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The Influence of Pretreatment Time, Type and the Concentration of Yeast on Ethanol Production from Rice Straw.

Akmal D[1]*, Mhd Riza Marjoni[2], and Friardi Ismed[1].

[1]Faculty of Pharmacy, University of Andalas, Padang 25163, West Sumatera, Indonesia.
[2]Dwi Farma Academy of Pharmaceutical, Bukittinggi 26121, West Sumatera, Indonesia.

ABSTRACT

Rice straw is one of the very abundant agricultural waste in Indonesia. The content of cellulose and hemicellulose are high on the straw can be used as feedstock for bioethanol production through fermentation with the help of yeast. This study uses two types of yeast is *Saccharomyces cerevisiae* and *Rhizopus sp*. which has the ability to change the straw hydrolyzate containing cellulose into ethanol through fermentation. The research was conducted by fermenting rice straw hydrolyzate pretreatment results with both the yeast at the same concentration. The purpose of this study was to obtain a pretreatment, the type and concentration of the best yeast to produce bioethanol. Variables examined included pretreatment time (1 hour, 2 hours and 3 hours), the type of yeast (S and R), and the concentration of yeast (3%, 5% and 10%). The parameters observed as a performance indicator production process is the change in pH during fermentation, biomass and ethanol produced after 72 hours of fermentation. The results showed that the highest biomass produced was 0.81 g with 7.74% ethanol using *Saccharomyces cerevisiae*. The best pretreatment time obtained after 2 hours of pretreatment with the concentration of 5% yeast *Saccharomyces cerevisiae*.
Keywords: rice straw, *Saccharomyces cerevisiae*, *Rhizopus sp*., bioethanol.

Corresponding author

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INTRODUCTION

According on the data of Agricultural Statistics conducted by the Ministry of Agriculture in 2013 can be seen that the vast rice fields in Indonesia is 8.1 million hectares [1]. Each hectare of agricultural land is capable of producing wet straw approximately 10-15 tons with moisture content of about 60% [2]. Research of Kim and Dale in 2004 states that every ton of dry rice straw yield of 1.4 tons and each kilo of dry straw can produce 0.28 L of bioethanol [3].

Chemically straw containing 10-15% lignin, 25-45% cellulose and hemicelluloses 20-30% [4]. The content ofcelluloseand hemicelluloses are high on the straw, allowing straw can be used for processed into a more useful products such as ethanol [5]. Celluloseand hemicellulose monomers prepared by the same sugars that make up starch sugars known as lignocellulosic [6]. Lignocellulose can be hydrolyzed into simple sugars for subsequent fermented into bioethanol with the help of microbes [7]. Microbes are widely used in fermentation are yeasts, fungi or bacteria. But not all yeasts, fungi or bacteria can be used directly, but required the selection of each order to assure a fermentation process in accordance with the objectives [8].

Bioethanol production process can be done through the conversion of raw materials by utilizing the appropriate microbes. One of the most common species of microbes used for fermentation is *Saccharomyces cerevisiae*. These microbes capable of converting carbohydrates into alcohol through fermentation. Besides *Saccharomyces cerevisiae*, the yeast *Rhizopus sp* also has capabilities similar to *Saccharomyces cerevisiae* to convert carbohydrates into bioethanol [5]. Based on this, then appeared an idea to make use of straw as araw material source of bioethanol by utilizing *Saccharomyces cerevisiae* and *Rhizopus sp* to convert sugar into ethanol by fermentation.

EXPERIMENTAL

Materials and Devices Research

The main ingredient in the form of rice straw fermentation obtained in the area of paddies in the area KamangAgam District of West Sumatra. Other materials used include 10% H_2SO_4, NaOH (E.Merck), ethanol pa (E.Merck), Nutrient Broth, the yeast *Saccharomyces cerevisiae* and *Rhizopus sp*, distilled water, urea, NPK. While the equipment used is a distillation aparatus, grinder, incubator shaker, analytical scales, centrifuge, Erlenmeyer 250 ml and 500 ml, pipette, timers, thermometers, pH meters, pycnometer (Iwaky Pyrex)

Study Design

Yeast used in this study is *Saccharomyces cerevisiae* and *Rhizopus sp*. The first factor is the variation Pretreatment time (1 hour, 2 hours and 3 hours) while the second factor is the variation of the concentration specified yeast (3%, 5% and 10%). Of the two factors obtained research design as shown in Table 1.

Table 1: Study Design

Type of Yeast	Yeast Concentration (%)	Pretreatment time with LHW method (Hours)			
			T1	T2	T3
Saccharomyces sereviceae	3	Y_3	Y_3T_1	Y_3T_2	Y_3T_3
	5	Y_5	Y_5T_1	Y_5T_2	Y_5T_3
	10	Y_{10}	$Y_{10}T_1$	$Y_{10}T_2$	$Y_{10}T_3$
Rizhopus sp.	3	Y_3	Y_3T_1	Y_3T_2	Y_3T_3
	5	Y_5	Y_5T_1	Y_5T_2	Y_5T_3
	10	Y_{10}	$Y_{10}T_1$	$Y_{10}T_2$	$Y_{10}T_3$

Note : T_1 = Treatment 1
T_2 = Treatment 2
T_3 = Treatment 3
Y_3 = yeast concentration 3%
Y_5 = yeast concentration 5%
Y_{10} = yeast concentration 10%

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Microorganisms and Medium

Saccharomyces cerevisiae and *Rhizopus sp* was used for the ethanol fermentation. Inoculum was prepared by transferring respectively 5 gram cells of *Saccharomyces cerevisiae* and *Rhizopus sp* into flask containing 500 ml of culture medium. Incubated at 30 °C for 48 h with an incubator shaker at 100 rpm. This was used to inoculate the fermentation medium. The inoculum at a concentration of 3%, 5% and 10% of the volume of hydrolyzate was used for fermentation purpose. Samples, for biomass and ethanol analysis were taken after 72 hours of fermentation [9].

Hydrolyzate fermentation

Samples in the form of dry straw consisting of stems, leaves, fruit and the rest of the fruit stalk chopped/diced small then smoothed with a grinder. 3 kg of straw is inserted into the pot hydrolysis, add water. Hydrolysis with variations within 1 hour, 2 hours and 3 hours using the method Liquid Hot Water (LHW).

Filter hydrolyzate obtained into Erlenmeyer was first sterilized. Adjust the pH of the hydrolyzate obtained becomes 4.5 to 4.8 and then inoculated with the inoculum according to treatment in experimental design (3%, 5% and 10% (v /v) of the hydrolyzate obtained). Added urea 0.13% and NPK 0.028% of the amount of water. The fermentation was carried out at room temperature with a shaker incubator at 100 rpm for 72 hours. Flowchart of the study are presented in Figure 1.

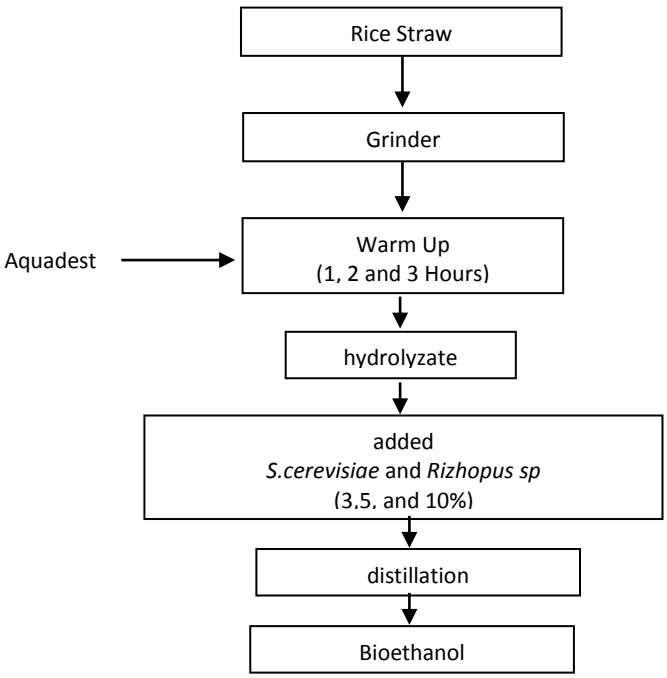

Figure 1: Schematic of bioethanol fermentation

Analysis procedures

Prepared a series of raw ethanol solution with a concentration of 5,10,15,20 and 25% by means of pipette ethanol pa respectively 5, 10, 15, 20 and 25 ml and its added with distilled water to 100 ml. Fill the pycnometer with distilled water, wipe with a tissue, weighed. Then Fill pycnometer with the standard solution of ethanol, cleared with a tissue, weighed and determined the relative specific gravity and subsequently made the relationship between the concentration and a specific gravity relative.

Quantitative analysis of ethanol made using the method by plotting the specific gravity of gravity of ethanol distillate to a linear regression equation of ethanol standard curve. Each fermentation culture was centrifuged at a speed of 400 rpm for 15 minutes. Supernatant were analyzed for levels of ethanol, while

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biomass determined gravimetrically. The parameters observed as a performance indicator production process is the change in pH during fermentation (pH-meter), Biomass (gravimetric) and ethanol (linear regression).

RESULTS AND DISCUSSION

From the graph the relationship between Specific gravity relative to each concentration of standard solution of ethanol in Figure 2 below, obtained curve regression equation Y=-0.00124x + 0.9986 with correlation coefficient (r^2) = 0.99585; standard deviation (SD) = 0.00027785; The limit of detection (LOD) = 0669%; System error 1.88%. The resulting linear regression curve is negative linear regression curve. The value ofthe coefficient b is negative, so the regression model also worth negative or not unidirectional. This means that the higher the specific gravity of ethanol, the lower the levels of ethanol produced.

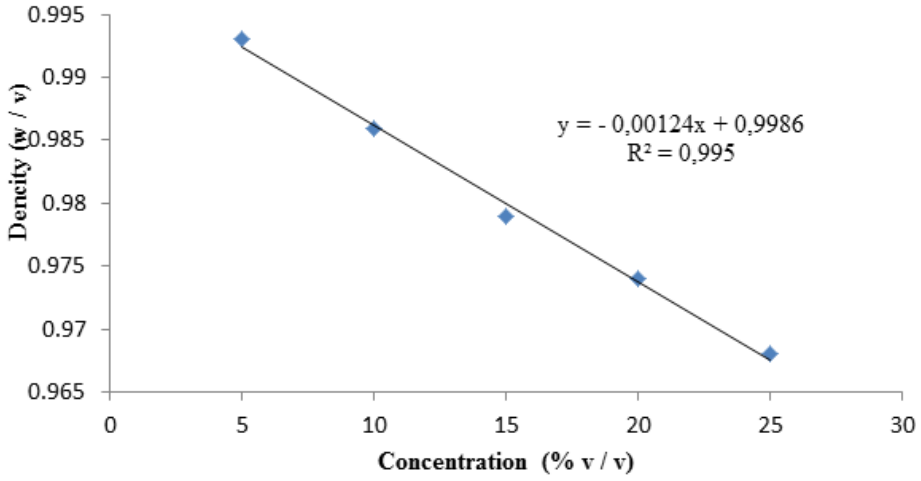

Figure 2. Graph Equation Regression Standard Solution of Ethanol

From the data obtained regression equation that the value of r near 1 proves that the regression equation is linear. Regression curve also shows that there is aclose relationship between the concentration of a specific gravity of ethanol. Of the value of R^2 (R Square) can be seen that there is a significant relationship between the closeness of ethanol concentration with a specific gravity relative to the degree of closeness of 0.99585 and a small standard deviation indicates a fairly high accuracy.

Experiment on pretreatment time, the type of yeast and yeast concentration used by pH, biomass and ethanol as an indicator of the data obtained are presented in Table 2.

Table 2: Results of measurements of pH, biomass and ethanol based on pretreatment time

Yeast	Yeast Concentration (%)	pH			Biomassa (g)			Ethanol (%)		
		P1	P2	P3	P1	P2	P3	P1	P2	P3
Sc	3	5.6	5.7	5.7	0.12	0.69	0.45	2.90	3.70	2.09
	5	5.6	5.7	5.7	0.69	0.81	0.80	2.90	7.74	4.51
	10	5.6	5.7	5.7	0.45	0.79	0.44	3.70	4.51	2.09
R	3	5.6	5.7	5.7	0.07	0.31	0.21	1.29	2.90	2.90
	5	5.7	5.7	5.7	0.29	0.68	0.51	2.90	5.32	4.51
	10	5.7	5.7	5.7	0.08	0.60	0.36	2.09	5.32	3.70

Sc. = *S. cereviseae* P1 = Pretreatment 1 hour
R =*Rizhopussp* P2 = Pretreatment 2 hours
P3 = Pretreatment 3 hours

In Table 2 it can be seen that the highest biomass was obtained at a concentration of 5% yeast with a pretreatment for 2 hours ie 0.81 g to 0.68 g of *Saccharomyces cerevisiae* and using *Rhizopus sp*. The highest

ethanol concentration was also obtained at the time of pretreatment 2 hours with the same yeast concentration of 5% as much as 7.74% to *Saccharomyces cerevisiae* and 5,32% for *Rhizopus sp*

After 1 hour pretreatment, levels of ethanol produced by using *Saccharomyces cerevisiae* higher levels of ethanol produced by *Rhizopus sp*. The yeast concentration of 10% (Figure 3). Biomass and ethanol by using two types of yeast, in pretreatment for 1 hour tends to increase because at this point, there is a growth of microbes and the decomposition of glucose to ethanol better.

Pretreatment in the process of change in biomass into bioethanol basically is to destroy the cell structure to be more easily processed in biological or chemical treatment [10]. Removal of hemicellulose can increase the pore volume and has a specific surface area, thus increasing the sugar obtained. Sugar obtained without pretreatment of less than 20% and after pretreatment was able to increase to 90% theoretically [11]. The main purpose of the process is the pretreatment of lignocellulose in order to liberate the cellulose structure becomes more accessible [12]

At a concentration of 3% yeas tin figure 3, it is seen that there has been a lag phase which is the adjustment phase microbial growth conditions in the new environment. In this phase of slow growth occurs because cells do prepare cleavage

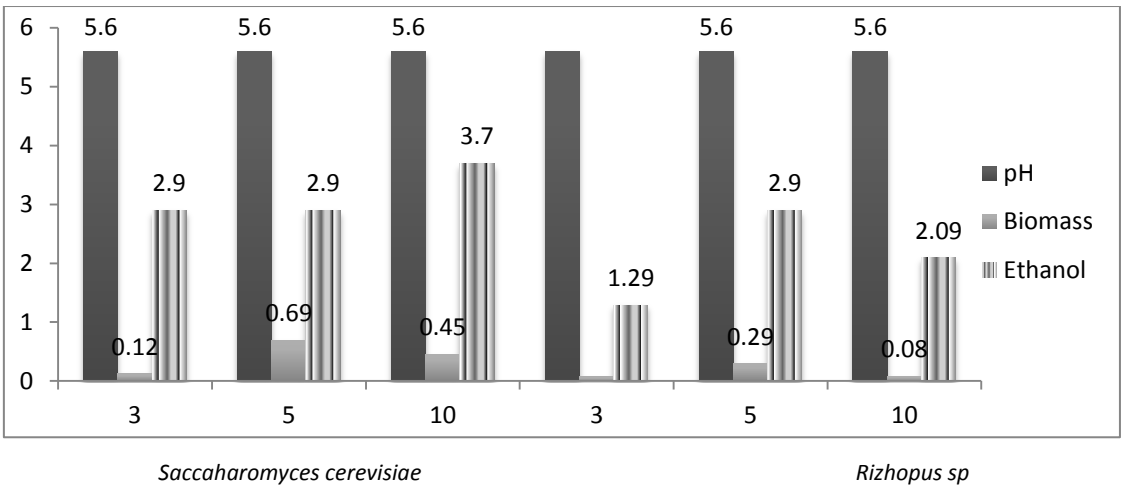

Figure 3: Graph of pH, biomass and ethanol between *Saccharomyces cerevisiae* and *Rizhopus sp*. At 1 hour Pretreatment

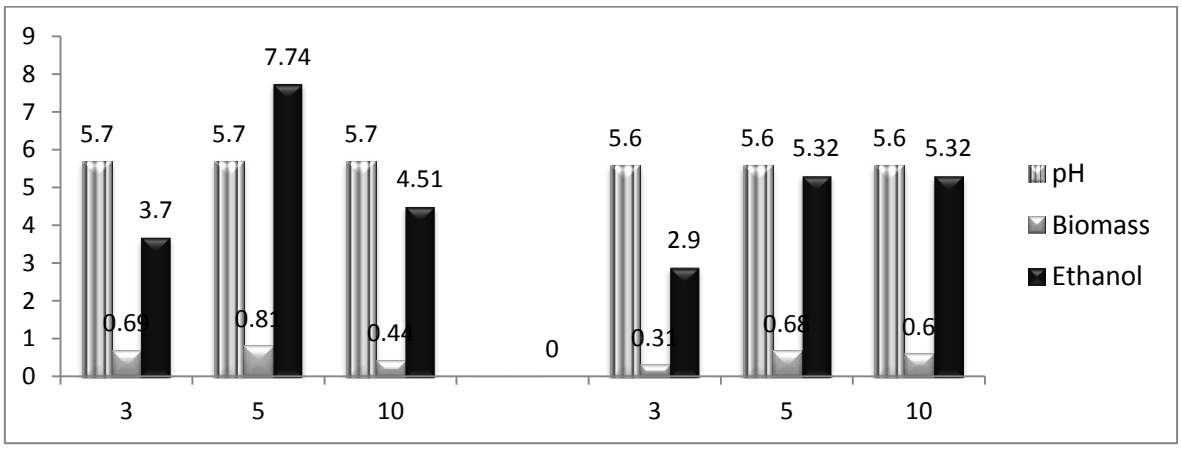

Figure 4: Graph of pH, biomass and ethanol between *Saccharomyces cerevisiae* and *Rizhopus sp*. At 2 hour Pretreatment

Figure 4 shows that the two types of yeast both biomass and the highest ethanol concentration was obtained at a concentration of 5%. This is because the yeast has experienced maximum growth. Biomass and

ethanol increased sharply compared to the first pretreatment. The highest levels of ethanol produced in this pretreatment as much as 7.74% to *Saccharomyces cerevisiae* and 5,32% to *Rizhopus sp*. Increasing concentrations of yeast used from 5% to 10% resulted in a decrease in levels of ethanol produced by *Saccharomyces cerevisiae* from 7.74% to 2,09%. This is caused by the increasing number of yeast given resulting in decreased glucose into ethanol decomposition. Meanwhile, the yeast *Rhizopussp*, occurred stationary phase in which the levels of ethanol produced is still stable at 5,32%.

In Figures 3 and 4 shown an association between levels of ethanol produced by the length of the pretreatment process, the concentration and type of yeast. Of the second graph, reflecting the trend of rising levels of ethanol produced by increasing the concentration of yeast used both in *Saccharomyces cerevisiae* and *Rhizopus sp*

Figure 5 shows that ethanol and biomass produced already tends to decrease from the previous pretreatment. This is caused by the decreasing amount of glucose that is hydrolyzed during the pretreatment process so that the process of decomposition of glucose was also decreased.

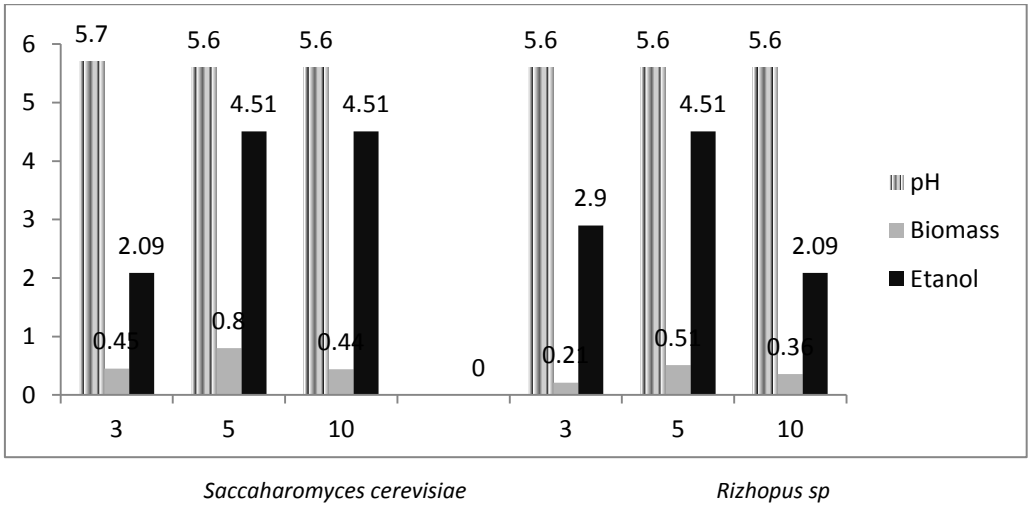

Figure 5: Graph of pH, biomass and ethanol between *Saccharomyces cerevisiae* and *Rizhopus sp*. At 3 hour Pretreatment

From the research has been done, it is found that the maximum ethanol concentration produced by *Saccharomyces cerevisiae* at concentrations of 5% with a pretreatment for 2 hours in the amount of 7.74%. This data is higher than the maximum ethanol content produced by *Rhizopus sp.,* concentration at the same pretreatment time is 5,32%. This is because when the fermentation of *Saccharomyces cerevisiae* is able to work better and more specific than *Rhizopus sp. Saccharomyces cerevisiae* has a high tolerance to temperature, pH and tolerance to ethanol produced. In addition, the yeast *Saccharomyces cereviceae* pure cultures which can be used as a"yeast starter" because it has a high ethanol production [13]. The results of the straw biomass measurements after fermented using *Saccharomyces cerevisiae* and *Rhizopus sp* conducted to determine the characteristics of the biomass on each type of yeast. Biomass measurement results obtained after 72 hours of fermentation is presented in Table 3. The graph biomass hydrolyzate fermented rice straw using *Saccharomyces cerevisiae* and *Rhizopus sp* can be seen in Figure 6 and 7.

Table 3: Biomass fermented rice straw

Concentration Of yeast (%)	Biomass *Saccharomyces cereviceae* (g)			Biomass *Rizhopus sp*(g)		
	Pretreatment Time (Hours)			Pretreatment Time (Hours)		
	1	2	3	1	2	3
3	0.12	0.69	0.45	0.07	0.31	0.21
5	0.69	0.81	0.80	0.29	0.68	0.51
10	0.45	0.79	0.44	0.08	0.60	0.36

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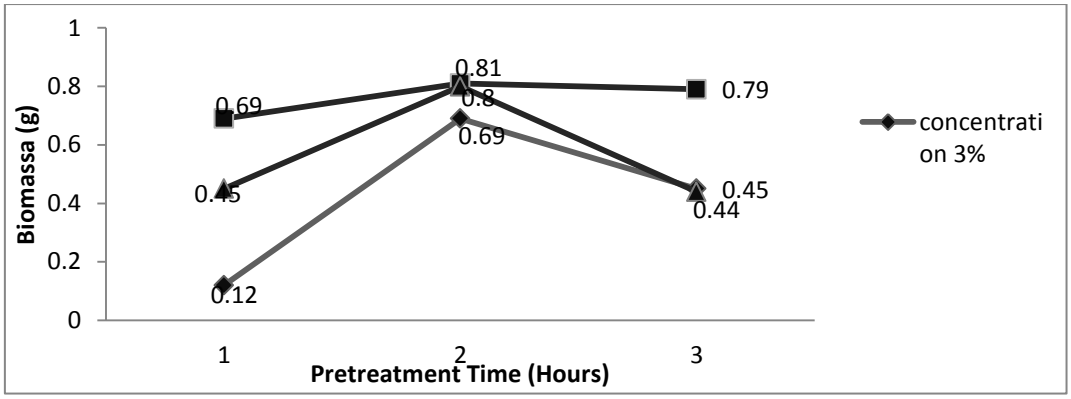

Figure 6: Biomass Fermentation by *Saccharomyces cereviseae*

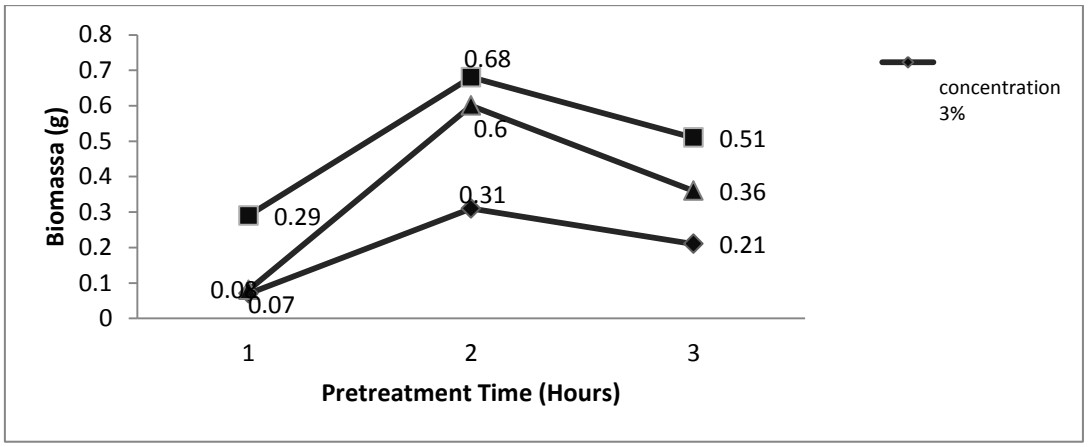

Figure 7: Biomass Fermentation with *Rizhopus sp*

Figures 6 and 7 show that the increase in biomass formation during fermentation is different for each hydrolyzate. Most biomass formation occurs in yeast concentration of 5%, in line with the levels of ethanol produced. The higher biomass is formed, then the ethanol produced is also higher due to the conversion of sugar into ethanol is also getting bigger. Increased pretreatment time to 3 hours with increasing concentrations of yeast from 5% to10% showed a tendency fall of biomass as it would have reduced glucose on hydrolysis.

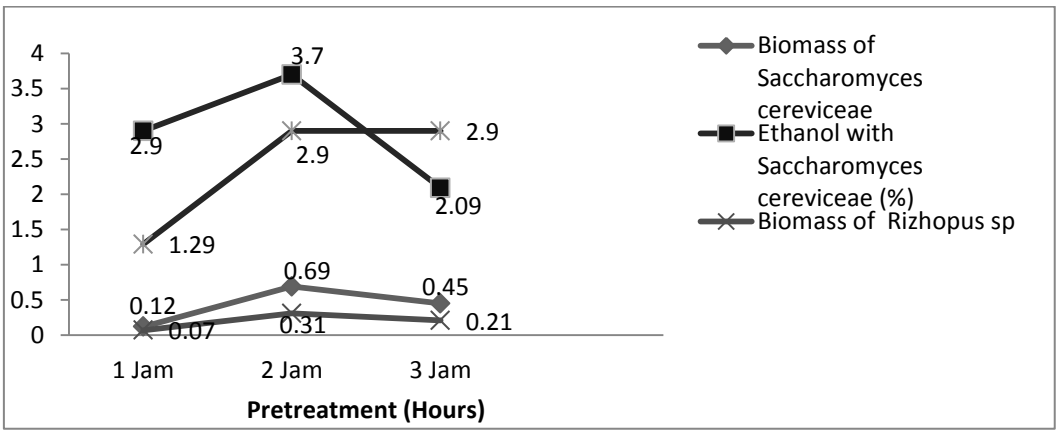

Figure 8: Graph of biomass and ethanol levels between *Saccharomy cescereviceae* and *Rizhopus sp* the concentration of 3%

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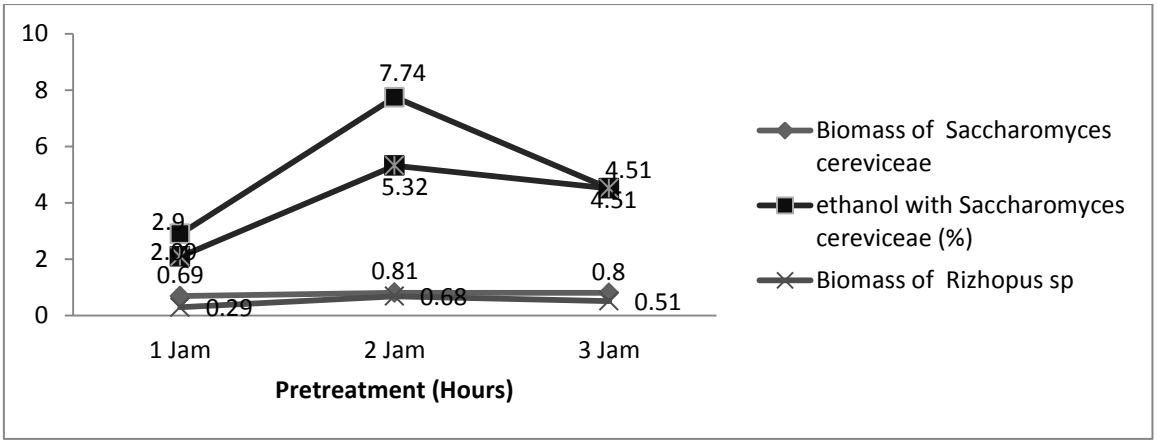

Figure 9: Graph of biomass and ethanol levels between *Saccharomyces cereviceae* and *Rizhopus sp* the concentration of 5%

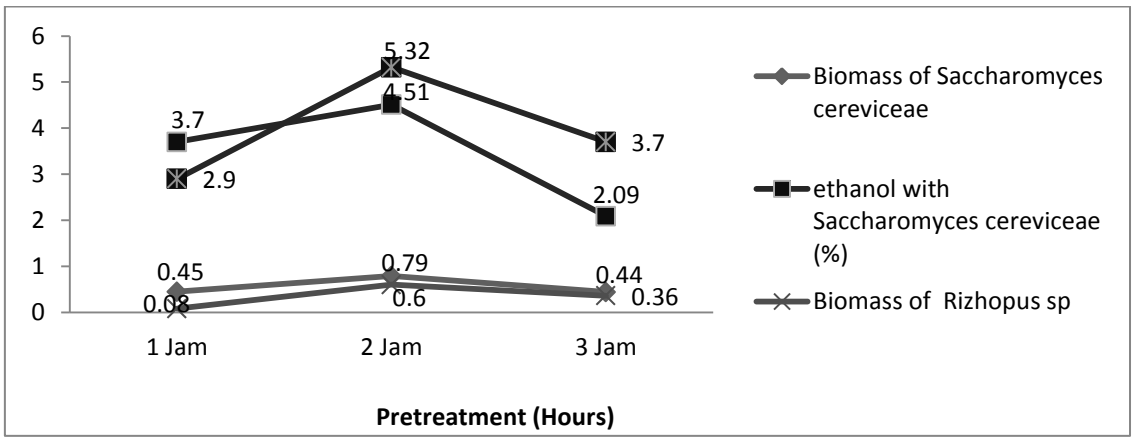

Figure 10: Graph of biomass and ethanol levels between *Saccharomyces cereviceae* and *Rizhopus sp* the concentration of 10%

Figure 8, 9 and 10 shows that increasing concentrations of each yeast from 3% to 5% resulted in an increase in the amount of biomass and ethanol produced. The highest bioethanol for *Saccharomyces cerevisiae* at 7.74% was obtained at the time of 2 hours pretreatment, whereas for *Rhizopussp* of 5.32% was obtained at the same time pretreatmet. So also with the amount of biomass. The highest biomass was obtained 0.81 g of *Saccharomyces cerevisiae* and 0.68% for *Rhizopus sp*. This increase is caused by microbial activity which grew better than the previous stages so that ethanol also increased.

Increasing concentrations of yeast from 5% to 10% showed a decrease in the amount of ethanol and biomass produced. This happens because microbes have undergone stationary phase so that no proliferation. The increase of time, the microba will decline or death phase.

In the fermentation process will occur revamp carbohydrates into glucose and fructose, as well as other compounds. The enzyme invertase produced by *Saccharomyces cerevisiae* will convert glucose into alcohol. The greater the longer the yeast and fermentation process, the more glucose converted into alcohol.

While it is influenced by the pH value of products produced during the fermentation process. The resulting product is a *Saccharomyces cerevisiae* alcohol acidic, so at that time will be more and more alcohol is formed. This condition causes the lower the pH of the substrate if the decrease in pH results in a more optimum substrate conditions, the yeast will grow better. However, if the decrease in pH results in optimum condition then the substrate will decrease [14, 15, 16]

Based on the data of this study note that, of the three variables pretreatment time, type and concentration of yeast used, the highest ethanol concentration was obtained at the time of pretreatment for 2

hours with a concentration of 5% *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is able to decipher the sugar into ethanol is better than *Rhizopus sp.* From all the data and the graph above, it is known that the concentration of yeast and yeast type and duration of pretreatment greatly affect the biomass and ethanol content hydrolyzate produced after fermentation

CONCLUSION

From the research conducted, it can be concluded that the highest biomass using *Saccharomyces cerevisiae*(0.81 g) and 0.68 g of using *Rhizopussp* with a pretreatment for 2 hours at a concentration of 5%. Produced the highest ethanol content was 7.74% using *Saccharomyces cerevisiae* with a pretreatment 2 hours at a concentration of 5%. The formation of biomass and ethanol from straw using *Saccharomyces cerevisiae* better than *Rhizopus sp* on pretreatment time and the same concentration.

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