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Bioethanol Production from Pineapple Peel Waste by A Fermentation Process Using *Saccharomyces Cerevisiae* Microorganisms in A Batch Type Fermentor

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Abstract. Bioenergy is the electricity which grows make through a process called photosynthesis. Bioethanol is a renewable energy that comes from plants. It may be easy for yeast from the Saccharomyces cerevisiae fermentation process to turn carbohydrates into bioethanol. Bioethanol is mostly made up of pineapple pulp (*Ananas comosus* L. Merr.). Since this food is high in carbs and low in sugar, it may be a good source of ethanol. Carbs are turned into ethanol and carbon dioxide gas during the fermentation process that makes bioethanol. The next step in preparing this material is to use methods like evaporation and extraction. The goal of this study is to find out how the amount of yeast (Saccharomyces cerevisiae) used and the length of time it ferments affect the final concentration of ethanol in bioethanol, which is made from pineapple peel waste. The results of the study show that the highest content that can be reached is 80% ethanol. A density of 0.7397 g/ml, a viscosity of 1.1432 cP, a water content of 0.793%, an ethanol pH of 7, and a yield of 10.2% were the best conditions.

Keywords : Bioethanol, Pineapple Peel Waste, Saccharomyces cerevisiae, Fermentation, Renewable Energy

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Introduction

Bioenergy is made when plant and animal matter breaks down without oxygen. Most biomass comes from plants and animals, with only a small amount from trash from farms, forests, and livestock. Photosynthesis is the process by which all living things turn into energy that can be used. Bioenergy can be supported by new technologies and tools that make the most efficient use of fossil fuels. Bioenergy is important for reaching the Millennium Development Goals (MDGs) because it makes the switch to green energy sources easier. Because of this, biofuel is one of the world's most efficient and hopeful energy industries [14].

Bioethanol, which is also called ethanol production by yeast, is made when *Saccharomyces cerevisiae* are used to process glucose. The burning liquid ethanol (C_5H_5OH) is a great option for an energy source because it has many of the same physical and chemical properties as gasoline. Bioethanol is made from a number of natural materials and farming raw materials, such as sugarcane, cassava, palm sap, sorghum, and others. These materials are made from carbohydrate-rich materials like sugar, starch, and cellulose [1] [16].

There is more than 99.5% alcohol by volume in fuel-grade ethanol, making it an ideal source of energy. Enhancing the flavour of food and drink, neutral-grade ethanol is employed because of its high alcohol concentration (96 to 99.5%). Additionally, the alcohol level of industrial-grade ethanol ranges from 90 to 94%.

There are many real-life situations where bioethanol is useful. We can use the bioethanol that comes from this study as a sustainable fuel or to add to other fuels to cut down on our use of fossil fuels and greenhouse gas emissions. In Indonesia, bioethanol has been investigated as a potential alternative fuel for vehicles and as a blend with gasoline to improve fuel performance and reduce pollutant emissions [4]. Additionally, it can serve as a solvent or as a raw material in chemical, pharmaceutical, cosmetic, and food industries, as cited in previous studies.

Pineapple peel waste is used as one of the raw materials for bioethanol production. Pineapples (*Ananas comosus* L. Merr) are a fruit commodity abundantly grown in Indonesia and widely distributed. In addition to being consumed fresh, pineapples are used as a processed ingredient in the food and beverage industry. From 2008–2010, the average national pineapple production reached 1.46 million tons annually. The increase in pineapple production has been accompanied by an increase in the volume of waste generated [6] [11].

Utilization of pineapple peel waste is currently limited, generally limited to animal feed. To provide added economic value, leftover pineapple peel can be used as a primary ingredient in ethanol production through hydrolysis and fermentation with the addition of yeast, followed by purification through distillation [13]. Pineapple consumption produces a lot of peel waste, which makes up about 34.61% of the total weight. The peel contains around 10.54% carbohydrates. This means pineapple peel waste could be a viable material for producing ethanol [6] [10].

Ethanol is produced by the bioconversion process by use of microorganisms. Fungus *Saccharomyces cerevisiae* is among the most prevalent microorganisms and produces the enzyme amylase. *Saccharomyces cerevisiae* consists of a single cell that ranges in size from 1 to 5 µm in length and 1 to 10 µm in width. Temperatures of 25–30°C and acidities of 4.5–5.5 are ideal for its growth. Among *Saccharomyces cerevisiae*'s numerous advantages include its rapid growth rate, tolerance to ethanol, strong metabolic resistance, and adaptability to novel environments. There are three different kinds of yeast: fresh, dry, or moist. The fermentation of ethanol and other industrial compounds often makes use of it. Due to its stability and ease of handling, active dry yeast is used in fermentation investigation. Cell development may be influenced by minerals, vitamins, and carbon and nitrogen derived from sources such as urea, ZA, ammonium, and peptone. The yeast employed and the duration of fermentation are two of several variables that influence the process.

Finding out how the pace of ethanol production and the quantity of pineapple peel garbage converted into alcohol are affected by variations in yeast dose and growth time is the primary objective of this research project. Building on previous work, we find the optimal yeast dosage and fermentation duration by using pineapple peel waste, which is readily available and inexpensive. However, nobody has investigated this yet. The primary objective of this research is to determine the optimal yeast dosage and processing time for bioethanol production from pineapple peel waste. You should also consider the ethanol's acidity, water content, stickiness, density, and other properties.

Experimental

The process used in this study to make bioethanol is shown in **Figure 1**. The first thing to do is look at the factors. The pretreatment clay comes next. The air is then cooled. The next step is to pick *Saccharomyces cerevisiae* as the organism that will process the sugar. Finally, you need to get the information out there. Hydrolysis and precipitation are both used to make more liquid that can be broken down. To do this, water breaks down the lignosulphonate molecule into a more stable carbohydrate. When ragi and nutrients are used in fermentation, they help the bacteria grow and reach their goals. For three, five, or seven days, the main testing factors are the yeast mass and the length of time that fermentation lasts. These factors change the amount of ethanol that is made and its quantity. The last step in cleaning ethanol is to test its quality with tests like yield, density, consistency, and water content. In this way, the bioethanol is kept as pure as possible. Other studies have also been able to make bioethanol from pineapple peel [6], and this process follows the same steps.

Materials

An analytical scale, measuring cups, thermometers, measuring flasks, blenders, and litmus paper are some of the tools that were used in this study.

Some of the things that are used are pure water, 2% NaOH solution, 1% H_2SO_4 solution, and pineapple peel trash.

Based on **Figure 2**, The batch-type fermenter used in this study was designed using AutoCAD software (**Figure 2**), with vessel geometry and layout based on established batch fermenter designs for ethanol production reported in the literature (e.g., a cylindrical reactor with conical bottom and appropriate mixing configuration) to support efficient fermentation performance. One such design concept is described for ethanol fermenter construction using a vertical cylindrical vessel with conical base to facilitate mixing and drainage in batch operations[16].

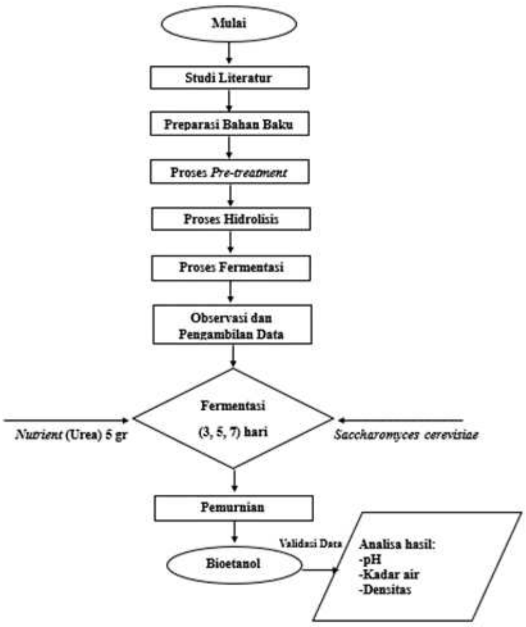

Figure 1. Research Stages

Bioethanol Production Process

Raw Material Preparation

Cut 3 kg of pineapple peel waste using a knife into small pieces to facilitate the grinding process. The pineapple peel is first cleaned using water to remove any dirt. Next, the pineapple peel is crushed using a blender with a little water to form a slurry.

Pre-treatment Process

Dissolve 20 grams of solid NaOH in 1 liter of distilled water. Then, add the NaOH solution to the slurry and stir until thoroughly mixed. The finished mixture is made by heating it for an hour at 60–80°C and then letting it cool to room temperature. Next, use a sieve to separate the solids from the pulp. Next, put the solids in a closed container and add

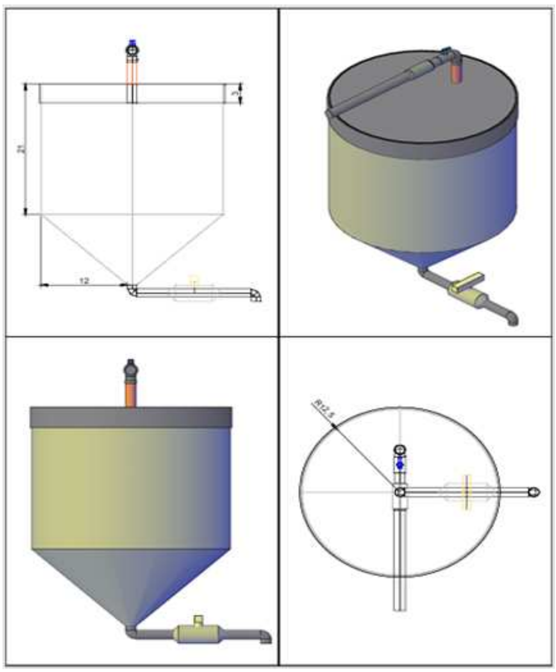

Figure 2. Batch type fermenter design

water to it. Throw away the rest of the liquid.

Hydrolysis Process

Sour sulfuric acid (20 ml) should be mixed with 880 ml of clean water. The solids that have already been handled (sediment) should be put in the hydrolysis box. Add the acid solution to the rest of the contents of the hydrolysis tank. Raise the temperature to 110 degrees Celsius. The process of hydrolysis is done in an hour. To start fermenting, open the valve and let the hydrolysate out. Filter the hydrolysate to get rid of any stray bits. A small refractometer can be used to find the starting sugar content of the hydrolysate before fermentation begins. 11–15 °Brix is a set of numbers that can be thought about. This is the beginning of the study into fermentation.

Fermentation Process

There are three parts, each with eleven, twenty-two, and thirty-three grams of yeast. 5 grams of urea, a nitrogen source, is added during fermentation to help Saccharomyces cerevisiae grow and do its biological work. To find the right amount, tests are done with high amounts of pineapple peel hydrolysate and yeast inoculum. This is necessary to make sure that yeast has enough nitrogen to work well. Add 100–200 ml of water (or clean water) and heat for a short time to 30°C to wake up the yeast. Now that you've stirred everything together well, cover the container with aluminum foil and let it sit for a while. The yeast is ready to use when foam forms on top of it. By opening the valve on the fermenter pump, the hydrolysate can be moved to the batch fermentation tank. When you're done adding the hydrolysate, add the active yeast and nutrients. The last step is to close the container so that air can't get inside. Figure 2 shows the outcome of creating a batch fermentation tank with AutoCAD software, which lets you get the exact sizes and layout for the best fermentation performance [16]. Because Saccharomyces cerevisiae grows best in a small temperature range, the fermentation process takes place at room temperature, which is about 30°C. Changes in yeast mass and fermentation time are some of the testing factors that will be used to see how they affect the production of bioethanol. Three, five, and seven days of fermentation were used. To see how different amounts of yeast affect how well fermentation works, we used three different yeast masses: 11 g, 22 g, and 33 g, which stand for low, middle, and high inoculum levels, respectively. But different stages of fermentation, from the beginning to the end, were shown by fermentation times of 3, 5, and 7 days, respectively. Previous studies on bioethanol fermentation have shown that the amount of yeast used and the length of the fermentation have a big effect on the amount of ethanol produced and how well the fermentation works. This experimental range was created using those studies as a guide [2, 8, 10]. Because of limited time, each fermentation setting was only tested once. The data are shown as experimental findings without any statistical analysis.

Purification Process

In the process of cleansing, distilling and evaporation are used to make ethanol. The ethanol is then used to get information about its density, viscosity, water content, and % return.

Data Analysis

Experiments were done to find out how yeast mass, fermentation time, ethanol concentration, and output are related. Microsoft Excel was used to find the best mix of yeast mass and growth time. It was also used to make **Figures 3** and **4**.

Raw Material Analysis

Cellulose Content Analysis

Analysis was conducted using the titrimetric method. Steps for analyzing α-cellulose, β-cellulose, and γ-cellulose content:

Sampling:

1.5 g sample was mixed with 75 mL of 17.5% NaOH and gently stirred for 10 minutes. Then, 25 mL of 17.5% NaOH was added, and the mixture was stirred for another 10 minutes. After 30 minutes, 100 mL of distilled water was added, and the solution stood for an additional 30 minutes. The mixture was then stirred, and 100 mL of the filtrate was collected.

α-cellulose content:

To 25 mL of the filtrate, add 10 mL of potassium dichromate and 50 mL of concentrated H_2SO_4. After 15 minutes, add 50 mL of distilled water, and cool the solution to room temperature. Add ferroine indicator, and titrate the solution with ferric ammonium sulfate until it turns purple. A blank test was done by replacing the filtrate with a mix of 12.5 mL of 17.5% NaOH and 12.5 mL of distilled water.

$$\alpha - \text{selulosa} = 100 - \frac{(6{,}85(V2 - v1)xNx20}{A\, x\, W} \quad (1)$$

Where:
V1 = titration in filtrate
V2 = titration blank (ml)
N = normality value of ferric ammonium sulfate solution
W = sample of oven-dried pulp sample (g)

γ-Cellulose Content:

A total of 50 mL of filtrate was mixed with 50 mL of 3 N H_2SO_4. The solution was heated at 70 to 90 °C for 15 minutes to separate the β -cellulose fraction. It then stood for 1.5 hours before separating the solid precipitate from the solution. To 50 mL of the resulting solution, 10 mL of potassium dichromate and 90 mL of H_2SO_4 were added. After 15 minutes, 50 mL of distilled water was added and the solution was cooled to room temperature. Ferroine indicator was added, and titration was performed using ammonium ferrous sulfate until the solution turned purple. A blank test was done by replacing the solution with a mix of 12.5 mL of 17.5% NaOH, 12.5 mL of distilled water, and 25 mL of 3 N H_2SO_4.

$$\gamma - \text{selulosa} = 100 - \frac{(6{,}85(V4 - v3) x N x 20}{A \, x \, W} \quad (2)$$

β-selulosa Content:

$$\beta \, selulosa \, (\%) = 100 - (\% \, \alpha - \text{selulosa} + \gamma - \text{selulosa} \quad (3)$$

Product Analysis

a. Density

Density is the relationship between weight and volume. When the density of a substance goes up, it means there is more mass in each unit of volume. The density of bioethanol is measured with the Pycnometric Method for Density.

The volume of the pycnometer was first checked, and a 10 mL pycnometer was used in this study. Weighed first when empty, the pycnometer was then filled with the bioethanol sample until it reached the required volume and sealed tightly. Next, we measured the mass of the pycnometer with the bioethanol inside. We calculated the mass of the bioethanol by subtracting the mass of the empty pycnometer from the filled one. After getting the results, perform the calculation using the following equation:

$$\rho \text{ bioethanol} = \frac{(Pycnometer\ mass + sample) - Pyicnometer\ mass}{Pycnometer\ Volume} \quad (4)$$

Where:

m (pycnometer + sample) = Total mass of pycnometer and sample (gr)

m pycnometer = Mass of pycnometer (gr)

V pycnometer = Volume of pycnometer (ml)

Viscosity

There is a way to measure how resistant fuel is to friction. It is possible for pressure and stress to change how thick a liquid is. When a liquid is thicker, it moves more slowly through a surface. Bioethanol often takes in water from the air. Since water is less sticky than fuel, adding it to a fuel blend might make it less fluid. How thick or thin a fuel mixture is depending on how much water is in the bioethanol. A Hoppler Viscometer was used by scientists to find out how thick bioethanol was. The first thing they did for their records was measure the densities of the ball and the liquid. After bioethanol was poured into the tube, the ball was put inside it. The length of time that the ball went up and down in the tube was written down. They also kept track of how long it took the ball to fall from its highest point. We can correctly figure out the bioethanol film thickness now that we have this information.

$$\eta \text{ Bioethanol} = K (\rho 1 - \rho 2) \times t \quad (5)$$

where:

η = Bioethanol Viscosity (Ns/m^2)

K = Spherical Constant (0,09 $mPa \cdot scm^3/g \cdot s$)

ρ1 = Spherical Density (2,2 g/cm^3)

ρ2,$cairan$ = Liquid Density (gr/cm^3)

T = Time (second)

Water Content

To find out how much water is in bioethanol, plot the weight loss during distillation against time. During the brewing process, this weight loss happens. Finally, look at the results in light of the starting weight. Use this method to figure out how much water is present.

The researchers made a sample of fermented bioethanol in a measuring cylinder. This amount was written down as A (mL). It was written down that there were B milliliters of liquid left in the distillation flask. They did the math later using these amounts.

$$Water\ Content\ (\%) \frac{A-B}{A} x\ 100\% \quad (6)$$

Yield (%)

$$Yield\ (\%) \frac{Distillate\ Volume}{Volume\ of\ Fermentation\ Solution} x\ 100\% \quad (7)$$

Result and Discussion

This study gives numbers for every step of making bioethanol, such as the qualities of the raw materials, chemical treatment, fermentation, and cleaning.

Table 1. Characteristics of Cellulose Content (α, β, γ)

Sample	Cellulose (%)		
	Alpha (α)	Betta (β)	Gamma (γ)
Pineapple peel waste	79,32	13,92	6,84

T

The results are given logically so that it can judge how well they address the study topic and meet the goals. The results of the cellulose test showed (**Table 1**) that the percentage value of α-cellulose in the pineapple peel waste used reached 79.32%, so this waste has the potential to be used for making bioethanol.

Table 2 shows The sugar content fell from an initial range of 11 to 15°Brix to a final level of 8 to 9°Brix. This remaining sugar shows that some fermentable substrate was still available during the observation period. This means that the changes in ethanol content were not restricted by a lack of sugar.

The Effect of Yeast Amount and Fermentation Duration on Bioethanol Percentage shown at **Table 3**. Changing the amount of yeast used and the length of time it ferments can cause different amounts of ethanol to be produced, as shown in **Figure 3**. Both of these factors were studied at the same time to see how they affect the production of ethanol. The best conditions for fermentation were 33 grams of yeast for three days. This created 80% ethanol, which was the highest concentration of all the conditions that were tried. The findings show that sugar can be turned into ethanol more efficiently if there are enough yeasts and the fermentation lasts long enough. In these settings, yeast cells keep up their biological activity, which lets fermentation happen without any stress. But when the fermentation time is increased to 5 or 7 days, the amount of ethanol drops. It's important that minerals are available, as shown in **Table 2**, and the leftover sugar stayed high at 8 to 9°Brix. So, it looks like the drop in ethanol production was more because the yeast wasn't able to use the leftover base as well as it used to than because it didn't have enough nutrients. The yeast may not have been able to fully turn the available sugars into ethanol because it became less viable or because inhibitory molecules

Table 3. Analysis data of fermentation time and yeast mass against % Yield

Fermentation Time (Day)	Yeast Mass (gr)	% Yield
3	11	9,1%
	22	9,3%
	33	10,2%
5	11	9,3%
	22	9,7%
	33	9,6%
7	11	10%
	22	9,8%
	33	9,6%

Table 2. Ethanol Content Data Research Results

No	Time (day)	Yeast Mass (gr)	Crude Ethanol Volume (ml)	Ethanol Volume (ml)	Initial Sugar (°Brix)	Residual Sugar (°Brix)	Ethanol Content (%)
1	3	11	2750	250	14	9	60
		22	2840	265	15	9	75
		33	2850	275	13	9	80
2	5	11	2800	260	11	8	65
		22	2845	275	11	9	75
		33	2800	270	12	8	76
3	7	11	2850	290	12	9	70
		22	2850	280	11	9	79
		33	2800	270	13	9	77

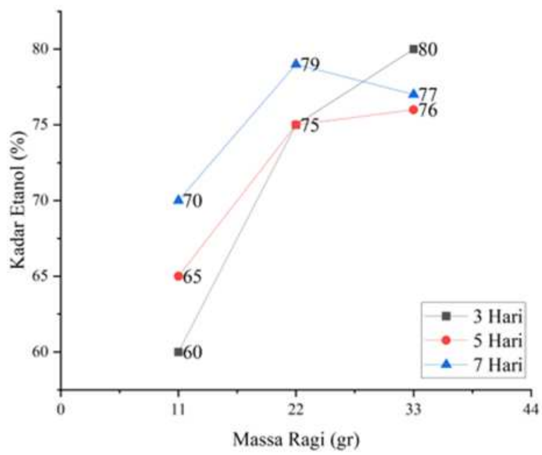

Figure 3. Graph of the effect of yeast mass and time on the level of bioethanol produced

built up during longer fermentation times. This would have made the fermentation less efficient in using the nutrients. On the other hand, smaller yeast masses led to less ethanol production, even after longer fermenting times. This shows that the available material wasn't changed properly because there weren't enough microbes. As long as the yeast stays in its active growth phase, adding to its mass up to a certain point may speed up fermentation and make more ethanol. As there was only one run of each condition, experimental trends will be looked at instead of statistical significance.

It can be seen in **Figure 4** that changes in yeast mass and fermentation time can affect the amount of bioethanol yield. The study says that both of these things affect how well the base is turned into ethanol. In the experiments that were done, the highest output rate (10.2%) came from using 33 grams of yeast for three days during fermentation.

When 22 grams of yeast were used, on the other hand, the fermentation worked consistently, as the yield value stayed the same even though the fermentation times changed. Based on these results, it looks like the right mix between yeast mass and fermentation time is needed to get a good bioethanol yield in the settings that were tried. According to SNI 06-3565:2021, this study checks the quality of the bioethanol that was made by measuring its density, viscosity, water content, and pH. Table 4 shows the findings. These values show that the density is between 0.7150 and 0.7802 g/mL, which is less than the SNI maximum of 0.7851 g/mL. The observed viscosity was between 1.1302 and 1.1556

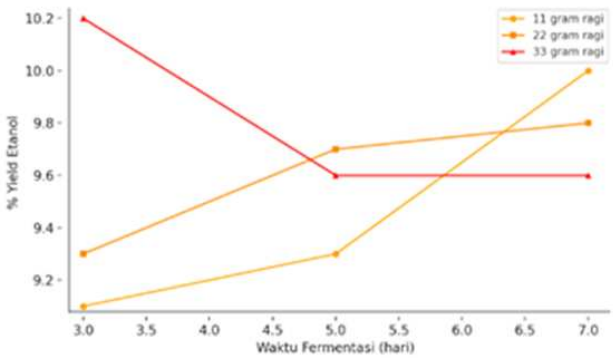

Figure 4. Graph of the effect of fermentation time and yeast mass on %Yield

cP, which is the same as the 1.17 cP level set by SNI. The pH stayed the same at 7.0, and the average amount of water was 0.79%, which is well below the maximum of 1%. In general, the bioethanol that is made fully meets the requirements set by SNI 06-3565:2021.

The very high purity of the raw material is the only reason for this high grade. Laboratory tests have shown that pineapple peel trash has 79.32% α-cellulose, which is a good source of carbon for turning it into fermentation sugar. This quantity, which is higher than what is usually found in some books, shows that the base was prepared well and that the local pineapple types are of good quality. Studies from the past have shown that a high cellulose content is important for making bioethanol that meets the needs of industry [6, 10]. Just as previously, their responses are absolutely correct. The assumption that pineapple peel is a good and reliable raw material for the development of green energy is supported by the fact that its physical qualities are consistent [4, 11, 13].

Conclusion

The fermentation process for making bioethanol from pineapple peel trash looks like it will work well. 79.32% of pineapple peel trash is made up of alpha-cellulose, which was found by looking at the cellulose content. This material is perfect for

Table 4. Data from the results of the analysis of bioethanol characteristics

Sample	pH	Water Content (%)	Density (gr/ml)	Viscosity (Cp)
1	7	0.78	0.7802	1.130211
2	7	0.81	0.7240	1.14391
3	7	0.79	0.7150	1.155609

making bioethanol because it has a lot of cellulose in it. Changes in the amount of yeast used and the length of time for fermentation have a big effect on the amount of ethanol present. There is a sweet spot where adding more yeast and letting the fermentation last longer will make the most ethanol. That's right, after three days of fermentation with 33 grams of yeast, the ethanol level hit an amazing 80%. After this point, the amount of ethanol dropped. The fact that 8 to 9 ° Brix of leftover sugar shows that this drop may be due to less active yeast rather than a lack of feedstock. When everything was perfect, the amount of crude bioethanol made ranged from 2,750 to 2,850 mL, with a high point of 10.2% yield. When these things weren't present, the amount of ethanol produced and the volume of ethanol produced tended to go down and stay the same. The bioethanol that had been produced also passed the physical tests that were needed by SNI 06-3565-2021. We found that the bioethanol is very good; it has a density of 0.782 g/mL, a viscosity of 1.13 cP, a water content of 0.78%, and an average pH of 7.2.

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Author Contributions

D.N.A. did the process and looked at the results inside the fermenter. I.R.O. looked at the results of the ethanol purification. I.R.S., I.F., and I.Y. oversaw the project, gave advice during the study, and helped make the paper better. All of the authors took part in the scientific discussion.

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