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Effect of Hydrolysis and Amount of Yeast on Banana Peel Fermentation into Bioethanol

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A B S T R A C T

Recently, renewable energy sources are needed to meet human energy needs, one of which is bioethanol. Bioethanol can be made from banana peels. Banana peel contains starch which has the potential to be converted into bioethanol through fermentation. There are factors that affect fermentation including the number of microorganisms and glucose levels. One method to increase glucose levels is hydrolysis. The purpose of this study was to determine the effect of hydrolysis and the amount of yeast on bioethanol levels in banana peel fermentation. The research variables used were hydrolyzed and non-hydrolyzed banana peel substrates, as well as variations in the amount of yeast as much as 3 g, 4.5 g and 6 g. From this research, it was found that hydrolysis causes an increase in glucose levels in the substrate due to the conversion of starch to glucose. Increased glucose levels can affect the yield of bioethanol. The bioethanol content of the hydrolyzed substrate fermentation is 9%-9.5% greater than the bioethanol content of the non-hydrolyzed substrate fermentation of 3%-3.5%. The difference in the amount of yeast used in banana peel fermentation has an effect on the bioethanol content but not significantly enough because the amount of yeast will depend on the glucose content in the substrate.

INTRODUCTION

The need for energy for consumption by humans is rising, due to the increasing number of human populations. However, the surge in energy demand is not accompanied by an increase in energy availability to cover this demand. Bioethanol is an alternative energy source. Bioethanol (C_2H_5OH) is produced by utilizing materials containing starch, cellulose or derived from lignocellulosic biomass through a fermentation process [1]. The octane number of bioethanol is relatively high, so bioethanol is used as a mixture of gasoline or used to increase the octane number in the transportation sector [2].

One material that has the potential to be used in the manufacture of bioethanol is banana peels. Banana peels have numerous ingredients that can be utilized, namely starch, cellulose, and hemicellulose, which can be exploited in the manufacture of bioethanol which is converted to glucose after being hydrolyzed [3]. The starch content in banana peels varies based on the ripeness of the bananas [4]. The green banana peel has starch of 58.6% and the yellow banana peel has about 2.6-6.3% starch [5].

Fermentation is a catabolic event to produce energy or a product formation process by utilizing the metabolic activity of microorganisms. Fermentation products include the production of cells, enzymes, metabolites, recombinant products, and transformation products. Bioethanol fermentation is a process of breaking down glucose into bioethanol and carbon dioxide by microorganisms. The changes that occur during the fermentation process is shown by the following reactions [6]:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbondioxide}}{2CO_2}$$

The fermentation process involves

yeast (*Saccharomyces cerevisiae*) which in the fermentation process undergoes metabolic processes in cells with a series of directed reactions. During the process, energy is produced from a series of reactions that break down certain materials. Yeast (*Saccharomyces cerevisiae*) will secrete enzymes first to break the glycosidic bonds in carbohydrate compounds which will then be fermented [7]. Saccharomyces cerevisiae is a type of yeast that has important role in the production of bioethanol. Optimal conditions for the growth of *Saccharomyces cerevisiae* are at temperatures of 28-35 ^{o}C and pH 3.5-6 and are inactive at temperatures above 40 ^{o}C [3]. Some factors can affect fermentation, including the number of microorganisms and glucose levels [8]. The amount of yeast can affect the level of bioethanol that can be produced, where the more amount of yeast used, the level of bioethanol produced will increase, but if the amount of yeast is too much it will reduce the level of bioethanol. This is because the amount of yeast is not proportional to the nutrients (carbohydrates) available. In addition to this, it should be noted that glucose levels in the substrate affect fermentation, where low glucose levels can cause fermentation to be not optimal because the activity of microorganisms is inhibited and some sugars become unfermentable whereas if the sugar concentration is too high it will inhibit the activity of microorganisms because enzyme activity slows down [8]. One method to increase glucose levels in the substrate is by hydrolysis, where hydrolysis can break down the starch contained in the substrate into glucose, with the help of a catalyst (enzyme or acid), causing the concentration of glucose in the substrate to increase [9].

Based on the research conducted by Wardefisni et al. (2020), the variation in types of acids used in the hydrolysis of banana peels results in different glucose levels. Specifically, citric acid produces a glucose level of 5%, acetic acid yields 6%, nitric acid results in 7%, and hydrochloric acid leads to an 8% glucose level [9]. Ardhiany (2019) conducted a study on the influence of varying amounts of yeast on the bioethanol content, revealing that the highest bioethanol content, at 13.54%, was obtained with the use of 0.0624 g of yeast [10]. Building upon the findings of these previous studies, this study aims to determine the effect of the hydrolysis process and the amount of yeast in banana peel fermentation on bioethanol levels.

METHODS

The materials used were Kepok banana peel (green and young) from Malang city, yeast (Fermipan), sugar, urea, NPK, HCl, NaOH, distilled water, glucose, Nelson A and B reagents, and Arsenomolybdate reagent. All other reagents were at analytical grade. The tool used are an erlenmeyer, refractometer, analytical balance, digital balance, beaker glass, dark bottle, measuring cup, hose, pH indicator, thermometer, blender, stove, autoclave, volumetric flask, test tube, hotplate, vortex, and UV-Vis spectrophotometer. This series of research tools can be seen in Figure 1.

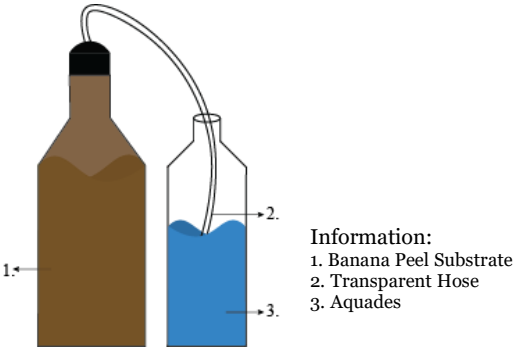

Figure 1. Fermentation Set

Preparation of starter

The starter was made by mixing several ingredients, 15% sugar, 0.075 g of urea, 0.03 g of NPK, and variations in the amount of yeast added by 3 g (R1), 4.5 g (R2), and 6 g (R3). The starter was allowed to stand for 3 hours at room temperature.

Preparation of fermentation substrate

Hydrolyzed substrate (H1) and non-hydrolyzed substrate (H2) were prepared by weighing 500 g of banana peel for each variable, then cleaning and grinding it into a banana peel pulp substrate. For the non-hydrolyzed (H2) substrates, the banana peel pulp is then filtered using gauze. Whereas the hydrolyzed banana peel substrate will be hydrolyzed by adding 5% HCl and hydrolyzed for 60 minutes at 100 ^{o}C.

Fermentation of Banana Peel

Before fermentation, the pH was adjusted to 6, then the hydrolyzed substrate (H1) and non-hydrolyzed substrate (H2)

was added as much as 100 mL of starter with yeast variations of 3 g (R1), 4.5 g (R2), and 6 g (R3). The mixture of substrate and starter was then fermented at room temperature for 168 hours.

Determination of Glucose Level

Determination of glucose levels was carried out using the Nelson-Somogyi method. A standard stock solution of glucose with a concentration of 10,000 ppm was diluted into several concentrations of 0, 10, 20, 30, 40, and 50 ppm to make a standard curve. 1 mL of each concentration will be taken to be put into a test tube and then added 1 mL of Nelson A and B reagents. Then heated for 20 minutes and cooled to room temperature. Then 1 mL of Arsenomolybdate reagent and 7 mL of distilled water were added, then vortexed until homogeneous. Absorbance measurements were carried out by UV-Vis spectrophotometer at a wavelength of 740 nm. After obtaining the standard curve, tests were carried out with the same procedure on hydrolyzed substrate (H1) and non-hydrolyzed substrate (H2). The concentration of glucose in the sample was calculated using the equation obtained from the standard curve.

Determination of Bioethanol Content

After the fermentation process, bioethanol levels were measured with an alcohol refractometer.

RESULTS & DISCUSSION

The Effect of Hydrolysis on Banana Peel Fermentation on Bioethanol Levels

Bioethanol levels in hydrolyzed substrate (H1) and non-hydrolyzed substrate (H2) with variations in the amount of yeast can be seen in Table 1. Based on Table 1, the levels of bioethanol produced from fermenting banana peel substrates that have been hydrolyzed are higher than those produced from fermented non-hydrolyzed banana peel substrates. This can happen because the hydrolysis process affects glucose levels which will also affect bioethanol levels, namely the greater the amount of glucose, the higher the level of bioethanol produced [11]. If the glucose concentration is too high, it can inhibit the activity of yeast (*Saccharomyces cerevisiae*) because the enzyme activity slows down, whereas if the sugar concentration is too low it causes fermentation to be not optimal because the activity of the yeast (*Saccharomyces cerevisiae*) is inhibited and some of the sugar becomes unfermentable. The reaction of converting glucose into bioethanol by yeast is as follows [8]:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{Ethanol}}{2C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_2}$$

Table 1. Levels of Bioethanol produced from Banana Peel Fermentation

Samples	Bioethanol level (%)
H_1R_1	9
H_1R_2	9
H_1R_3	9.5
H_2R_1	3.5
H_2R_2	3
H_2R_3	3

Information:
H_1R_1: Hydrolyzed Substrate by 3 g of yeast
H_1R_2: Hydrolyzed Substrate by 4.5 g of yeast
H_1R_3: Hydrolyzed Substrate by 6 g of yeast
H_2R_1: Non-Hydrolyzed Substrate by 3 g of yeast
H_2R_2: Non-Hydrolyzed Substrate by 4.5 g of yeast
H_2R_3: Non-Hydrolyzed Substrate by 6 g of yeast

The process of hydrolysis is a reaction of decomposition or breakdown of a compound with the help of water [12]. In this study the hydrolysis process can help break down starch compounds (carbohydrates) into glucose which will be fermented into bioethanol. Water as a hydrolyzing agent will attack the 1,4-α glycosidic bonds in starch to produce dextrin, syrup, or glucose which depends on the degree of breakdown of the polysaccharide chains in starch. The reaction that occurs between starch and water is slow, requiring a catalyst in the form of an acid, base or enzyme catalyst [5]. In this study, hydrolysis was carried out using an acid catalyst, namely HCl. The addition of HCl can damage the polysaccharide bonds contained in the substrate by cutting the starch molecules that occur randomly into smaller parts so that the amount of hydrolyzed polysaccharides is greater and the amount of reducing sugars is higher. The hydrolysis reaction that occurs is [5], [13]:

$$\underset{\text{Starch}}{(C_6H_{10}O_5)x} + \underset{\text{Water}}{H_2O} \rightarrow \underset{\text{Glucose}}{x(C_6H_{12}O_6)}$$

Hydrolysis in acidic conditions will break the glucose bonds which takes place in three stages, namely [14]:

I. In the early stages, the oxygen glycosides that link the two glucose

(sugar) units will react with protons which act as acid catalysts.

II. In the second stage will form conjugate acid.

III. In the third step, the C-O bond will slowly break and produce a cyclic Carbonium cation intermediate. The Carbonium cations quickly add to the water molecules and produce stable glucose. In the process, there are protons released.

The hydrolysis reaction mechanism is presented in Figure 2.

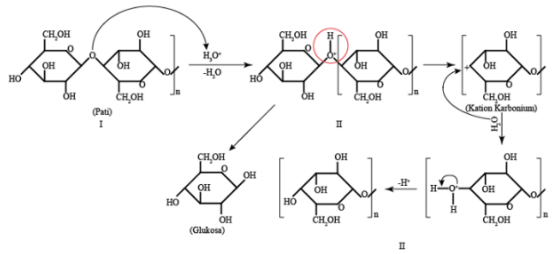

Figure 2. Hydrolysis Mechanism [14]

The glucose levels of the hydrolyzed and non-hydrolyzed samples can be seen in Table 2. Based on Table 2, the glucose levels of the hydrolyzed substrate were greater than those of the non-hydrolyzed samples. This shows that starch has been converted into glucose after hydrolysis with an acid catalyst, causing an increase in glucose levels in the substrate.

Table 2. Glucose level on banana peel substrate

Samples	Glucose Level (ppm)
H_1	73.202
H_2	25.837

Information:

H_1: Hydrolyzed Banana peel substrate

H_2: Non-hydrolyzed Banana peel substrate

Effect of Yeast Amount on Banana Peel Fermentation on Bioethanol Levels

Based on Table 1, the bioethanol content increased with the increase in the amount of yeast on the hydrolyzed banana peel substrate, although the increase was not so significant. This is because it is based on the fermentation factor, namely, the amount of yeast affects the speed of fermentation. If the amount of yeast added is not appropriate, the fermentation speed will decrease due to the lack of mass of microorganisms that can convert glucose into fermentation products [8].

Whereas in the banana peel substrate without hydrolysis, the resulting bioethanol content decreased which was not significant as the amount of yeast was increased, this was due to the amount of yeast being greater than glucose available as a carbohydrate source. Based on the data in Table 2, the amount of glucose on the substrate without hydrolysis has a lower concentration than the amount of glucose on the hydrolyzed substrate, so the yeast uses the glucose as a nutrient to survive and does not break down the glucose into bioethanol [15]. Bioethanol is included in the primary metabolite; this primary metabolite is produced by yeast (*Saccharomyces cerevisiae*) in the exponential phase. Where in this exponential phase the microorganism cells will grow at the highest and most stable speed and will produce primary metabolites [16].

In the fermentation process there are two stages namely [17]:

I. The first stage takes place in the presence of oxygen (aerobic) on the dissolved surface. This is the stage of yeast multiplication which is characterized by the presence of CO_2 gas, with the reaction that occurs, namely:
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 36$ ATP
At this stage, very little or no bioethanol is produced.

II. In the second stage, fermentation occurs anaerobically or in the absence of oxygen. At this stage, a lot of yeast and enzymes are produced so that fermentation will take place, namely the conversion of glucose into bioethanol with the reactions that occur as follows:
$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + CO_2 + 2$ATP
In the alcoholic fermentation process, yeast (*Saccharomyces cerevisiae*) will produce enzymes that are useful for hydrolyzing substrates into simple sugar components which will later be converted into ethanol, the enzyme is the zimase enzyme. The work of the zimase enzyme is only specific for sugar so that not all carbohydrates can be converted into ethanol. Alcoholic fermentation can occur in disaccharides such as sucrose or maltose ($C_{12}H_{22}O_4$) and then hydrolyzed to hexose ($C_6H_{12}O_6$) by the invertase or maltase enzymes found in microorganism cells. Then the resulting hexose will be converted by the enzyme zymase into ethanol and carbohydrates [1].

Fermented banana peels in this study produced the highest bioethanol content of

9.5%. This shows that banana peels have competitive potential compared to other natural materials used as raw materials for bioethanol production. Table 3 shows a comparison of the results of this study's bioethanol with other studies with different substrates.

Table 3. Comparison of bioethanol yields

Researcher (Year)	Substrate	Bioethanol Levels (%)
Simanjuntak dkk. (2013) [18]	Aren sap	8
Litya dkk. (2014) [19]	Sweet Potato	9
This Research	Banana Peel	9.5

CONCLUSION

The hydrolysis process can increase the level of glucose contained in the substrate so that it can increase the level of bioethanol produced in the fermentation process. The levels of hydrolysed bioethanol were higher on hydrolysed substrates, namely 9% and 9.5%, while on non-hydrolyzed substrate, the resulting bioethanol levels were 3% and 3.5%. On the other hand, the amount of yeast has an effect on the level of bioethanol produced but not significant enough, because the amount of yeast will depend on the level of glucose contained in the substrate. For further research, it is necessary to optimize hydrolysis to increase glucose levels in the substrate and carry out fractional distillation to produce higher levels of bioethanol.

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