



The Effect of *Bacillus subtilis* on Bioethanol Production from Ambon Banana (*Musa paradisiaca* var. *sapientum* Linn) Peels by Using Fermentation Process

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Abstract - The breakthrough to optimize Indonesia's energy is by utilizing abundant renewable energy sources. Its geographic location has the potential to become a country with the largest renewable energy source in the world. The potential energy that comes from vegetables is called bioethanol. Bioethanol is an organic fuel produced by bioethanol fermentation. It can be an alternative material for making sanitary products amid the increasing spread of COVID-19 in Indonesia. So the authors try to innovate how to use Ambon Banana (*Musa paradisiaca* var. *sapientum* Linn) peels to become bioethanol. This research aimed to investigate the effect of *Bacillus subtilis* in the fermentation of bioethanol production from Ambon banana peels. The methods used were pretreatment, hydrolysis, fermentation, and distillation. *Bacillus subtilis* and *Saccharomyces cerevisiae* ratio (10: 5 and 5: 5) use for obtaining high ethanol yields, as well as variations in pH 2 and 6 in the fermentation starter. The variable were designed by using Factorial Design. The result shows the yeast ratio of *B. subtilis* and *S. cerevisiae* (10: 5) resulted the highest concentration of bioethanol (6%) in 6 days. In acidic conditions (pH 2), the bacteria don't grow optimally. The higher concentration of bioethanol (6%) was reached in pH 6. The research supports the Government Program Reducing the dependency of fossil fuels and innovation to produce sanitary product from bioethanol.

Keywords- Ambon Banana Peels, *Bacillus subtilis*, Bioethanol, Fermentation, pH

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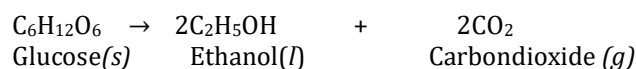
1. Introduction

The world's energy demand has recently increased significantly due to the growth in population. According to projections of the World Energy Agency by 2030 will increase world energy demand by 45% or an average of 1.6% per year [1]. Fossil fuels supply 80% of the world's energy right now. There are numerous of innovations that develop the renewable energy, such as biodiesel, bioethanol, and other products derived from renewable materials to overcome the problem.

At the conclusion of 2019, the coronavirus strain was at first found in Wuhan City, China. In Indonesia the number of covid-19 cases increases rapidly. Consequently, Indonesia take a decision Large-scale Social Limitation (PSBB -Pembatasan Sosial Berkala Besar). [2]. This policy

has an impact on the economic sector and makes ethanol prices increase

Bioethanol is ethanol or ethyl alcohol (C₂H₅OH) that produced from the fermentation of glucose (C₆H₁₂O₆) from vegetable raw materials. Bioethanol is one of the biofuels is required to be used for alternative energy (PP No. 5 of 2006)[3]. The reaction formation of ethanol [4] :



Biofuels are normally produced by fermentation of agricultural wastes, fruit wastes, and industrial wastes using *Saccharomyces cerevisiae* as nutrition for the microorganisms. The complexity of the manufacturing technique relies upon on the feedstock [5].

The major challenge of production ethanol is to convert the biomass into ethanol using fast, cheap and efficient methodologies. In industrial system of ethanol production operate under non-steril condition that slow down the fermentation process [6]. The fermentation process takes starch and cellulose from plants to make Bioethanol. Bioethanol also can be made from waste materials such as potato peels, sugar canes, rice, and banana peels. The high human population causes high waste generated [7]. The major problem is the waste in the city accumulates causing an environmental problem [8]. Banana peels are organic wastes that have economic value.

The main ingredient used to make bioethanol in this study is Ambon Banana Peels. The high level of banana consumption shown from 90 million tons of bananas are produced mainly in tropical areas such as in Africa (13%), Central and South America (28%), and Southeast Asia (47%). Of the banana production, 40% is in the form of banana peel waste. This waste is decomposed easily because of the high water content. The high water content allows microorganisms to grow and accelerate decomposition. Banana peels also have various ingredients such as vitamins and minerals, besides having a lot of water content [8]. The existing research shows that banana peels contain high antioxidant activity (94,25% at concentration of 125 mg/ml) [9].

Banana peels contain monosaccharides, especially glucose (8.16%) [10]. Chemical Composition of *Musa sapientum* (Banana) peels shows that 10 grams of banana peel samples contain 59% carbohydrate [11]. Banana peels contain specific nutrition which depends on the stage of maturity and the cultivar. Banana peels contain an average of 6-9% protein and 20- 30% fibre. [12]

The fermentation process uses *Saccharomyces cerevisiae* and *Bacillus subtilis* bacteria to support the fermentation process and upgrade the quality of ethanol. The the common probiotic concept states probiotics are one or more microorganisms that have beneficial effects on the host, able to persist in the digestive tract due to their tolerance to different conditions (acid & bile salts)[13].

Wild-*Bacillus subtilis* genus ferments 20 g/liter glucose for 48 h, producing lactate and butanediol, but not ethanol or acetate. To create the ethanologenic formula *B. subtilis*, reconstitution was used to disrupt the natural lactate dehydrogenase (LDH) (ldh) gene by chromosomal insertion of the *Zymomonas mobilis pyruvate decarboxylase gene* (pdc) and dehydrogenase II gene (adhrom) liqueur under adhrom. natives of ldh. *B. subtilis* 35 produced ethanol and buta nediol. Cell growth and glucose utilization rates have been reduced by 70% and 65%. [14].

2. Methodology

2.1. Materials

In this research, The main materials for this research are Ambon banana peels. The supporting materials to complete this research are Probiotic that contain *B.subtilis*, Aquadest, Urea, NaOH, *S.cerevisiae*, pH meter, Amylum,

CuSO₄, Luff Schrool Solvent, H₂SO₄, Na₂S₂O₃, Methylene Blue, Pb Asetat, Potassium Iodida (KI), Citric Acid, Backing Soda.

2.2. Methods

The operating variables during the research consisted of control variables and independent variables.

- Control Variables

The control variables are Hidrolysis time (30 min), concentration of H₂SO₄ (2%), mass of Urea (12 g), Fermentation time (6 days), Destilation temperature (78 °C), and Destilasi time (90 min).

- Independent Variables

The independent variables including the ratio of sample and water, pH, and ratio *B. subtilis* and *S.cerevisiae* shown in Table 1

Table 1 Independent Variables

Run	S : W (g)	pH	Bs : Sc (ml)
1	200 : 600	2	5 : 5
2	300 : 600	2	5 : 5
3	200 : 600	2	10 : 5
4	300 : 600	2	10 : 5
5	200 : 600	6	5 : 5
6	300 : 600	6	5 : 5
7	200 : 600	6	10 : 5
8	300 : 600	6	10 : 5

*S:W (sample:water ratio)

*Bs:Sc (*B.subtilis*:*S.cerevisiae* ratio)

- Experimental Process

Material Pretreatmnet

- Prepare banana peels (2 kg), wash banana peels with water
- Smooth the banana peel with a ginder or blender.
- Prepare eight beaker glasses, add 200/300 g of smooth banana peel according to the design and add water with a ratio of 1: 3 or 1: 2 to each beaker glass
- The sample is ready for hydrolysis
- Analysis of glucose Level

Fermentation

- Put in 100 ml Erlenmeyer filtrate, then add 12 grams of Urea as nutrition and stir until homogeneous
- Variation of pH (2 and 6) by adding H₂SO₄
- Add Fish Probiotic containing *B.subtilis* 5 or 10 ml according to the variables in each sample
- Make a growth medium for fermentation.
- Fermentation in six days.

Destilation

- Separate water and alcohol
- Destination the Liquid containing alcohol to obtain a higher purity
- Arrange the destilation tools
- Heat to a temperature of 78 °C (keep the temperature constant) for 90 minutes)

Analysis

Sample (Ambon Banana Peels) will be analyzed the percentage of glucose (%) and the product will be analyzed the physical characteristic including product volume, product yield, product density, and product Specific Gavity. Therefrence used to analyzed the level of glucose on sample is SNI 01-2892-1992 by Luff Schrool method. The percentage of Glucose is obtained by calculating based on the following equation

$$\% \text{ glucose} = \frac{V2 \times FP}{W} \times 100\%$$

- V2 : Glucose (from Luff Shcrool Glucose Determination Table based on Na₂S₂O₃ used (mg))
- FP : Dilution Factor
- W : Sample mass (mg).

3. Results and Discussion

3.1. Glucose Percentage Analysis of Ambon Banana Peels

From glucose concentration analysis by using Luff Schrool Method, it is resulting in an average glucose concentration of 0.436% per 100 g sample with 3 times of titrations (titrant volume is 3.2, 3.4, and 3.2 ml). Based on the article[15], the glucose concentration in the tested banana peels was obtained from 3 types of bananas (A, B, and C). The process of analysis of glucose concentration uses the calculation and conversion of the refractive index obtained from the refractometer during the hydrolysis test. The results of the conversion of the refractive index value of banana type A obtained glucose concentration of 0.47608%, type B banana of 0.47711%, and banana type C of 0.47667%. In the results of glucose concentration in reference and the results of research show that the values are not far apart. In the reference the average concentration of glucose from three types of bananas was 0.47662% and in the research was 0.436%.

Table 2 Sample Glucose Percentage (%) per 100 g sample

No	Sample Volume (ml)	Glucose (%)
1	3,2	0,432 %
2	3,4	0,428 %
3	3,2	0,450 %
Average		0,436 %

3.2. Product Analysis Volume

Table 3 shows the volume results from the distillation. In the ratio sample: water (1: 2) resulted the highest volume was 91 ml from 4 experiments with an average of 65.75 ml. Whereas in the ratio sample: water (1: 3) resulted the highest volume was 69 ml from 4 experiments with an average of 64.25 ml. From the results obtained that the more the banana sample ratio, resulting the more volume of product. The more samples used will enrich the glucose for fermentation. Ambon banana peel as containing 68.9 g of water per 100 g. [16] Another ingedient that produces the higher volume is the water content of the sample, the more samples used will make the

water involved in the fermentation process and also the possibility to pass the distillation process.

Table 3 Product Volume Analysis

Sample : Water (g)	Volume (mL)
1 : 3 / 200:600	59
1 : 3 / 200:600	62
1 : 3 / 200:600	67
1 : 3 / 200:600	69
1 : 2 / 300:600	49
1 : 2 / 300:600	91
1 : 2 / 300:600	51
1 : 2 / 300:600	72

3.3. Product Density Analysis

Table 4 shows the density analysis result of two types of variables (pH 2 & pH 6). The density values obtained in four experiments with a pH of 6 (0.9852 g/ml) are lower than the density values in the pH 2 (the lowest is 0.9872 g/ml.). The alcohol fermentation process can run optimally and produce a higher alcohol conversion ranges from pH 4 to 6 [17]. The density data obtained can be converted into alcohol content, where the lower the density resulted in a higher level of alcohol. The density standard of bioethanol is 0.790 g / ml [18]. From the research, the density value obtained is still far from the bioethanol density standard due to the distillation process. The study still uses simple distillation and manual temperature indicator, so that the possibility of the temperature exceeding the boiling point of alcohol increases and makes water enter the product.

Table 4 Density Analysis

pH	Density (g/mL)
2	0,989
2	0,988
2	0,989
2	0,987
6	0,985
6	0,986
6	0,986
6	0,985

3.4. Ethanol Concentration Analysis

This research using the conversion table for the density - ethanol concentration of the Badan Standardisasi Nasional to obtain the ethanol concentration. The results are shown in Figure 4.3 that shows the highest ethanol concentration obtained in the two variables was 6%. However, the average ethanol concentration obtained for the variable Sc: Bs (10: 5) ratio was higher (4.75%) compared to the Sc: Bs (5: 5) variable (4.5%). The more *B. subtilis* bacteria made the product have a higher average ethanol concentration. In the anaerobic fermentation process *B. subtilis* contains pads and adhB genes, which can help ethanol production, but the presence of the gene reduces the growth rate of *B. subtilis*, and increases glucose

consumption. It requires more glucose-containing materials to produce ethanol [14].

Table 5 Density - Etanol Concentration Conversion

Bs : Sc (mL)	Density (g/mL)	Ethanol Concentration (%)
5 : 5	0.9896	3
5 : 5	0.9888	4
5 : 5	0.9852	6
5 : 5	0.9864	5
10 : 5	0.9892	3
10 : 5	0.9872	5
10 : 5	0.9864	5
10 : 5	0.9856	6

*Bc (*Bacillus subtilis*)

*Sc (*Saccaromyces cerevisiae*)

3.5. Specific Gavity Analysis

Specific Gavity is a ratio between fluid density and water, standard density at the same temperature. In this research, the result of Specific Gavity obtained an average value of 0.992. The result indicates that the yield of product distillation has not been maximum, because the Specific Gavity of the fuel from organic waste ranges from 0.734 - 0.829 [19]. From the results obtained in the research, the average value of Specific Gavity was 0.992. These results indicate that the quality of bioethanol products is still not maximum. For this reason, it is necessary to do several distillations to get maximum result, because the fuel that has a high Specific Gavity value will reduce the quality of the fuels.

Table 6 Specific Gavity Analisis

No	Product Density	Water Density	Specific Gavity
1	0.9896	0.995	0.994
2	0.9888	0.995	0.993
3	0.9892	0.995	0.994
4	0.9872	0.995	0.992
5	0.9852	0.995	0.990
6	0.9864	0.995	0.991
7	0.9864	0.995	0.991
8	0.9856	0.995	0.990
Average	0.9873	0.995	0.992

3.6. Relationship Between Product Volume and Ethanol Concentration

Figure 1 shows that the highest of the volume of bioethanol products does not always produce more ethanol content. The results show that the ethanol content fluctuates with the increasing volume of bioethanol products. Based on the result of linear equation ($y = 0.25x + 3.5$), it shows that a positive gradient value can make it possible to produce higher ethanol content as volume increases. However, the fermentation process also depends on the type of yeast or bacteria used, fermentation temperature, pH, sugar concentration, nutrient concentration, and other factors that can affect bacterial activity.

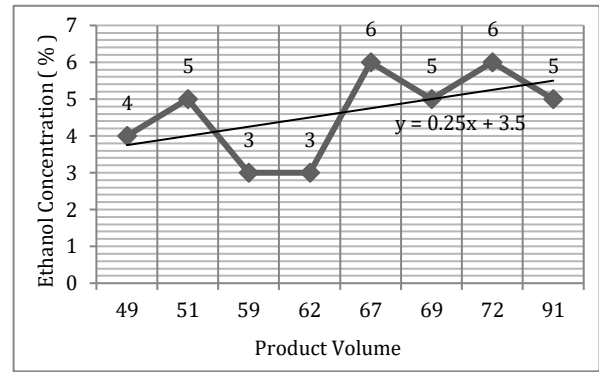


Figure 1 Relationship Between Product Volume and Ethanol Concentration

3.7. Relationship Between Product Volume and Product Density

Figure 2 shows that the higher the density value, the ethanol content will decrease. This is shown in the linear equation obtained ($y = -0.4643x + 6.7143$). The linear equation obtained has a negative gradient value, so there is a possibility that the higher the density, the lower the ethanol concentration. High density indicates a high moisture content in the product because the density of the research results is closer to the density of water than the bioethanol density standard used (0.790 g / ml) [18]. A higher density value will make the consumption level of the fuel increase and a higher density will result in a smaller calorific value, thus reducing the quality of the fuel according to [20]. The alcohol concentration produced in the study shows a decrease when there is an increased density and also shows that there is a decrease in the quality of the bioethanol product and the smaller the purity value.

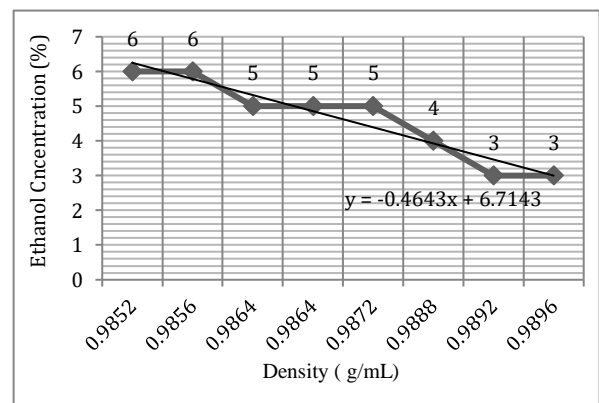


Figure 2 Relationship Between Product Volume and Product Density

3.8. Relationship Between Density and Ethanol Concentration

Figure 3 shows the relationship between product density and product volume. The figure shows that as the volume of the product produced from the distillation process increases, the density value tends to decrease. The tendency of the density value to decrease is represented by

a linear line with the equation $y = -0.0003x + 0.9988$. The data obtained shows a fluctuating value, but a gradient value of -0.0003 shows a tendency for the density value to decrease. High-density values (above 0.98 g/ml) indicate that the results of the fermentation and distillation processes are not optimal. To get a high density and purity value requires several times the distillation process. According to [20] shows that the distillation process takes 14 times to produce high purity and low density. So that further research is needed multilevel distillation or several distillations to get high ethanol concentrations and high volumes.

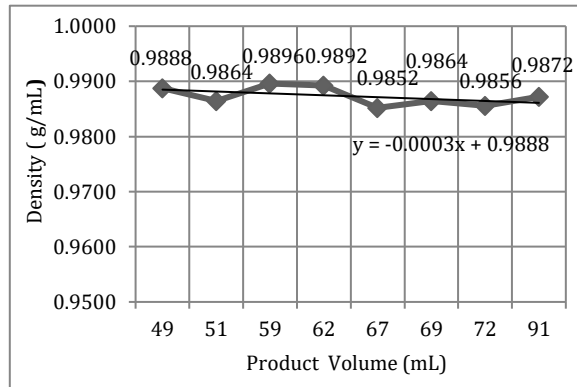


Figure 3 Relationship Between Product Density and Product Volume

4. Conclusion

The results can add references to the research and development of renewable biofuels to keep energy safe. In this context, to make bioethanol use the material from Ambon banana peels with variable types of yeast (*B. subtilis* from fish probiotics) and the pH variable fermentation. *B. subtilis* from probiotics affect the results of the alcohol concentration obtained. The ratio of *B. subtilis* and *S. cerevisiae* (10: 5) resulted in the highest concentration (6%) ethanol concentration and pH affects the yield of bioethanol products. In acidic conditions (pH 2), bacteria cannot grow and develop optimally, so they cannot produce fermentation products maximally, at alkaline conditions (pH 6) where the environment supports fermentation, obtained with higher concentration ethanol of 6%.

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