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UTILIZATION OF BIOETANOL FERMENTATION WASTE PINEAPPLE AND COCONUT WATER AS DISINFECTANTS WITH BACTERIA *SACCHAROMYCES CEREVISIAE*

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ARTICLE INFO	ABSTRACT
Received: **May, 25th 2021** Revised: **June, 4th 2021** Approved: **June, 15th 2021**	*Research analysis of sugar content and the effect of ethanol content on bioethanol from old coconut water and pineapple peel with the help of Saccharomyces Cerevisease bacteria. The condition of the spread of the Corona Virus or COVID-19 in Indonesia, thus making bioethanol produced from fermenting pineapple peel waste and old coconut water for disinfectant products to spray around homes and public places to reduce bacteria and viruses. The production of bioethanol is carried out by pre-treating coconut water and pineapple peel, the fermentation stage with Saccharomyces cerevisiae yeast and the distillation stage. The result of the highest bioethanol content was 32% with a mass of 5 g yeast with a time of 24 hours. The highest calorific value at 72 hours was 211.95 kcal/kg. The result of the highest specific gravity at 24 hours and the mass of yeast 4 g is 0.98 g/ml. Based on the bioethanol quality requirements, the bioethanol produced is not in accordance with the bioethanol quality requirements, this is due to the absence of nutrient decomposing bacteria so that it is less than optimal in converting glucose into bioethanol.*
KEYWORDS	Bioethanol, Disinfectant, Fermentation, Distillation

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INTRODUCTION

In 2019, a new disease caused by a virus appeared and attacked the respiratory system, this disease is called the Corona Virus or COVID-19 (Ramesh, Siddaiah, & Joseph, 2020). The COVID-19 cases started with pneumonia or mysterious pneumonia. Coronaviruses are a large family of viruses that cause illness ranging from mild to severe symptoms (Putri, 2020). There are at least two types of coronavirus that are known to cause diseases that can cause severe symptoms such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) (Timah, 2021). Coronavirus Disease 2019 (COVID-19) is a new type of disease that has never been previously identified in humans (Bahtiar & Ariyanti, 2021).

Common signs and symptoms of COVID-19 infection include acute respiratory symptoms such as fever, cough and shortness of breath. The average incubation period is 5-6 days with the longest incubation period of 14 days (Yuzar, 2020). Clinical signs and symptoms reported in the majority of cases were fever, with some cases having difficulty breathing, and X-rays showed extensive pneumonia infiltrates in both lungs (Nurhayatun & Prabowo, 2020). Appropriate action has been taken to prevent and limit this spread to a wider and faster pace (Nicola et al., 2020). Presence of inhibitory chemicals in lignocellulose hydrolysates is a major hurdle for production of second-generation bioethanol. Especially cheaper pre-treatment methods that ensure an economical viable production process generate high levels of these inhibitory chemicals (Vanmarcke, Demeke, Foulquié-Moreno, & Thevelein, 2021).

Bioethanol as renewable fuel addresses elevated production costs, as well as food security concerns (Nwaefuna, Rumbold, Boekhout, & Zhou, 2021). The right precaution is to use natural disinfectants from the manufacture of bioethanol produced by fermented pineapple peel waste and old coconut water (Prasetyo, 2011). In bioethanol, this is useful for a disinfectant mixture so as to take advantage of the surrounding waste (Novia, Windarti, & Rosmawati, 2014). Using coconut water as bioethanol is one of the ingredients for making bioethanol. In coconut water, natural chemical content is very good for the body, including: Vitamin C which has a description of the content in it, such as nicotinic acid, folic acid, pantothenic acid, bitin, and riboflavin (Andari, Mulyadi, & Puspawiningtyas, 2015). Besides containing minerals, coconut water also contains sugar in the range of 1.7-2.6%, 0.07-0.55% protein and contains potassium (Faizal, Zuhandri, & Andrio, 2011).

In addition to the old coconut water, use of waste can be used as alternative fruit is pineapple skin (Noviandi, Yaman, & Rinidar, 2018). The content of pineapple skin that can be processed and used as a basic ingredient in the manufacture of bioethanol. The content of pineapple skin contains carbohydrates by 10.54%, ash by 0.48%, water by 86.7%, fat, by 0.02%, and wet fiber by 1.66%. According to (Roni, Susanto, Pratama, & Herawati, 2020), the presence of carbohydrate content means that pineapple skin can be used as a base material for bioethanol production, so that coconut water and pineapple skin have the potential to make bioethanol.

RESEARCH METHODS

The main ingredients are old coconut water and honey pineapple skin, the supporting materials are distilled water, HCl, Urea NPK, Saccharomyces cerevisiae yeast, Glucose

Anhydrous, Anthrone Sulfate. In this study, using the method of fermentation with the beginning of the process of pre-treatment of raw materials, the analysis of glucose, fermentation and distillation.Method used factorial design, the control variables used are sample volume 5 ml, Anthrone 20 mg, 1 mg anhydrous glucose, wavelength uv-vis spectrophotometer 630nm, sample volume 150 ml coconut milk, pineapple filtrate volume of 150 ml, distillation temperature 78^0C - 80^0C. The independent variables used were fermentation time of 24 hours and 72 hours, temperature of 26^0C and 30^0C, yeast concentration of 5 grams and 2 grams, urea NPK 3 grams.

Table 1 The experimental design with a factorial design

No.	T	pH	k	tpH	Tk	pHk	tpHk
1.	-	-	-	+	+	+	-
2.	+	-	-	-	-	+	+
3.	-	+	-	-	+	-	+
4.	+	+	-	+	-	-	-
5.	-	-	+	+	-	-	+
6.	+	-	+	-	+	-	-
7.	-	+	+	-	-	+	-
8.	+	+	+	+	+	+	+

Description of table 1:
Time (t): (-) 24 and (+) 72 hours
pH: (-) 4 and (+) 5
Yeast content (k): (-) 5 and (+) 2 grams

The research process can be observed in Figure 1 analyzes were performed on the samples to determine the glucose level analysis with Quicker-Method method, and product analysis is performed to determine the density, calorific value, ethanol content, and Specific Gravitv.

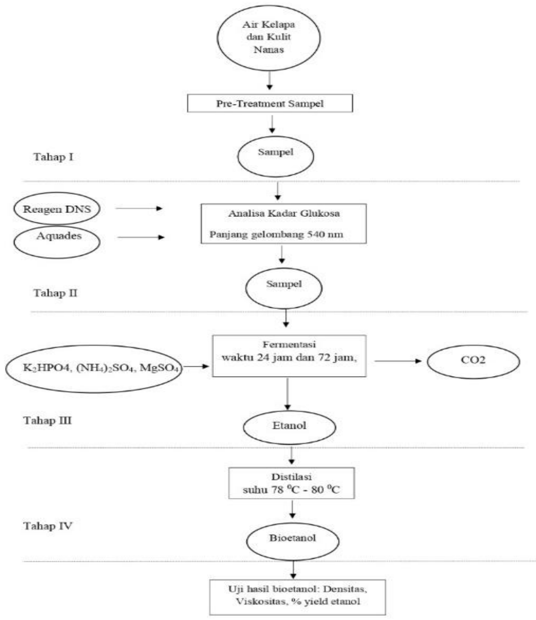

Figure 1. Flowchart of Research

The Athrone method was used to determine the sugar content in honey and old coconut water samples, and the analysis of variants using the Quicker – Method.

RESULTS AND DISCUSSION

A. Analysis of Glucose Levels Using the Anthrone Method

The standard solution is made of 6 concentrations, namely 0 mg / ml; 0.2 mg / ml; 0.4 mg / ml; 0.6 mg / ml; 0.8 mg / ml; 1 mg / ml made from the dilution of 0.2 mg glucose standard solution and then take 10 ml dissolved in 100 mL volumetric flask. The following chart calibration curve.

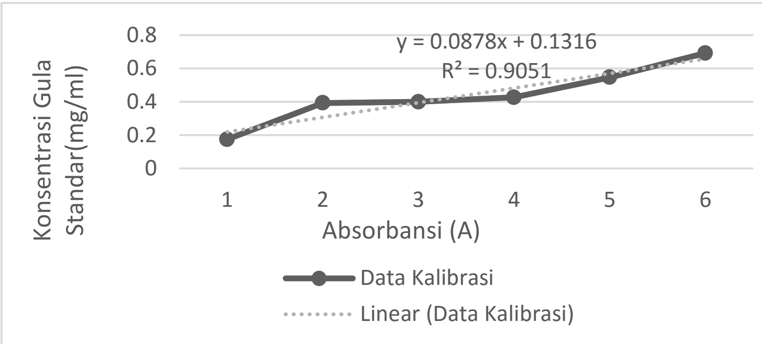

Figure 2. Calibration Curve Graph

From the graph, obtain standard solutions absorbance measurement results are then used to create a standard curve. Standard curves need to be created to determine the regression equation. So that the regression equation can be determined the sample concentration. The regression equation obtained is $y = 0.08x + 0.13$ with $R^2 = 0.905$.

Based on the regression equation that has been made, it can be determined the glucose level in honey pineapple skin, old coconut water and honey pineapple skin + old coconut water. The glucose level in the sample of honey pineapple peel as a result of the experiment was 8.24 mg / mL, the sample of old coconut water was 8.23 mg / mL, and in the sample of pineapple honey + old coconut water was 8.11 mg / mL.

While the glucose content in honey pineapple peel is 8.53%, the glucose content in old coconut water is 3%. In this study, the percentage of glucose levels for honey pineapple peel was 0.08% and in old coconut water was 0.08%. The small glucose level in the experimental sample is likely due to the influence of human error and the error in the absorbance measurement of the standard solution as discussed earlier, so that the standard curve equation is not good and affects the determination of glucose levels in the sample.

B. Analysis of Variants Using the Quicker Method

a. Effect of Density Variables on Bioethanol

Table 2 Calculation Results Main Effects and Interactions Effects of Ethanol Density

Effects	Results
t	0,01 → Main Effects
P	0,01
m	0,04
tP	0,02
tm	-0,03
Pm	-0,03 → Interactions Effects
tPm	0,01

Table 2 shows that the main effect for the density value in this study is the fermentation time (t) with a value of 0.01 with an interaction effect in the form of pH and nutrient mass with a value of -0.04.

Table 3. Determination of Variables Influencing Bioethanol Density

P (%)	Effects	Identity Effects
7,14	0,04	M
21,42	0,01	P
35,71	0.01	T
50	0,02	tP
64,29	-0,03	Tm
78,58	0,01	tPm
92,86	-0,03	Pm

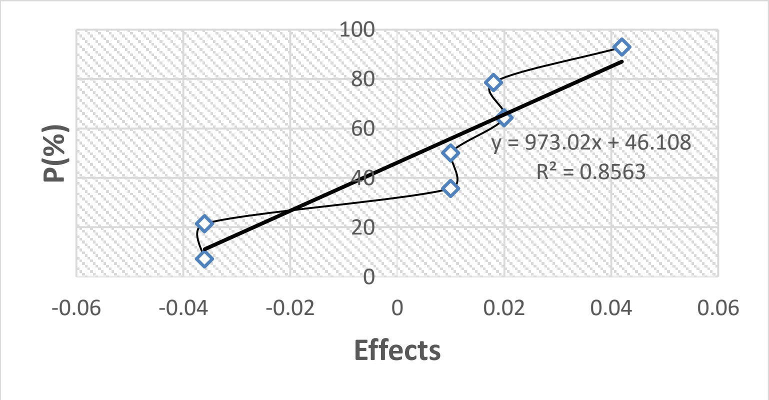

Figure 3. Normal Probability Density Plot Against Bioethanol for Factorial Design 2^3

Figure 3 shows the Normal Probability Plot graph between the P value and the effect obtained by regression (R^2) of 0.85 by activating the Trendline feature in Microsoft Excel. This means that 85.63% of the total variation in the model can be represented by a regression equation. The equation that shows the correlation between the value of bioethanol density and the parameters of the research process (nutrient mass and operating conditions) is y = 973.02x + 46.10.

b. Effect of Variable Ethanol Levels on Bioethanol

Table 4. Calculation Results of Main Effects and Interaction Effects on Ethanol Bioethanol Levels

Effects	Results
t	-0,05 → Main Effects
P	-0,09
m	-0,27
tP	-0,15
tm	0,23
Pm	0,23 → Interaction Effects
tPm	-0,07

Table 4 shows that the main effect for the value of ethanol content in this study is the fermentation time (t) with a value of -0.05 with an interaction effect in the form of pH and nutrient mass with a value of 0.23.

Table 5 Determination of Influential Variables on Bioethanol Ethanol Levels

P (%)	Effects	Identity Effects
7,14	-0,27	M
21,42	-0.09	P
35,71	-0.05	T
50	-0,15	tP
64,29	0,23	Tm
78,58	-0,07	tPm
92,86	0,23	Pm

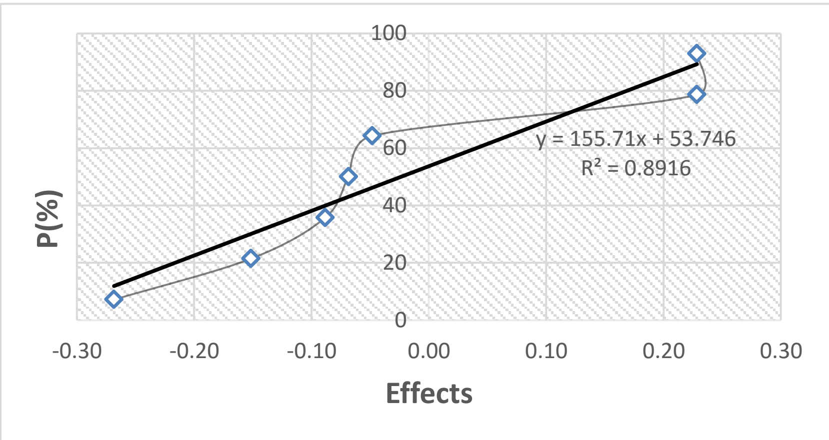

Figure 4. Normal Probability Plot Graph of Ethanol Bioethanol Content for Factorial Design 2

Figure 4 shows the Normal Probability Plot graph between the P value and the effect obtained by regression (R2) of 0.89 by activating the Trendline feature in Microsoft Excel. This means that 89.16% of the total variation in the model can be represented by a regression equation. The equation that shows the correlation between bioethanol ethanol content and the parameters of the research process (nutrient mass and operating conditions) is y = 155.71x + 53.74.

c. Effect of Variable Specific Gravity (*sg*) on Bioethanol

Table 6 Calculation Results of Main Effects and Interaction Effects on Specific Gravity (sg) Bioethanol

Effects	Results
t	0,01→ Main Effects
P	0,02
m	0,04
tP	0,02
tm	-0,04
Pm	-0,04→ Interaction Effects
tPm	0,01

Table 6 shows that the main effect for the specific gravity (sg) value in this study is the fermentation time (t) with a value of 0.01 with an interaction effect in the form of pH and nutrient mass with a value of 0.01.

Table 7 Determination of Variables Affecting the Specific Gravity (sg) of Bioethanol

P (%)	Effects	Identity Effects
7,14	0,04	M
21,42	0,02	P
35,71	0,01	T
50	0,02	tP
64,29	-0,04	Tm
78,58	0,01	tPm
92,86	-0.04	Pm

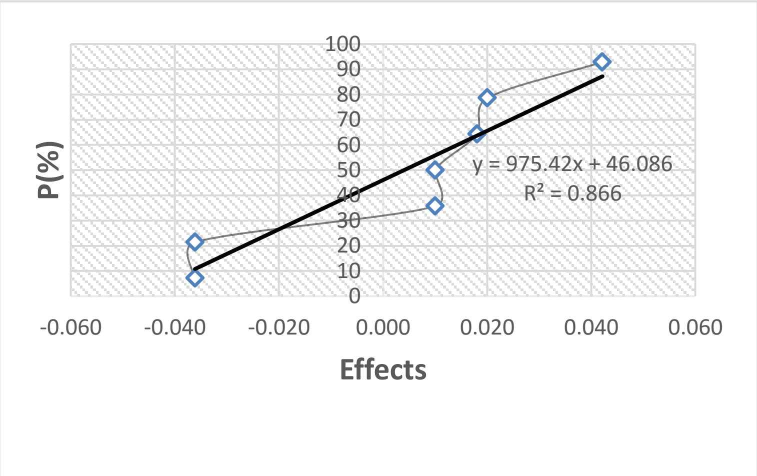

Figure 5. Normal Probability Plot Against Specific Gravity (sg) Bioethanol for Factorial Design 2^3

Figure 5 shows the Normal Probability Plot graph between the P value and the effect obtained by regression (R2) of 0.87 by activating the Trendline feature in Microsoft Excel. This means that 86.6% of the total model variation can be represented by a regression equation. The equation showing the correlation between the specific gravity (sg) of bioethanol and the parameters of the research process (nutrient mass and operating conditions) is y = 957.42x + 46.08.

d. Effect of Variable Calorific Value on Bioethanol

Table 8 Calculation Results of Main Effects and Interaction Effects on Calorific Value of Bioethanol

Effects	Results
t	32,07 → Main Effects
P	-109,33
m	-190,14
tP	-133,69
tm	54,86
Pm	169,16 → Interaction Effects
tPm	-33,24

Table 8 shows that the main effect for the calorific value of bioethanol in this study is the fermentation time (t) with a value of 32.07 with an interaction effect in the form of pH and nutrient mass with a value of 169.16.

Table 9 Determination of Variables Affecting the Calorific Value of Bioethanol

P (%)	Effects	Identity Effects
7,14	-190,14	M
21,42	-109,33	P
35,71	32,07	T
50	-133,69	tP
64,29	54,86	Tm
78,58	33,24	tPm
92,86	169,16	Pm

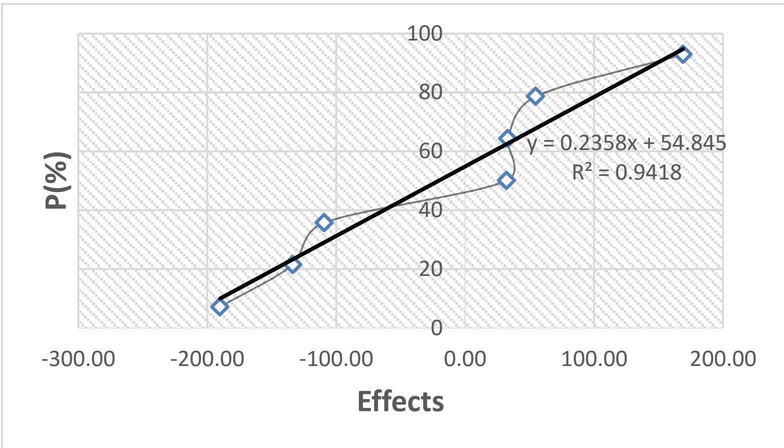

Figure 6. Normal Probability Plot Graph of the Calorific Value of Bioethanol for Factorial Design 2^3

Figure 6 shows the Normal Probability Plot graph between the P value and the effect obtained by regression (R2) of 0.94 by activating the Trendline feature in Microsoft Excel. This means that 94.18% of the total model variation can be represented by a regression equation. The equation that shows the correlation between the calorific value of bioethanol and the parameters of the research process (nutrient mass and operating conditions) is y = 0.23x + 54.84.

C. Relationship between Yeast Mass and Time on Bioethanol Density

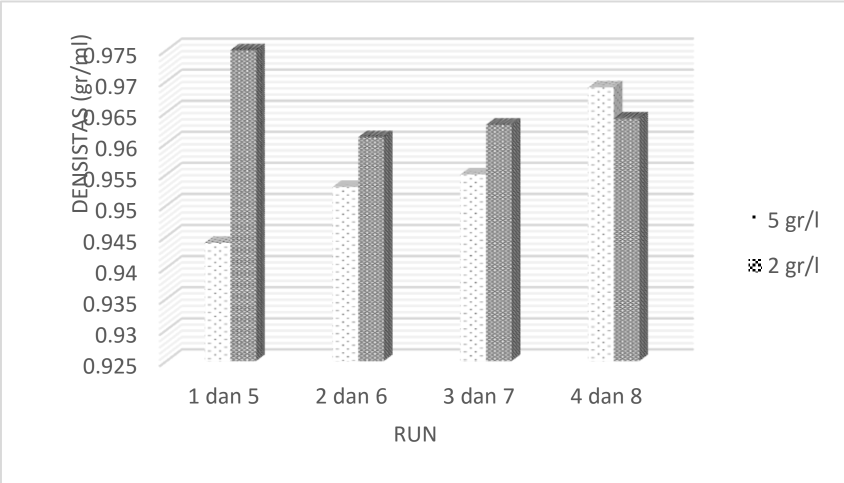

Figure 7. Graph of Yeast Mass Relation to Bioethanol Density

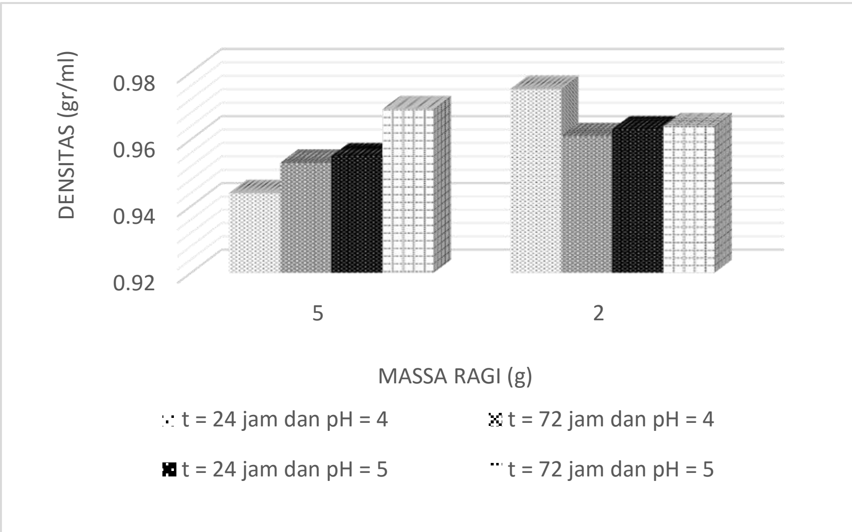

Figure 8. Graph of the Relationship between Operating Conditions and Density of Bioethanol

Figure 7, it can be seen that the density of bioethanol obtained is 0.96 gr / ml, where the density exceeds the requirements for the quality requirements of bioethanol, namely 0.82 gr / ml. This shows that the ethanol produced is still not pure because ethanol is mixed with water. This is because the distillation does not maintain the stability of the distillation temperature, so that the ethanol that comes out has been mixed with water. In Figure 8, it can be seen that 24 hours and 72 hours of fermentation using Saccharomyces Cerevisiae yeast. This shows that 24-hour ethanol with a pH of 4 contains more ethanol than 72 hours with a pH of 5. This is due to the lack of density in the fermenter bottle, the theory is that the longer the fermentation time, the more ethanol content is due to the activity of

Saccharomyces Cerevisiae experiencing a phase stationary, where there is a process of breaking down glucose on a large scale. The results of the breakdown of glucose by Saccharomyces Cerevisiae produce ethanol.

D. Relationship between Yeast Mass and Fermentation Time on Bioethanol Levels

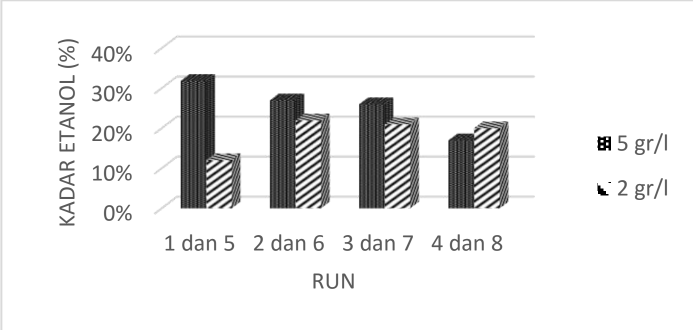

Figure 9. Graph of Yeast Mass Relationship to Bioethanol Ethanol Content

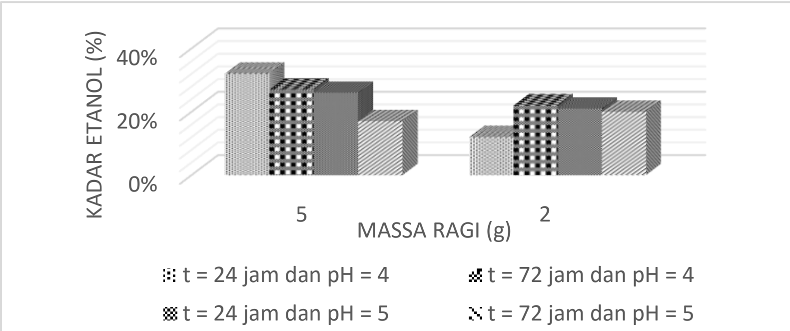

Figure 10 Graph of the Relationship between Operating Conditions and Bioethanol Ethanol Content

Figure 9 and Figure 10, it can be seen that the highest bioethanol content is 32% with a yeast mass of 5 grams for 24 hours. However, at 72 hours with a mass of 5 g of yeast it has a bioethanol content of 27%. This shows that the bioethanol content is not in accordance with the provisions of the bioethanol quality requirements, namely 94.1% minimum due to the lack of density in the fermenter bottle and less maintaining temperature stability in distillation. This is not in accordance with the theory, the real theory is that the longer the fermentation time, the higher the bioethanol content.

E. Relationship between Yeast Mass and Fermentation Time on Bioethanol Calorific Value

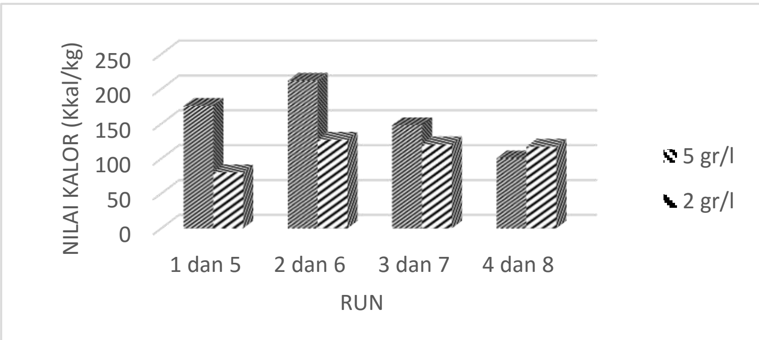

Figure 11 Graph of Yeast Mass Relationship to Bioethanol Calorific Value

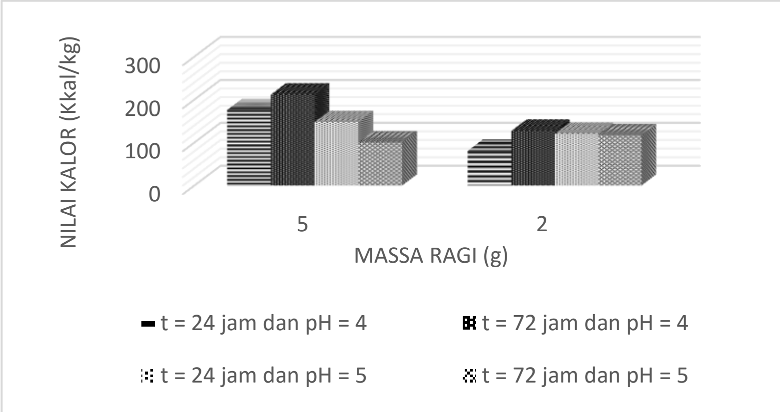

Figure 12. Graph of the Relationship of Operating Conditions to the Calorific Value of Bioethanol

In Figure 11 and Figure 12, the highest heating value at 72 hours is 211.95 kcal / kg. The calorific value obtained in bioethanol produced from pineapple skin honey and old coconut water is still small compared to bioethanol from different raw materials, including the calorific value of organic waste ranging from 10,000-11,000 kcal / kg. A higher calorific value will make it more flammable so that the quality of bioethanol is more flammable. This shows that the quality of bioethanol produced in this study is still low, this is due to the lack of density in the fermenter bottle and the lack of temperature stability in the distillation.

F. Relationship between Yeast Mass and Fermentation Time Against Specific Gravity Bioethanol

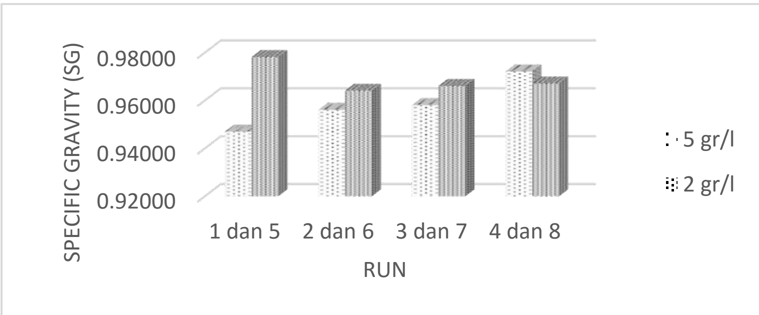

Figure 13 Graph of Yeast Mass Relationship to Bioethanol Specific Gravity

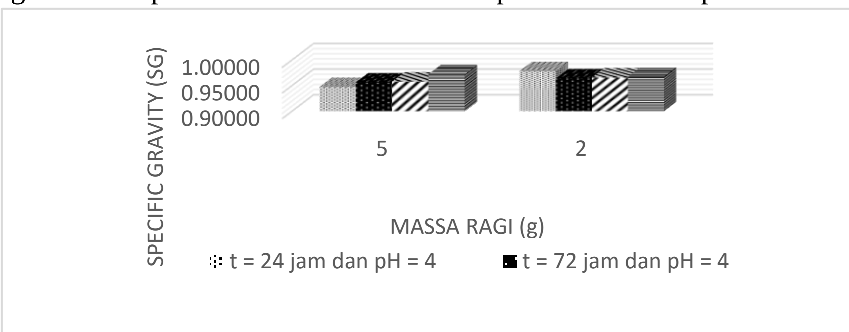

Figure 14 Graph of the Relationship of Operation Conditions to the Specific Gravity of Bioethanol

In Figure 13 and Figure 14 the results of the highest specific gravity at 24 hours and the mass of 4 gr yeast are 0.98 gr / ml, where the specific gravity exceeds the requirements for the quality requirements of bioethanol, namely 0.82 gr / ml. From the research conducted, the length of fermentation has an influence on the specific gravity of the alcohol being tested, this effect is in the form of a decrease in the specific gravity value with increasing time. The theory should be that the longer the fermentation lasts, the number of microbes needed in the process will also increase, so that with the increasing number of microbes, the more carbohydrates are broken down into alcohol.

With the increase in the amount of alcohol, automatically the weight or density of the alcohol-water mixture will be lower, which also causes the specific gravity of the mixture to have a low value. In addition, temperature also has an influence on specific gravity, the relationship between specific gravity and temperature, the higher the temperature, the higher the value of specific gravity. This is because when the distillation is carried out at a high temperature, the amount of water that accompanies the alcohol will also be high and vice versa and therefore the density of the distillation product will also be high.

G. Comparison of Bioethanol Quality Standards

Table 10 Quality Requirements for Bioethanol

Parameter	Unit	Bioethanol Standard Quality	Pineapple Skin + Coconut Water Bioethanol	Information
Ethanol Content	% v/v	Min 94,1	32	Not appropriate
Fusel Oil	mg/L	Max 15	-	-
Aldehid	mg/L	Max 30	-	-
Metanol	mg/L	Max 30	-	-
Density	gr/ml	Max 8,21	0,97	Not appropriate
Specific Gravity	-	Max 8,21	0,98	Not appropriate
Calorific Value	kkal/kg	Max 5000	211,95	Not appropriate
Acidity (as acetic acid)	mg/L	Max 30	-	-
Water Content	% b/b	Max 2	-	-

Source: Badan Standar Nasional, Etanol Nabati, SNI 3565

Based on the bioethanol quality requirements, the bioethanol produced in this study is not in accordance with the predetermined bioethanol quality requirements, this is due to the absence of nutrient provision to decomposing bacteria so that it does not work optimally in converting glucose into bioethanol. In addition, the accumulation of products can accelerate the death of bacteria during fermentation. In this study, the lack of attention was paid to bioethanol for the purity of the yeast used and for the suboptimal purification process.

CONCLUSION

The results of this study are the results of the glucose content in honey pineapple peel is 0.08%, the glucose content in old coconut water is 0.08%, but based on the theory, the glucose content of honey pineapple peel is 8.53% and in old coconut water. 3%. So the results of glucose levels are not in accordance with this theory. The yield of bioethanol density was 0.96 gr / ml, but the density exceeded the requirements for the quality of bioethanol, namely 0.82 gr / ml. The highest yield of bioethanol content is 32% with a yeast mass of 5 grams for 24 hours. However, at 72 hours with a mass of 5 g of yeast it has a bioethanol content of 27%.

The result of the highest heating value at 72 hours is 211.95 kcal / kg. The result of the highest specific gravity at 24 hours and the mass of 4 gr yeast is 0.98 gr / ml, where the specific gravity exceeds the requirements for the quality of bioethanol, namely 0.82 gr / ml. Based on these bioethanol quality requirements, the bioethanol produced in this study is not yet in accordance with the predetermined bioethanol quality requirements, this is due to the absence of nutrient provision to decomposing bacteria so that it is not optimal in converting glucose into bioethanol.

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