UTILIZATION SOFT STEM OF KEPOK BANANA WASTE (*Musa paradisiaca formatypica*) AS A BASIC MATERIAL FOR MAKING BIOETANOL WITH ACID HYDROLYSIS METHOD AND FERMENTATION

Sulfiani[1*], Abd. Karim[1], Hasnah Natsir[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Perintis Kemerdekaan Street KM 10, Tamalanrea Makasssar, Indonesia 90245
*Corresponding author: Fhanisulfia@gmail.com

Abstrak. Bioenergi, diantaranya bioetanol, adalah salah satu bentuk energi alternatif yang dapat memenuhi kebutuhan energi masa depan. Bioetanol adalah etanol yang dihasilkan melalui proses fermentasi terhadap glukosa dari bahan alam yang mengandung komponen pati dan selulosa. Penelitian ini bertujuan untuk memanfaatkan batang pisang kapok, yang memiliki kandungan selulosa sebesar 64%, guna menghasilkan bioetanol dengan cara hidrolisis asam dan fermentasi bakteri menggunakan bakteri *Clostridum acetobutylicum*. Variasi yang digunakan pada penelitian ini adalah penambahan jumlah asam (HCl) dalam proses hirolisis (penambahan 15 mL, 20 mL, 25 mL, 30 mL, dan 35 mL) dan lama proses fermentasi (3 hari, 6 hari, 9 hari, 12 hari, dan 15 hari). Hasil penelitian menunjukkan kadar selulosa dalam batang pisang kepok sebesar 46,38%, dan hasil bioetanol tertinggi didapatkan pada perlakukan dengan penambahan asam 15 mL dan lama waktu optimum fermentasi
6 hari.

Kata kunci: bioetanol, batang pisang kepok, selulosa, *Clostridium acetobutilicum*

Abstract. Bioenergy, such as bioethanol, is one of the alternative options in order to fulfil the energy demands in the future. Bioethanol is ethanol which resulted from the fermentation process of glucose from natural sources that contains starch and cellulose. This research are aimed to utilize soft stem of kapok banana (*Musa paradisiaca formatypica*), which known to have cellulose content approximately 64%, in order to produce bioethanol undergoes acid hydrolysis and bacterial fermentation using *Clostridium acetobutylicum*. There are two main variables that being investigate in this research such as, acid (HCl) volume of adding (varied from 15 mL, 20 mL, 25 mL, 30 mL, and 30 mL) and length of fermentation time (3, 6, 9, 12, and 15 days respectively). Results shown that the maximum concentration of cellulose in soft stem of kepok banana is 46.38% and the bioethanol concentration/volume were achieved on the addition of 15 mL acid chloride and optimum fermentation time of 6 days.

Keywords: bioethanol, soft stem of kepok banana, cellulose, *Clostridium acetobutylicum*

INTRODUCTION

Nowadays, fuel oil needs are increasing because it is a vital requirement for humans, most or almost all technologies use fuel oil as an energy source. However, the fuel we use today is increasingly scarce, this causes the oil in the earth's layer to continue to thin. One of the disadvantages of petroleum is that it cannot be approved and the formation process takes millions of years. The price of petroleum is increasing (Simamora, 2008).

Alternative materials are needed which can be used as a substitute for petroleum for fuel, one of which is bioethanol. Bioethanol is ethanol derived from biological sources, including potential carbohydrates as raw materials such as sugar cane, sorghum juice, cassava, arrowroot, sweet potato, sago, corn, straw, corncob and wood (Novia, 2014). Bioethanol can too be produced from plants that contain a lot of cellulose compounds using aid from microbial activity (Seftian, et al., 2012). Cellulose is an abundant polysaccharide on earth that can be converted into glucose by hydrolysis (Groggins in Sari, 2009).

One of the plants that is often found in Indonesia which is still underutilized is waste of banana stems. According to Lisnawati (2000), banana stems have lignin levels (5%), cellulose (63-64%), hemicellulola (20%), and relatively long fibers around 4.29 mm. Hydrolysis is needed so that the raw materials used are converted into glucose solutions which are ready to be fermented into bioethanol. This process aims to break down lignin bonds, eliminate the content of lignin and hemicellulose, damage the crystal structure of seulose and increase porosity of the material (Sun and Cheng, 2002). This is the basis for further research and study on bioethanol production from banana stem waste by acid hydrolysis and fermentation using *Clostridium acetobutylicum* bacteria.

MATERIALS AND METHODS

Tools

The tools used are the equipment used in this study include knives, crucible, oven, needle oce, analytic balance, autoclave, furnace, incubator, thermometer, rotary shaker, filter cloth, reflux, distillation apparatus, hotplate, water bath, refractometer, 2010 Shimadzu gas chromatography and glassware commonly used in laboratories.

Materials

The main materials in this study were stems of Kepok Banana from banana stem gardens in Sudiang Makassar area, 72% H_2SO_4 solution, 0.1 N HCl, 5% NaOH solution, 3% H_2O_2, Glucose/cornstarch, 10% bean sprout extract, KH_2PO_4, Na_2SO_4 anhydrous, yeast extract, Bacto agar, phosphate buffer, 0.3% meat extract, cotton, $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, $MgSO_4.7H_2O$, $MnSO_4$, NaCl, $CaCl_2$ anhydrate, $FeSO_4.7H2O$, $Co(NO_3)_2.6H_2O$, Cysteine, Asparagine, Casein, aquades, absolute ethanol, Tissue roll, Aluminum Foil, pH paper, gauze, filter paper.

Procedures

1. **The Process of Pre-Treatment of Banana Stem Samples**

Preliminary experiments carried out by pretreatment of 50 g of banana stem flour using 5% b/b NaOH solution were carried out by immersion for 24 hours. Then filtered with filter paper or filter cloth.

The residue is washed with distilled water until all black lignin comes out. The washing process is stopped after the washing liquid is clear or neutral pH. The washed residue is put back into a plastic container with 3% H_2O_2 added until all residues are submerged, stirred and left overnight in a closed state until the remaining lignin attached to the residue is released (the sample is white) then the sample is dried in an oven at 110 ºC (Seligh, et. al, 2009).

2. Analysis of Lignin and Cellulose

Analysis of cellulose and lignin was carried out by the Chesson method (Datta, 1981). As much as 1 g of dry sample as heavy (a), added 150 mL of distilled water, refluxed at a temperature of 100 ^{o}C for 1 hour. The result is filtered, the residue is washed with hot water. The residue was then dried in an oven and then weighed, obtained by weight (b). The residue added 150 mL H_2SO_4 1 N then refluxed for 1 hour at 100 ^{o}C. The result is filtered and washed with distilled water until neutral and then dried, obtained by weight (c). Dry residue added 10 mL of 72% H_2SO_4 and soaked at room temperature for 4 hours. 150 mL of 1N H_2SO_4 was added and refluxed for 1 hour in the reverse coolant. The residue is filtered and washed with distilled water until it is neutral and then heated in an oven at 105 ^{o}C and the results are weighed until the weight is fixed, obtained by weight (d), then the residue is ignored and weighed, obtained by weight (e). Calculation of cellulose levels and lignin levels as follows (Ruso, 2011):

$$\text{Cellulose level} = \frac{c - d}{a} \times 100\% \quad \text{.....} \quad (1)$$

$$\text{Cellulose level} = \frac{d - e}{a} \times 100\% \quad \text{.....} \quad (2)$$

Where is :

a = sample weight (gram)
c = weight of residue at the third weighing (gram)
d = weight of residue at the fourth weighing (gram)
e = ash weight (gram)

3. The Effect of Addition of Acid in the Hydrolysis Process

The results of the pre-treatment process were divided into 5 and each weighed 5 g, put into erlenmeyer, then added 0.1 N HCl as much as 15 mL for the first erlemeyer. The suspension is then refluxed at 90 ^{o}C for 30 minutes to form a pulp. The same treatment for adding 0.1 N HCl to Erlenmeyer 2 to 5, with additional volume of 20 mL, 25 mL, 30 mL and 35 mL respectively.

4. The Effect of Optimum Time in the Fermentation Process

The porridge of the hydrolyzed banana stem was added with 15 mL of pure culture stock of Clostridium acetobutylicum bacteria and stirred at 150 rpm until homogeneous. Furthermore, the solution was fermented in an incubator at 37 ^{o}C for 3 days, 6 days, 9 days, 12 days and 15 days (according to treatment). The fermentation process is carried out under anaerobic conditions. Then the solution formed is separated by a slurry of banana stems so that you can get liquid alcohol and water.

RESULT AND DISCUSSION

In this study there were 2 samples analyzed for cellulose and lignin levels, there were samples before delignification and delignified samples.

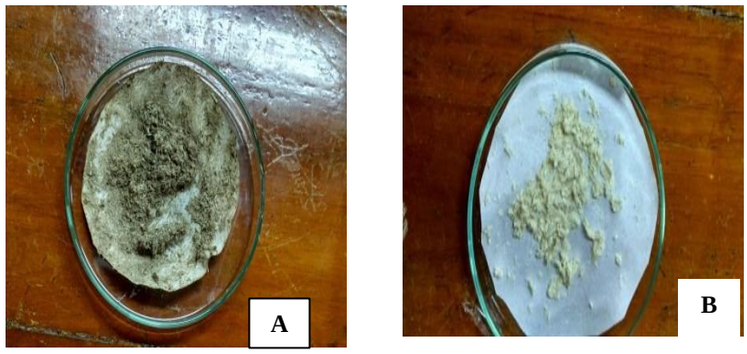

Figure 1. Banana stem flour before delignification (A) and Banana stem flour after delignification (B)

In accordance with the calculation of cellulose and lignin levels according to the chesson method, the results obtained in the form of data in Table 1.

Table 1. Data from sample delignification analysis

Sample	Cellulose level (%)
Before delignification	6,82
After delignification	46,38

The results of the analysis in Table 1 showed that cellulose levels before delignification had differences with cellulose levels after delignification, which were 6.82% and 46.38%. The difference in percentage levels from the results of the analysis in Table 1 is due to the cellulosic content which is still influenced by lignin which is still bound to cellulose, which is characterized by its color still blackish brown, and if observed the flour fiber is rather coarse and small, while the sample delignified already contains no lignin, which is characterized by the color of the yellowish-white sample and the fiber is rather fine, and large, this causes the cellulose content of the sample after delignification to increase.

The Effect of Addition of Acid in the Hydrolysis Process

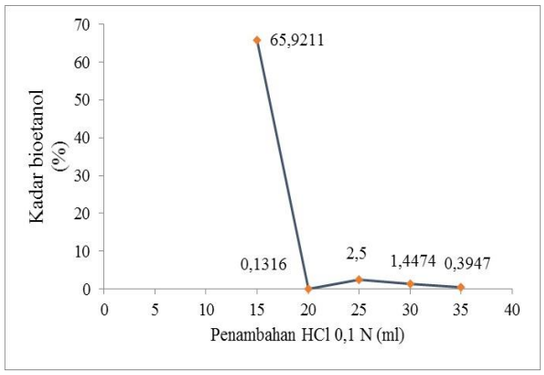

Figure 2. The Effect of acid addition on bioethanol levels

The analysis of the effect of acid addition on bioethanol levels in Figure 2 shows that the highest percentage of ethanol content was in the addition of 15 mL of HCl which was 65.9211%, indicating that the cellulose contained in the sample had been completely hydrolyzed by HCl resulting in high bioethanol levels , but at the addition of 20 mL of HCl, a very drastic decrease in bioethanol levels, the bioethanol content produced was only 0.1316%. At the addition of 25 mL, there was a slight increase, but only as much as 2,500%, after that at the addition of 30 mL and 35 mL the resulting bioethanol level decreased. This is due to excessive HCl acid does not break down cellulose, but also there are several other compounds that react with acids to

produce other byproducts, thereby reducing the levels of bioethanol produced.

The results of this study indicate that in the hydrolysis process, the more acid is added to the hydrolysis process, the lower the bioethanol content produced, this is because excessive acid does not break down cellulose anymore, but also reacts with other compounds to produce side products. So that the addition of 15 mL of acid produces a high level of bioethanol as much as 65.9211%.

The Effect of Optimum Time in the Fermentation Process

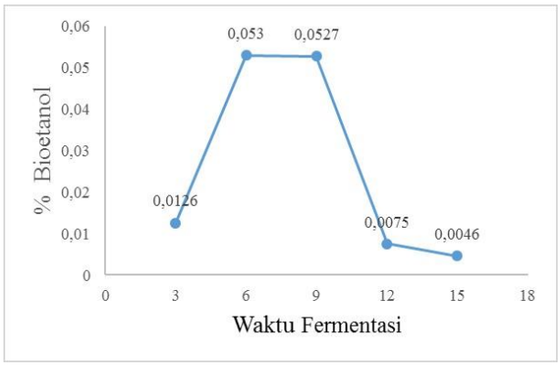

Figure 3. Effect of fermentation time on bioethanol levels

On the third day the levels of bioethanol produced are still very small, this is due to the possibility that on the 0th to 3rd day at this stage there will be a lag phase which is the phase where microbes are still adjusting to environmental conditions so that microbial activity is not optimum. During this phase the cell mass increases very little without the addition of the density of the cell number, therefore the cell growth rate can only be zero. The duration of the lag phase in bacteria varies greatly, depending on the composition of the media, pH, temperature, aeration, number of cells in the initial inoculum and physiological properties of microorganisms in the previous media. When the cell has adapted to the new environment, the cell begins to divide until it reaches the maximum population. This phase is called the logarithmic phase or exponential phase. The exponential phase is characterized by a period of rapid growth. Each cell in the population divides into two cells.

The variation in the degree of bacterial growth in this exponential phase is strongly influenced by the genetic traits that it inherits. In addition, the degree of growth is also influenced by nutrient levels in the media, incubation temperature, pH and aeration conditions. In this study this phase occurred on the 3rd to 6th day, marked by an increase in high levels of bioethanol, on the 6th day there was a very high level of bioethanol.

On the 6th day to the 9th day, the bacteria undergo a stationary phase which shows that the bacteria are no longer working optimally. The stationary phase occurs when the rate of bacterial growth equals the rate of death, so that the total number of bacteria remains. The balance of the total number of bacteria is due to a reduction in the degree of cell division. This phase is caused by diminishing glucose and nutrient levels, accumulation of toxic products that interfere with cell division, and the occurrence of fermentation byproducts which are not related to bacterial growth and productivity, so that on the 9th day the enzyme produced is slightly reduced and the bioethanol levels are decreases too.

On the 9th to 12th day, the concentration of bioethanol produced has dropped very dramatically. At this stage the bacteria have experienced a phase of death as well as the 15th day, which is characterized by an increase in the death rate that exceeds the growth rate, so that overall a decline in the bacterial population.

This occurs in all time variations, so it can be said that the optimum time of performance of *Clostridium*

acetobutylicum bacteria in the bioethanol fermentation process from kepok banana stem waste is on the 6th day.

CONCLUSIONS

Based on the research that has been done, it can be concluded that the levels of cellulose contained in kepok banana stems before and after delignification amounted to 6.82% and 46.38%, the effect of fermentation time on bioethanol levels is that *Clostridium acetobutylicum* can actively carry out fermentation for 6, seen from the highest bioethanol level with 6th day fermentation time which is as much as 0.0530% and the optimum time for fermentation on day 6, Effect of adding acid catalysts in the process of making bioethanol by utilizing kepok banana stem waste is the more acidic added, the less bioethanol produced and high levels of bioethanol in the addition of 15 mL HCl as much as 65.9211%.

Suggestions for further research are hydrolysis with other acids, varying the pH used in hydrolysis and using other types of bacteria. More use of samples to get more distillate and can be tested for quality. And if using a banana stem sample, in order to compare the levels of bioethanol from each layer of the banana stem.

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