Pretreated of banana pseudo-stem as raw material for enzymatic hydrolysis and bioethanol production

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Abstract. Development of alternative energy is needed to solve the energy problem, including bioethanol. Banana pseudo-stem is a lignocellulose material that can used to produce bioethanol. Banana pseudo-stem has 28.83% cellulose and 19.39% lignin. The amount of lignin will reduce by pretreatment process. Variations of pretreatment methods by autoclaving of banana-pseudo stem in a steam, 0.5N, 1N, 1.5N, 2N NaOH solutions for 90 minutes were employed. Then the preteated samples were further enzymatic hydrolysed for 24, 48, 72 hours. The fermentation method of simultaneous saccharification and fermentation (SSF) was applied using cellulase enzyme and yeast of *Saccharomyces cerevisiae* for 120 hours. The variation of the pretreatment process by increasing of NaOH concentration solutions led to decreased the lignin content while increased in cellulose content. The lowest lignin content was 11.44% and the highest cellulose was 51.66%. The highest sugar content was 29.8 g/L (at pretreatment 2N NaOH solution, 72 hours hydrolysis). The highest bioethanol amount (4.32 g/L) was produced from pretreated banana stem using 2N NaOH solution.
Keywords: bioethanol, banana pseudo-stem, alkali pretreatment, enzymatic hydrolysis, SSF, *Saccharomyces cerevisiae*

1 Introduction

One of the problems that appear in Indonesia is decreasing crude oil as a main source of energy. Development of renewable energy is needed to solve the problem of energy limitation, one way is a bioethanol development.

Bioethanol is made from raw materials such as fibers, molasses, fruits, and other materials that containing reduction sugar which can be fermented [1]. Second generation bioethanol is produced from lignocellulose material. The process of conversion lignocellulose material to bioethanol generally consists of pretreatment, hydrolysis, and fermentation [2].

Alkaline pretreatments are more effective to dissolve lignin than acid and hydrothermal process [3], due to decrease of cellulose crystallinity and increasing access for enzymes [4]. The most alkaline used are NaOH, KOH, $Ca(OH)_2$, and ammonia [5].

Banana pseudo-stem is one of lignocelllulosic material that could be fermented to ethanol. According to [6], banana pseudo-stem 44% cellulose, and 8.1% lignin on a dry basis.

Saccharomyces cerevisiae is generally used in ethanol production industries. *S. cerevisiae* has tolerance to substrate concentration, high ethanol content, low pH, and low oxygen levels [1].

The aims of this study were to investigate the influence of pretreatment variation (autoclaving pretreatments of banana stem in steam, 0.5N, 1N, 1.5N, 2N NaOH solutions) on the cellulose, hemicellulose, and lignin contents of the pretreated materials. Subsequently, the effect of the pretreatments on glucose from hydrolysis process and bioethanol contents from fermentation were also evaluated.

2 Materials and Methods

2.1. Pretreatment

Dry banana pseudo-stem was cut to a size 5 cm, milled and sieved up to 60 mesh then dried at 140 °C to reduce the remaining moisture content. Alkaline solution (99% NaOH, Merck) was added to 25 gr of dry banana pseudo-stem placed in a 250 mL beaker glass to form a slurry. The concentrations of NaOH were 0.5 N, 1N, 1.5 N, and 2N. Pretreatment of the slurries was carried out for 90 minutes in the autoclave at 121 °C.

2.2 *Saccharomyces cerevisiae* preparations

S. cerevisiae was cultivated on agar slant containing 10 g/L glucose, 5 g/L peptone, 3 g/L yeast extract, 20 g/L agar at 28 °C [7]. The inoculum of *S. cerevisiae* was cultivated on culture medium containing 50 g/L glucose, 1 g/L $(NH4)_2HPO4$, 0.5 g/L KH_2PO_4, 0.25 g/L $MgSO_4.7H_2O$, 10 g/L peptone, 10 g/L yeast extract, at 30 °C for 24 hours [8].

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2.3 Enzymatic Hydrolysis

5 grams of pretreated banana pseudo-stem was added with citrate buffer solution at pH 5 (solid:solution ratio 1:10) then added with one gram of cellulase (SQzyme CS; 20,000 u/g). The hydrolysis process was carried out in rotary shaker water bath at 50°C, 150 rpm for 24, 48, and 72 hours.

2.4 Ethanol Fermentation

Ethanol fermentation was carried out by simultaneous saccharification and fermentation (SSF) process. The treated banana pseudo-stem was added with 100 mL of citrate buffer at solid concentration 10% (w/v), then sterilized at 121°C for 15 minutes. Cellulase enzim at a dosage 20 FPU/g $_{\text{dry solid}}$ and 10% (v/v) yeast *S. cerevisiae* was added to the samples of the pretreated banana-stem solution. The ratio of cellulase and yeast 1:5 (w/v). SSF process was performed at 37.5°C for 120 hours.

2.5 Distillation

After the fermentation process is complete, the fermentation product is then distilled to separate the ethanol from impurities.

2.6 Analysis

Analysis of lignin content was done by Klason method [9]. 1 gr dry sample (A) was extracted with alcohol benzene (ratio 1:2). The sample was transferred to a 50 mL beaker glass and 15 mL of 72% sulfuric acid was added at 20ºC with stirring for 2-3 minutes and then idle for 2 hours. Then it was added with 300 mL of distilled water in a 100 mL erlenmeyer flask.The sample then was added to distilled water up to 575 mL (3% sulfuric acid concentration). It was boiled and leave for 4 hours at low heating. Sample was washed until the lignin acid-free was obtained then dried at 105ºC, cool in desicator and weigh up to constant weight (B).

$$\text{lignin content} = \frac{B}{A} \times 100\% \quad (1)$$

Analysis of cellulose and hemicellulose content was done by Chesson method [10]. 1 gr dry sample (A) refluxed with 150 mL of distilled water at 100ºC for 2 hours. The result is filtered, the residue is washed until neutral and dried (B). Dried residue refluxed with 150 mL of 0.5M sulfuric acid at 100ºC for 2 hours. The result is filtered and dried (C). Dried residue treated with 10 mL of 72% sulfuric acid at room temperature for 4 hours, then diluted to 0.5 M sulfuric acid and refluxed at 100ºC for 2 hours. The result is filtered, the residue washed and dried (D).

$$\text{hemicellulose content} = \frac{B - C}{A} \times 100\% \quad (2)$$

$$\text{cellulose content} = \frac{C - D}{A} \times 100\% \quad (3)$$

Analysis of glucose content was done by photometric method. Sample of 0.01 mL added with 1 mL of glucose reagent then incubated at photometer (BA-88A, Mindray) for 1 minute at 37ºC.

Analysis of ethanol content was done by Gas Chromatography (GC) method. On the GC tool (Clarus 680, Perkin Elmer), the injector temperature is set to 130ºC, the detector temperature 150ºC, initial temperature 80ºC, initial time 1 minute at heating rate 5ºC/minute, final time 5 minutes.

3 Results and Discussion

3.1 Effect Different NaOH Concentration of Pretreatments on Lignin, Cellulose, and Hemicellulose Contents

Pretreatment process was carried out to decrease lignin content and increase cellulose content on banana stem. Physical and chemical processes were carried out by using grinding and autoclaving the banana stem using a steam or NaOH solution.

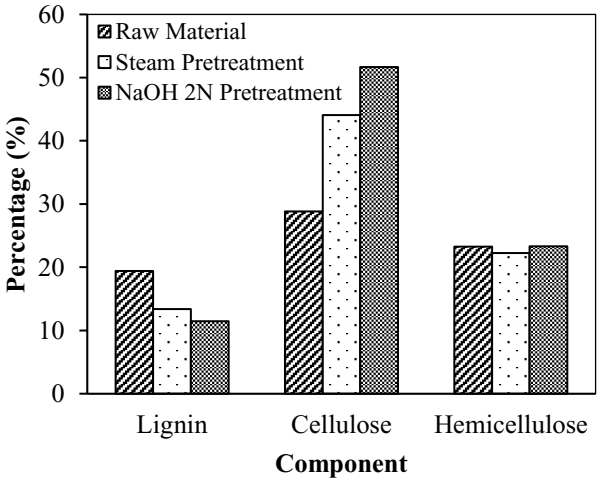

Fig. 1. Effect of pretreatments on lignin, cellulose, and hemicellulose contents. Pretreatment conditions at banana pseudo-stem: liquid ratio (1:4) autoclaved for 90 minutes.

Fig. 1 shows the optimal pretreatment was obtained using autoclaving banana stem in 2N NaOH solution that resulted lignin content 11.44%, cellulose 51.66%, and hemicellulose 23.29%. Lower cellulose was observed for steam pretreatment sample that yielded 13.37% lignin and 44.10% cellulose. While, for untreatment banana stem resulted the lowest cellulose that yielded 19.39% lignin and 28.83% cellulose. Physical pretreatment causes some hemicellulose degrades and lignin decomposes, while chemically is degrading the hemicellulose and dissolving lignin [11].

In the study by [12], pretreatment using alkali can increase cellulose content up to 8-10%. Moreover, the increase of NaOH concentration can give a significant influence to lignin degradation [13].

3.2 Effect of Different Pretreatments and Hydrolysis Time to Glucose Content

The hydrolysis process is aimed to convert cellulose into glucose which is then converted to bioethanol. The enzyme hydrolysis was carried out using 2% (w/w) cellulase enzyme with 5 gr of pretreated banana stem at 50 °C. Different pretreatment and time of hydrolysis on glucose content were investigated.

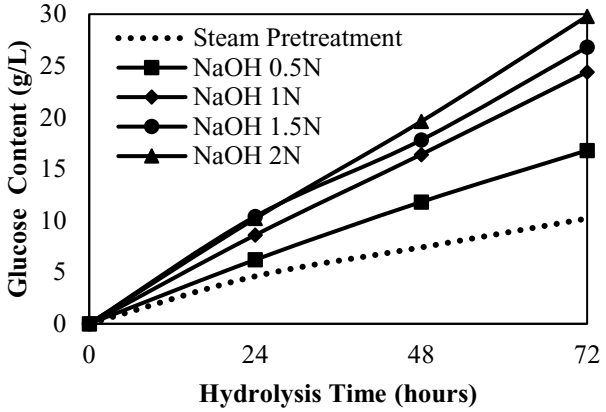

Fig. 2. Effect of different pretreatments and hydrolysis time on glucose content. Enzymatic hydrolysis at solid: liquid ratio 1:10 (w/v), 50 °C, 150 rpm, pH 5.

Fig. 2 shows the optimum glucose content obtained at banana stem pretreated with autoclaving in 2N NaOH solution for 72 hours hydrolysis that resulted 29.8 g/L glucose. Different pretreatments show that the glucose increased with increasing time. However the highest glucose were obtained at 72 h hydrolysis time that resulted 10.2 g/L for stem pretreatment, 16.8 g/L for NaOH 0.5 N pretreatment, 24.4 g/L for NaOH 1N pretreatment, 26.8 g/L for NaOH 1.5N pretreatment. The higher NaOH solution increased glucose from hydrolysis was similar with studied reported by [14].

3.3 Effect of Different Pretreatments on Bioethanol Concentration

The fermentation process was performed by Simultaneous Saccharification and Fermentation (SSF) method on 10 gram banana pseudo-stem by adding 100 mL of citrate buffer, 2% (w/w) cellulase enzyme, and 1:1 (w/v) *S. cerevisiae* at 37.5 °C for 120 hours.

The highest bioethanol was observed on banana stem pretreated using NaOH 2N solution that yielded 4.32 g/L ethanol. Ethanol content increased with increasing NaOH concentration in the pretreatment of banana stem. The increasing of ethanol due to cellulose content of banana increased with increasing NaOH concentration. The cellulose was converted to reduction sugar at hydrolysis process followed by fermentation of the sugar to bioethanol. Alkali pretreatment can increase ethanol concentration was also reported by a study by [15].

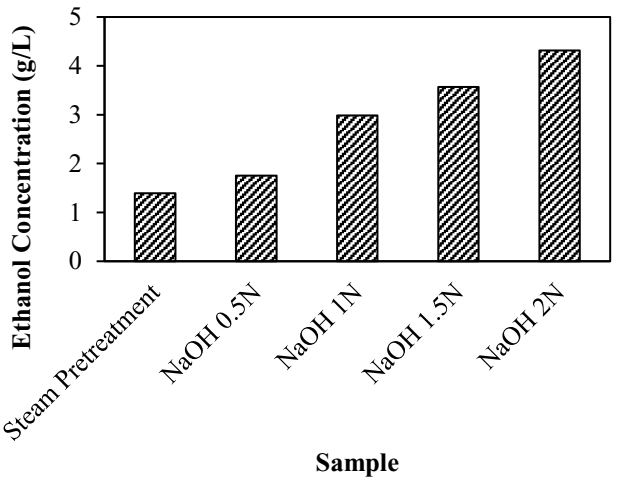

Fig. 3. Effect of NaOH concentration on bioethanol concentration. SSF conditions at 1:10 (w/v) raw material and citrate buffer ratio, 2% (w/w) cellulase enzyme, 1:1 (w/v) *S. cerevisiae*, 37.5°C, pH 5, 120 hours.

4 Conclusions

Different alkaline concentration of pretreatment can affect to the lignin, cellulose, and hemicellulose contained in banana pseudo-stem. It can also affect the ethanol content that is obtained from fermentation. Hydrolysis time also affected to glucose content from hydrolysis. The glucose content increased with increasing of hydrolysis time. The best result from pretreatment process were 11.44% lignin, 51.66% cellulose, and 23.29% hemicellulose from sample that pretreated by autoclaving banana stem in 2N NaOH . The highest result from hydrolysis process was 29.8 g/L glucose at 72 hours hydrolysis using sample pretreated by autoclaving using 2N NaOH that resulted 4.32 g/L ethanol.

The authors would like to thank the Directorate of Research and Community Service, Ministry of Education, Technology and Higher Education Indonesia (Kemenristekdikti) for funding this Research (PB Kompetensi 2017).

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