Bioethanol Production from Molasses by Varying Sugar and Yeast Concentration

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Abstract. When 2020 began the outbreak of new pneumonia, namely the coronavirus disease 2019 (COVID-19), alcohol suddenly became less available because it was the most sought-after item. This is because 70% alcohol can be used as an antiseptic and disinfectant solution to overcome coronavirus transmission. The manufacture of 70% alcohol can use alternative materials from sugar industry waste, namely molasses. This study aimed to determine the effect of Brix and yeast concentration variations on the level and volume of alcohol made from molasses and how much Brix and yeast concentration can obtain optimal bioethanol yield. The research method used is the experimental method. The variations of Brix used were 12%, 15%, and 18%, while the yeast concentrations used were 0.5%, 1%, and 1.5% of the total sample weight. Bioethanol production is carried out by fermentation process for 48 h against 30 kg of molasses solution, but firstly, the propagation process is carried out for 2 h. Then it is distilled to get bioethanol from the fermentation of molasses. The results of this study indicate that the two factors affect the volume and level of bioethanol produced. Still, the variation factor Brix is the dominant factor in determining the volume and alcohol content. The optimal treatment to deliver the highest volume and content of bioethanol is Brix 15% and yeast concentration of 1%, with an average volume of bioethanol produced as much as 1882 ml and the resulting alcohol content of 84%.

Keywords: bioethanol · molasses · brix · yeast concentration · alcohol volume · alcohol content

1 Introduction

One of the alternative materials for the manufacture of alcohol is derived from industrial organic waste. Alcohol produced from organic waste is commonly called bioethanol. One of the potential industrial organic wastes for the manufacture of bioethanol is molasses (cane drops). Molasses is usually obtained from sugar factory waste. Molasses contains a relatively high sugar content, so it can potentially be used as material for making bioethanol. The process of making bioethanol from molasses can vary by several factors. Variations in sugar concentration (Brix), namely 12%, 14%, 16%, 18%, 19% and 20%, resulted in different levels and volumes of alcohol [1] from research conducted

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by Maryana et al. [2] on bioethanol production using variations in yeast concentrations, namely 0.4%, 0.6% and 0.8% by weight, producing different levels and volumes of alcohol as well. But the study only focused on yeast concentrations. According to Jayus et al. [3], the aeration process can increase bioethanol production from molasses. According to Hidayati and Puspita [4] the optimal fermentation time in bioethanol production is 48 h (2 days).

2 Material and Methods

2.1 Bioethanol Production

The research method used is experimental research, is a method used to find the effect of specific treatments. The stages in the implementation used in this study include the initial preparation stage, the research design stage, the equipment and material preparation stage, the testing and data collection stage, and the data analysis stage. In the initial preparation stage, the research and literature study location determined. Next is the research design stage. This research is an experimental study using Completely Randomized Block Design. In this study, there were two variation factors: Brix and yeast concentration. There are three Brix used, namely 12%, 15%, and 18%, while the addition of yeast, there are three, namely 0.5%, 1% and 1.5% of the sample weight of 30 kg. So, there were 9 treatments with 3 repetitions in each, so the total number of samples used in this study was 27. In the preparation stage of tools and materials, the tools used are, fermentation tank, continuous distiller reflux, refractometer brix, alcoholmeter, heating tank, measuring cup, pH meter, digital scale, aerator, syringe and pipette, water drum, filter tool, tool stirrer, thermometer, and pump. The materials used are molasses, baker's yeast, ammonium sulfate (ZA fertilizer), and water.

2.2 Experimental Setup and Testing

In the testing and data collection stage, the research procedure was carried out, namely molasses dilution by mixing with water to obtain 12%, 15%, and 18% brix, then the heating process was carried out, the molasses solution was heated to a temperature of ± 85 °C and then cooled to a temperature of ± 30 °C. Furthermore, in the propagation process, in this process the molasses solution was added 0.5%, 1%, and 1.5% of the sample weight and ammonium sulfate was added in a ratio of 1:1 from the amount of yeast, then aeration was carried out using an aerator for 2 h. Furthermore, the fermentation process was carried out for 48 h at room temperature ranging from 23–33 °C and in a closed (anaerobic) state. To even out the yeast spread during the fermentation process using a circulation system. After the fermentation process, then the distillation process is carried out using a Continuous Distiller Reflux. In the testing and data collection stage, the research procedure was carried out, namely molasses dilution by mixing with water to obtain 12%, 15%, and 18% brix, then the heating process was carried out, the molasses solution was heated to a temperature of ±85 °C and then cooled to a temperature of ±30 °C. Next is the propagation process, in this process the molasses solution is added yeast. In this study there were several data collections carried out, namely, brix data collection in the propagation process by taking a few drops of molasses solution with a syringe or pipette and then dripping onto the brix refractometer and getting brix data, retrieval of brix data in the fermentation process by taking a few drops of molasses solution with a syringe or pipette and then dripping onto the brix refractometer and obtaining brix data, taking pH data in the fermentation process by taking 30 ml of molasses solution, dipping the pH meter, wait a few minutes, pH data were obtained. Data collection of volume and alcohol content by pouring alcohol into a measuring cup, inserting an alcoholmeter, and taking GC-MS test data. At the data analysis stage, the two-way ANOVA method will be used, to determine whether there is an effect of the concentration of Brix and the addition of yeast on the volume and alcohol content.

3 Result and Discussion

3.1 Yeast Propagation

From the results of the study, taking brix data once every hour with three repetitions during the propagation process for two hours, the data obtained on the average value of brix reduction in each treatment as shown in Table 1.

Based on Table 1, it can be seen from the results of the study that at higher concentrations of yeast, there was a faster decline in brix because the yeast Saccharomyces Cerevisiae carried out the propagation process more. During the 2-h propagation process, aerobic respiration will occur, the goal is to make the yeast adapt to its environment and prepare the seeds to be able to carry out the process of converting sugar into alcohol [4]. There was a 1% decrease in brix in all treatments during the propagation process, this means the yeast is active and consumes less sugar for the yeast to breed.

Table 1. Brix concentration throughout propagation.

Brix (%)	Yeast concentration (%)	Brix (%) hour		
		0	1	2
12	0,5	12	12	11
	1	12	12	11
	1,5	12	11	11
15	0,5	15	15	14
	1	15	15	14
	1,5	15	14	14
18	0,5	18	18	17
	1	18	18	17
	1,5	18	17	17

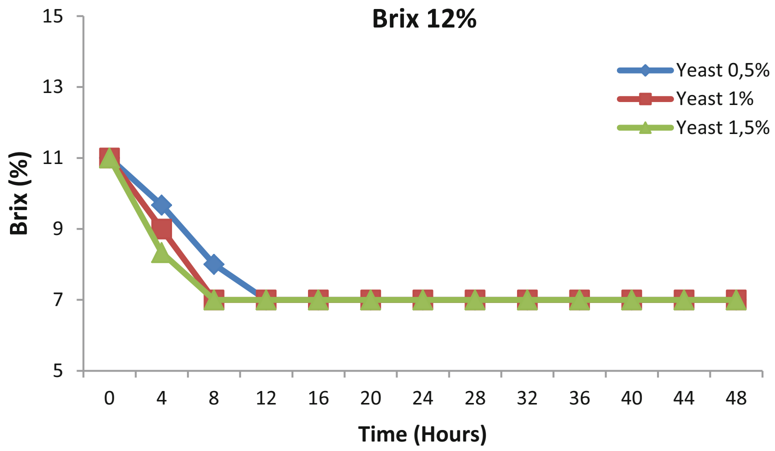

Fig. 1. Brix 12% profile throughout the time of fermentation.

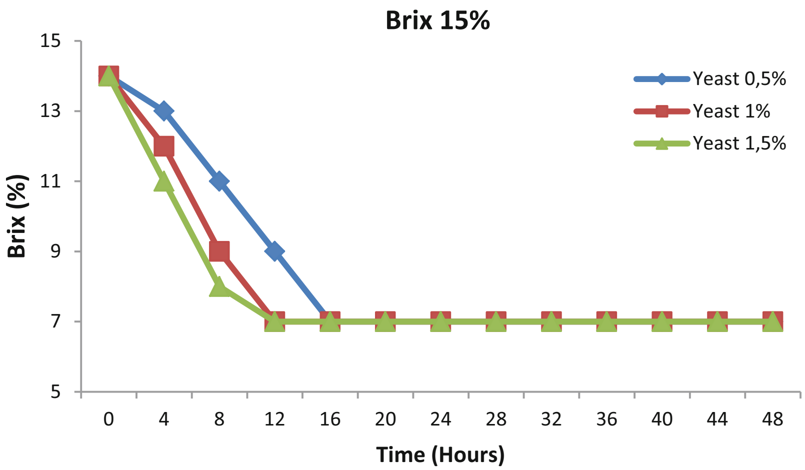

Fig. 2. Brix 15% profile throughout the time of fermentation.

3.2 Brix Fermentation

From the results of the study, taking brix data every four hours with three repetitions during the fermentation process for two days (48 h), the data obtained on the average value of brix reduction in each treatment as shown in the graphic below.

Based on Figs. 1, 2 and 3 it can be seen from the results of the study that the higher the brix, the longer the fermentation time needed so that the brix value can be constant tends to be longer, this is because the amount of sugar that will be broken down into alcohol is also increasing. And for the more yeast concentration, the faster the fermentation time needed so that the brix value can be constant, but at 15% and 18% brix with a yeast

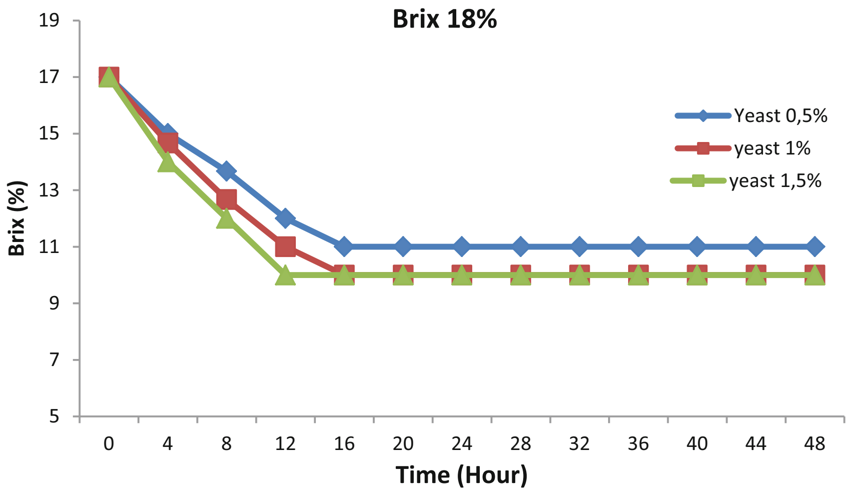

Fig. 3. Brix 18% profile throughout the time of fermentation.

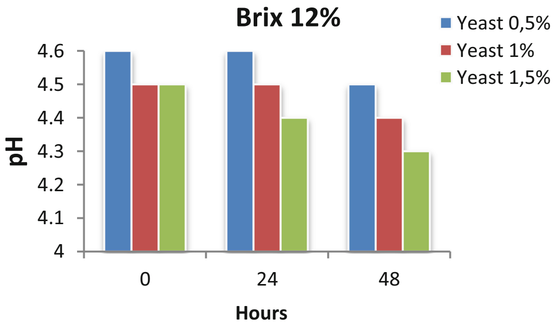

Fig. 4. pH throughout the time of fermentation Brix 12%.

concentration of 1.5% the fermentation time needed to keep the brix constant is the same, because in this study yeast only able to break down sugar a maximum of 7% only.

3.3 PH Fermentation

From the results of the study, taking pH data once a day (24 h) with three repetitions during the fermentation process for two days (48 h), the results obtained data on the average pH decrease value in each treatment as shown in Table 2.

Based on Figs. 4, 5 and 6 it can be seen from the results of the study that the greater the brix value, the smaller the initial pH value of fermentation, it is because the ratio of molasses to water is more with the nature of molasses being more acidic than water. And the greater the concentration of yeast, the faster the decrease in pH. According to Setiawati et al. [5], if too much addition of yeast will also produce a lot of acid. The change in pH value is due to the formation of acids such as pyruvic acid during the fermentation

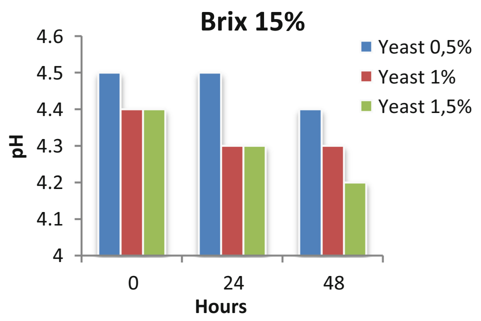

Fig. 5. pH throughout the time of fermentation Brix 15%.

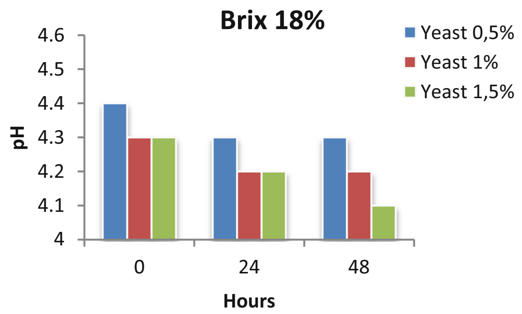

Fig. 6. pH throughout the time of fermentation Brix 18%

process. The degree of acidity (pH) is an important factor that can affect the growth of yeast and ethanol production. The optimal initial pH during fermentation is between pH 4 − 5. The use of pH below 4 and above 5 will result in decreased fermentation activity and alcohol yield [6]. Setting and controlling pH during fermentation is also aimed at reducing the possibility of contamination during fermentation. In this study, it is no longer necessary to set the initial pH because it is already in the 4 − 5 value interval.

3.4 Alcohol Volume

Based on Fig. 7 it can be seen from the results of the study that as the brix increases, the volume of alcohol tends to increase. But at too high a brix can reduce the volume of alcohol produced. This is because the higher the brix, the more viscous and concentrated the molasses solution, it can interfere with the metabolic activity of the yeast.

At the same brix with the addition of a high concentration of yeast will result in a decrease in the volume of alcohol produced, this is because the addition of more yeast will reduce the productivity of Saccharomyces Cerevisiae because the small amount of sugar does not match the amount of yeast, resulting in a lack of nutrients for yeast. This is also explained by Setiawati et al. [5] which states that at the addition of a yeast dose that is too high, the alcohol produced decreases because the productivity of microorganisms

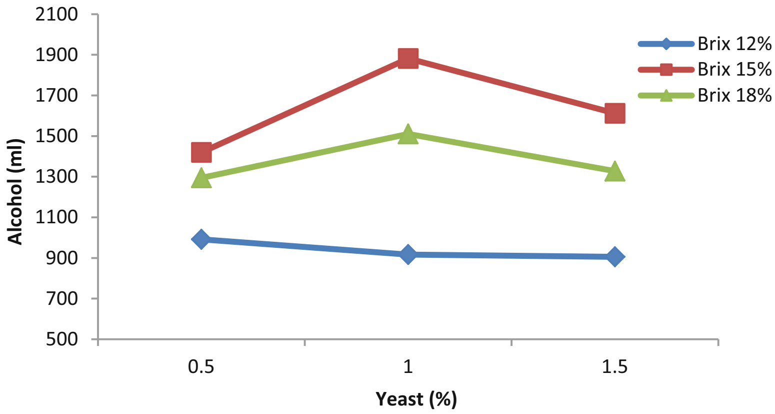

Fig. 7. Alcohol production variation of yeast.

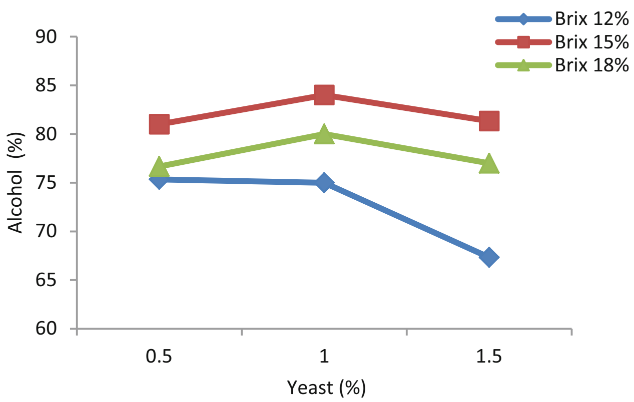

Fig. 8. Alcohol concentration variation of yeast.

decreases due to a lack of nutrients needed by these microorganisms. Similarly, high brix with less yeast will cause the volume of alcohol produced to decrease as well, this is because less yeast is not optimal for breaking down large amounts of sugar. So that 15% brix with 1% yeast concentration produces the most alcohol, that's because the amount of yeast is in accordance with the amount of sugar in the sample, so the productivity of Saccharomyces Cerevisiae to break down sugar into alcohol will be very optimal.

3.5 Alcohol Content

Based on Fig. 8 it can be seen from the results of the study that the effect of brix and yeast concentration on alcohol content was not much different from the effect of brix and yeast concentration on alcohol volume. Which if the increase in brix then the alcohol content tends to increase but at too high a brix can reduce the alcohol content produced.

This is because too high a brix can cross the tolerance limit of the live area of the yeast. At the same brix with the addition of a high concentration of yeast, the resulting

Table 2. GC-MS results.

Treated	Ethanol (%)	Methanol
Brix 12%, Yeast 1%	77,22	0
Brix 15%, Yeast 0,5%	82,17	0
Brix 15%, Yeast 1%	84,15	0
Brix 15%, Yeast 1,5%	81,18	0
Brix 18%, Yeast 1%	78,21	0

alcohol content will decrease. This is also explained by Setiawati et al. [5] which states that the addition of too much yeast dose will produce a lot of acid and the resulting alcohol content will be less. At 1% yeast concentration, the highest alcohol content was obtained because the amount of yeast was in accordance with the amount of sugar in the sample. At high brix also tend to be more acidic, this acid can interfere with yeast productivity to produce alcohol. Therefore, even though at high brix there is a lot of sugar, yeast is not optimal in breaking it down into alcohol because yeast activity is hampered by the acid. In this study, the most optimal treatment to produce the highest alcohol content was 15% brix and 1% yeast concentration with an alcohol content of 84%.

3.6 Alcohol Content

This test is carried out to determine the content in alcohol. Because in this study, the production of alcohol (ethanol) is used for antiseptics and disinfectants, it is required that there are no methanol compounds, because methanol is toxic to humans. In this test, the researchers only tested five samples that were considered the best, namely the 12% brix treatment, 1% yeast; brix 15%, yeast 0.5%; brix 15%, yeast 1%; brix 15%, yeast 1.5% and brix 18%, yeast 1% in the second repeat sample.

In Table 2 it can be seen that of the five treatments tested, none contained methanol. The five treatments also meet the requirements to be antiseptic and disinfectant because the ethanol content reaches 70%. If the excess alcohol content can be lowered by adding distilled water to the alcohol.

4 Conclusion

The concentration of brix and the concentration of yeast affect the volume and content of bioethanol produced from molasses fermentation. It can be proven by the two-way ANOVA statistical analysis technique that there are significant differences in the volume and content of bioethanol produced from molasses fermentation by varying the Brix and yeast concentration, but the brix variation factor is the dominant factor in determining the volume and alcohol content. The concentration of brix and yeast concentration in order to obtain the optimal yield of bioethanol from molasses fermentation is by using 15% brix and 1% yeast concentration.

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