Alkaline Pretreatment Effect on 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 feedstock for the production of energy and chemicals. The bagasse residue of sweet sorghum can be use utilized as raw material to alternative energy such as bioethanol. Bioethanol production consist of pretreatment, saccharification, fermentation and purification process. The pretreatment process is 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 separate 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 shows that maximum cellulose conversion to total glucose plus xylose were found to be greatest with NaOH 10% for 30 min. Maximum ethanol yield 92.19% and high concentration of ethanol 66.88g/L were obtained at SSF condition after 24 h.

Key words: Bagasse Sorghum, pretreatment, saccharification,fermentation, bioethanol.

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 sucrose, glucose and fructose 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 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).

Like other sources of lignocellulosic biomass, bagasse sorghum requires pretreatment to improve the efficiency of enzymatic hydrolysis. Several pretreatment methods have been developed to increase the enzymatic hydrolysis sugar yields from lignocellulosic biomass (Monsier et al, 2005; Taherzadeh et al., 2008). Alkaline pretreatment is one approach 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 mechanisms 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, Y., & Cheng, J. 2002).

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

Materials and Methods

Materials

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 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 $FPU \cdot g^{-1}$ cellulose (measured by NREL method), while the activity of Cellic®HTec2 is 240 $CBU \cdot g^{1}$ (reported by Novozyme). In this study, the saccharification applied Cellic®CTec2 of 30 $FPU \cdot g^{-1}$ 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 are used in this study were analytical grade, except the reagent of the pretreatment process, i.e. sodium hydroxide is industrial grade.

Procedure

Alkaline (NaOH) Pretreatment

Pretreatment was conducted using 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. Bagasse Sorghum in small size was heated using NaOH solution 6 and 10% (kg/L) at 130ºC for 10 and 30 minutes. A solid liquid ratio was 1:5. The pressure was controlled four bars at early heating. Bagasse Sorghum treated was washed until wash water turned to pH 7 and dried in the oven at 50 – 60 ºC overnight. The composition of materials component after pretreatment was analyzed according to National Renewable Energy Laboratory (NREL) standard procedures (Sluiter et al., 2012).

Saccharification and Fermentation

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, pretreated 15% (g/ml) in the 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 were measured as a product in this hydrolysis. After saccharification, 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 the each flask. Ethanol content, glucose, and xylose were monitored every 24 hour.

Simultaneous Saccharification and Fermentation (SSF)

SSF experiment were performed by duplicate in 250 mL flasks. Fifteen percent (g/ml) of pretreated EFB in 0.05 M buffer citrate in erlenmeyer flask was sterilized by autoclave at 121^{o}C for 20 minutes. The 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 hour. 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] folowing formula :

$$Yield_{ethanol} = \left[\frac{(C_{ethanol,f} - C_{ethanol,i})}{0.51f.\, C_{biomass}}\right] x100\%$$

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 from cellulose to ethanol.

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 maintain 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.

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. The main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass.

The precipitation bagasse sorghum from the sodium hydroxide pretreatment was quantified for lignocellulose components. The degradation of lignin and hemicelluloses in the bagasse sorghum fiber after NaOH pretreatment are represented as the component loss of lignin and hemicelluloses, shown in Table 1.

Table 1. The component content of sweet sorghum bagasse fiber 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 bagasse sorghum 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).

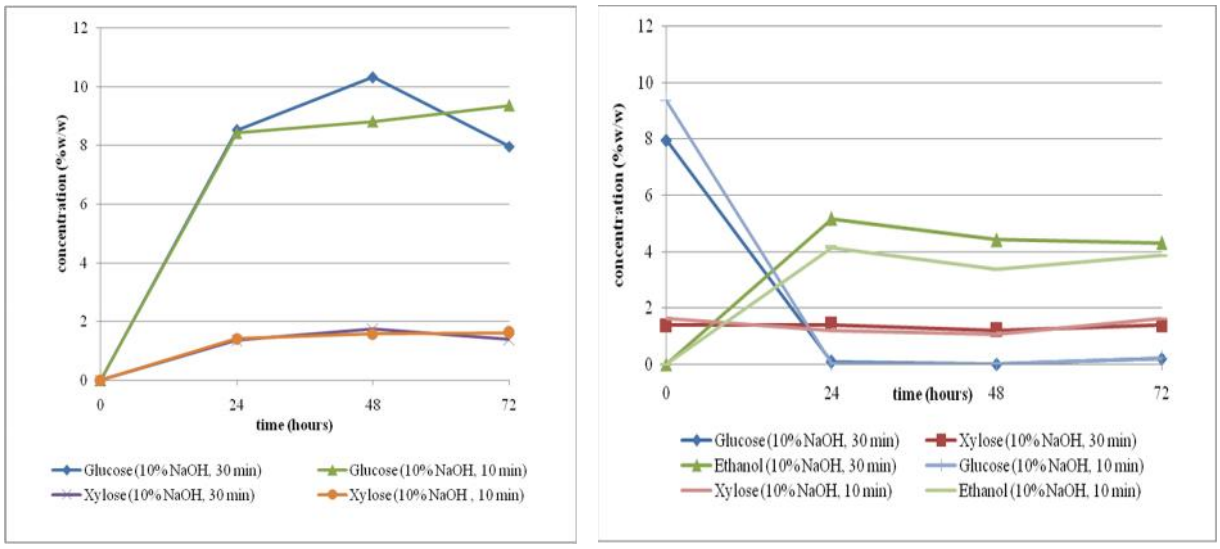
In this study increase residence time and alkali concentration, increased the loss of solids during NaOH pretreatment as shown in Table 1, the optimum pretreated BS fiber contains 77.80% cellulose, 8.19% hemicellulose and 4,90% lignin. Compared wit h the chemical components in the initial BS fiber, it was clear that NaOH pretreatment increased cellulose by 2 times and decreased lignin by 77.09 %. The increase of cellulose content and the decrease of lignin content 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 effects on hemicelluloce and cellulose content after pretreatment it was proven by p Value <0.05. Compared to Deliana et al., (2014) 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. During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol.

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 pretreated bagasse of Sweet Sorghum 10% NaOH, 30 min and at 72 h for pretreated bagasse of Sweet Sorghum 10% NaOH, 10 min. As expected, higher glucose concentration results in higher yield of hydrolysis.



(a) (b)

Figure 1. SHF Process (a) hydrolysis process; (b) fermentation process

The ethanol concentration trend line (Fig. 1b) was very similar as 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 pretreated bagasse of Sweet Sorghum 10% NaOH, 30 min.

While in SSF process, the enzymes and yeast simultaneous added at the beginning process. Glucose produced from hydrolysis is simultaneously metabolized by microorganism. Figure 2 showed the result of SSF process. From Fig. 2, ethanol content was tending to decrease after 48 hours, it could be said that, optimum time reaction in SSF process was 48 hours. SSF process is more efficient than previous processes (SHF) because only with 24 hours, ethanol has been formed.

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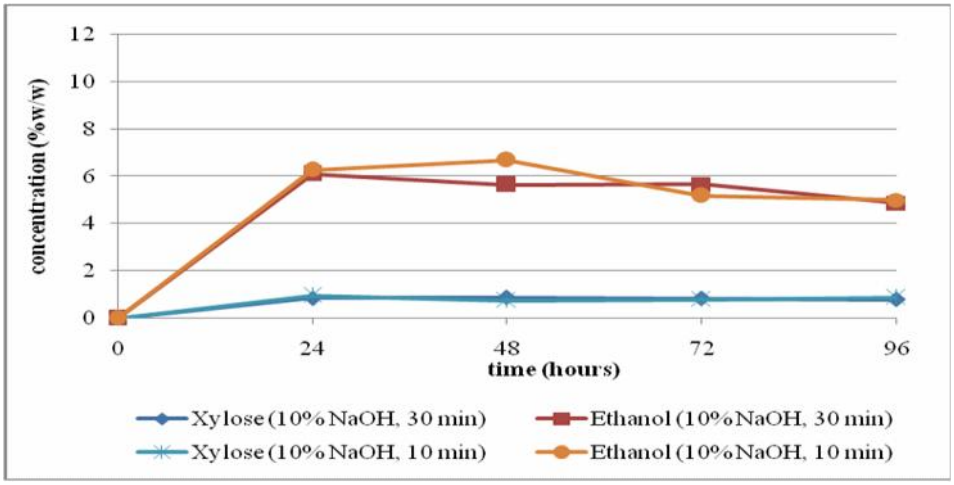

Figure 2. Ethanol concentration of SSF Process

The comparison of ethanol yield between SHF and SSF process can be seen in Table 2. The data showed that SSF process can produce ethanol concentration higher than SHF process. Moreover, pretreatment at 10% NaOH, 30 min produce higher ethanol concentration than

pretreatment at 10% NaOH, 10 min. The optimum ethanol concentration was 66.88 g/L, which produced in SSF process at 24 h (pretreatment 10% NaOH, 30 min) and at 48 h (pretreatment 10% NaOH, 10 min).

Tabel 2. Comparison of ethanol yield between SHF and SSF process

Process	Substrate (Bagasse sorghum-treated)	Time (h)	Final Ethanol conc. (g/L)	Ethanol yield
SHF	10% NaOH, 30 min	72 h saccharification & 24 h fermentation	51.62	78.07
	10% NaOH, 10 min	72 h saccharification & 24 h fermentation	41.50	65.20
SSF	10% NaOH, 30 min	24 h SSF	66.88	92.19
	10% NaOH, 10 min	48 h SSF	66.88	100.00

Conclusions

Pretreatment with alkaline NaOH result in high saccharification yield of bagasse sorghum associated with the reduction in lignin and hemicelluloses content and the increase in cellulose content.

Acknowledgements

This work was financially supported by PT. Panen Energy, Indonesia. The author thank to Hendris Hendarsyah, Irni Fitriani and Novita Ariani for assistance work done.

References

Ballesteros, M., Oliva, J.M, Negro, M.J., Manzanares, P., Ballesteros., I. (2004). Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces 39:1843 marxianusCECT 10875. Process Biochemistry. 39: 1843–1848.

Carvalheiro, F.,Duarte,L.C., Girio,F.M. (2008) hemicellulose biorefineries: a review on biomass pretreatments.J.Sci Ind. Res 67.849-864.

Deliana, D. Tasum, Sri Oktavia. Triwahyuni, E. Nurdin, Muhammad. Abimanyu,H. (2014). Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. Energy Procedia 68: 107-116.

Havannavar, R. B. & Geeta, G. S. (2007). Pre-treatment of agroresidues for release of maximum reducing sugar. Karnataka J. Agric. Sci. 20 (4) : 771-772.

Kumar, R., Wyman, C.E. (2009). Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. Biotechnol. Prog. 25:302–314.

Lijun Wang, zhenglin Luo, Abolghasem Shahbazi. (2013). Optimization of simulteneous saccharification and fermentation for the production of ethanol from sweet sorghum (Sorghum bicolor) bagasse using response surface methodology. Industrial Crops and products. 42 : 280-291.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol . 96: 673–686.

Shen,F., Saddler, J.N.,Liu,R.,Lin,L., Deng,S., Zhang,Y., Yang,G., Xiao,H., Li,Y. (2011). Evaluation of Steam pretreatment on sweet sorghum bagasse for enzymatic hydrolysis and bioethanol production. Carbohydrate Polymers. 86:1542-1548.

Sluiter, B., Hames, R., Ruiz, C., Scarlata, J., Sluiter, D., Templeton, M., and Crocker, D. (2011). Determination of structural carbohydrates and lignin in biomass. Technical report NREL/TP-510-Int.

Sun, Y., Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review, J Bioresource Technology. 83 : 1–11.

Taherzadeh, Muhammad J. and Karimi, Keikhosro. (2008). Pretreatment of Lignocellulosic Waste to Improve Bioethanol and Biogas Production, Int. J. Mol. Sci, 9: 1621-1651.

Zhao, Y., Wang, Y., Zhu, J.Y., Ragauskas, A. & Dengl, Y. (2008). Enhanced Enzymatic Hydrolysis of Spruce by Alkaline Pretreatment at Low Temperature. Biotechnol. Bioeng. 99 (6).