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Bioethanol Production from Sugarcane Bagasse by Simultaneous Sacarification and Fermentation using *Saccharomyces cerevisiae*

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Abstract. Sugarcane bagasse (SCB) is most abundant agricultural wastes in the world. It is an attractive feedstock for the large-scale biological production of bioethanol. However, the limitation in bagase use is its high degree of complexity because of its mixed composition of extremely inhomogeneous fibers. Therefore, ethanol production from bagase is often complex, with three main steps, i.e pretreatment, sacharification, and fermentation. Here we used alkali pretreatment using delignification reactor with NaOH 1N and 1.5 bar for 2 hours. Followed by Simultaneous Sacarification and Fermentation (SSF) using *Saccharomyces cerevisiae* in addition of cellulase and β-glucosidase enzyme. We found that the alkaline pretreatment can decrease cellulose crystallinity, decrease lignin content up to 84.83% and increased cellulose content up to 74.29%. SSF using cellulase enzymes and combination of cellulase enzymes and β-glucosidase derived bioethanol levels respectively 5.87±0.78% and 6.83±0.07%. In conclusion these results strongly suggest that addition of β-glucosidase enzyme on alkali-pretreated bagasse increased the bioethanol production.

INTRODUCTION

Sugarcane bagasse (SCB) is most abundant agricultural wastes in the world, creating 540 million tons of biomass per year [1]. As an example of the sugar cane processing factory Madukismo in Bantul, Yogyakarta, which is the largest supplier of sugar cane in Yogyakarta, resulting in a solid waste such as baggase. Sugarcane raw material needs nearly 400,000 tonnes per year for the sugar production process, resulting bagase as by product 70-90%. Bagase is an attractive feedstock for the large-scale biological production of bioethanol, because of the abundance and concentration of low-cost raw materials, contributing to the reduction of greenhouse gas emissions and the improvement of food security.

However, the limitation in bagase use is its high degree of complexity because of its mixed composition of extremely inhomogeneous fibers. Bagase typically contains approximately 40% cellulose, 24% hemicelluloses, and 25% lignin [2]. Therefore, ethanol production from bagasse is often complex, with three main steps, i.e pretreatment, sacharification, and fermentation. Pretreatment may damage the crystalline structure and lignin of bagasse. It is necessary to reduce the lignin content to facilitate carbohydrate hydrolysis by enzyme systems. Several pretreatment methods have been studied for various biomass sources [3,4,5], including steam explosion, solvent extraction, and thermal pretreatment using acids or bases [6,7].

Although various physical (comminution, hydrothermolysis), chemical (acid, alkali, ozone, solvents), and biological pretreatment methods have been investigated over the years [8], thermo-chemical pretreatment of biomass has been the pretreatment of choice to enhance substrate accessibility for efficient enzymatic hydrolysis [9]. Alkaline pretreatment dissolves most of lignin and various uronic acid substitutions responsible for inhibiting

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accessibility of the cellulose to enzymatic saccharification [10] and removes part of hemicellulose, swelling cellulose micro fibrils [11].

The aim of this study was to evaluate the bioethanol production by *Saccharomyces cerevisiae* in simultaneous saccharification and fermentation of alkaline delignificated sugarcane bagasse. The effects of enzymatic load (20 FPU/g cellulose and mixed combined cellulose and β-glucosidase were evaluated.

EXPERIMENTAL WORKS

Alkaline Pretreatment

Sugarcane bagasse (SCB) was provided by PG/PS Madukismo, a sugar cane factory processing in Yogyakarta province, Indonesia. The bagasse was washed and dried, then milled to size 40 mesh. Alkaline treatment was followed Maryana et al [6] with upscaling scales, 2500 g of bagasse was weighted and placed at 120 L reactor. Thirty litter of sodium hydroxide 1N was added to the reactor (solid to liquid ratio was 1:12). Cooking time with sodium hydroxide 1N was performed by heating the reactor at 100ºC and 1.5 bar pressure for 45 minutes. Before and after treatment, lignin, cellulose and hemicelluloses content of bagasse has been analyzed using Chesson method [12]. One g (a) of dry sample was added with 150 ml H_2O and refluxed at 100 °C in water bath for 1 h. The result was filtered, and the residue washed with 300 ml hot water. The residue was then dried until constant in an oven then weighed (b). Residue was added 150 ml of 1 N H_2SO_4 then refluxed in a water bath temperature of 100° C for 1 h. The results are filtered to neutral (300 mL) and dried (c). Dried residue was added with 10 ml of 72% H_2SO_4 and soaked at room temperature for 4 h. Residue then added 150 ml of 1 N H_2SO_4 and refluxed on a water bath for 1 h. Residue was filtered and washed with H_2O to neutral (400 mL) and then dried with a temperature of 105ºC and the results weighed (d), the residue weighed and ashed in the furnace (e). Lignin content = [(d-e)/a]x 100%; Cellulose content = [(c-d)/a] x 100%; Hemicellulose content = [(b-c)/a] x 100%.

Sugarcane bagasse characterization

Characterization of bagasse before and after treatment has been conducted by using difractometer X-ray (Shimadzu). The samples were exposed to x-ray radiation (Cu Kα) at wavelength of 1.5406 A; 40kV; 30mA; and scanning range 3-80º. The rate of the scanning was 0.6^o/min at a range of 5–50° 2θ. Samples were measured on a low background quartz plate in an aluminum holder. Furthermore, characterization of functional groups has been performed by Fourier Transform Infra Red (FTIR) spectrometer Shimadzu, wavelengeth 400-4000cm^{-1}.

Simultaneous saccharification and fermentation

Simultaneous saccharification and fermentation *(SSF)* had been done according optimized method by Wahono *et al* [13] with slight modification, Medium for SSF as much as 20 mL consists of delignificated bagasse samples (1 g); nutrients (NH4)2HPO4 (3.44 mL), MgSO4.7H2O (0.17 mL), yeast extract (6.88 mL); citrate buffer (pH 5.0); 25 % ($v \cdot v^{-1}$) of yeast *Saccharomyces cerevisiae*; cellulose enzymes (20 fpu) and mix combined cellulose enzyme (20 fpu) and β-glucosidase (20 fpu). SSF was conducted on ambient temperature and pressure with two variables incubation period of 3 d and 5 d. Ethanol concentration was determined using Gas Chromatography (GC) type HP 5890 FID col-CBP 525 m with n-propanol as internal standard, at Chemistry Laboratory-Universitas Gadjah Mada

TABLE 1. Chemical compositions of the sugarcane bagasses

Component	Raw Sugarcane baggase	Alkaline Pretreatment
Cellulose	44.43	77.37
Hemicellulose	22.90	9.45
Lignin	17.52	2.53

RESULT

In this work, alkaline pretreatment was chosen as the first step to pretreat the sugarcane bagasse. The chemical compositions of sugarcane bagasse are shown in Table 1. After alkaline pretreatment, the percent hemicellulose and lignin content decreased from 22.90% and 17,52 to 9.45% and 2,53%, respectively. The percentage concentrations of cellulose increased from 44.43% to 77.37%. Lignin still was a protective physical barrier to enzymatic attack for it could be effectively removed by alkaline delignification (NaOH) pretreatment.

After alkaline delignification, the recovery of solids was 56,55%. As result of the alkaline delignification, the content of lignin in the delignificated bagasse was approximately 84.83% lower than in the raw bagasse. On the other hand, due to lignin solubilization, cellulose content increased in 74.29%. Lignin solubilization was higher than that obtained by Maryana [6] for alkaline delignification (NaOH) of sugarcane bagasse (60%).

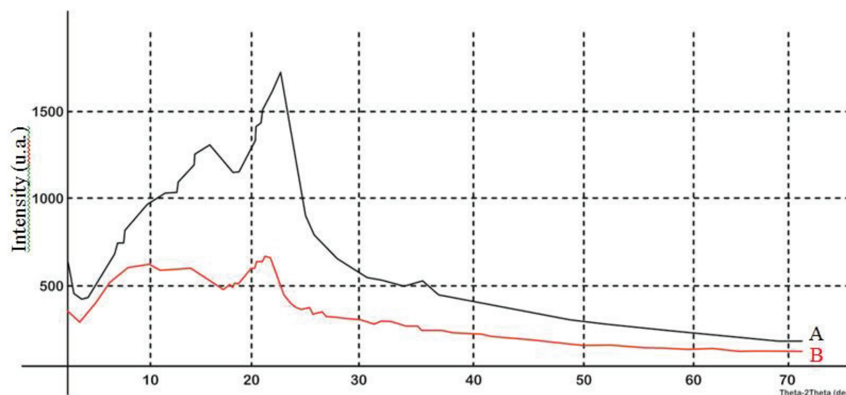

FIGURE 1. Diffractogram of raw bagasse (A); diffractogram of delignificated bagasse (B)

Alkaline pretreatment may damage the crystalline structure of bagasse. It is necessary to reduce the crystalinity of the bagasse to facilitate carbohydrate hydrolysis by enzyme systems. The crystallinity of bagasse before and after treatment has been analyzed by using X-ray Diffraction (XRD). The result is presented in Figure 1. Crystallinity index of the delignificated bagasse was around 56.67% lower than the raw bagasse. These results are consistent with the aims of pretreatments, which are to reduce crystallinity and thus improve the enzymatic conversion of cellulose [14]. Sodium hydroxide causes swelling, decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption [15]. Sodium hydroxide could alter the morphology and conformation of cellulose fibers. During the alkaline treatment process, the cellulose chain expands due to diffusion of base into the crystalline form. Furthermore, the cellulose polymer chain will undergo re-arrangements that result in damage to the structure of crystalline cellulose [6]. Enzyme accessibility should be affected by crystallinity, but it is also known to be affected by the concentration of lignin and hemicelluloses, particle size, and porosity of the native cell wall sample [16].

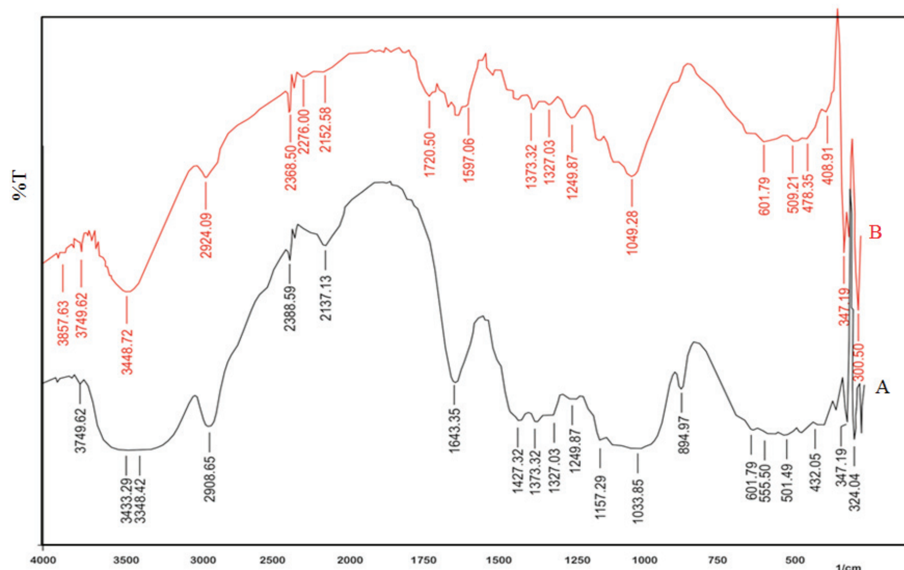

FIGURE 2. FTIR spectrum of raw bagasse (A); diffractogram of delignificated bagasse (B)

FTIR spectroscopy has been used in this work. Infra red spectroscopy is used to determine the chemical changes that occur during delignification by NaOH based on functional groups that occur in the lignocellulosic material. The result obtained is presented in Figure 2. Based on Figure 2, there was a significant difference between the spectra of raw bagasse and delignificated baggase. Spectra contained broad peak 3400 to 3500 cm^{-1} due to the hydroxyl group and a peak around 2924 cm^{-1} derived from the CH stretching. Peak appeared around 1033 cm^{-1} in the spectra of delignificated bagasse, and the peak around 1049 cm^{-1} at the spectra of raw bagasse, due to the C-O-C stretching of glycoside bond β-(1- 4) [17]. At the spectra of raw bagasse contained peaks at 2924 cm^{-1} derived from the -CH2 stretching and shifted to 2908 cm^{-1} on delignificated bagasse. Increased peak intensity that occurs between 900-1150 cm^{-1} indicated that the presence of elevated levels of cellulose [6,17].

TABLE 2. Bioethanol concentration after SSF

Sample	Bioethanol content (%)	
	3 d	5 d
Cellulose enzyme	3.80 ±0.96	5.87 ±0.78
Cellulose + β-glukosidase enzyme	5.62 ±0.03	6.83±0.07

In our studies, SSF was conducted on ambient temperature and pressure with two variables incubation period of 3 d and 5 d in addition cellulose enzyme and mixed combined cellulose and β-glucosidase enzyme. During the SSF proceses cellulose from sugarcane bagasse was converted into glucose by cellulose enzymes and β-glucosidase enzyme and then simultaneously glucose was converted into ethanol by *Saccharomyces cerevisiae*. The result obtained is presented in Table 2. Based on the Table 2, bioethanol concentration of SSF product with mixed combined enzyme cellulose and β-glucosidase higher than cellulose enzyme, both during fermentation 3 or 5 days. This is due to β -glucosidase is the key enzyme component present in cellulase and completes the final step during cellulose hydrolysis by converting the cellobiose to glucose [18].

CONCLUSION

This study proposes a combined process between alkaline pretreatment and SSF. Alkaline pretreatment can decrease cellulose crystallinity, decrease lignin content up to 84.83% and increased cellulose content up to 74.29%. SSF using cellulase enzymes and combination of cellulase enzymes and β-glucosidase derived bioethanol levels respectively 5.87±0.78% and 6.83±0.07%. In conclusion these results strongly suggest that addition of β-glucosidase enzyme on alkali-pretreated bagasse increased the bioethanol production.

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