The Effect of Type and Concentration Yeast with Fermentation Time and Liquifaction Variations on the Bioethanol Concentration Resulted by Sorgum Seeds with Hydrolysis and Fermentation Processes

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RESEARCH ARTICLE

The Effect of Type and Concentration Yeast with Fermentation Time and Liquifaction Variations on the Bioethanol Concentration Resulted by Sorgum Seeds with Hydrolysis and Fermentation Processes

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Sorghum is one of the plants that can be used as raw material for making bioethanol. Sorghum has seeds with a starch composition of 73.8%, which is potential as a raw material for making bioethanol. Sorghum starch can be converted into bioethanol through the hydrolysis process (the process of converting carbohydrates into glucose) which consists of liquefaction and saccharification processes and is followed by a fermentation process. The hydrolysis method is carried out enzymatically. In this study alpha amylase and gluco amylase enzymes were used with various types of yeast including *Saccharomyces cerevisiae*, Rhizopus oryzae, Acetobacter xylinum, Mucor sp, and Aspergilus niger which varied with liquefaction temperatures including 80, 85, 90, 95, and 100 °C. Obtained the most optimal yeast is *Saccharomyces cerevisiae* with an optimal temperature of 95 °C resulting in a bioethanol concentration of 4.3%. After getting the optimal yeast and temperature, the fermentation step of the two variables is used in the next step. In the fermentation process, variations of yeast concentration and duration of fermentation were used, the optimum yeast concentration was at 2.5% with 48 hours of fermentation resulting in bioethanol concentration of 11%.

Keywords: Bioethanol, Hydrolysis, Fermentation, Sorghum, *Saccharomyces cereviceae*.

1. INTRODUCTION

Energy is an important aspect of daily life. Energy needs always increase with the rapid rate of population growth and the rapid industrialization of the world which results in the depletion of large amounts of energy reserves, especially fossil energy, which is the world's main energy source. Global economic recovery driven by high economic growth in Asia is accompanied by an increase in energy demand for industry and consumption, contributing to the increase in world energy prices. The proportion of petroleum as the main source of energy currently reaches 40% of the world's total energy demand, but its reserves continue to decrease. In 2011 world oil demand growth reached 1.7%. The increase in production which only reached 0.9% and the diminishing global petroleum reserves caused countries including Indonesia to be vulnerable to the risk of the world energy crisis. Petroleum reserves are currently proven in Indonesia to be 9 billion barrels, with an average production rate of 0.5 billion barrels per year, and are expected to run out within 18 years. Gas reserves are estimated at 170 TSCF (trillion standard cubic feeds) while production capacity reaches 8.35 BSCF (billion standard cubic feeds). Meanwhile, coal reserves are estimated at 57 billion tons with a production capacity of 131.72 million tons per year [1]. The depletion of fossil energy reserves is of particular concern. Indonesia, which has been making fossil energy as the main energy, must start implementing alternative energy or new and renewable energy (RE). The government wants to increase the share of RE in the energy mix to 23 percent by 2025. At present, the portion is still below 15 percent [2]. This is in line with Regulation Number 5 of 2006 and Presidential Instruction Number 1 of 2006 dated January 25, 2006, on the National Energy Policy to develop alternative energy sources as a substitute for fuel oil [3]. Indonesia as a rich country in natural resources has a wide opportunity for the development of bioethanol to replace the fewer fossil energy sources. At this time bioethanol has begun to be produced [4]. Bio ethanol is ethanol produced

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from biological raw materials with biochemical technology, through the fermentation process of raw materials [5]. Indonesia has natural resources that have the potential as raw materials for bioethanol production. One of them is sorghum. Sorghum has a high adaptability to climate change because of its efficient use of water. When other crops such as rice and corn fail, sorghum can thrive and give good results. Sweet sorghum plants have a high yield, which is 80 tons/ha/year [6]. Sorghum has seeds with a composition of 73.8% starch, which is potential as a raw material for making bioethanol. From the results of *4–6* t/ha seeds can be produced 3.6 tons of starch flour or 1,800 liters of ethanol/ha [7]. Sorghum starch can be converted into bio ethanol through the hydrolysis process (the process of converting carbohydrates to glucose) and fermentation. The hydrolysis method can be carried out with an acid catalyst and enzymatically. Enzymatic hydrolysis method is more often used because it is more environmental friendly [5]. In a study conducted by ErniNurFitriana, fermentation was carried out yeast *Saccharomyces cerevisiae* with variations of 2, 4, 6, 8 and 10% (w/w) for 7 days. The level of bioethanol without hydrolysis results without acid followed by fermentation of 3.33; 3.19; 3.99; 3.79 and 3.49%, and direct fermentation of 1.00 and 0.81% in the use of yeast 4 and 6% (w/w) [8, 9]. In the research conducted by Nova [10]. With the liquefaction temperature variables of 75 °C, 85 °C and 95 °C. For the temperature of 75 °C the highest productivity value is obtained at 24 hours fermentation time, which is 3% or 30 g/L. At a temperature of 85 °C the highest productivity value is obtained at 24 hours, which is equal to 5% or 50 g/L. Meanwhile, at the liquidation temperature of 95 °C the highest productivity was obtained at an ethanol concentration of 5% or 50 g/L with a 24-hour fermentation time [9]. Research by Jhonprimen HS et al., about the effect of yeast types on bioethanol fermentation process for 3 days using variables in bread yeast with masses of 5, 7, 10 grams yielding ethanol levels which tend to increase ie successively 13.73%, 19.22%, and 20.37%. In tape yeast with successive periods of 5, 7, 10 gr, the ethanol content tends to increase, which are 18.05%, 19.67%, and 24.01% respectively [10]. From the research that has been done, there are important variables that can affect the yield of bioethanol. Therefore, this study aims to obtain the most optimal type of yeast and its concentration and obtain liquefaction temperature and duration of fermentation.

2. RESEARCH METHODS

Bioethanol is made in several stages, namely hydrolysis (liquifaction and saccharification), fermentation, and distillation (see Fig. 1).

The ingredients used in this study were sorghum seeds, alpha amylase enzyme, glucosyl amylase enzyme, luffschrool solution, H_2SO_4, aquadest, *Saccharomyces cerevisiae*, rhizopusoryzae, acetobacterxylinum, mucorsp, and aspergilusniger. While the equipment used in this study is a set of fermentors, a set of distillation tools, 10 ml and 25 ml measuring pipettes, rubber balls, universal indicators, 250 ml and 500 ml beakers, 100 ml measuring cups, drip pipettes, stirrers, pumpkin necks three, 100 ml and 250 ml pumpkin, watch glass, 250 ml and 1000 ml erlenmeyer, hot plate, magnetic stirer, 100 °C thermometer, boiling stone, and analytical balance.

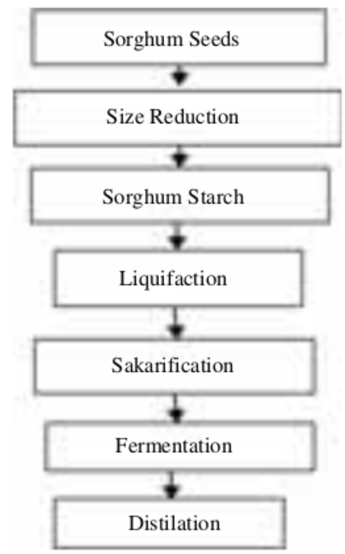

Fig. 1. Bioethanol making scheme.

3. RESULTS AND DISCUSSION

In the manufacture of bioethanol from sorghum seeds, before the fermentation stage is carried out, a sugar concentration test is carried out using a luff-schoorl solution. The results of the analysis can be seen in the following table.

From the Table I above it can be seen that in the temperature range 80–95 °C the sugar concentration increases in concentration, whereas when the temperature is raised to 100 °C the sugar concentration decreases. It can be said that the optimum liquefaction temperature in the formation of sugar is at a temperature of 95 °C which is equal to 13.998%. Visually the increase and decrease in sugar concentration can be seen in the Figure 2.

Table I. Concentration of sugar in starch sorghum.

Liquifaction temperature (°C)	Sugar concentration (g/L)
80	11.254
85	11.879
90	12.424
95	13.998
100	12.872

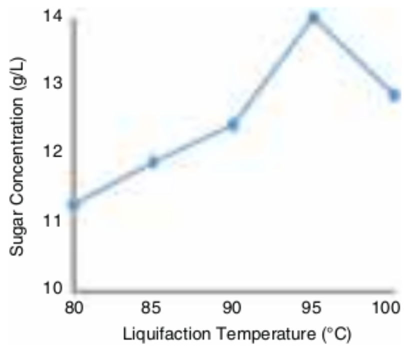

Fig. 2. Effect of liquifaction temperature on sugar concentration.

3.1. Effect of Yeast Type on Bioethanol Concentration with Liquifaction Temperature Variation

The liquefaction results are then carried out in the fermentation stage. Each variable has different results. Bioethanol acquisition data which is influenced by the type of yeast and the temperature of the liquefaction can be seen in Figure 3.

From Figure 3, it can be seen that in liquefaction process with temperatures of 80 °C and 85 °C, the highest concentration of bioethanol is 2% using *Saccharomyces cereviceae* yeast, at liquefaction temperature 90 °C and 95 °C, bioethanol concentration is increased, each of which is obtained 3% bioethanol concentration and 4.3% using *Saccharomyces cerevisiae* yeast. But when liquefaction temperature is increased by 5 °C, the bioethanol yield obtained decreases. This happens because at a temperature of 100 °C, the amount of sugar concentration decreases. Sugar is a transition product before the product turns into biethanol. When the sugar breaking activity that occurs at temperatures of 100 °C occurs so slowly. This occurs because the activity of enzymes that break down complex carbohydrates into simple carbohydrates becomes more decreased because the enzymes used work optimally at a temperature of 95 °C (see Table II).

Table II. Obtaining bioethanol with variations in yeast types and liquifaction temperature.

Types of yeast	Concentration of bioethanol (%) with liquifaction (°C) temperature variation				
	80	85	90	95	100
SC	2	2	3	4,3	3,7
RO	1	1	1,2	2	1,8
AX	0,6	0,65	0,8	1	0,9
M	1	1,5	2	2	1,7
AN	1,1	1,2	1,2	1,4	1,2

Information: SC: *Saccharomyces cerevisiae*, RO: Rhizopus oryzae, AX: Acetobacter xylinum, M: Mucor sp., AN: Aspergilus niger.

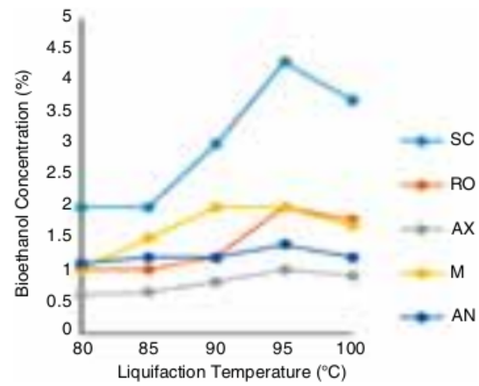

Fig. 3. Effect of yeast type variation and liquifaction temperature on bioethanol concentration.

3.2. Effect of Yeast Concentration on Bioethanol Concentration with Variations in Fermentation Time

The fermentation process is done anaerobically in the fermenter column. Based on the results of the research on the variety of yeast types above, *Saccharomyces cerevisiae* yeast was taken as the most optimal yeast in the process of determining the optimal time for fermentation. Yeast *Saccharomyces cerevisiae* functions as a decomposing bacterium of glucose into bioethanol in the fermentation process. The reactions that occur during this fermentation process are as follows:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

The variation of yeast concentrations used in this study were 1, 1.5, 2, 2.5, and 3%, while the variation of fermentation time was 24, 32, 40, 48, and 56 hours. In this research process the following data were obtained (see Table III).

In the fermentation process, the concentration of bioethanol is determined by an alcohol meter. The process of forming bioethanol using *Saccharomyces cerevisie* yeast begins with the hydrolysis process. This hydrolysis process ims to break down carbohydrates into glucose. Two stages occurred, namely liquifaction and saccharification stage.

Table III. Bioethanol yield with yeast concentration variation and fermentation time.

Yeast concentration (%)	Bioethanol concentration (%) with fermentation time variation (hours)				
	24	32	40	48	56
1	2	6	7,8	9	7,3
1,5	3	4,5	6	8,3	8
2	3,2	6,7	7,2	8,5	7,8
2,5	3	6	8	11	9
3	2,5	5,2	7,5	9,3	7,1

At the liquifaction stage occurs at an optimal temperature of 95 °C for 90 minutes. The liquifaction process which is part of the hydrolysis process aims to convert starch in sorghum to sucrose with the help of the alpha amylase enzyme catalyst. Whereas the saccharification stage aims to break down the sucrose obtained in the previous stage with the help of the gluco amylase enzyme catalyst. This process takes place at a temperature of 60 °C for 90 minutes. Sucrose is broken down into simple sugars, glucose, which is then adjusted to bioethanol in the fermentation process with the help of metabolism from *Saccharomyces cerevisiae*. The fermentation process takes place at room temperature which is 30 °C with a time variation of 24, 32, 40, 48, and 56 hours. The concentration of bioethanol produced in this time variation and yeast concentration can be seen in Figure 4.

It can be seen in Figure 4, that in all variations of the fermentation time, the highest bioethanol concentration was obtained at the yeast concentration of 2.5% in 48 hours which is 11% but decreased in addition to the yeast concentration to 3%. From the data obtained it can be concluded that the higher the yeast concentration used, the higher the concentration of bioethanol obtained. This occurs because of the increasing metabolic activity of microorganisms that takes place during the fermentation process, so that glucose is converted into more ethanol. But in this condition the optimal yeast concentration is at 2.5%. This statement is in accordance with Murray (2009) in Ilham [5], 2016 which states that the more enzymes, to a certain extent, the more substrates are converted because the higher the enzyme activity, but excessive enzyme concentration will also affect the rate of enzymatic reactions [3, 5]. Similar research states that substrate concentrations that are too high can inhibit the

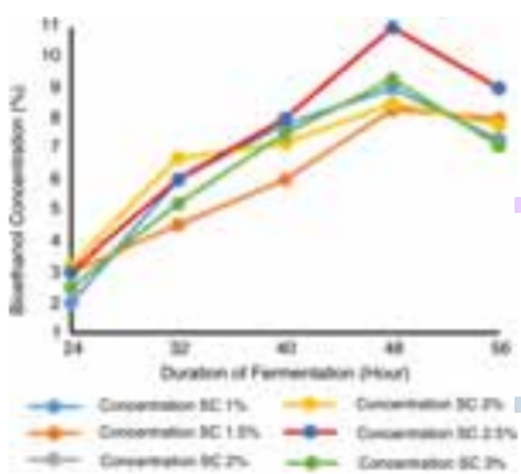

Fig. 4. Effect of variation in yeast concentration and fermentation time on bioethanol concentration.

growth of microorganisms so the conversion rate becomes slow [11, 12]. This is consistent with the data obtained in this study that the most optimal yeast concentration is at 2.5% and the activity of *Saccharomyces cerevisiae* has decreased at 3% yeast because under these conditions, *Saccharomyces cerevisiae* is no longer working optimally. In addition to yeast concentration, fermentation is also influenced by fermentation time. Similar to yeast concentration, fermentation time also cannot be said the longer the process takes place, the higher the concentration of bioethanol obtained. From Figure 4 it can be seen that the most optimal fermentation time is at 48 hours and decreases in the next 8 hours. This happens because at the 56th hour the concentration of sugar decreases because it has been converted to bioethanol. Another thing that causes this decrease in bioethanol concentration is if the fermentation time is too long then over time bioethanol can be converted to acetic acid. Like the following reaction [5].

$$C_1H_5OH + O_2 \longrightarrow CH_2COOH + H_2O$$

4. CONCLUSION

Based on research that has been done, it can be concluded that the optimal liquefaction temperature is 95 °C with the resulting sugar concentration of 13.998 g/L and the type of yeast used is *Saccharomyces cereviceae* with the yield of a bioethanol concentration of 4.3%. In the fermentation process, the most optimal concentration of yeast *Saccharomyces cereviceae* is 2.5% with a fermentation time of 48 hours obtained a bioethanol concentration of 11%.

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