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Alkaline Pretreatment of Sweet Sorghum Bagasse for Bioethanol Production

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ABSTRACT. Lignocellulosic material, which consist mainly of cellulose, hemicelluloses and lignin, are among the most promising renewable feedstocks for the production of energy and chemicals. The bagasse residue of sweet sorghum can be utilized as raw material for alternative energy such as bioethanol. Bioethanol production consists of pretreatment, saccharification, fermentation and purification process. The pretreatment process was of great importance to ethanol yield. In the present study, alkaline pretreatment was conducted using a steam explosion reactor at 130^{0}C with concentrations of NaOH 6, and 10% (kg/L) for 10, and 30 min. For ethanol production separated hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) process were conducted with 30 FPU of Ctec2 and Htec2 enzyme and yeast of *Saccharomyces cerevisiae*. The results showed that maximum cellulose conversion to total glucose plus xylose were showed greatest with NaOH 10% for 30 min. The highest yield of ethanol is 96.26% and high concentration of ethanol 66.88 g/L were obtained at SSF condition during 48 h process. Using SSF process could increase yields and concentration of ethanol with less energy process.

Keywords: Bagasse Sorghum, bioethanol. pretreatment, saccharification, fermentation,

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1. Introduction

The demand of ethanol as a substitute of gasoline is rapidly increasing due to the recent increase imbalance in oil market and interest in environmental issues. Currently bioethanol which is derived mainly from food crops generate many problems such as net energy losses, green house gas emission, and increased food price. Bioethanol can also be produced from abundant and renewable biomass resources such as agriculture residues, plantation and forest residues and energy crops, are still today a challenging proposition.

The sweet sorghum (*Sorghum bicolor* (L.) Moench) is one of the most attractive biomass resources for fuel ethanol production due to its adaptability to adverse conditions and it has high fermentable sugar content in its juice and high yield of green biomass. The juice extracted from the fresh stem is composed of sucrose, glucose and fructose that it can be readily fermented to alcohol, known first generation bioethanol (G1). The residue after extracting the juice from the sweet sorghum is solid fraction left behind, so-called bagasse is lignocellulosic material, can be hydrolyzed into sugar and further can be fermented to ethanol (Shen *et al*., 2011; Lijun *et al*., 2013), and called as second generation bioethanol (G2).

In the conversion of lignocellulosic biomass to fuel, the biomass needs to be treated so that the cellulose in the plant fibers is exposed (Kumar *et al*., 2009). The major constraint to the development of successful bioconversion process is the physical protection of cellulose by lignin against cellulolytic enzymes (Havannavar *et al*., 2007). Therefore, for the utilization

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of lignocellulosic materials in a bioconversion process involving enzymatic hydrolysis followed by fermentation, pretreatment is required in order to break down the complex structure of lignocellulose, to reduce the lignin content, cellulose crystallinity and to increase the surface area for enzymatic reactions (Zhao *et al.*, 2008).

Several pretreatment methods have been developed to increase the enzymatic hydrolysis sugar yields from lignocellulosic biomass (Monsier *et al*, 2005; Taherzadeh *et al*., 2008). Concentrated acid pretreatment can produce high yield of monoric sugar but they have some disadvantages such as corrosive nature and the need of acid recycle in order to reduce the cost of pretreatment (Chaturvedi & Verma, 2013). Alkaline pretreatment is one approaches that has several potential advantages compared to other pretreatment processes including low operation cost, reduced degradation of holocellulose, and subsequent formation of inhibitors for downstream processing (Monsier *et al*.,2005). The main mechanism of alkaline pretreatment are the degradation of ester bonds and cleavage of glycosidic linkages in the lignocellulosic cell wall matrix, which lead to the alteration of the structure of lignin, the reduction of the lignin–hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose (Sun & Cheng, 2002).

Many studies on the ethanol production from cellulose, the simultaneous saccharification and fermentation (SSF) process has attracted many revise. investigators. The SSF process offers benefits such as improved ethanol yield by reducing the product inhibition exerted by saccharification products and also eliminates the need for separate reactors for saccharification and fermentation, which results in cost reduction. Optimization of the fermentation process requires an efficient pre-treatment to remove lignin and to release cellulose and hemicellulose from lignocellulosic complex of plant fibre.

The objective of this study was to investigate the effect of alkaline (sodium hydroxide, NaOH) pre-treatment with provide highest cellulose to glucose conversion during enzymatic hydrolysis for ethanol production. The importance of both pretreatment and process conditions (chemical concentration and duration time) was investigated.

2. Materials and Methods

2.1 Material

Bagasse of Sweet Sorghum (*Sorghum bicolor* (L.) Moench) was obtained from PT. Panen Energy Malang East Java, Indonesia. After air-dried, physical pretreatment i.e. chipping and milling until 2 mm was conducted to maximize contact area of the substrate. The moisture content of 10-12% was measured by Moisture Analyzer OHAUS MB 45 and stored in a dry place. After drying then it was stored in sealed plastic bag at room temperature until be used for chemical pretreatment.

The enzymes used for the saccharification were provided by Novozyme Denmark. Two cellulase enzymes, Cellic®CTec2 and Cellic®HTec2 were used for hydrolysis (saccharification) process. The activity of Cellic®CTec2 is 144 FPU·g^{-1} cellulose (measured by NREL method), while the activity of Cellic®HTec2 is 240 CBU·g^{1} (reported by Novozyme). In this study, the saccharification applied Cellic®CTec2 of 30 FPU·g^{-1} dry biomass and one-fifth of Cellic® CTec2 (v/v) for Cellic®HTec2.

Dry yeast (*Saccharomyces cereviceae*) was employed for the fermentation process. All reagents used in this study were analytical grade, except the reagent of the pretreatment process, i.e. sodium hydroxide was an industrial grade.

2.2 Procedure

Alkaline (NaOH) Pretreatment

Pretreatment was conducted using a bench scale reactor CHEMEX at the Research Center for Chemistry, Indonesian Institute of Sciences (LIPI). This reactor was equipped by cyclone, belt press, washing tank, and buffer tank. Sweet Sorghum Bagasse (SSB) in small size was heated using NaOH solution 6 and 10% (kg/L) at 130^{o}C for 10 and 30 minutes. A solid liquid ratio was 1:5. The pressure was controlled at 4 bars at early heating. Sorghum bagasse treated was washed until the wash water turned to pH= 7 and dried overnight in the oven at 50 – 60^{o}C. The composition of materials component after pretreatment was analyzed according to National Renewable Energy Laboratory (NREL) standard procedures (Sluiter et al., 2012).

Separate Hydrolysis and Fermentation (SHF)

The SHF process was carried out in each step, which hydrolysis was followed by fermentation process. Both of the processes, enzymatic hydrolysis (saccharification) and fermentation, was carried out for 72 hours. Duplicate process was arranged to get the best approach. The samples, pre-treated 15% (g/ml) in an erlenmeyer flask containing 0.05 M the buffer citrate with pH 4.8, were autoclaved at 121 ^{o}C for 20 minutes. After cooling, 30 FPU of Cellic® CTec2 per gram dry biomass and 20% Cellic® HTec2 was added for each. All of the samples were placed in the shaking incubator at temperature 50 ^{o}C and 150 rpm agitation. Sampling every 24 hour was employed to monitor producing sugar, glucose and xylose. After sacharification, fermentation process was conducted for 72 hours. Thus the total time processes were 144 hours. The

temperature of shaking incubator was changed into 32 ^{o}C. In the constant temperature, one percent (g/ml) of dry yeast, *S. cereviceae*, was put in each flask. Ethanol content, glucose, and xylose were monitored every 24 hours.

Simultaneous Saccharification and Fermentation (SSF)

SSF experiment were performed by duplicate in 250 mL flasks. Fifteen percent (g/ml) of pretreated SSB in 0.05 M buffer citrate in Erlenmeyer flask was sterilized by autoclave at 121^{o}C for 20 minutes. Each enzyme concentration as described in SHF was added together with 1% (g/ml) dry yeast, *S. cereviceae*. The process was conducted in the shaking incubator under temperature condition 32^{o}C with velocity agitation 150 rpm during 72 hours. Sugar and ethanol were monitored every 24 hours. All fermentations were done without any yeast nutrient suplementation.

The calculation percentage of yield in fermentation is as same as those in saccharification process i.e by comparing measured ethanol weight with the theoretical weight of ethanol. The anhydro correction is 0.51 (for glucose to ethanol) (Ballesteros, et al. 2004)(Eq 1) :

$$Yield_{ethanol} = \left[\frac{\left(C_{ethanol,f} - C_{ethanol,i}\right)}{0.51 \cdot f \cdot 1.11 \cdot C_{biomass}} \right] x100\% \quad \textbf{(1)}$$

Where $C_{ethanol,f}$: ethanol concentration at the end of the SSF (g/L), $C_{ethanol,i}$: ethanol concentration at the beginning of the SSF (g/L), $C_{biomass}$: dry biomass concentration at the beginning of the SSF (g/L); f: cellulose fraction of the dry biomass (g/g); 0.51: conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast, 1.111 is conversion factor of cellulose to equivalent glucose

Product Analysis

Glucose, xylose and ethanol product were measured by High Performance Liquid Chromatography (HPLC) waters, USA. The mobile phase of this equipment is 5 mM H_2SO_4 at 0.6 ml/min and was equipped with AMINEX HPX 87H column, a guard column, an automated sampler, a gradient pump. A Relative Index (RI) was used as the detector. The oven temperature was maintained at 65^{o}C.

Statistical analysis

The content of chemical component of cellulose, hemicellulose, lignin, sugar and ethanol were analized with ANOVA procedurs using the software Minitab 17 and and statistical significance (CI) of 95% to evaluate the significant differences among mean treatments.

3. Results and Discussion

The effect of Alkaline NaOH pretreatment on lignocellulosic components

Alkaline pretreatment of lignocellulosic biomass is one of the most effective pretreatment methods which predominantly affect lignin content of biomass in the present study, alkaline pretreatment with high pressure and temperature was chosen. The characteristic of the process is almost same as Ammonia Fiber Explosion (AFEX), which lignocellulosic materials are dissolved in ammonia solution at high temperature and pressure for a period (Talebnia, 2010). Using alkaline pretreatment would increase cellulose digestibility and effective for lignin solubility on agricultural waste than wood materials (Carvalheiro, 2008; Kumar, 2009). During alkali pretreatment, lignin and hemicellulose are solubilized and/or decomposed in the aqueous phase result in a soluble fraction containing hemicelluloses and lignin degradation products, while cellulose remain in the solid fraction result in an insoluble cellulose-rich fraction (Sun,Y., & Cheng,J. 2002; Klinke*et al*., 2004).

The precipitation bagasse sorghum from the sodium hydroxide pretreatment was quantified for lignocellulose components as shown in Table 1.

Table 1
The component content of sweet sorghum bagasse fibre before and after NaOH pretreatment at 130^{0}C for 10 and 30 minutes

NaOH (%)	Time (min)	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Untreated		21.39	14.72	34.09
6	10	6.79	13.98	68.50
	30	5.09	13.50	67.37
10	10	7.67	8.67	74.89
	30	4.90	8.19	77.80

In the present study, alkaline pretreatment SSB was aimed to alter the structure of cellulosic biomass by removing lignin and hemicelluloses, so that the cellulose became more accessible to the enzymes that convert carbohydrate polymers into fermentable sugars. During alkali pretreatment, lignin and hemicellulose are solubilized and/or decomposed in the aqueous phase result in a soluble fraction containing hemicelluloses and lignin degradation products, while cellulose remain in the solid fraction result in an insoluble cellulose-rich fraction (Carvalheiro *et al*., 2008).

In this study increasing residence time and alkali concentration lead to an increase of the loss of solids during NaOH pretreatment. Table 1 shows that the optimum pretreated SSB fiber contains 77.80% cellulose, 8.19% hemicellulose and 4,90% lignin. Compared with the chemical components in the initial SSB fiber, it was clearly shown that NaOH pretreatment

increase cellulose by 2 times and decrease the lignin by 77.09 %. The increase of cellulose and the decrease of lignin contents can facilitate the process of enzymatic hydrolysis. Results of this research showed that the optimum pretreatment for sweet sorghum bagasse was obtained by using 10 % NaOH for 30 min. From the statistical analysis using two way anova showed that NaOH concentration give significant effects on hemicelluloce and cellulose contents after pretreatment ($p<0.05$). Compared to Dahnum *et al.* (2015) which pretreatment using NaOH 10% for EFB resulted only 38% lignin removal, these results clearly showed higher lignin removal.

Enzymatic saccharification

The insoluble cellulose-rich fraction or residual solid of pretreated bagasse of Sweet Sorghum was treated with a commercial cellulase preparation, at enzyme loadings of 30 FPU/g in SHF process. Cellulase is a mixture of several enzymes i.e cellulose and xylanase that consist of Cellic®CTec2 and Cellic®HTec2. Cellic® CTec2 is complex enzymes that consist of cellulase, β-glucosidase, and hemicellulase whereas Cellic® HTec2 consists of endoxylanase with high specificity toward soluble hemicellulose and cellulase. So, beside glucose a main product in saccharification process, hemicellulose also can be converted to xylose. The result of glucose and xylose during saccharification process obtained from HPLC analysis was shown in Figure1. This analysis predicted the sugar released by enzyme performance. Cellulose and hemicellulose were converted into glucose and xylose respectively using combined enzyme. As shown in Fig.1, glucose and xylose concentration reached the highest concentration at 48 h process for pre-treated SSB 10% NaOH, 30 min and at 72 h for pre-treated of SSB 10% NaOH, 10 min. As expected, higher glucose concentration results in higher yield of hydrolysis.

Fermentation

The process of fermentation was conducted using two methods: 1) Separated Hydrolysis and Fermentation (SHF) and 2) Simultaneous Saccharification and Fermentation (SSF). A series of experiments was first carried out to study the effect of variation of enzyme loading, i.e; 20, 30 and 40 FPU of Cellic® CTec2 per gram dry biomass on the rate and extend of hydrolysis of treated SSB. In these experiment the best hydrolysis process was obtained using enzyme cellulose complex 30 FPU.

Separate hydrolysis and fermentation process (SHF)

SHF is one method of saccharification and fermentation process that involves two sequential steps: enzymatic saccharification and fermentation. First the cellulose in substrate was enzymatically hydrolyzed to glucose. Second, the glucose in the hydrolyzed was subsequently fermented to ethanol. Both steps were conducted under their optimal condition (i.e. temperature 50^0C, pH 4.8 for hydrolysis and temperature 37^0C for fermentation). In SHF process, after 48 hours of enzymatic hydrolysis, process was continued by fermentation. Fermentation was monitored 72-hour, the results of SHF can be depicted in Figure 2.

Fig. 2 shows the existing compound the three components i.e., xylose, glucose, and ethanol during the fermentation process. The xylose appeared constant in the beginning until the last, this due to xylose was not used by *S. cereviceae* to produce ethanol in this fermentation process. *S. cereviceae* only convert glucose, while xylose needs other microbes such as *Clostridium thermohydrosulfuricum*, *Zymomonas mobilis,* etc, to convert themselves to ethanol (Olsson,1996). In this study, xylose was obtained as co-product at the end of fermentation.

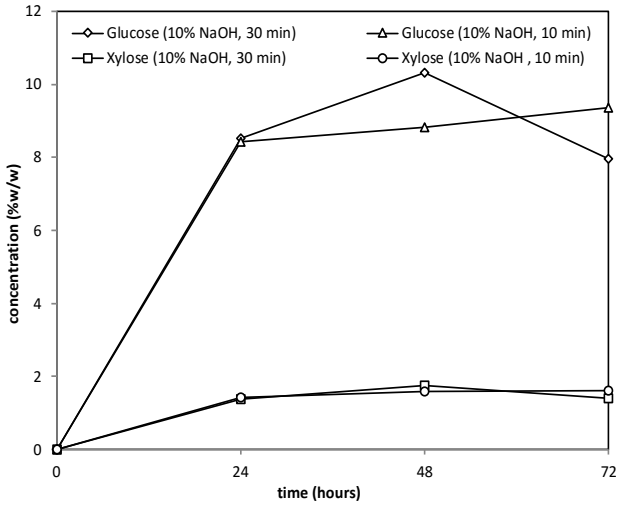

Fig.1 Hydrolysis process of SSB

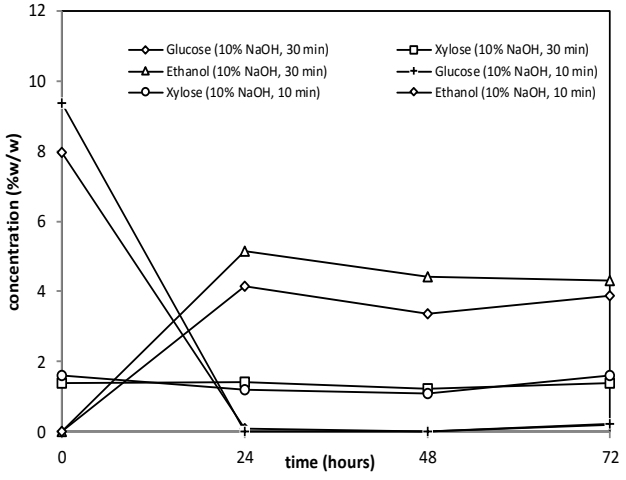

Fig. 2 Ethanol production from SSB by SHF Process

The ethanol concentration trend line (Fig. 2) was very similar to the dependent enzymatic hydrolyzability of bagasse substrate. This indicated that the generation rate of ethanol was consistent with the generation rate of glucose. The highest ethanol concentration was obtained from pre-treated bagasse of Sweet Sorghum 10% NaOH, 30 min

Simultaneous Saccharification and Fermentation Process (SSF)

SSF process allowed enzymes and yeast simultaneous added at the beginning of process. In this study, the temperature was the same as fermentation i.e., 32 ^{o}C. More saving energy is one of the advantages of the SSF process. Time of producing ethanol becomes shorter because the hydrolysis and fermentation were carried out at the same time. Once enzyme has produced glucose, the yeast, *S. cereviceae* changed the glucose to ethanol directly. It is a reason of the concentration of glucose which is always zero in the 24, 48, 72 hours fermentation. SSF process was conducted using various substrate bagasse of sweet sorghum treated with NaOH 10% for 10 and 30 min. The highest ethanol after 24 h fermentation reached by using SSB treated with NaOH 10% for 30 min (Fig 3).

The comparison of ethanol production from two methods of saccharification and fermentation process (SHF and SSF) can be seen in Table 2.

Table 2.
Comparison Yield Ethanol of SHF and SSF Processes of Sweet sorghum bagasse

Process	Substrate (Bagasse sorghum-treated)	Time (h)	Glu-cose yield	Final Ethanol conc. (g/L)	Ethanol yield (%)	Ethanol produc-tion (L/ton)
SHF	10% NaOH 30 min	72 h sacch. & 24 h ferment.	93.57	51.62	68.83	137.66
	10% NaOH 10 min	72 h sacch. & 24 h ferment.	86.30	41.50	59.73	110.66
SSF	10% NaOH 30 min	24 h SSF	-	66.88	81.28	162.57
	10% NaOH 10 min	48 h SSF	-	66.88	96.26	178.34

From the result, SSF was preferred than SHF in the hydrolysis and fermentation of lignocellulose process because rapidly ethanol production and the highest concentration of produced ethanol. SHF involves two sequential step, enzymatic saccharification and fermentation. Glucose was produced in saccharification process that glucose yield can be seen in Table 2. After saccharification glucose was converted into ethanol in fermentation process. Whereas in SSF, glucose produced from hydrolysis is simultaneously metabolized by microorganism to produce ethanol, so that the value of glucose yield can not be calculated.

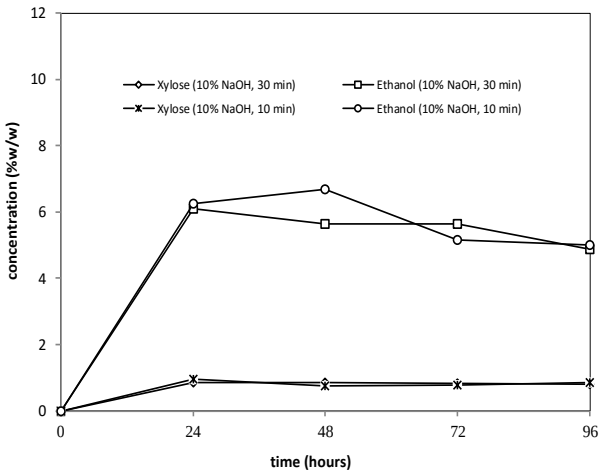

Fig. 3 Ethanol concentration from SSB of SSF Process

The yield of ethanol after fermentation from SHF process lower than in SSF process. It may be caused by the accumulation of hydrolysis product in the enzymatic process which causes feedback inhibition of the cellulolytic enzyme system. The highest yield of ethanol is 96.26% and high concentration of ethanol 66.88 g/L were obtained at SSF condition during 48 h process. Using SSF process could increase yields and concentration of ethanol with less energy process.

4. Conclusion

A number conclusion can be made from this study, alkali pretreatment using steam explosion is an effective pretreatment for SSB. Both time, temperature pretreatment affects the overall of hydrolysis and yield of fermentation. The SSF method was considered as a better process than SHF due to rapidly ethanol production and the highest concentration of produced ethanol.

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