Effect of pH fermentation on production bioethanol from jackfruit seeds (Artocarpus heterophyllus) through separate fermentation hydrolysis method

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Abstract. Bioethanol is one of the alternative energy sourced from natural products containing carbohydrates through hydrolysis and fermentation process. Jackfruit seeds is one of the feedstock that contain high carbohydrate content but less utilized. The aims of this study to determine the effect of pH hydrolysis in the process of production bioethanol from jackfruit seeds (Artocarpus heterophyllus) through separate fermentation hydrolysis (SHF) method. The hydrolysis process uses H2SO4 as a hydrolyzing agent. The fermentation process used Saccharomyces cerevisiae as a fermentor with a variation of pH 2, 3, 4 and 5 for 70 hours. The results showed that glucose content of 75% and pH 3 was the optimum pH of fermentation with the content of bioethanol 57.94%. The fermentation stage has an important role in increasing the levels of glucose and bioethanol in linear. The content of glucose and bioethanol of jackfruit seeds showed a great potential for development as the feedstock in bioethanol production.

1. Introduction

Bioethanol is the product of the sugar fermentation process from carbohydrate sources (starch) such as cassava, sugar cane, sap, sorghum and sweet potato. However, the use of carbohydrate sources is also used as food sources cause a new problem that is the occurrence of competition with human needs. The solution of the problem is to search for raw materials that are underutilized but rich in carbohydrates [1].

Jackfruit (Artocarpus heterophyllus) is a multifunctional tropical fruit plant that can be grown in tropical areas less than 1,000 meters above sea level from South India. In Indonesia jackfruit is widely used as food and basic ingredients in making traditional cakes. However, the underutilized of jackfruit is in the seeds. Jackfruit seeds contain 36.7% of carbohydrates. High carbohydrate content in jackfruit seeds is very potential to be used as a raw material in the production of bioethanol by fermentation [2].
The fermentation process is one of the stages in bioethanol synthesis. The fermentation process is used a bread yeast (Saccharomyces cerevisiae), because this yeast highly produce and stable in alcohol with high content [3]. In addition, the pH factor of fermentation solution determines the process of sugar reaction to bioethanol. Fermentation solutions that are very acidic or alkaline can inhibit the fermentation process, thus reducing the amount of bioethanol produced [4]. This study aims to examine the effect of fermentation pH in bioethanol production from jackfruit seeds through a separate fermentation hydrolysis method.

2. Material and Methods

2.1 Materials
The materials used in this research are jackfruit seeds, Saccharomyces cerevisiae, aquadest, Whatman No. 42 filter paper, H$_2$SO$_4$ 0.3 M, NaOH, NPK fertilizer, urea, Nelson-Somogyi reagents.

2.2 Preparation of Jackfruit
Jackfruit seeds are cleaned and then dried under the sun. The dried jackfruit seeds are crushed using a flour mill. Jackfruit seed flour is continued at the hydrolysis stage.

2.3 Chemical Hydrolysis
Jackfruit seed powder is hydrolyzed with H$_2$SO$_4$ 0.3 M at a ratio of 1:10, then refluxed at 100 °C for 1 hour. The sample is cooled and then filtered. Glucose levels of filtrate were analyzed by Nelson-Somogyi method [5].

2.4 Fermentation

2.4.1 Making a Stater. Yeast bread weighed as much as 9 grams and then put in a solution of hydrolysis result as much as 9% of total volume. The pH of the starter solution was varied at pH 2, 3, 4 and 5. Urea and NPK were added to a solution of 3 g/L. The mixed solution is then shaken for 30 minutes [5].

2.4.2 Fermentation Process. The hydrolysis solution is added with NaOH to adjust the pH. Starter Saccharomyces cerevisiae as much as 9% of the volume of the solution is mixed into the hydrolysis solution. The mixed solution is introduced into the fermentation reactor. The fermentation process is carried out anaerobically for 1 - 5 days at a temperature of 30 °C. The same process was performed for pH 3, 4 and pH 5 [6].

2.5 The Process of Distillation and Dehydration of Bioethanol. The fermented product is filtered and then fed into the distillation flask, the temperature is kept at 78-88 °C. Distillation is carried out for 6 hours until bioethanol stops dripping. The bioethanol fraction is dried by CaO. Bioethanol content was analyzed using Gas Chromatography [7].

2.6 Gas Chromatography Analysis. Measurement of bioethanol content using gas chromatography. Bioethanol concentration was obtained from the calculation of area area ratios in which the area of the sample bioethanol was divided by the area of pure ethanol which was the comparison [8].

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\text{ethanol content} = \frac{\text{area of ethanol sample}}{\text{standard area of ethanol}} \times 100\% \quad (1)
\]
3. Results and Discussion

3.1 Preparation sample
In the process of making jackfruit seeds powder as much as 4.5 kg washed to remove dirt. The jackfruit seeds have been cleaned and dried for 12 days in the light sun. Dry jackfruit seeds are crushed into jackfruit seed powder. Jackfruit seed flour obtained as much as 2.5 kg of turbid white as in figure 1.

![Figure 1. (a). Jackfruit seed clearance, (b) Seeds of jackfruit, (c) Jackfruit seeds powder.](image)

3.2 Chemical Hydrolysis Process
The hydrolysis process is carried out by using sulfuric acid with a ratio of 1:10 (w/v). Samples of 30 grams were dissolved with 0.3 M sulfuric acid in 300 mL and heated at 100 °C for 1 hour. Sulfuric acid is used as a catalyst because it is effective for breaking the glycoside bond on starch to glucose. The reaction mechanism can be seen in figure 2.

![Figure 2. Mechanism of reaction of carbohydrate hydrolysis with sulfuric acid.](image)

In the process of hydrolysis occurs a physical change of the solution from a turbid white to a dark reddish brown. The changes indicate the compound in the jackfruit seed flour has decomposed. The result of the hydrolysis process was cooled and then filtered using filter paper whatman no 42. The filtrate obtained was 298 mL. The same treatment is used for all pH. Glucose level of the filtrate of hydrolysis result determined by the Nelson Somogy method. Based on the measurement obtained glucose conten of 75%.

3.3 Making a Starter
Starter media serves to activate bacteria before the fermentation process. The addition of NaOH serves to increase the pH. the pH of the initial solution is at pH 1 so that NaOH is added until it reaches pH 2. The pH setting to optimize the activation process because bacteria can not work optimally in a solution that is moreover acidic. After reaching pH 2, nutrient NPK and Urea were added 3 g/L. The addition of nutrients
serves as a source of food for yeast *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* as much as 9 grams was added in the solution and stirred until homogeneous and then allowed to stand for 30 minutes. The same treatment is applied for pH 3, 4 and 5.

### 3.4 Fermentation Process

The fermentation process lasts for 1-5 days. At pH 2 incubated only 1 day because it is still too acidic solution, so the bacteria *Saccharomyces cerevisiae* can not survive while pH 3, 4, and pH 5 incubated for 2, 4 and 5 days. After the fermentation is complete, the volume of the solution is reduced to 280 mL. This happens because the formation of residues in the form of CO$_2$ gas in the process of fermentation [9]. After the fermentation process is complete, the color of the sample solution changes from brownish yellow to brownish red and forms 2 layers. According prasetyo [5] the formation of these two layers resulting from the process of separation of bioethanol by impurities.

The sample distillation was carried out at a temperature range of 78-88 °C for 6 hours. The temperature range used is due to the difficulty of obtaining bioethanol distillate if used the boiling point of ethanol. Each pH produces a different volume of the distillate, the result can be seen in table 1.

| No | pH | Volume of distillate |
|----|----|----------------------|
| 1  | 2  | 3 mL                 |
| 2  | 3  | 3 mL                 |
| 3  | 4  | 4 mL                 |
| 4  | 5  | 6 mL                 |

Distillate bioethanol is added CaO to bind water. Distillates were filtered and analyzed qualitatively using gas chromatography (GC). The bioethanol distillate chromatogram can be seen in figure 3.

The retention time for pH 2 was 1.902, pH 3 of 1.910, pH 4 and pH 5 with retention times of 1.894 and 1.907. The standard ethanol retention time difference between the four samples can be affected by the purity rate of the bioethanol content. According to Brexlo, [10] Uncultible samples can occur due to impurities in the form of by products formed in the form of acetic acid or other organic acids formed from bioethanol that undergo further reactions or the dehydration process of bioethanol is not good so that it affects the separation process of the components contained in the distillate.

The sample content of bioethanol can be determined by using chromatograms from pure ethanol as standard. The determination of the sample content was determined by comparing the area between the standard ethanol and the sample bioethanol. The results showed the standard ethanol area (Rt: 1.875) = 66587191, area of bioethanol pH 2 (Rt: 1.902) = 10004822, area of bioethanol pH 3 (Rt: 1.910) = 38657956, area of bioethanol pH 4 (Rt: 1.894) = 34386838, area of bioethanol pH 5 (Rt: 1.907) = 31874859. Bioethanol content of each sample can be seen in table 2.

| No | pH | Bioethanol Content (%) |
|----|----|------------------------|
| 1  | 2  | 14, 99                 |
| 2  | 3  | 57,94                  |
| 3  | 4  | 51,53                  |
| 4  | 5  | 47,77                  |
Figure 3. The chromatogram of (a) ethanol standard, (b) bioethanol at pH 2, (c) bioethanol at pH 3, (d) bioethanol at pH 4 (e) bioethanol at pH 5.

Figure 4 showed that the difference in pH affects the glucose and bioethanol levels produced. The optimum pH was obtained at pH 3 with bioethanol content of 57.94%. The smallest content of bioethanol was obtained at pH 2 with a value of 14.99%. The condition of the solution that is still too acid cause yeast does not last long the process of transformation of glucose into bioethanol is not optimal.

Figure 4. Glucose and bioethanol content during various pH fermentations.
Jackfruit seeds as the feedstock of bioethanol is very potential to be developed refers to the content of glucose content of 75% and bioethanol content obtained by 57.94%. Several other studies on bioethanol exploration from several sources such as seaweed gracilarià sp yielded 5.50% bioethanol content [11] and woodflour acid hydrolyzate of 6.77% [12]. When compared to the amount of bioethanol content produced from the two studies, the potential of jackfruit seeds as the basic ingredients of bioethanol production is very prospective to be developed in the future.

4. Conclusions
Bioethanol production process from jackfruit seed flour can be done by hydrolysis of H2SO4 and anaerobic fermentation. Total carbohydrate content in hydrolysate result is 75% while based on gas chromatography analysis obtained high bioethanol content at pH 3 equal to 57,94% while at pH 2, 4 and pH 5 bioethanol content obtained equal to 14,99%, 51,53 % and 47.77%. The results showed that high carbohydrate content in jackfruit seeds could potentially be developed as the feedstock of bioethanol production on a large scale.

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