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UTILIZATION OF STARCH WATER AS BIOETHANOL USING FERMENTATION METHOD AND BIOACTIVATOR WITH NPK AND UREA

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ABSTRACT

Researchers are looking for sustainable alternative fuels that may be utilized as substitutes for petroleum-based materials due to the problem of dwindling petroleum fuels, rising energy demand, and concerns about rising environmental pollution. The solution to this problem is to produce renewable energy. Bioethanol is a product that has a lot of potential in terms of its utility renewable sources of energy In this project, bioethanol will be generated from household waste, namely starch water (rice boiled water), which includes a significant amount of starch and hence has the potential to be used as a raw material for generating bioethanol, as well as reducing household waste, Better still. Fermentation, hydrolysis, neutralization, and distillation are the processes employed in this study. The starch water is used because it has a significant amount of starch (rice cooking water). Because the starch content is not extracted perfectly during the boiling process, it is compared to typical rice washing water. Bioethanol is the end product, and it is intended to be a sustainable energy that will help to solve the energy issue while also reducing and repurposing household trash.

KEYWORDS
Bioethanol, Starch Water, Saccharomyces Cerevisiae

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INTRODUCTION

In 2016, the demand for fuel oil (BBM) in Indonesia was recorded at 4,.7 percent. Because of decreased production and rising local demand, crude oil imports remained at 283 million barrels in 2018, meeting only 65 percent of domestic demand. Indonesia's oil supplies are expected to run out in 2030 if current consumption patterns do not. As a result of the impact of these issues, namely a global shortage of fuel or energy, all countries that are obliged to create and make renewable energy would be under pressure. The solution to this challenge was to create renewable energy, specifically bioethanol, where families consume 11,6 percent of energy and have a good possibility of subsequently turning this waste into renewable energy (Kementerian, 2012). Because of its widespread use as an energy source, the possibility of oil and its derivatives depletion is a cause for concern. Furthermore, there are numerous environmental issues associated with the excessive usage of these sources, as the combustion of fossil fuels increases greenhouse gas emissions, causing global warming to worsen (Mohapatra, Mishra, Bhalla, & Thatoi, 2019). Bioethanol and biodiesel stand out among biofuels, as they are produced by carbohydrate fermentation and lipid transesterification, respectively (Dasan, Lam, Yusup, Lim, & Lee, 2019).

Starch water, also known as (rice cooked water), is a white, somewhat thickened water that comes from boiling rice. Given that our country, Indonesia, is the world's third largest rice grower, we may take use of this. Starch processing creates a relatively clean glucose stream that Saccharomyces yeasts digest into ethanol (Gray, Zhao, & Emptage, 2006). Which contains soluble carbohydrates and starch in this starch water, and from which we get the starch essence that we need to make bioethanol later, the content of starch is about 5,28 g (per 100 mL) according to (Faizati, 2018). Stain water or rice washing water of 7,3 g (Rachmatika, 2018).

With a problem like this, the government issued Presidential Regulation of the Republic of Indonesia Number 5 of 2006 concerning the National Energy Policy, with the goal of making innovations related to the manufacture of renewable energy to overcome these problems, and it is hoped that a study on the manufacture of renewable energy will be conducted is expected. This alternative energy can be mass-produced and scaled to replace the dwindling fuel energy source (Prihandana, 2011).

Bioethanol is ethanol produced by a fermentation process derived from plants. Bioethanol, along from being an alternative energy source for replacing BBM, can also be used to reduce CO2 emissions. Materials that can be utilized to make bioethanol include those that include starch as well as glucose (Hikmiyati & Yanie, 2009). Bioethanol has already been widely adopted in Brazil, the United States, and Europe [2-3]. Because many countries seek to reduce oil imports, enhance rural economies, and improve air quality, production has risen dramatically. In 2007, global ethyl alcohol production was estimated to be over 51000 million liters. The substrate is the most important component in ethanol synthesis, therefore it is critical that ethanol production be done with a low-cost substrate like starch or cellulose (Eliasson, Hofmeyr, Pedler, & Hahn-Hägerdal, 2001).

In most ethanol fermentations, a higher substrate load results in higher ethanol concentrations, which improves downstream processing efficiency. Furthermore, the capacity to function at high solid concentrations is a critical component in the enzymatic hydrolysis process since it affects the energy balance and economic sustainability of

bioethanol production (Yanuar & Amrullah, 2015).

RESEARCH METHOD

Materials and Tools

The basic ingredient in the production of bioethanol in this study is starch water, which is derived from boiling rice water. 600 mL water, preheating temperature of 80°C, heating duration sufficient to exit the bubble are the variables utilized in the sample preparation stage. It takes 0.1,2,3,4 days for the fermentation stage, with 70 mL of HCl and enough NaOH to reach pH 4-5, Saccharomyces 3 and 5 grams of cerevisiae, 2 and 5 grams of NPK, and 2 and 5 grams of urea Using a heating temperature of 78oC and a distillation period of - 1 hour in the distillation stage.

Analytical balance, hot plate, basin, thermometer, three neck flask, leibig cooler, erlenmeyer, jar, plastic, spoon, stirrer, measuring cup, pycnometer, alcoholmeter, rag, watch glass, dropper, and a set distillation apparatus were among the instruments used.

Methods

In the procedure, prepare 200, 300 grams of rice (for two variables) by washing it completely with water, putting it in a saucepan with 600 mL of water, heating it until it boils while stirring, and removing it from heat if it is somewhat thickened. Bring the rice water to a boil, then strain the rice from the water. At this point in the procedure, add 70 mL of HCl to a starch hydrolysis solution that has been produced up to 600 mL (for each variable), heat to 80°C to boil out the bubbles, and then allow the solution to cool. The pH of the cooled solution is checked at this stage of the neutralization process; if the pH is excessively acidic, add a few drops of NaOH until the pH reaches 4-5. At this stage of the fermentation process, take a 600 mL sample, add 3 and 5 grams of Saccharomyces Cerevisiae, then 2 and 5 grams of NPK and urea as nutrients, stir until evenly distributed, cover tightly with plastic, and allow to stand at room temperature for the specified variables, namely 0.1,2,3,4 days. The distillation process is stopped when the distillate is no longer dripping at this stage of the distillation process, which includes assembling a distillation apparatus, pouring the fermented solution into a three neck flask and turning on the heater, heating the fermented solution at a temperature of 78oC for approximately 1 hour until the distillate no longer drips. the distillation process is stopped when the distillate is no longer dripping, measuring the volume of the resulting distillate.

RESULT AND DISCUSSION

A. *Results of Glucose Level Analysis by Luff-Schoorl Method*

Table 1. Observation Result of The Glucose Level Analysis by *Luff-Schoorl* Method

No.	Sample	Glucose
1	Starch Water	4,73 %

The Luff Schoorl method is a reduction method that uses reducing sugars, such as monoxide, lactose, and maltose. CuO monosaccharides present in the solution of Luff Schoorl will be reduced by an excess of KI and released into the I2 in this study for the determination of glucose or carbohydrate compounds based on changes CuO monosaccharides present in the solution of Luff Schoorl into Cu_2O Excess will be

reduced by an excess of KI and released into the I_2. I_2 liberatedwill be titrated by a solution of $Na_2S_2O_3$ [9].

The determination of glucose levels in starch water was carried out in this study before the hydrolysis process in order to determine how much glucose was present in the starch water sample, and the samples collected would then be evaluated for glucose levels using the method chosen Luff School. Because this approach is commonly used to excuse has a 10% mistake in measuring glucose levels, but it is easier and saves money bahan. The sample will be titrated using a solution of $Na_2S_2O_3$ that has been standardized by KIO_3 after it has reacted with the Luff Schoorl reagent. The volume of the Natitrant $Na_2S_2O_3$ is the difference between the volume of $Na_2S_2O_3$ in the blank titration and the volume of $Na_2S_2O_3$ in the sample titration, which will be used to calculate glucose levels in the starch water sample later by computation. Sample preparation was done initially, before the glucose levels in the sample were examined. The samples were cooked to extract greater glucose levels, and then several mL were taken to be examined. A solution of 10% Pb Acetate was applied to the samples that had been taken. The addition of Pb Acetate is intended to precipitate the protein present in the starch water sample so that it does not interfere with the glucose level determination [19].

The titration of the blank solution was carried out three times in my research, with the titrant volumes being 24,36, 24,42, and 24,41, respectively, and the glucose level reached being 4,73 percent.

B. Results of Starch Level Analysis by Method *Luff-Schoorl*

Tabel 2. Observation Result of The Starch Level Analysis by *Luff-Schoorl* Method

No.	Sample	Starch
1	Starch Water	9,30 %

We will use the Luff Schoorl method to determine the starch concentration in starch water in this investigation. The starch content is determined by hydrolyzing it with HCl, which is nearly identical to the process for determining glucose levels. Luff Schoorl differs solely in the way the components are prepared and the calculating formula. If Pb acetate is added to the assessment of glucose levels in the processing of materials, then Pb acetate is not added to the analysis of starch content. For the titration utilizing $Na_2S_2O_3$, this sample determination involves calculating the amount of Cu_2O (cuprooxide) in the solution before it is reacted with reducing sugar (blank titration) and after it is reacted with a sample on reducing sugar (sample titration), The difference between the blank and sample titrations in the presence of Cu_2O produced and the amount of reducing sugar present in the material or starch itself will be noticed later.

The following stage is sample preparation, which involves obtaining a sample of the material, diluting it with distilled water, adding HCl, heating it, and finally neutralizing it with NaOH to pH 7. The weight of starch is equal to the weight of glucose multiplied by 0.9. [9]. Even though it is already liquid, it should be filtered to remove the carbs included in the soluble material as well as the starch's insoluble nature in water. The material in the filter paper is then removed and mixed with distilled water and HCl before being heated.

Heating is used to hydrolyze the starch in the sample by breaking the glycosidic bonds in it, resulting in the formation of shorter starch molecules such as monosaccharides, disaccharides, or polysaccharides with shorter chains such as maltodextrin, as evidenced by the sample turning yellowish white [21]. Furthermore,

heating starch can cause it to lose its characteristics, such as gelatinization, and become more water soluble, making it easier to test [21].

The sample is neutralized with NaOH after the aforementioned process so that it is not too acidic. If the sample is excessively acidic, the titration process will take a long time or be problematic. After that, the sample

solution was mixed with Luff Schoorl's solution and heated. This reaction produces Cu_2O, and it is carried out in this case in order for reducing sugars to convert copper Cu^{2+} to Cu^{+}. Furthermore, the $Cu^{2+,}$ which is not reduced or can be considered to be (remaining), is then continued with the Iodometry method, namely by adding a solution of KI and also H_2SO_4, followed by titration with $Na_2S_2O_3$, which changes color to pale yellow.

Following the steps outlined above, the difference in volume between the blank titration and the sample is referred to as the amount of carbs in the sample. The result is then multiplied by 0.9, yielding the weight of starch, which describes the relationship between the amount of reducing sugar and the amount of starch [21].

The blank solution was titrated three times in my research, with the titrant volume being 23.59, 24.28, and 24.30, respectively, and the starch content being 9.30 percent.

C. Density Analysis Results

Table 3. Density Results

Nutrition	Rice	Sacchar omyces	Fermentation Time (Days) (gr/mL)			
(gr)	(gr)	(gr)	0	1	2	3	4
2	200	3	0,00	1,6919	1,5063	1,5023	0,8638
2	200	5	0,00	1,5593	1,5085	1,5030	0,8553
2	300	3	0,00	1,5301	1,5062	1,5013	0,8137
2	300	5	0,00	1,5301	1,5061	1,4991	0,8108
5	200	3	0,00	1,5286	1,5093	1,4867	0,780
5	200	5	0,00	1,5260	1,5074	1,4860	0,7844
5	300	3	0,00	1,5259	1,5049	1,4680	0,4971
5	300	5	0,00	1,5244	1,5027	1,3776	0,4503

Table 4. Distillate Volume Results

Fermentation Time (Days)				
0	1	2	3	4
0 Ml	31 mL	52 mL	88 mL	102 mL
0 Ml	33 mL	50 mL	94 mL	115 mL
0 mL	35 mL	57 mL	98 mL	109 mL
0 Ml	36 mL	66 mL	103 mL	114 mL
0 Ml	36 mL	64 mL	101 mL	126 mL
0 mL	34 mL	79 mL	100 mL	138 mL
0 Ml	37 mL	83 mL	102 mL	146 mL
0 mL	42 mL	87 mL	107 mL	167 mL

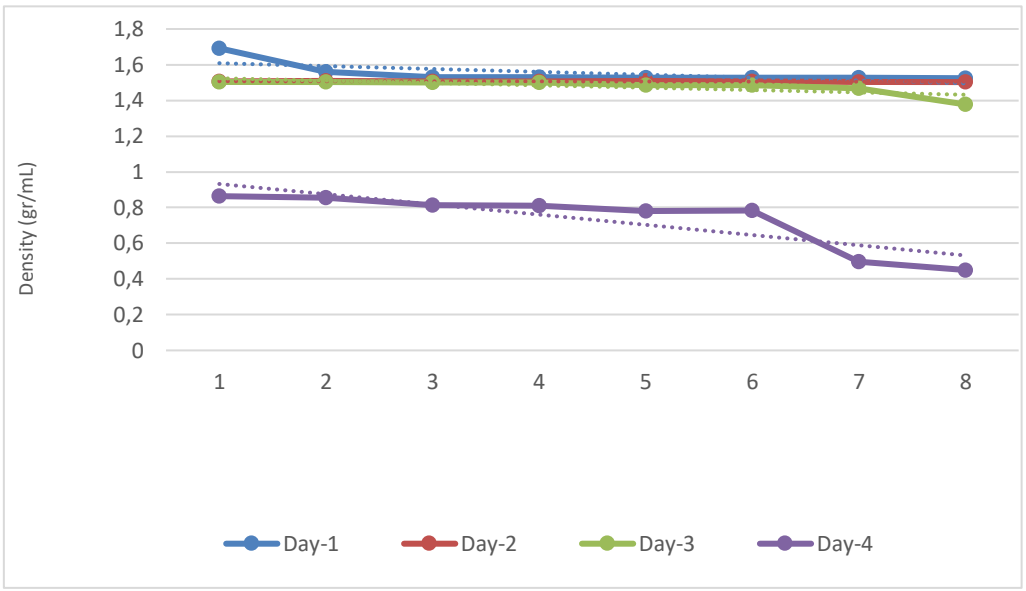

Figure 1. Density Result Graph

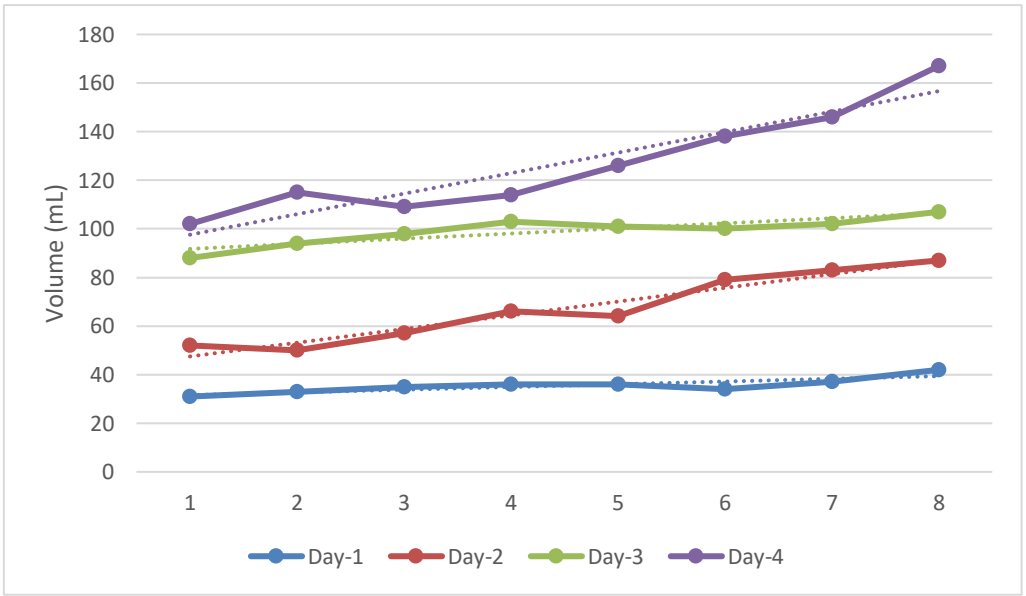

Figure 2. Distillate Volume Result Graph

From Figures 1 and 2 graphs of the relationship between volume and density, we can conclude that the larger the volume, the smaller the density value produced. As we know where the longer the time in the fermentation process, the more the number of microbes needed in the process so that the more the number of microbes, the more carbohydrates will break down into ethanol, therefore the higher the amount of alcohol, the lower the density. resulting from. Because basically the density of water with alcohol is lower than alcohol.

D. Relationship of Time with Distillate Volume

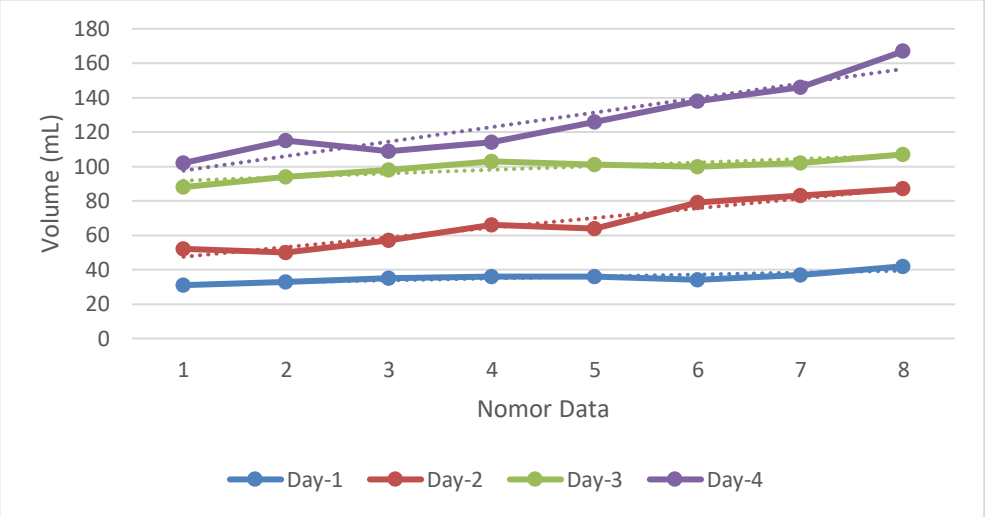

Figure 3. Graph Relationship of Time with Volume of Distillate

From Figure 3 Graph of the Relationship between Time and Volume, we can conclude that the longer the fermentation time, the greater the volume. That the fermentation time can affect the acquisition of bioethanol where the longer the time we use for the fermentation process, the volume that will be obtained will increase over a certain time and will experience a decrease in the process. The decrease was caused by the function of the bacteria having decreased and running out of nutrients or could be said to be entering the death phase because the microbes were able to convert carbohydrates into ethanol.

E. Relationship Between Density and Bioethanol Content

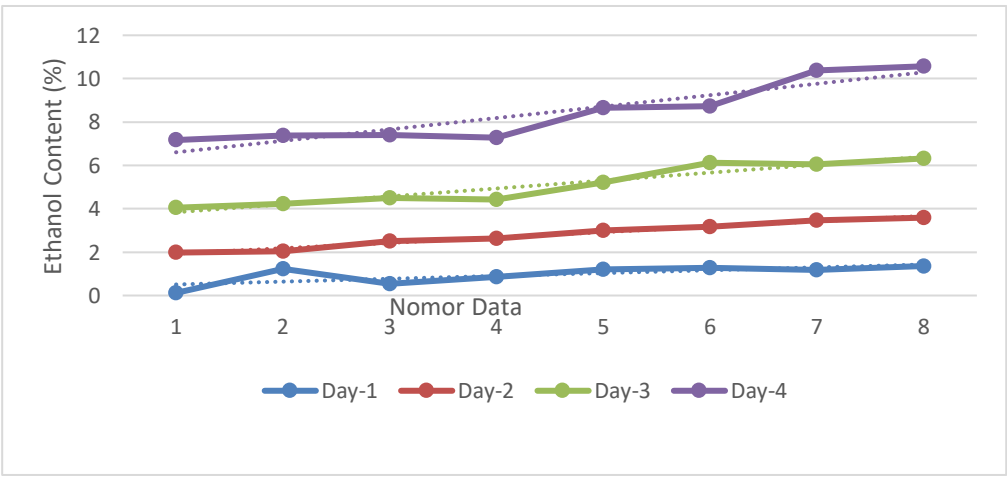

Figure 4. Graph of The Result of Bioethanol Levels

From Figures 1 and 4 graphs of the relationship between density and ethanol content, we can conclude that the lower the density value, the higher the ethanol content produced. This is because ethanol has been produced from the fermentation process will undergo an evaporation caused by the transport of gas CO_2, if the density of high value resulting mixed solution will be increasingly difficult for its evaporation process. Because there is still a mixture with water, it means that the density value is high and the purification process will take longer. As we know where fuel or we can call ethanol here is good, namely by having a low density value with a high octane number.

F. The Results of The Analysis of Bioethanol Levels

Table 4. Results of Bioethanol Levels

Nutrition	Rice	Saccharomyces	Fermentation Time (Days)				
(gr)	(gr)	(gr)	0	1	2	3	4
2	200	3	0,00%	0,12 %	1,98%	4,05%	7,17%
2	200	5	0,00%	0,22 %	2,04 %	4,23%	7,38%
2	300	3	0,00%	0,54%	2,50%	4,51%	7,40%
2	300	5	0,00%	0,85 %	2,63 %	4,43%	7,29%
5	200	3	0,00%	1,21 %	3,01 %	5,22%	8,65%
5	200	5	0,00%	1,27 %	3,18 %	6,12%	8,74%
5	300	3	0,00%	1,18%	3,47%	6,06%	10,39%
5	300	5	0,00%	1,36%	3,59%	6,32%	10,57%

In the research I did, the usual time used for starch water fermentation was 0, 1, 2, 3, 4 days. With a variety of nutrients in the form of NPK and Urea as much as 2 and 5 grams and Saccharomyces Cerevisiae as much as 3 and 5 grams with a water volume of 600 mL with a distillation temperature of 78^{o}C-80^{o}C.

The results obtained were taken the best 2 for the ethanol content every day and the best results were 1,27% and 1,36% on day 1st and day 2nd 3,47% and 3,49% on day 3th 6,12% and 6,32% on the 4th day 10,39% and 10,57%.

The fermentation process depends on the amount of yeast added in the material. The more the amount of yeast that is given means the more the amount of yeast involved, so that the ethanol content increases. Because as we know that the characteristics of the yeast Saccharomyces Cerevisiae have a fermentation rate and a fast growth rate as a microbe in the formation of ethanol, besides that it is also resistant to high salt concentrations.

When used during fermentation, Saccharomyces Cerevisiae experienced a rapid growth phase so that the process of reshuffling or changing sugar into ethanol was faster so that the pH value increased. Although there was an increase in the pH value, the value achieved at the end of the fermentation process was still around the optimum pH of 5,5-6,0 which could be adapted by Saccharomyces Cerevisiae involved in the fermentation process (Nikulin et al., 2020). The length of fermentation greatly affects the high and low levels of ethanol formed. According to, the incubation time affects the fermentation results because the longer the incubation, the higher the ethanol content (Kundiyana, Wilkins, Maddipati, & Huhnke, 2011). In the fermentation process before ethanol is formed, it will form glucose first so that the formation of ethanol takes longer than the formation of glucose. However, if the fermentation is too long, the nutrients in the substrate will run out and the yeast cannot ferment the material.

In this study, starch water bioethanol samples were fermented for a certain time, which then entered the distillation process at a temperature of 78-80^{o}C. stated that the

principle of distillation is where the liquid will evaporate and condensation occurs again where the steam is will be at the boiling point (Yumas & Rosniati, 2014). The boiling point of a liquid is the temperature at which its vapor pressure equals atmospheric pressure. Then the condensed liquid is called the distillate again. The purpose of this distillation process is to purify the liquid at its boiling point, and separate the liquid from dissolved solids or from other liquids that have different boiling points of pure liquids. In ordinary distillation, atmospheric pressure (normal boiling point) is the vapor pressure above the liquid vapor pressure. For pure compounds, the temperature obtained on a thermometer placed at the place where the distillation process occurs is the same as the boiling point of the distillate obtained.

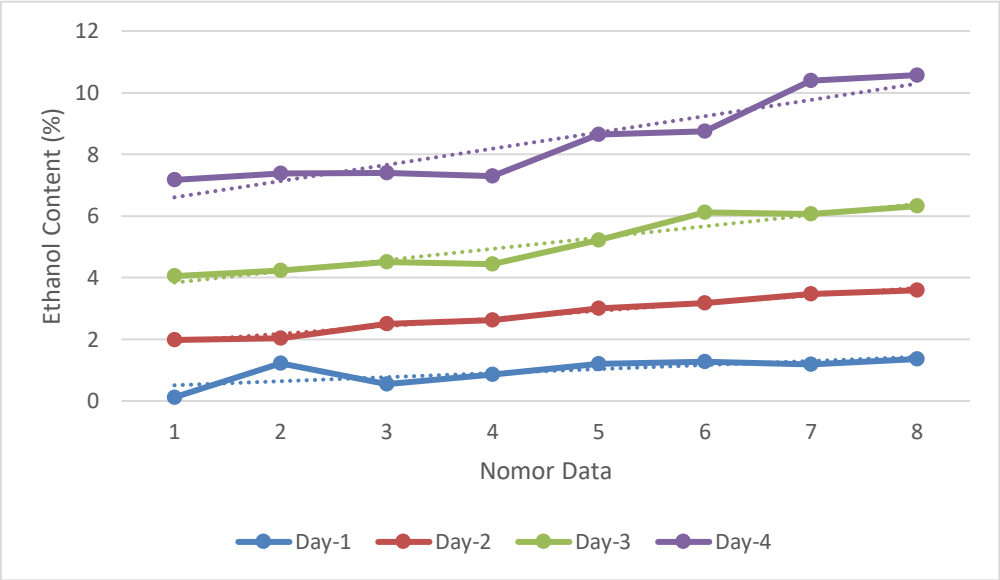

Figure 5. Bioethanol Content Results Graph

From Figure 5, it can be seen that the longer the fermentation time, the higher the ethanol content, where the longer the fermentation time, Saccharomyces Cerevisiae undergoes a rapid growth phase so that the process of reshuffling or changing sugar into ethanol is faster so that the pH value increases, besides that the value of the density will decrease and the ethanol content will increase because the amount of water is less than ethanol.

G. Michaelis Menten Fermentation Kinetics

$$\frac{1}{V} = \frac{1}{Vmaks} + \{\frac{Km}{Vmaks}\}\frac{1}{[S]}$$

t = Fermentation Time (Hours)
[S] = Substrate Concentration / Glucose (gr/mL)
[P] = Product Contentration / Alcohol (gr/mL)
V = Reaction Velocity (gr/mL.jam)

Table 5. Michaelis Menten Fermentation Kinetics

t	[P]	1/[P]	V	1/[V]
0	0	0	0	0
0	0	0	0	0
24	1,21	0,826446281	0,05041667	19,83471074
24	1,36	0,735294118	0,05666667	17,64705882
48	3,47	0,288184438	0,07229167	13,83285303
48	3,59	0,278551532	0,07479167	13,37047354
72	6,12	0,163398693	0,085	11,76470588
72	6,32	0,158227848	0,08777778	11,39240506
96	10,39	0,096246391	0,10822917	9,239653513
96	10,57	0,094607379	0,11010417	9,08230842

Intercept = 5,551234479
Slope = 19,17934687
$1/V_{maks}$ = Intercept
V_{maks} = 0,180140112
Km/Vmaks = Slope
Km = 3,45496969

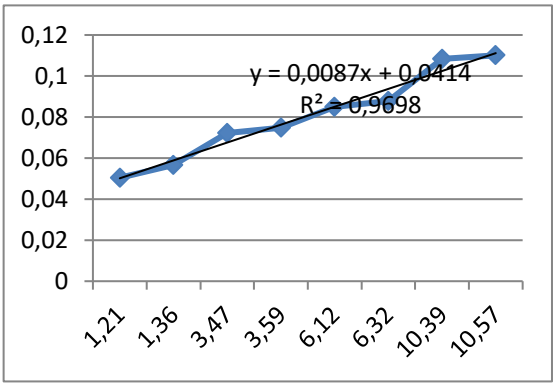

Figure 6. Michaelis Menten Fermentatiton Kinetics Graph [P] vs [V]

From Figure 6. above, the results of Michaelis Menten's fermentation kinetics can be seen that the higher the concentration, the more reactant molecules are available, thus the possibility of collisions will also increase so that the reaction speed increases. So the higher the concentration, the faster the reaction rate. We can know that one that affects the speed or rate of a reaction is concentration, so we can see that the higher the reaction rate, the higher the concentration and vice versa. And in this ethanol concentration, it can accelerate the reaction rate because of the starch content, namely as an amylase enzyme, while when the reaction rate is high, the concentration will automatically be high because the ingredients in it react more quickly. Where the reaction rate is the change in the concentration of the reactants or products per unit time.

In my research, Michaelis Menten fermentation kinetics obtained for starch and glucose levels in the sample of 9,30% and 4,73% From the determination of glucose fermentation into bioethanol obtained: Maximum reaction speed (Vmax) = 0.1801 gr/mL.hour Michaelis Menten constant (Km) = 3.4549 gr/mL (Rahmasari, 2013).

Spesific Gravity Analysis

Table 6. The Results of Aquadest Density Measurement

Ingredients	Volume (mL)	Density (gr/mL)
Aquadest	25	0,9621
Aquadest	25	0,9634
Aquadest	25	0,9637
Rata-rata	25	0,9630

Table 7. Specific Gravity Results

Fermentation (Days)	Bioethanol (gr/mL)	Density	Spesific Gravity
1	1,5260		1,5846
1	1,5259		1,5845
1	1,5244		1,5829
2	1,5074		1,5653
2	1,5049		1,5627
2	1,5027		1,5604
3	1,4860		1,5430
3	1,4680		1,5244
3	1,3776		1,4305
4	0,7844		0,8145
4	0,4871		0,5161
4	0,4503		0,4676

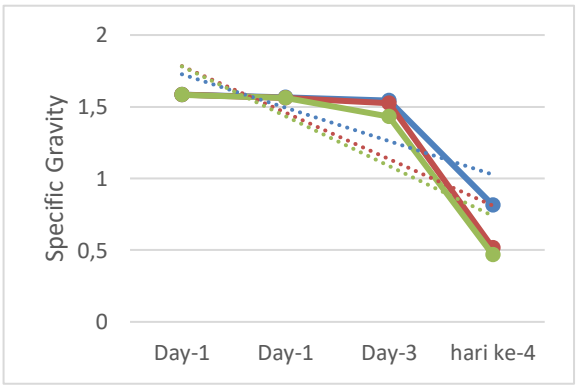

Figure 7. Graph of The Effect of Time on Specific Gravity

The results in Table 4. above are the effect of the length of fermentation time on thevalue specific gravity of the ethanol produced. From the data above, it can be seen that the length of fermentation time greatly affects the value of the specific gravity, namely where thevaluedecreases specific gravity of the tested ethanolwith increasing time.

Where we know that the longer the fermentation time, the more the number of microbes needed for the process, and that is related to the amount of carbohydrates that will slowly break down into alcohol if the number of microbes increases. In this way, indirectly, with the increase in the amount of alcohol, the value of density will also be lower which will result in the value of specific gravity decreasing (Christensen, Yanowitz, Ratcliff, & McCormick, 2011).

CONCLUSION

From the results of the discussion, it can be concluded that in this study, the factorial design approach was used in the design experimental with 40 experiments being carried out. In the glucose level test, the result was 4,73%, where the blank solution titration was carried out 3 times and the titrant volume was 24,36, 24,42 and 24,41, while the starch content test was 9.30%. The blank solution titration was carried out 3 times and the titrant volume was 23,59, 24,28 and 24,30. The density is in accordance with the theory where the larger the volume, the smaller the density value and the longer the fermentation time, the greater the volume obtained. For the ethanol content test, the best 2 were taken, namely on the 1st day it was 1,27% and 1,36% on the 2nd day 3,47% and 3,49% on the 3rd day 6,12% and 6,32 % on the 4th day 10,39% and 10,57 % and is in accordance with the theory where the longer the fermentation time, the higher the ethanol content. For Michaelis Menten kinetics, the starch and glucose levels in the sample were 9,30% and 4,73% from the determination of glucose fermentation into bioethanol, the maximum reaction speed (Vmax) = 0,1801 gr/mL.hour and also Michaelis Menten constant (km)= 3,4549 gr/mL. The specific gravity obtained is in accordance with the theory because the length of fermentation time greatly affects the specific gravity value, where the longer the fermentation time, the higher the amount of alcohol which will result in low density and decreased specific gravity. This research is a new study because it uses household waste raw materials, namely starch water (rice boiled water) which has never been done before and added NPK and Urea nutrients which are used for bacterial nutrition during the fermentation process so that the results are maximized, besides that it also adds value. added to my research namely Michaelis Menten fermentation reaction kinetics. The method used in this research is fermentation and hydrolysis and using the calculation of the luff-school method. Lack of supply of fuel or energy in the world which will result in pressure on all countries in the world which are required to produce and make renewable energy. As well as the lack of utilization of waste from household products that can be used better so as not to pollute the environment. With the existence of an innovation in making bioethanol from starch water, this problem can be overcome and can utilize household waste so that it can become a quality product. For this research article, it includes experimental research because no one has made it before

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