharomyces cerevisiae* (yeast) is on the 24th to 72nd hours. In this phase microbes remodel the substrate into nutrients for growth [17].

Whereas in the variation of *Saccharomyces cerevisiae* concentration 8 g/L and 10 g/L it was seen that the number of cells decreased as decreasing sugar concentration. This is because microbes in these variations use substrates to produce bioethanol. In addition, the length of time of fermentation, the nutrients in the medium decreases with the increasing number of cells can lead to competition and eventually enter the phase of death. In this death phase, the number of microbes that divide is less than the number of microbes that die, so the number of cells as cell dry weight decreases throughout the fermentation time [13]. When entering the death phase, the primary metabolites (bioethanol) produced are toxic to *Saccharomyces cerevisiae*. In addition, after 72 hours of fermentation began to run less effectively due to the decreasing acidity (pH) of the fermentation solution. The pH reduction is caused by the presence of organic acids such as lactic acid, acetate and pyruvate which are formed during the fermentation process [17].

### 3.3. Effect of Decreasing Sugar Concentration on Bioethanol Concentrations

Sugar solution obtained from the hydrolysis process is used as a carbon source by *Saccharomyces cerevisiae* to multiply cells and produce bioethanol. The relationship of sugar concentration to the level of bioethanol produced from the fermentation process is shown in Figure 2. Based on Figure 2, overall sugar concentration decreases all the time and bioethanol concentration increases. This condition occurs because sugar in the fermentation substrate is consumed by microorganisms and converted into bioethanol. However, not all sugar in the substrate is converted to bioethanol. Sugar 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 [18].

During the first 24 hours fermentation, the decrease in sugar concentrations was not matched by the production of bioethanol. This is because the sugar is used by microbes to multiply cells [17]. At the time of fermentation up to 72 hours, bioethanol has not been produced optimally for variations in *Saccharomyces cerevisiae* concentrations of 4 g/L and 6 g/L. In this variation, the number of microbial cells that exist still continues to multiply itself so that it is quite a bit to consume sugar in the substrate and convert it to bioethanol. Whereas in the variation of the concentration of *Saccharomyces cerevisiae* 8 g/L and 10 g/L, the number of microbes that consume more sugar thus produces higher concentrations of bioethanol.

At the concentration of 8 g/L *Saccharomyces cerevisiae* obtained the greatest bioethanol content, which is 7% (v/v) at 96 hours fermentation. In these conditions, there is still a lot of glucose in the media so that the process of cleavage and fermentation of *Saccharomyces cerevisiae* cells goes well and the alcohol produced is also high [15]. The longer the fermentation process and the more yeast is given to the same substrate, the higher concentration of bioethanol produced. This is because more and more microbes are involved in the fermentation process. Fermentation time also affects bioethanol productions. The longer fermentation time will provide an opportunity for microbes to do more decomposition of the substrate [19]. But at 120 hours fermentation time, bioethanol concentrations decreased to 5% (v/v). This is because the number of microbes is decreasing and will go to the death phase because more bioethanol is produced and the nutrients are getting thinner. In addition, the bioethanol produced has been further oxidized to acetic acid [20].

At a concentration of 10 g/L *Saccharomyces cerevisiae* produced the highest bioethanol content of 6% (v/v) at 72 hours fermentation, but bioethanol concentrations decreased at 96 hours of
Fermentation and so on. Fermentation is the process of breaking down simple sugars (glucose and fructose) into bioethanol and CO$_2$ by involving enzymes produced in yeast to work at optimum temperatures. The fermentation process depends on the least amount of yeast addition in the substrates [19]. However, the greater number of microbes in the concentration of 10 g/L Saccharomyces cerevisiae causes the faster nutrients to be converted to bioethanol, so that nutrients soon thin out and microbes are faster to the death phase. This is because the amount of available nutrition is not comparable to the number of Saccharomyces cerevisiae which is more, so Saccharomyces cereviceae lacks food which results in the performance of Saccharomyces cerevisiae decreasing and resulting in a decrease in the amount of bioethanol produced [21]. In addition, in the bioethanol production, the number of cells that are too high causes the process to weaken faster and reduce cell viability after the growth phase. Growth conditions and metabolism in high cell populations are not expected, because they interfere with access to nutrients, limited space and cell interaction [22].

3.4. Bioethanol Concentrations in the Best Conditions
This research was done to determine the best conditions in the fermentation process of oil palm fronds using Saccharomyces cerevisiae. Based on the research, it is known that the concentration of Saccharomyces cerevisiae which gives the best results is 8 g/L. The bioethanol content analyzed using gas chromatography (GC) at each fermentation time as shown in Table 3 below.

| Fermentation time (hour) | Bioethanol concentrations (%) v/v | Bioethanol concentrations (g/L) |
|--------------------------|----------------------------------|---------------------------------|
| 24                       | 0.83                             | 6.55                            |
| 48                       | 2.05                             | 16.18                           |
| 72                       | 2.39                             | 18.86                           |
| 96                       | 3.29                             | 25.97                           |
| 120                      | 1.00                             | 7.89                            |

This GC analysis aims to determine whether there is bioethanol produced from the fermentation process. The samples analyzed were fermented at Saccharomyces cerevisiae concentration of 8 g/L at 24 hours to 120 hours of fermentation. Based on Table 3, it can be seen that bioethanol levels are influenced by fermentation time. The longer the fermentation time, the bioethanol level increases. The optimum ethanol content was obtained at 96 hours fermentation, which was 3.29% (v/v) or 25.97 g/L and decreased at 120 hours of fermentation. The longer the fermentation time, the concentration of bioethanol produced is also increasing. However, after optimum conditions have been reached, bioethanol produced tends to decrease. This is because the nutrients that exist as microbial foods also decrease [23]. In addition, the fermentation occur further bioethanol so that the bioethanol produced decreases with increasing fermentation time after passing the optimum conditions. This further reaction occurs because bioethanol is oxidized to acetic acid [24].

In Table 4 below is a comparison of bioethanol produced from several studies that have been carried out.

| Researcher | Raw materials | Microorganisms | Microorganism concentrations | Initial sugar concentrations | Bioethanol concentrations |
|------------|---------------|----------------|-------------------------------|-------------------------------|--------------------------|
|            | Pineapple skin | Saccharomyces cerevisiae | 6 g/L | 283.74 g/L | 31.40% (v/v) |
|            | Sugarcane     | Saccharomyces cerevisiae | 4% (v/v) | 50.23% | 8.79% (b/v) |
|            | Cassava       | Saccharomyces cerevisiae | 7.5 g/L | 220 g/L | 83.44 g/L |
|            | Oil palm frond| Saccharomyces cerevisiae | 8 g/L | 117.55 g/L | 25.97 g/L |
Based on Table 4, the results of bioethanol fermentation in this study were quite low compared to the study of [25] and [27]. This is because the raw material in the study contains a lot of glucose. In [25] which use pineapple skin as a substrate not through the process of hydrolysis. In addition, the level of glucose contained in the substrate of the pineapple skin is quite high, so that it can produce a higher product too. Likewise research conducted by [27] who use raw materials from cassava. Glucose content contained in the substrate is also higher than this study, so it can produce higher bioethanol products.

In a study conducted by [26] produced a lower bioethanol level than this study, which was 8.79% (b/v) or 8.79 g/L, whereas in this study produced bioethanol with a level of 25.97 g/L. This happened because of the research conducted by [25] using substrate from sugarcane resulting from dilution with a glucose concentration of 50.23%. If the glucose density is 1.54 g/ml, then glucose levels in the study conducted by [26] amounted to 77.35 g/L, which means it is smaller than this study. In addition, the concentration of *Saccharomyces cerevisiae* used is also smaller, so the number of microbes that convert glucose to bioethanol is also less.

4. Conclusions

Based on the results of this study, it can be concluded the following points. First, bioethanol can be produced from palm fronds raw materials through a process of Separated Hydrolysis and Fermentation (SHF) using *Saccharomyces cerevisiae* with the highest bioethanol content of 25.97 g/L. Second, the higher the concentration of *Saccharomyces cerevisiae*, the greater the level of bioethanol produced. The optimum *Saccharomyces cerevisiae* concentration for the palm frond fermentation process through the process of SHF is 8 g/L. The fermentation time affects the level of bioethanol produced, with the optimum time of fermentation of the oil palm frond through the process of SHF using *Saccharomyces cerevisiae* for 96 hours.

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