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Kiagus Ahmad Roni[1], Muhammad Erlangga Pangestu[2], Ani Melani[3], Netty Herawati[4], Zahrul Muhrodi[5], Sri Martini[6]

[1,2,3,4,5,6] Department of Chemical Engineering, Universitas Muhammadiyah Palembang, Indonesia

[5] Department of Chemical Engineering, Universitas Ahmad Dahlan, Indonesia.

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Abstract:
One of the plants used as a producer of bioethanol is sorghum . Sorghum has seeds with a starch composition of 80.4 %, which is potential as a raw material for making bioethanol. Sorghum starch can be converted into bioethanol through a hydrolysis process (the process of converting carbohydrates into glucose) which consists of a liquefaction and saccharification process and followed by a fermentation process. The hydrolysis method is carried out enzymatically. This research used alpha amylase and gluco amylase enzymes with various types of yeast including *Lactobacilus Casei, Rhizopus Oligosporus, Candida Glabrata, Saccharomyces Cerevisiae, and Neurospora Crassa* which varied with liquefaction temperature covering 95 o C. After getting yeast which is optimal at that temperature , then the second fermentation stage of the variables used in the next phase. In the fermentation process, variations in percent yeast levels are used with variations of 1%, 1.5%, 2%, 2.5% and 3% . Obtained the most optimal yeast is *Saccharomyces Cerevisiae* with optimal yeast concentration at 2.5% with 48 hours of fermentation which results in a bioethanol concentration of 4.3% in the initial sugar concentration of 11,998 g / L. After analysis, the most optimal bioethanol product has physical properties, namely the density of 0.783 gr / mL with a refractive index of 1.3589.

Keywords: *Bioethanol, hydrolysis, fermentation, sorghum, yeast, Saccharomyces Cereviceae , Lactobacilus Casei.*

PRELIMINARY

In the current millennial generation, energy can not be separated from everyday life. Increased population growth has an impact on increasing energy consumption. Various human activities are very dependent on the availability of energy resources.

Meeting energy needs still depend on fossil energy, especially petroleum. The resources of fossil energy resources are non-renewable (non reneweable energy) which, if explored continuously be thinned. With these conditions, an unlimited number of renewable energy efforts are needed, one of which is bioethanol. In addition, the use of fuel oil (BBM) raises environmental problems such as the threat of air pollution and global warming. Therefore, people are required to be more wise in using energy. One of the efforts made is to substitute gasoline or premium with bioethanol (Prihandana et al, 2008)[6]

Along with the depletion of fuel energy reserves, in recent decades, an interesting study to find out the potential of renewable energy sources. This is in line with the instructions of the Minister of Energy and Mineral Resources No. 12 of 2015, the types of sectors that are required to implement include

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micro businesses, fisheries businesses, agricultural businesses, transportation and public services / PSO (Public Service Obligation); non PSO transportation; industrial and commercial; and power plants. The mixing program of Biodiesel into diesel by 20% (B20) has been implemented well in the transportation sector (PSO) since 2016. In accordance with the direction of the President of the Republic of Indonesia, starting on September 1, 2018 the B20 mandatory was carried out massively in all sectors.

Government support for implementing biohydrocarbon biofuel that has the same or even better characteristics than fossil-based hydrocarbon / BBM compounds has encouraged researchers to get alternative fuels as a fuel chaser from petroleum. Alternative fuels that are developed are those that are renewable and environmentally friendly and efficient, especially those from natural and plant-based materials. One type of vegetable fuel that is feasible to be developed is bioethanol, ethanol obtained through the process of glucose fermentation using enzymes produced by the microorganism Saccharomyces cerevisiae. The bioethanol is a biofuel substitute for premium or biokerosin called Biofuel (BBN).

The potential of sorghum plants is used as a raw material for making bioethanol, because the source of raw materials can be taken from starch, sap and pulp from sorghum. Sorghum has a starch composition of 80.42%. From the results of 4-6 t / ha seeds can produce 3.6 tons of starch or 1,800 liters of ethanol / ha . The composition of sorghum starch is very potential as a source of biofuel, bioethanol. Sorghum starch can be converted to bioethanol through hydrolysis and fermentation processes. Sorghum is considered capable of becoming a source of raw materials that can survive and be renewed to produce bioethanol (Barcelos et al, 2011)[2]

The fermentation process was carried out by Emmanuela M. Widyanti using a column reactor at a substrate flow rate of 6 mL / min and a residence time of 41.03 minutes with a continuous system. The results were analyzed every 30 minutes, from 0 to 240 minutes, the following results were obtained, sucrose experienced a decrease of 8.64-7.88% and ethanol formation from 0 to 3.11%, whereas a decrease in glucose from 8.45 to 7.08 % and ethanol increase of 0–4.46%, liquid sugar decreased from 8.45 to 6.94% and ethanol increase from 0–4.46%, testing was carried out using a brixmeter and ethanol sensor. The results showed that the difference in substrate reduction was not very significant, while the formation of ethanol in glucose and liquid sugar substrate was not much different, because the liquid sugar used had high purity. (Emanuela M. Widyanti, 2016)[7]

Other microorganisms such as lactic acid bacteria (BAL) are known to potentially break down sugar into bioethanol. Research related to the use of LAB as a bioethanol-producing microorganism has not been done much, but several studies report that LAB has the potential to produce bioethanol. Erten (2000) reported that Leuconostoc mesenteroides can break down glucose into products of lactic acid and ethanol. Lara et al. (2016) strengthen the suspicion that Lactobacillus casei is able to survive in the condition of the media with ethanol, and produce the final product in the form of ethanol after fermentation. It is suspected that Lactobacillus casei is able to break down simple sugars on the corn dregs substrate with environmental modification to produce ethanol. (Tri Nur Utami. 2017)[5]

Research by Jhonprimen HS et al., About the effect of yeast type on bioethanol fermentation process during 3 days recidence time using variables in bread yeast with successive masses of 5, 7, 10 grams resulting in ethanol levels which tend to increase ie successively 13.73%, 19.22% and 20.37%. In yeast tape with consecutive time 5, 7, l0 gr produce ethanol content that is likely to increase in a row 18:05%, 19.67%, and 24.01% (H onprimen HS et al, 2017)[3]

From the research that has been done, it is seen that important variables can influence the acquisition of bioethanol. Therefore, this study aims to obtain the most optimal type of yeast and concentration and to obtain the recidence time of fermentation.

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RESEARCH METHODS

Bioethanol production is carried out in several stages, namely hydrolysis (liquifaction and saccharification), fermentation, and distillation.

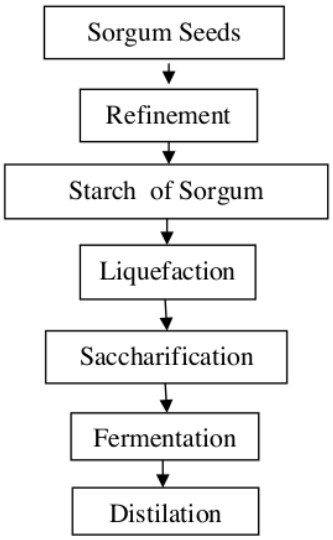

Figure 1. Schema of Making Bioethanol

The materials used in this study is grain sorghum, the enzyme alpha amylase , an enzyme gluko amylase , the solution luff-schrool , H_2SO_4 , distilled water , Lactobacillus Casei, oligosporus Rhizopus, Candida glabrata, Saccharomyces cerevisiae and Neurospora crassa . While the equipment used in this study is a set of fermentors, a set of distillation devices, 10 ml and 25 ml measuring pipettes , rubber balls, universal indicators, 250 ml and 500 ml beakers, 100 ml measuring cups, drop pipettes, stirrers, neck flasks 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 .

RESULTS AND DISCUSSION

In the manufacture of bioethanol from sorghum seeds, prior to the fermentation stage, a hydrolysis process is carried out to increase glucose levels by testing the sugar concentration using a luff-schoorl solution.

Establishment sugar used in this research that is by 11.998 % derived from sorghum solution liquefaction process at a temperature of 75 °C.

The liquefaction results are then carried out the fermentation stage. Each variable has a different result.

Sugar is a transition product before the product turns into biethanol. When the activity of breaking down sugar that occurs at temperatures that are too hot 100 °C occurs so slowly. This happens 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.

Effect of Yeast Concentration on Bioethanol Concentration by Recidence Time Fermentation Variations

The anaerobic fermentation process is carried out in the fermenter column . Based on the results pe nelit i 's to variations in the type of yeast on top, then take yeast Saccharomyces cerevisiae as yeast most optimal da lam the process of determining the optimum time to ferment.

Yeast Saccharomyces cerevisiae functions as a glucose-decomposing bacterium 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 variations in the concentration of yeast used in this study were 1 , 1.5 , 2, 2.5 , and 3%, while for the fermentation time variations were 12, 24 , 3 6 , 48, and 72 hours. In this research process the following data were obtained.

Table 3. Obtaining Bioethanol with Variations in Yeast Concentration and Fermentation Time

Konsentrasi Yeast (%)	Konsentrasi Bioetanol (%) dengan Variasi Waktu Fermentasi (jam)				
	12	24	32	48	72
RO	0	2	6	9	7,3
LC	1	3	4,5	8,3	8
CG	1	3,2	6,7	8,5	7,8

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SC	2	3	6	11	9
NC	1	2,5	5,2	9,3	7,1

Noted :
RO : *Rhizopus Oligosporus*
LC : *Lactobacilus Casei*
CG : *Candida Glabrata*
SC : *Saccharomyces Cerevisiae*
NC : *Neurospora Crassa*

In this fermentation process, the concentration of bioethanol is determined by means of an alcohol meter. The process of formation of bioethanol using yeast Saccharomyces cerevisiae begins with the process of hidrolysis. This hydrolysis process aims to break down carbohydrates into glucose. Two stages occurred, namely liquifaction and saccharification stage. 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 support of metabolism from *Saccharomyces cerevisiae* .

The fermentation process takes place at room temperature that is 30 °C with a variation of recidence time 12 hours, 24 hours, 36 hours, 48 hours, and 72 hours. The concentration of bioethanol produced by this time variation and yeast concentration can be seen in Figure 3.

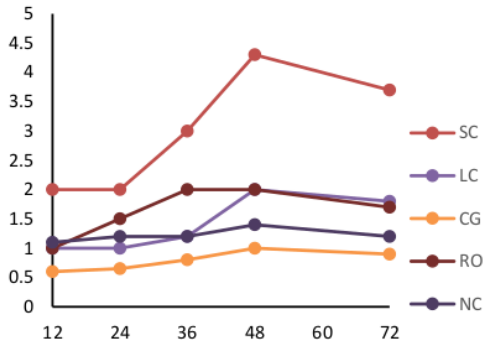

Figure 3. Effect of Yeast Type Variation and Recidence Time on Bioethanol Concentration

From Figure 3 can be seen that the yeast of Saccharomyces cerevisiae has the highest concentration of bioethanol at the same temperature. Whereas Lactobacilus Casei bacteria has a relatively stable value.

It is happening because of the recidence time of fermentation in line with the amount of yeast that is getting increased. Most of the yeast that is isolated in fermented food carries out fermentation activities by producing varying percentages of ethanol. The type of yeast capable of producing ethanol is depicted in phylogenetic construction which only leads to the Ascomycetes Division, which is five clades.

Isolation and identification of yeast in fermented foods shows the diversity of yeast that plays a role in the fermentation process of food. Fermented food samples isolated by modification of the basic method affect the type of isolated yeast. (I Nyoman Sumerta : 2016)[4]

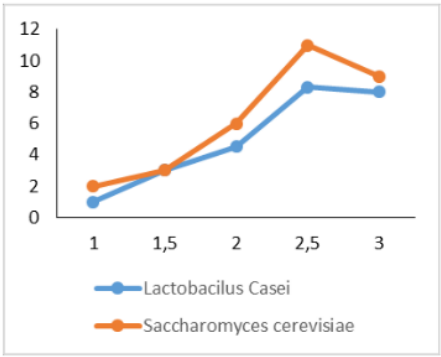

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Figure 4 . Bioethanol levels graph obtained from a concentration of 2.5% yeast within 48 hours.

Can be seen in Figure 4, that on the whole variation of fermentation time, obtained with modern bioethanol concentration on microbe *Saccharomyces cerevisiae* with Konse ntrasi yeast 2.5% in recidence time of 48 hours is equal to 11% but decreased in addition yeast concentration becomes 3%. As for the *Lactobacilus Casei* microbe, it has a relatively stable value. That is because *Lactobacillus casei* is included in the kingdom of microbial bacteria that can break down simple sugars on the substrate of sorghum with environmental modification to produce ethanol. From the data obtained it can be concluded that the higher the concentration of yeast used, the higher the concentration of bioethanol obtained. This happens because of the increasing number of metabolic activities of microorganisms that take place during the fermentation process, so that more glucose is converted to ethanol . But in this condition the optimal yeast concentration is at 2.5%.

This statement is in accordance with Murray (2009) in Ilha m 1 , 2016 which states the more enzyme, to a certain extent, then se more and more substrates which have been converted for se the higher is also the activity of the enzyme, but the enzyme concentration, excessive will also affect the rate of enzymatic reactions (Ilham et al , 2016)[1]

This is consistent with the data obtained in this study , the most optimal concentration of yeast is at 2.5% and the activity of Saccharomyces cerevisiae has decreased at yeast 3% 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 (Ilham et al, 2016) [1]

$$C_2H_5OH + O_2 \longrightarrow CH_3COOH + H_2O$$

CONCLUSION

Based on the research that has been done, it can be concluded that in the fermentation process, the most optimal concentration of Saccharomyces cereviceae yeast is 2.5% with a recidence time of fermentation that is 48 hours obtained by bioethanol concentration of 11% . Whereas the most stable yeast is Lactobacilus Casei.

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