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Hydrolysis Optimization of Beneng Taro Tubers (*Xantoshoma undipes K. Koch*) as Bioethanol Raw Material

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Abstract. This study conducted to assess the most appropriate method or hydrolysis of beneng taro tuber to optimize the production of bioethanol to investigate the effect of pHs, times, and temperatures on reducing sugar produced. The concentration of reducing sugar was determined using a UV-Vis spectrophotometer. The result obtained indicated that the optimum amount of reducing sugar (910,875 mg/L) achieved at pH 10, hydrolysis time of 3 hours, and the temperature of 90 °C. Fermentation of reducing sugar using the powdered bark of raru plant produced 0,18765 % (v/v) of bioethanol and fermentation using *Saccharomyces cerevisiae* produced 0,2116 % (v/v) of bioethanol.

Keywords*:* Beneng taro tuber, bioethanol, hydrolysis, raru plant, reducing sugar, Saccharomyces cerevisiae.

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Introduction

Bioethanol is still one of the priority energy sources in the development of alternative and renewable energy sources [1]. The use of bioethanol is quite extensive as a fuel either directly or as a mixture of gasoline known as gasohol [2][3]. Besides, bioethanol can also be used as raw material for biodiesel by a transesterification reaction[4]. Bioethanol is an environmentally friendly fuel because it quickly broke down biologically[5] [6][7]. Bioethanol can also reduce carbon monoxide (CO) exhaust gas and has a high octane value [8]. The bioethnol industry in Indonesia, the raw materials used, are still limited to molasses and cassava [9]. In addition to the raw materials above, in Indonesia, there are a lot of potential starchy materials that have not used optimally, one of which is taro[10][11]. Taro has high potential as a bioethanol feedstock because taro tubers contain about 70-80% starch [12][13]. Beneng taro (*Xantoshoma undipes K. Koch*) has a large size with a high enough starch content but less utilized as a food plant because of its high oxalate content[14].

For Bioethanol produce, taro tubers must hydrolyze first to produce reducing sugars, which then fermented to produce ethanol [15]. Several hydrolysis methods have developed, namely the technique of acid[16][17], alkaline[18], and enzymatic [19][20][21] hydrolysis. In this study, an attempt to determine the hydrolysis conditions suitable for Beneng taro was carried out by hydrolysis treatment of Beneng taro using acids and bases condition at various temperatures and times. Reducing sugar produced from the hydrolysis of taro tubers is then fermented using Saccharomyces cerevisiae and Raru bark powder to produce bioethanol.

Materials and Methods

Materials

The tools used are water batch (Precisterm), analytical balance (Wiggen Houser), UV-VIS spectrophotometer (variant Cary 100), autoclave (Kleinfeld-Germany HV-L25), laminar water flow (ESCO AVC4A1), gas chromatography (ESCO AVC4A1), gas chromatography (GC-2010 AF Shimadzu), oven, centrifuge, and tools commonly used in laboratories. The materials used are taro beneng tubers, sulfuric acid (H_2SO_4) (Merck), glucose (Merck), NaOH (Merck), distilled water, Fehling A and B solution (Merck), Na_2SO_3 (Merck), *Saccharomyces cerevisiae*, raru bark powder, phosphate buffer pH 5 (Merck), NaCl 0.85% (w/v), $K_2Cr_2O_7$ (Merck), filter paper (Whatman), and aluminum foil.

Methods

Taro Bulbs Preparation. Taro tubers are peeled and crushed with a shredder until they become pulp. Taro tuber porridge was dried in oven at 110 ^oC for 24 hours. The dried taro pulp was mashed again with a blender until it becomes flour.

Beneng Taro Bulbs Hydrolysis

Determination of Optimum pH. Hydrolysis carried out at different pH 2, 4, 6, 8, and 10. Hydrolysis was carried out on Beneng taro tubers as much as 20 grams. The starch suspended in 500 mL of distilled water, and the pH was determined using a 0.1 M H_2SO_4 solution for acidic pH and 0.1 M NaOH for basic pH. Hydrolysis carried out for 3 hours at 80 ^oC.

Determination of Optimum Time. The optimum time determines with the prepared sample as a previous experiment with the pH of the sample adjusted to the optimum pH obtained of earlier tests. The samples were then hydrolyzed at 80 ^oC at different times 1, 3, 5, and 7 hours.

Determination of Optimum Temperature. The sample prepared as a previous experiment with the pH of the sample set to the optimum pH. The example then hydrolyzed at different temperatures 60, 70, 80, and 90 ^oC during the optimum time obtained from previous experiments.

Reducing Sugar Analysis

Qualitative Analysis. The Fehling method used to determine the presence of reducing sugars in the hydrolyzate[22].

The Fehling method was carried out by inserting 1 mL of Fehling A and Fehling B solutions into a test tube, then adding 2 mL of the sample and heating it in a boiling water bath for 10 minutes. The presence of reducing sugar indicated by the formation of red brick Cu_2O deposits [23].

Quantitative Analysis. Samples were prepared the same as standard glucose in a previous experiment. The absorbance of the sample obtained used to determine the level of reducing sugars in the example, which calculated using a line equation derived from a glucose standard curve 200 – 1000 (mg/L).

Bioethanol production

***Saccharomyces cerevisiae*.** Bioethanol production with *Saccharomyces cerevisiae* yeast using Song et al. methods modified [24]. Hydrolyzate, which determined to reduce sugar levels as much as 100 mL put into Erlenmeyer, the mixture was pH adjusted to 5. A 5 mL phosphate buffer added as much as 5 mL and 0.1 gram Saccharomyces cerevisiae dissolved into 10 mL of 0.85 % NaCl solution and incubate for 1 hour in advance. Erlenmeyer's mouth then gagged with cotton, which is rolled up in gauze and wrapped in aluminum foil so that the system becomes semi-anaerobic, then left at 30 ^{o}C for 72 hours.

Raru bark. Hydrolyzate, which determined to reduce sugar as much as 100 mL added to the Erlenmeyer, then 5 mL juice is added. The pH of the mixture then adjusted to 5, then 5 mL of phosphate buffer is added. Into the mix, then add 5 grams of r bark powder. The stages of fermentation and analysis of bioethanol are then carried out in the same manner as was done with Saccha-

Qualitative and Quantitative Analysis of Bioethanol. Analyze the presence of bioethanol results based on Salim et al. modified method [25]. $K_2Cr_2O_7$ solution as much as 1 ml of 0.05 M plus 1 mL of aqueous H_2SO_4 solution was put into a test tube and homogenized, then added 2 ml of sample. Next, the test tube is closed with aluminum foil, then heated for 10 minutes on the bath. The existence of ethanol shown by changing the color of the solution, which was initially yellow to green. For quantitative analysis of bioethanol using gas chromatography.

Result and Discussion

Quantitative analysis of reducing sugars begins with determining the maximum wavelength for optimal conditions of absorbance measurement of reducing sugars. The maximum wavelength for reducing sugar measurements obtained at λ 494 nm. Rusin et al. describe for detecting glucose can be done at a wavelength in the UV-Vis Region 460 – 530 nm [26]. The maximum wavelength spectrum for reducing sugar measurements presented in Figure 1.

Standard curves obtained at glucose concentrations of 200 - 1,000 mg / L against the absorbance value. The standard curve presented in Figure 2.

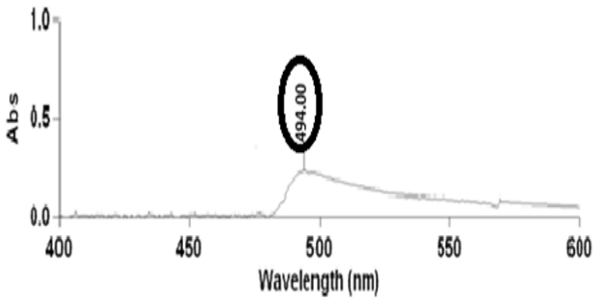

Figure 1. The maximum wavelength spectrum UV-Vis of reducing sugars

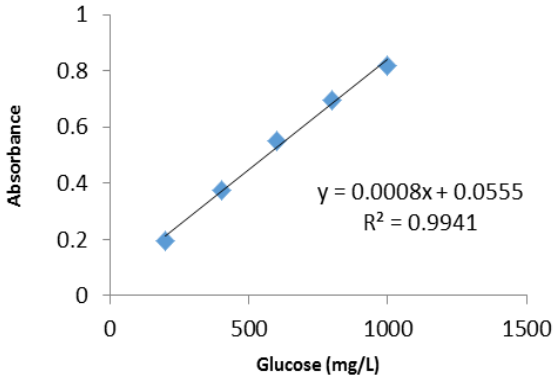

Figure 2. Standard curve for determining the level of reducing sugar

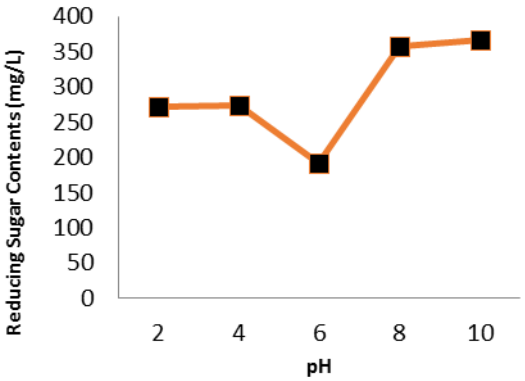

Figure 4. Reducing sugar contents at various pH

Beneng Taro Bulbs Hydrolysis

The optimum pH. Hydrolysis was carried out at 80 °C for 3 hours with a pH variation of 2, 4, 6, 8, and 10. Acidic pH conditions of 2, 4, and 6 conditioned using sulfuric acid (H_2SO_4) 0.01 M, while the base pH conditions were 8 and 10 conditioned using 0.1 M NaOH. The results of the qualitative analysis of reducing sugar hydrolyzate samples using Fehling reagents presented in Figure 3. The absorbance of the hydrolyzate sample used to measure reducing sugar contents. The results at different pH presented in Figure 4.

The highest reducing sugar contents obtained in the pH ten hydrolyzate sample that is equal to 365.765 mg / L. Based on the results obtained, the optimum pH for the hydrolysis of beneng taro tubers is pH 10. Palacios-Fonseca et al. inform in their research about the effect of the alkaline and acid treatments on the physicochemical propertiesof corn starch, describe that alkaline is the optimum condition on hydrolysis starch[27].

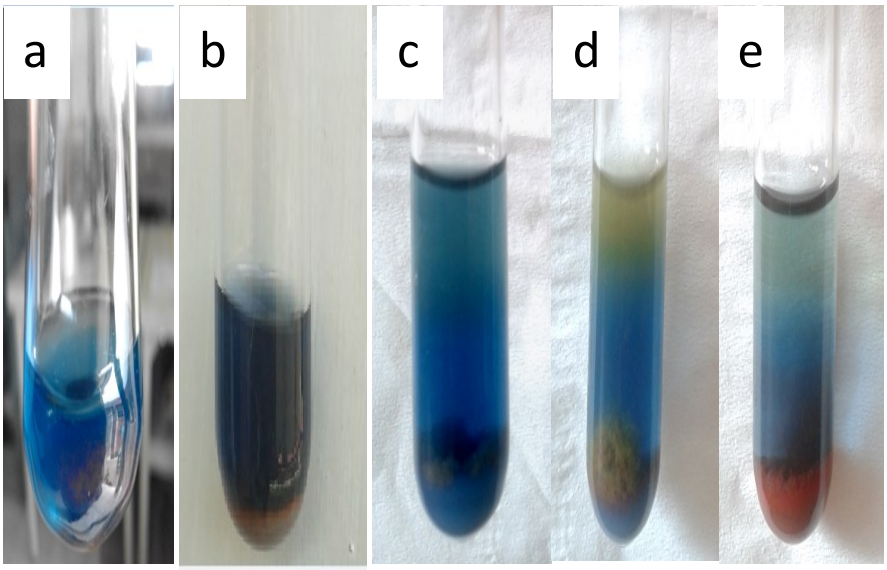

Figure 3. Reducing sugar qualitative analysis result from hydrolysate at various pH (a) pH = 2, (b) pH = 4, (c) pH = 6, (d) pH = 8, and (e) pH = 10

Optimum Time. Hydrolysis carried out at the optimum pH that has been obtained pH 10, with a temperature of 80 and time variations of 1, 3, 5, and 7 hours — the results of the qualitative analysis of hydrolyzate samples using Fehling reagents presented in Figure 5 and 6.

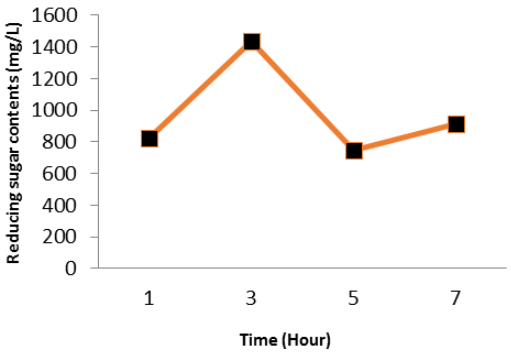

Figure 6. Reducing sugar contents at various hydrolysis time

Reducing sugar levels increased significantly from 1 to 3 hours, then reducing sugar contents decreased again within 5 hours, and yet, there was an increase in reducing sugar contents at 7 hours hydrolysis but not too significant.

The decrease in reducing sugar contents after the hydrolysis time of 3 hours is likely because the reduced sugar that has formed decomposes again due to longer heating time. Based on the results obtained, the optimum time for hydrolysis of beneng taro tubers is 3 hours, with the resulting reducing sugar of 1439 mg / L.

Determination of optimum temperature is done by hydrolysis of beneng taro tubers at the optimum pH of pH ten during the optimum time of 3 hours with different temperatures 60, 70, 80, and 90 ^{o}C — the results of the qualitative analysis of hydrolyzate samples using Fehling reagents presented in Figure 7 and 8.

The graphic pattern in Figure 8 shows that the higher the hydrolysis temperature, the reducing sugar produced increase, so the optimum hydrolysis temperature in this experiment cannot be determined yet, but the highest reducing sugar content provided at 90 ^{o}C, which is 910.875 mg / L.

Bioethanol Qualitative and Quantitative Analysis. Hydrolyzate at optimum conditions with reducing sugar levels of 910.875 mg/L fermented using Raru bark, which has the potential as an alternative fermentation agent and Saccharomyces cerevisiae as a comparative fermentation agent to produce bioethanol

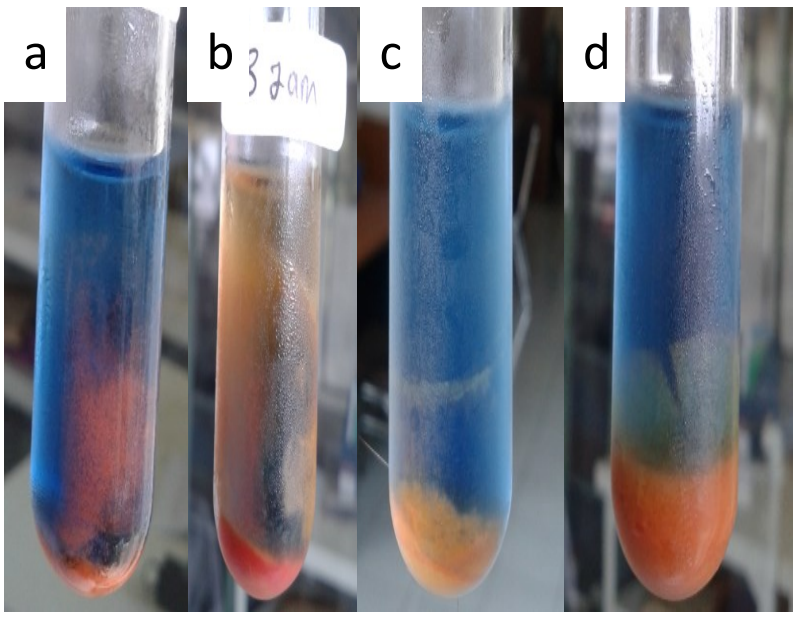

Figure 5. Fehling test results at various hydrolysis time (a) 1, (b) 3, (c) 5, and (d) 7 hour.

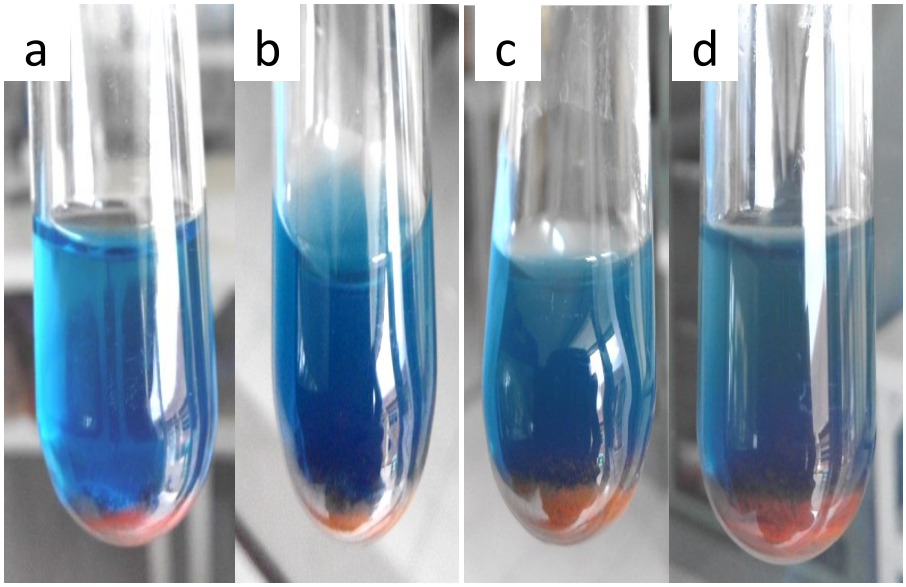

Figure 7. Fehling test result at various temperature (a) 60, (b) 70, (c) 80, and (d) 90 ^{o}C.

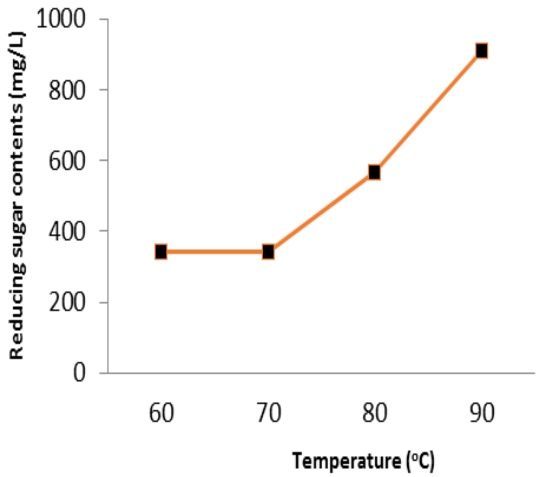

Figure 8. Reducing sugar contents at various temperatures.

The results of the bioethanol qualitative analysis presented in Figure 9. Fermentation using Saccharomyces cerevisiae showed almost the same green color as the positive control, while fermentation using Raru bark showed a green color mixed with the brown color derived from Raru bark pigments. These results indicate that both fermentation products have produced bioethanol.

Quantitative ethanol analysis using gas chromatography obtained ethanol levels from fermented samples using Raru bark powder of 0.187 % (v/v), whereas for fermented samples using Saccharomyces cerevisiae contained ethanol of 0.211 % (v/v).

Conclusion

Based on the results of this study, optimum conditions for Beneng taro tubers hydrolysis reached at pH 10, 3 hours, and a temperature of 90 ^{o}C. This condition resulted in reducing sugar levels of 910.875 mg/L. Fermentation using Raru bark produces ethanol of 0.187% (v / v), while fermentation using Saccharomyces cerevisiae produces ethanol of 0.211% (v / v). Raru bark has the potential to be developed as an alternative fermentation agent because it can produce ethanol with levels that are not much different from the fermentation agent *Saccharomyces cerevisiae*.

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