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Effect of Hydrolysis Time and Sulfuric Acid Concentration on Reducing Sugar Content on Corn Cob Hydrolysis

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ABSTRACT

Energy demand in Indonesia continues to increase, while the source of petroleum energy is a non-renewable energy source. This problem encourages people to seek alternative renewable and environmentally friendly fuels. Bioethanol is an alternative to replace petroleum energy that is environmentally friendly because it can be made from organic waste. The utilization of corncobs waste currently is not optimal. Corncobs are known to contain lignocellulose compounds, namely cellulose, hemicellulose, and lignin which are suitable as raw materials for making bioethanol. One of the conversions of lignocellulose into bioethanol is through hydrolysis. So in this study, the effect of hydrolysis time and sulfuric acid concentration (H_2SO_4) will be analyzed on the reducing sugar levels that will be produced because high reducing sugars will produce more bioethanol. The method of determining reducing sugar used is Nelson - Somogyi. The independent variables used were hydrolysis times that is 45 minutes, 90 minutes, 135 minutes, and 180 minutes, and the acid concentrations that are 5%, 10%, 15%, and 20% (1:20 w/v). The fixed variables used were corncob mass and hydrolysis volume, while the dependent variable was the reducing sugar content.

1. Introduction

The use of non-renewable energy fueled by fossil fuels is almost used in all activities, both from the household, and transportation to industrial scale, which if used continuously will affect the number of fossil fuels, which are increasingly scarce and depleting [1,2]. The depletion of fossil fuels is a reference for producing biofuels [3]. Biofuel can be produced by utilizing lignocellulosic waste so that it does not interfere with food conditions and is instead environmentally friendly, one of the uses is by producing bioethanol [4].

Bioethanol is the result of a sugar-carbohydrate fermentation process assisted by microorganisms to produce a liquid product [1,2,5,6]. Carbohydrates can be produced from plants and some carbohydrates are reducing sugars [7]. One of the alternatives for making bioethanol is by utilizing lignocellulosic waste such as corn cobs [6,8].

Corn cobs are the remains of plant parts that are disposed of by burning or left to pollute the road in an environmentally unfriendly way, even though it is one of the most abundant agricultural wastes, about 16% - 19% of the total part of the corn [9]. Corn cobs consist of high hemicellulose (39.8%), high cellulose (32.3–45.6%), and low lignin content (6.7-13.9%) so corn cobs are very suitable to be used as raw materials to make bioethanol [6,10,11]. To produce bioethanol from lignocellulose, it is necessary to go through four stages, namely pretreatment, hydrolysis, fermentation, and distillation [2,6]. The result of this hydrolysis process contains reducing sugars. Reducing sugar is a type of sugar contained in carbohydrates that serves to reduce oxidizing agents or electron acceptor compounds [7]. The sugar content is directly proportional to the bioethanol yield, namely the higher sugar content will produce more bioethanol yield after going through the fermentation process. One of the measurements of reducing sugar can use the Nelson – Somogyi method [12,13].

Based on research by Devi, et al. [14], the use of strong acids in hydrolysis at low concentrations can avoid reducing sugars because glucose is easily decomposed. The disadvantage is that it requires a longer processing time. While the use of acids with high concentrations will more quickly decompose cellulose and hemicellulose into other types of glucose, the possibility of inhibitors produced is greater [12]. The hydrolysis process can be carried out both at low temperatures and high temperatures if the low temperature is 80 - 140 ^oC and the high temperature is 160 - 240 ^oC [2].

Testing of reducing sugar content in corn cobs hydrolysis has not been widely informed so this study aims to determine the hydrolysis time and concentration of sulfuric acid which gives the highest reducing sugar content using the Nelson – Somogyi method.

2. Research Methodology

2.1. Materials

The tools used are 60 mesh sieve, glass bottle with rubber cap, glass funnel, beaker glass, Ika hot plate magnetic stirrer C-mag Hs 7, cuvette, measuring flask, Memmert Oven Laboratory Un 55, analytical balance OHAUS PA224, glass stirrer, measuring pipette, dropper, volume pipette, Genesys 10 UV – Vis Scanning spectronic, test tube and Thermo Cimarec Hotplate Stirrer SP131635.

The materials used were aluminum foil, distilled water, corn cobs, glucose, H_2SO_4 95-97%, filter paper, arsenomolybdate reagent, Nelson A reagent and Nelson B reagent.

2.2. Raw Material Preparation

Corn cobs were cleaned in running water and then cut into small pieces and then baked at 100 ^oC for 120 minutes or until dry. Then the corn cobs are mashed with a blender to form a fine powder. The powder is then filtered through a 60 mesh sieve to obtain a homogeneous powder in size.

2.3. Hydrolysis

Corn cob powder was weighed using an analytical balance as much as 2.5 grams in a beaker then added 50 ml of sulfuric acid with variations of 5%, 10%, 15% and 20% (1:20 w/v). Then the solution was hydrolyzed on a hot plate at a temperature of 100 ^oC for 45, 90, 135 and 180 minutes with a stirring speed of 200 rpm.

2.4. Quantitative Analysis of Reducing Sugar Test

1) Creation of a Glucose Standard Curve

Glucose 10 mg is diluted with 100 ml of distilled water so that it will become a glucose solution with a concentration of 100 ppm. Then the dilutions were made to 2 mg/100 ml, 4 mg/100 ml, 6 mg/100 ml, 8 mg/100 ml and 10 mg/100 ml. In the test tube, put 1 ml of each concentration that has been made, then add Nelson's reagent (the ratio is 25 Nelson A and 1 Nelson B), and one other tube is filled with distilled water as a blank. The five tubes containing the standard glucose solution were heated for ± 20 minutes in boiling water in a beaker. After being heated, the test tube was immediately cooled with cold water in a beaker so that the temperature of the test tube became the ambient temperature.

After cooling, 1 ml of arsenomolybdate was added into five tubes containing a standard solution of glucose and then shaken until all the Cu_2O precipitates were dissolved or homogeneous. After everything was dissolved, 7 ml of distilled water was added to the tube and the tube was shaken again until all the solutions were well mixed. Furthermore, the absorbance value was measured at a wave length of 540 nm on a spectrophotometer (Genesys 10 UV-Vis Scanning). From the absorbance results obtained, it is made into a standard curve which is the relationship between absorbance and standard glucose concentration so that a linear equation will be obtained.

2) Determination of Reducing Sugar Concentration

The resulting hydrolysis solution was measured for glucose concentration by the Nelson-Somogyi method. In a dry and clean test tube, add 1 ml of the hydrolyzed solution, then add 1 ml of Nelson's reagent (the ratio is 25 Nelson A and 1 Nelson B), mixed until homogeneous, and then heated in boiling water in a beaker for ± 20 minutes. Then the test tube was cooled to 25 °C.

After the test tube has cooled, 1 ml of the arsenomolybdate solution is put in the tube and shaken until all the Cu_2O precipitate is dissolved. Then 7 ml of distilled water was added into the tube and shaken again until all dissolved. Furthermore, by using a spectrophotometer, the absorbance value was measured at a wavelength of 540 nm. Based on the absorbance value of the hydrolysis results, the concentration of reducing sugar can be determined after being entered into the regression equation from the curve of the glucose standard solution.

3. Results and Discussion

3.1. Absorbance of Standard Glucose Solution

Preparation of 100 ppm standard glucose solution which was diluted into concentrations of 2, 4, 6, 8, and 10 mg/100 ml. This solution is used to obtain a linear equation where the value of x is the dilution concentration and y is the absorbance. This equation is used to find the reducing sugar content in the hydrolysis solution of corn cobs after passing the analysis of the absorbance value determined from the results of the spectrophotometer. The results of the absorbance of standard glucose solutions are presented in Figure 1.

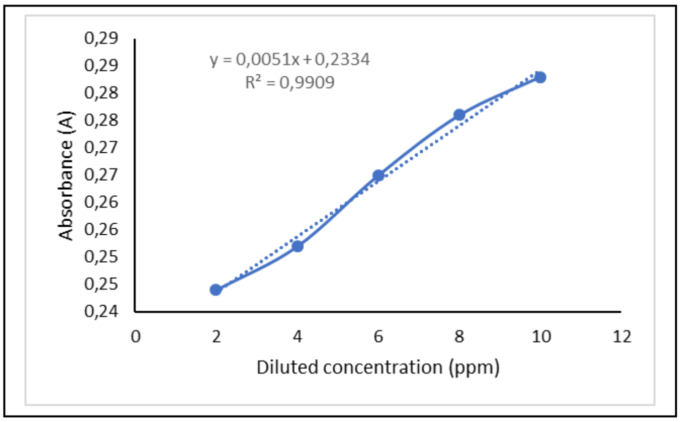

Fig. 1. Graph of the absorbance curve of standard glucose solution

Based on Figure 1, it can be seen that the glucose concentration has an effect on the absorbance value, which is directly proportional where the higher the glucose concentration used, the higher the absorbance value. The linear equation obtained is $y = 0.0051x + 0.2334$ where x is the concentration of standard glucose solution (mg/100 ml) y is absorbance. Based on statistical calculations obtained the value of a (slope) = - 0.234; b (intercept) = 0.0051 and r (correlation coefficient) = 0.9909. The value of this correlation coefficient is close to 1 or almost linear so that the results of the curve can be used to calculate the concentration of reducing sugars in the hydrolysis solution of corn cobs.

3.2. Effect of hydrolysis time on reducing sugar content

Hydrolysis can be influenced by several factors including the ratio of raw materials, hydrolysis temperature, hydrolysis time and concentration of catalyst used. Some of these factors will shift to the right of balance if one of the reactants is set in excess so that more reducing sugars are formed [15]. In this study, variations of hydrolysis time were used, namely 45 minutes, 90 minutes, 135 minutes and 180 minutes. The results of reducing sugar content based on variations in hydrolysis time are presented in Figure 2.

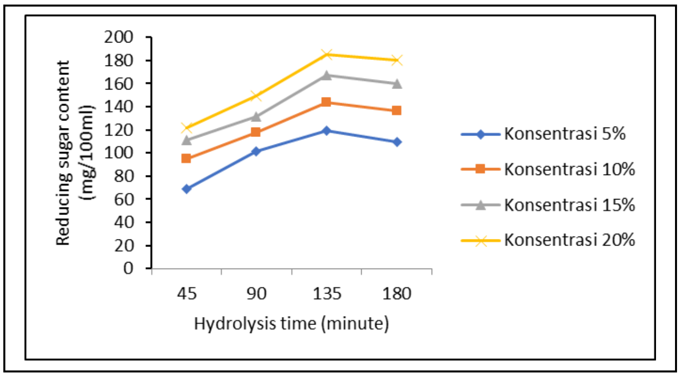

Fig. 2. Graph of the relationship between hydrolysis time and reducing sugar content

The results obtained are the relationship between the hydrolysis time is directly proportional to the reducing sugar content, namely the longer hydrolysis heating time, the higher the reducing sugar content. However, at 180 minutes of hydrolysis there was a decrease in reducing sugar content. This is because the hydrolysis time is too long so that the reactants have started to react and form inhibitors [15].

The highest yield was obtained at a hydrolysis time of 135 minutes, which was 166.98 mg/100 ml because the surface contact between the particles and the liquid was getting wider and flatter, while the hydrolysis time of 45 minutes produced the lowest reducing sugar content, which was 68,549 mg/100 ml.

3.3. Effect of sulfuric acid concentration on reducing sugar content

The concentration of the catalyst is one of the factors that affect the hydrolysis process. Sulfuric acid was chosen as the catalyst here because sulfuric acid is a strong acid so it will accelerate the reaction, while a low concentration is chosen to avoid reduction of reducing sugars because glucose is easily decomposed but has the disadvantage that it takes a longer hydrolysis time [2]. The concentrations of sulfuric acid used were 5%, 10%, 15% and 20% (1:20 w/v). The reducing sugar content obtained is presented in Figure 3.

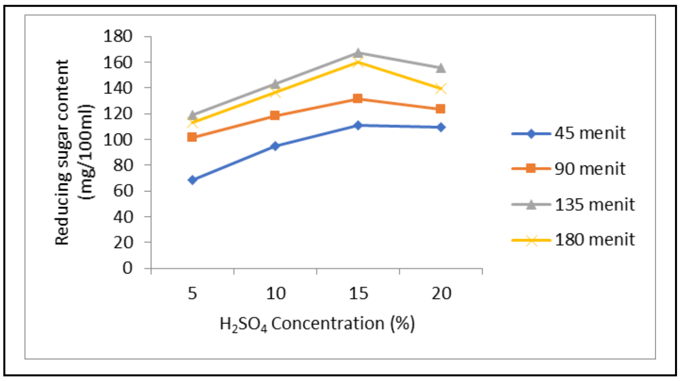

Fig. 3. Graph of the relationship between hydrolysis time and reducing sugar content

The results obtained show that the concentration of sulfuric acid is directly proportional to the levels of reducing sugars, namely the higher the concentration used, the more reducing sugars formed. This result is in line with the statement of Sun and Cheng [16], that a high acid concentration will facilitate the degradation of hemicellulose and cellulose turning into glucose and other forms of sugar compounds because the greater the contact between acid and cellulose in the hydrolysis reaction. The disadvantage is that an inhibitor can be formed by increasing the concentration of the acid used. Then it was strengthened by Ratnawati's statement [17], that the higher the concentration of acid used, the greater the number of collisions between reactant molecules so that more products will be produced. The lowest reducing sugar content produced at a concentration of 5% was 68.55 mg/100ml and the highest was at a concentration of 15%, which was 166.98 mg/100ml. Meanwhile, at a concentration of 20%, there was a decrease in reducing sugar levels due to the higher acid concentration components will be easier to form inhibitors [12].

4. Conclusion

Based on the research that has been carried out, the hydrolysis time and sulfuric acid concentration affect the hydrolysis of corn cobs, which is directly proportional. The longer the hydrolysis heating time is carried out, the more reducing sugar produced, as well as the higher the acid concentration used, the higher the reducing sugar content will be to a certain level. However, if the hydrolysis time is too long and using too high a concentration of sulfuric acid can accelerate the formation of inhibitors, causing reducing sugar levels to decrease. The highest reducing sugar content was produced at a hydrolysis time of 135 minutes and an acid concentration of 15%, which produced 166.98 mg / 100 ml.

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