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Effect of Brix Concentration and pH of Molassses Media on Ethanol Yield in The Fermentation Process at PT. Indo Acidatama Tbk

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Abstract
PT. Indo Acidatama Tbk. is a company that produces ethanol. Ethanol is produced by fermentation using molasses derived from sugar processing waste. To get the optimal ethanol content, it can be determined from the yield produced, it is necessary to have effectiveness in the fermentation process. In this experiment, the fermentation process was carried out with variations in molasses concentration and pH. The aim was to determine the combination of brix concentration and molasses pH in producing the most effective ethanol yield. The concentration of molasses used was 16 °brix, 20 °brix and 24 °brix. While the pH used was 4, 5 and 6. In the determination of the ethanol content, the separation was carried out by a distillation process, then the distilled ethanol was tested using a spindle alcoholmeter and a chromatographic test was carried out using a Gas Chromatography type 6890N. After getting the ethanol content, the next step is to determine the yield by using the alcohol content calculation. The results of the chromatography test obtained samples of 24 °brix variations and pH 4, getting the highest concentration, which was 3.05%. From the calculation of alcohol content, the sample variation of 16 °brix and pH 5 got the highest yield value, with an average of 26.74. From the results of the Two-Way ANOVA calculation, it was found that obrix and pH had a significant effect on ethanol yield.

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INTRODUCTION

In Indonesia, ethanol is one of the industrial sectors that has great potential in Indonesia, because ethanol itself has many uses, including as a fuel, synthetic medicine, cosmetic ingredient and beverage ingredient. Therefore, ethanol is needed by many people, both at home and abroad. Ethanol can be produced from materials containing glucose (Kang & Lee, 2015). Glucose can be found in molasses waste (Sabrina, 2018). Molasses is the raw material of choice because it is abundant and still contains quite a lot of sugar (Hartina et al., 2014). According to Rochani et al., 2015 molasses has a high sugar compound content ranging from 50 - 65%. Ethanol is made by a fermentation process (Rochani et al., 2015). Ethanol is made by a fermentation process. Fermentation is a process of chemical change in an organic substrate through the activity of enzymes produced by microorganisms (Usmana et al., 2012).

One of the ethanol industries developing in Indonesia is PT. Indo Acidatama Tbk. Molasses is the main raw material used by PT. Indo Acidatama Tbk. Molasses is fermented using Saccharomyces cerevisiae and has been done since 1989. The effectiveness of the fermentation process is something that needs to be considered to obtain optimal ethanol levels which can be seen from the yield produced. Yield is the ratio of the amount of ethanol product produced to the initial glucose during the fermentation process (Kurniawan et al., 2018). Therefore, efforts are needed to achieve higher yield levels. The fermentation process is influenced by various factors, including molasses concentration, pH, time and temperature during the fermentation process (Wagestu et al., 2015). Hendrawan et al., 2017 revealed that pH is an important factor in fermentation because pH influences microbial growth conditions. Each microbe has a different level of survival in certain pH conditions (Hendrawan et al., 2017).

Previous research conducted by Khak & Rohmatiningsih, 2014 examined the effect of molasses concentration and pH on ethanol production at PG-PS (Sugar Factory) Madukismo. This research states that increasing the molasses concentration can produce high ethanol levels, but too high a molasses concentration can inhibit the activity of Saccharomyces cerevisiae. This can be used as a development for further research with different variations in pH and Brix concentration so as to get more optimal results (Khak & Rohmatiningsih, 2014). According to the results of research conducted by Wardani and Eka Pertiwi, 2013, the higher the addition of Saccharomyces cerevisiae, the higher the yield produced and the better the quality of the product produced. Based on the background above, this research aims to examine the effect of molasses concentration and pH on ethanol yield in the fermentation process at PT. Indo Acidatama Tbk, by considering variations in Brix concentration and pH of the molasses media which can increase ethanol yield levels, and also not inhibit the activity of *Saccharomyces cerevisiae* (Wardani & Eka Pertiwi, 2013).

METHOD

The ingredients used in this research were molasses, *Saccharomyces cerevisiae,* Diammonium hydrogen phosphate, urea, peptone from meat, extract of yeast powder, H_2SO_4, NaOH and Aquadest. The equipment used in this research was an ATAGO Hand-refractometer, a series of distillation tools, a fermentation flask, a Gas Chromatography type 6890 N, a Hirayama type Autoclave, an Incubator and a Spindle Alcoholmeter (range 0 -7%).

The sample used in this research was molasses taken from PT. Indo Acidatama Tbk. Samples were taken as much as 10 liters in 1 collection. The aim of taking 10 liters in one shot is to represent the condition of the molasses at PT. Indo Acidatama Tbk. In this research, the molasses used came from the same molasses to ensure the homogeneity of the molasses content. Molasses used as raw material must meet the % Brix parameters. Good quality sugar cane molasses must have a % Brix between 85% - 95% (Fitria, 2017). The concentration of molasses taken was 85.4 ^{o}brix and had a TS (Total Sugar) content of 55.024%. The molasses is diluted according to the concentration that will be used for growth media and fermentation media with a total volume of 600 mL. Calculation for making molasses media to find out how much the molasses sample will weigh.

The data processing used in this research was a Completely Randomized Design (CRD). Data in the form of the effect of Brix molasses concentration and pH were analyzed using ANOVA at a confidence level of 95%.

Preparation of Molasses Solution as a Fermentasion Medium

Before making the molasses solution, the culture of *Saccharomyces cerevisiae* was prepared. Saccharomyces cerevisiae cultures were inoculated using Laminar Air Flow to prevent contamination and direct air contact. Then the *Saccharomyces cerevisiae* culture was incubated for 24 hours at 30 ^{o}C. Then the *Saccharomyces cerevisiae* culture was inoculated into the fermentation medium aseptically so that there was no bacterial contamination. After preparing the *Saccharomyces cerevisiae* culture, molasses solution was prepared with a concentration of

16 °Brix, 20 °Brix and 24 °Brix with the formulation as shown in Table 1. Molasses together with nutrients is dissolved with distilled water up to a volume of 500 mL in a measuring flask, and put into a 1000 mL fermentation flask. The measuring flask is then filled with 100 mL of distilled water. The solution is stirred until it is homogeneous. The pH of the solution was adjusted to 4, 5 and 6 by adding H_2SO_4 if it was too alkaline and adding NaOH if it was too acidic. The fermented flask is then wrapped in a silicone plug and covered with aluminum foil. The solution was sterilized using an autoclave at 120 ºC 1 atm for 20 minutes, then cooled.

Table 1. Preparation of Molasses Solution

Brix Molasses Concentration (ºBx)	Total Added Molasses (grams)	Total Diammonium Hydrogen Phosphate (grams)	Total Pepton from Meat (grams)	Total Urea (grams)
16	112,412	0,25	2,5	0,5
20	140,515	0,25	2,5	0,5
24	168,630	0,25	2,5	0,5

Ethanol Production by Saccharomyces cerevisiae

Saccharomyces cerevisiae which had been incubated for 1 day was inoculated into the fermentation medium. The fermented flask lid was replaced with a gooseneck muffler, then 100 mL of distilled water was added and incubated for 3 days at room temperature. The fermentation stage was carried out in various concentrations of brix 16, 20 and 24 and pH 4, 5 and 6.

Fermented Distillation

250 mL of fermented media was taken, put into a distillation flask and 200 mL of distilled water was added. The sample is distilled, the distillate is collected in an erlenmeyer with a volume of 250 mL. The distillate was transferred to a 500 mL measuring flask and diluted with distilled water to a volume of 500 mL, shaken until homogeneous, then stored in the freezer until the temperature was below 20 °C. The cooled sample is then put into a 250 mL volume measuring cup. The sample is read precisely at 20 °C using a spindle alcoholmeter.

Calculation of Fermented Ethanol Yield

The final yield of ethanol is calculated by:

$$y = \frac{250}{600 \text{ x medium molasses weight}}$$

$$\text{Yield} = \frac{\text{\% spindle alcoholmeter x correction factor x 500}}{y}$$

Chromatography Test

Distilled samples were injected using a Gas Chromatography type 6890 N. The results to be obtained were the concentration of ethanol in the sample tested in ppm, then converted to percent.

Statistical Analysis of Data

Processing of the data used is Completely Randomized Design (CRD). Data in the form of the effect of molasses brix concentration and pH were analyzed using ANOVA at the 95% confidence level.

RESULTS AND DISCUSSION

Determination of Ethanol Concentration using a Spindle Alcoholmeter

Distilled samples were analyzed using a spindle alcoholmeter 0 – 7 % at 20 °C at 3 repetitions, to determine the concentration of ethanol present in the sample. The sample research results can be seen in Table 2.

Table 2. Ethanol Concentration Using a Spindle Alcoholmeter

°Brix	pH	Ethanol Concentration (%)
16	4	2,05
16	5	2,26
16	6	1,88
20	4	2,62
20	5	2,98
20	6	2,10
24	4	3,03
24	5	2,92
24	6	3,02

Control Sample = 2,2%

The results obtained, where there are one variations that have the highest ethanol concentration results compared to the others, are variations of 24 °brix, pH 4. This variation has an average of 3.03 %. For the variation that has the lowest concentration compared to the others, namely in the 16 °brix pH 6 variation sample, where this variation obtained an average result of 1.88 %. It was found that on average samples that had a high molasses Brix also had a high ethanol concentration. In previous research conducted by Widyanti & Moehadi, 2016 it was stated that the higher the addition of molasses, the higher the concentration of ethanol produced (Widyanti & Moehadi, 2016).

Analysis of Ethanol Concentration Using Gas Chromatography (GC)

Analysis using GC aims to determine the concentration of ethanol in the distillation sample and can be referred to as a comparison of the results of the analysis using a spindle alcoholmeter. The comatogram results in this study can be seen in Figure 1 and Table 3.

Table 3. Chromatography Test Results using Gas Chromatography

°Brix	pH	Ethanol Concentration (ppm)	Ethanol Concentration (%)
16	4	$2{,}014 \times 10^4$	2,01
16	5	$2{,}320 \times 10^4$	2,32
16	6	$1{,}937 \times 10^4$	1,94
20	4	$2{,}582 \times 10^4$	2,58
20	5	$2{,}669 \times 10^4$	2,67
20	6	$2{,}789 \times 10^4$	2,79
24	4	$3{,}045 \times 10^4$	3,05
24	5	$2{,}829 \times 10^4$	2,83
24	6	$2{,}937 \times 10^4$	2,94
Control Sample		$2{,}118 \times 10^4$	2,18

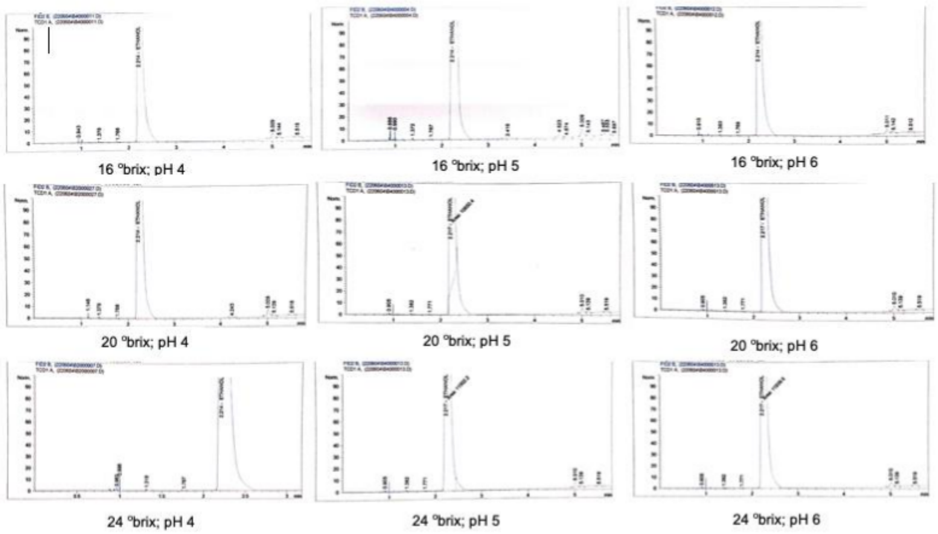

Figure 1. Chromatogram of Ethanol Concentration

In the chromatographic test results, it was found that the 24 °brix and pH 4 samples obtained the highest results, namely 3.05 %, while the 16 °brix and pH 6 variations obtained the lowest results, namely 1.94 %. The concentrations of the distillated samples using Gas Chromatography did not differ much from the concentration results using a spindle alcoholmeter, meaning that the distillated samples had valid data on concentration testing using these two tools and continued with the calculation of the ethanol yield.

Calculation Results Alcohol Content to Determine The Yield Value of Ethanol

After getting the ethanol concentration data, then the data can be used to determine the ethanol yield using the Alcohol content formula. The calculation results are in Table 4.

Table 4. Ethanol Yield Data

°Brix	pH	Yield
16	4	25,11
16	5	26,74
16	6	23,26
20	4	24,02
20	5	26,37
20	6	20,21
24	4	22,41
24	5	21,75
24	6	22,62

Control Sample = 27,10

In previous research conducted by Ningsih, 2009 it was stated that the optimal conditions for ethanol fermentation which produce optimal ethanol levels were variations of 22.32 – 24.0 brix and pH 4-5 (Ningsih, 2009). The sample variation of 16 °brix and pH 5 got the highest yield value for 3 repetitions, namely with an average of 26.74, while the sample variation of 20 °brix with pH 6 got the lowest yield value for 3 repetitions, namely with an average of 20.21. So a sample variation of 16 °brix with pH 5 is declared as the most optimal variation. This is in accordance with previous research conducted by Liu et al., 2014 using *Saccharomyces cerevisiae* and pH 5 media, obtaining a maximum ethanol yield of 24.26% (Liu et al., 2014).

Two Way ANOVA Test

After obtaining ethanol yield data for all samples analyzed, a Two Way ANOVA test was then carried out to determine whether there were significant differences between the averages of the various samples analyzed. The Two Way ANOVA test in this study is in Table 5.

Table 5. Two Way ANOVA Test

Source of Variation	Degrees of Freedom (DF)	Sum of Squares	Middle square	F Cal.	F Tab (0,05)
Treatment	8	112,0215053			
°*brix* (B)	2	34,763414	17,381707	31,04193	3,55455715
pH (P)	2	39,10885267	19,55442633	34,92218	3,55455715
Interaction BP	4	38,14923867	9,537309667	17,03265	2,92774417
Error	18	10,07897133	0,559942852		
Total	26	234,121982	47,03338585	82,99676	10,0368585

°brix (B) has F. Calculate > F. Table (0.05). This shows that °brix molasses has a significant effect on ethanol yield. pH (P) has F. Calculate > F. Table (0.05). This shows that the pH of molasses has a significant effect on ethanol yield. The BP interaction has F. Count > F. Table (0.05). This shows that the molasses BP interaction has a significant effect on ethanol yield.

CONCLUSION

The concentration of molasses has the most dominant effect in determining the concentration of ethanol both in the use of a spindle alcoholmeter and in the chromatography test. The results of handling the ethanol concentration using the spindle alcoholmeter are not much different from using the chromatography test. The results of the chromatography test obtained samples of 24 °brix variations and pH 4, getting the highest concentration, which was 3.05%. From the calculation of alcohol content, the sample variation of 16 °brix and pH 5 got the highest yield value, with an average of 26.74. From the results of the Two-Way ANOVA calculation, it was found that obrix and pH had a significant effect on ethanol yield.

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