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Citation: AIP Conference Proceedings **1803**, 020015 (2017); doi: 10.1063/1.4973142
View online: http://dx.doi.org/10.1063/1.4973142
View Table of Contents: http://aip.scitation.org/toc/apc/1803/1
Published by the American Institute of Physics

# Optimization Pretreatment Condition of Sweet Sorghum Bagasse for Production of Second Generation Bioethanol

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**Abstract.** The bagasse residue of Sweet sorghum (*Sorghum bicolor* (L.) Moench) consist of cellulose 39.48%; hemicellulose 16.56% and lignin 24.77% that can be converted to ethanol. Pretreatment is of great importance to ethanol yield. In this study, pretreatment process was conducted in a 5-liter reactor using NaOH 10% at various temperature 110, 130, 150$^{o}$C and reaction time 10, 20, 30 minutes and optimizing severity parameter (log $R_0$ between 1.3 - 2.9). The statistical analysis using two way anova showed that third variations of temperature give different effects significant on lignin, hemicellulose and cellulose content at 95% the confidence level. The optimum pretreatment of bagasse sorghum were obtained with Log $R_0$ value between 2.4-2.9. High severity value in pretreatment condition reduce lignin almost 84-86%, maximum reducing lignin content was 86% obtained at temperature 150$^{o}$C for 20 minutes reaction time and cellulose increased almost two times the initial content.

## INTRODUCTION

Today bioethanol has become one of prospective biofuels in the transportation sector. Most of bioethanol produced in Indonesia currently is from molasses, or starchy raw materials, such as cassava and corn, known first generation bioethanol (G1). These materials are important raw materials for producing food and supporting food industries, so will not be sufficient to meet the increasing demand for the fuel ethanol [1]. On the other side, there are large amount of abundantly lignocellulosic biomass wastes of agricultural, estate crops and forestry industries that has not been used, can be converted to ethanol, called as second generation bioethanol (G2), is a possible candidate for a cheap and renewable source of energy.

The bagasse residue after extracting the juice from the sweet sorghum stalk is lignocellulosic material that consist of cellulose hemicellulose and lignin, these material can be hydrolyzed into sugar and further fermented to ethanol [2]. On the conversion of lignocellulosic to ethanol, need three key steps i.e. pretreatment to modify the lignocellulose structure, enzymatic hydrolysis to obtain sugars and fermentation process. Pretreatment of lignocellulose has received considerable research globally due to its influence on the technical, economic and environmental sustainability of cellulosic ethanol production. For developing countries, alkali-based methods are relatively easy to deploy in decentralized, low-tech systems owing to advantages such as the requirement of simple reactors and the easy of operation.

*International Symposium on Applied Chemistry (ISAC) 2016*
AIP Conf. Proc. 1803, 020015-1–020015-7; doi: 10.1063/1.4973142
Published by AIP Publishing. 978-0-7354-1471-6/$30.00

Alkaline pretreatment is the target to reduce lignin content to increase enzyme access to holocellulose [3,4], reduction of cellulose crystallinity [5,6], and increase in the surface area [7,8] and porosity [9,10] of pretreated substrates, resulting in increased hydrolysis rate. The mechanism of alkaline pretreatment is saponification of intermolecular ester bonds crosslinking xylan, hemicelluloses and other component [11,12].

To fully utilize bagasse residue of sweet sorghum as a feedstock for ethanol production, optimal pretreatment is required to render the cellulose fiber more amenable to the action of hydrolize enzyme. The main goal of this work is to study the effect pretreatment of alkaline NaOH 10% concentration, reaction time and temperature on the sweet sorghum bagasse as raw material for ethanol production, function of pretreatment time and temperature was investigated to optimize the operating parameter of pretreatment, while glucose production is the parameter for evaluating allow enzymatic performance.

# MATERIALS AND METHODS

## Materials

Bagasse of Sweet Sorghum (*Sorghum bicolor* (L.) Moench) called (BS) was obtained from PT. Panen Energy, Malang East Java, Indonesia. After air-dried, physical pretreatment i.e. chipping and milling until 3 mm was conducted to maximize contact area of the substrate.

## Alkaline Pretreatment

In this work optimization process of pretreatment was conducted using bench scale reactor a 5-liter at the Research Centre for Chemistry, Indonesian Institute of Sciences (LIPI). 250 g of BS (10% moisture content) was heated using NaOH 10% (kg/L) at 110,130 and 150$^{o}$C for 10, 20 and 30 minutes. A solid liquid ratio was 1:5. The pressure was controlled four bars at early heating. After pretreatment, the material was pressed in order to separate solidity from liquid fraction. Solidity of BS treated was washed until wash water turned to pH 7 and dried in the oven at 50-60$^{o}$C overnight. The composition of materials component before and after pretreatment was analyzed according to National Renewable Energy Laboratory (NREL) standard procedures [13].

The treatment severity was quantified by a semi-empirical parameter called severity parameter, log $R_0$, combining pretreatment time and temperature acording to the equation Overend, 1987, [14].

$$R_0 = t.e^{\left[\frac{T-100}{14.75}\right]} \quad (1)$$

where t is the time in minute and T the temperature in degree Celcius.

**TABLE 1**. Severity Factor of Experiment

| Pretreatment | | Log $R_0$ |
|---|---|---|
| Temperature ($^oC$) | Time (min) | |
| 110 | 10 | 1.3 |
| 110 | 20 | 1.6 |
| 110 | 30 | 1.8 |
| 130 | 10 | 1.9 |
| 130 | 20 | 2.2 |
| 130 | 30 | 2.4 |
| 150 | 10 | 2.5 |
| 150 | 20 | 2.8 |
| 150 | 30 | 2.9 |

## Enzymatic Hydrolysis

Two Enzyme sellulace Cellic®CTec2 and Cellic®HTec2 were applied for hydrolysis (saccharification) process of pretreated BS. 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. Hemicellulose could be converted to xylose because Cellic® CTec2 has hemicellulase and Cellic® HTec2 has endoxylanase using combined enzymes. The activity of Cellic®CTec2 is 144 $FPU \cdot g^{-1}$ cellulose (measured by NREL method) [15], 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. The optimum hydrolysis condition for enzyme provided by company of Novozyme was temperature and velocity agitation was set $50^oC$ and 150 rpm respectively.

Duplicate process was arranged to get the best approach. The samples, containing pretreated 15% (g/ml) in the erlenmeyer flask containing 0.05 M the buffer citrate with pH 4.8, were autoclaved at $121^oC$ 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. Sampling every 24 hour was employed to monitor producing sugar, glucose and xylose were measured as a product in this hydrolysis.

## Statistical analysis

The content of chemical component of cellulose, hemicellulose, lignin, sugar and ethanol were analized with Two Way ANOVA procedurs using the software SPSS 16 and statistical significance confidance of 95% to evaluate the significant differences among mean treatments.

# RESULT AND DISCUSSION

## Pretreatment Condition

Pretreatment using alkali solution such as NaOH, CaOH or amonia can remove lignin and a part of hemicelluloses that caused weight loss [14,15]. In these study pretreatment process of BS was conducted with NaOH 10% (kg/L), biomass quantity each pretreatment was 250 g. After treatment and followed wash neutralization and dried, the mass was measured to calculate biomass recovery, the result as shown in Table 2.

**TABLE 2**. Pretreated Samples Results

| Pretreatment | | After Treatment Charged Dry Matter (g) | Weight Loss (g) | Yield % | Recovered Biomass (%) |
|---|---|---|---|---|---|
| Temperature ($^oC$) | Time (min) | | | | |
| 110 | 10 | 150.27 | 99.73 | 39.89 | 60.11 |
| 110 | 20 | 149.28 | 100.72 | 40.29 | 59.71 |
| 110 | 30 | 103.51 | 146.49 | 58.60 | 41.40 |
| 130 | 10 | 134.28 | 115.72 | 46.29 | 53.71 |
| 130 | 20 | 149.34 | 100.66 | 40.26 | 59.74 |
| 130 | 30 | 139.17 | 110.83 | 44.33 | 55.67 |
| 150 | 10 | 154.43 | 95.57 | 38.23 | 63.44 |
| 150 | 20 | 158.59 | 128.60 | 51.44 | 65.56 |
| 150 | 30 | 163.89 | 86.11 | 34.44 | 63.44 |

After pretreatment, showed that some pretreatment results in terms of pretreatment efficiency is expressed as recovered biomass in percentage compared to initial biomass charged into the reactor. Dilute NaOH application loosens the biomass structure, separate the bonds between the lignin and the carbohydrates, increases the internal surface, decreases the degree of polymerization and crystallinity, and disrupts the lignin structure, that caused weight loss [15,16]. Highest recovered biomass was observed at temperature 150$^o$C.

## Composition components of Pretreated substrates

Before pretreatment the carbohydrate fraction of BS (cellulose and hemicellulose) were 56.04% of total biomass, and the major component were cellulose (39.48%). Meanwhile, lignin content of BS was 24.77%, comparable to the lignin content of empty of fruit bunch (25.83%) [17]. Lignocellulosic biomass requires pretreatment, mainly because the lignin in plant cell walls forms a barrier against enzyme attack. An ideal pretreatment reduces the lignin content and crystallinity of the cellulose and increase surface area [18]. The loss of lignin in the pretreatment is one of the most important indicators of pretreatment effectiveness because the presence of lignin impedes enzymatic hydrolysis of the carbohydrates [15]. In order to degrade and reduce the lignin and non-cellulose component, BS was treated using alkaline solution NaOH 10%, nine samples were selected for the folowing stage, in the range Log $R_0$ 1.3-2.9. The effect of NaOH 10% at different temperature and different times pretreatment. After treatment at all condition, the BS was quantified for lignocellulose components, the results as shown in Tabel 3.

Pretreatment changed the BS composition. From Table 3 it can be seen during pretreatment delignification was occured and the process was effective for reduction of hemicellulose, so the proportion of lignin decreased, and the cellulose content after pretreatment was increased in all pretreatment condition. The results shows the optimum pretreatment condition was NaOH 10% at 150$^o$C for 10-30 minutes, with the optimum loss of lignin and hemicellulose was 86%, and 55% respectively. Further increasing reaction time also affected in increase of cellulose content from 10 to 20 minutes and there was no significant effect reaction time after 20 minutes. The optimum cellulose content was 86.85% was achived at 150$^o$C for 20 minutes. Comparing the two parameter, we observe an approxymately constant trend which does influence of recovered biomass in line with cellulose content after treatment. The increase of cellulose content and the decrease of hemicellulose and lignin content can facilitate the process of enzymatic hydrolysis.

**TABLE 3**. Chemical Composition of Bagasse Sweet Sorghum

| Pretreatment Temperature ($^oC$), Time (min) | Cellulose % | Hemicellulose % | Lignin % | Ash % |
|---|---|---|---|---|
| Untreated | 39.48 | 16.56 | 24.77 | 4.20 |
| 110, 10 | 76.52 | 10.03 | 8.61 | 0.16 |
| 110, 20 | 78.06 | 9.94 | 8.31 | 0.32 |
| 110, 30 | 84.03 | 9.93 | 8.08 | 0.28 |
| 130, 10 | 81.74 | 8.87 | 6.16 | 0.25 |
| 130, 20 | 83.32 | 9.37 | 6.11 | 0.37 |
| 130, 30 | 88.25 | 8.57 | 5.45 | 0.55 |
| 150, 10 | 86.72 | 7.37 | 3.76 | 0.25 |
| 150, 20 | 86.85 | 7.45 | 3.51 | 0.52 |
| 150, 30 | 86.44 | 7.50 | 3.49 | 0.53 |

Further investigation for pretreatment parameters were carried out, using the analysis of two way ANOVA, to identify which parameter significantly affect the component cellulose, hemicellulose and lignin.

**TABLE 4**. ANOVA

| Source | DF | Lignin | | | | Hemicellulose | | | | Cellulose | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | MS | F | Sig | SS | MS | F | Sig | SS | MS | F | Sig |
| Corrected Model | 8 | 68.16 | 8.52 | 2479 | 0.00 | 21.43 | 2.68 | 419.25 | 0.00 | 268.83 | 33.60 | 100.89 | 0.00 |
| Intercept | 1 | 636.40 | 636.40 | 185200 | 0.00 | 1380.17 | 1380.17 | 216000 | 0.00 | 125646.25 | 125646.25 | 377200 | 0.00 |
| Temperature | 2 | 67.19 | 33.59 | 9774 | 0.00 | 20.74 | 10.37 | 1623 | 0.00 | 159.75 | 79.87 | 239.80 | 0.00 |
| Time | 2 | 0.73 | 0.36 | 105.51 | 0.00 | 0.20 | 0.10 | 15.83 | 0.00 | 68.67 | 34.33 | 103.08 | 0.00 |
| Tempera-ture*Time | 4 | 0.25 | 0.06 | 18.33 | 0.00 | 0.49 | 0.12 | 18.97 | 0.00 | 40.42 | 10.11 | 30.34 | 0.00 |
| Error | 9 | 0.03 | 0.00 | | | 0.06 | 0.01 | | | 3.00 | 0.33 | | |
| Total | 18 | 704.60 | | | | 1401.66 | | | | 125918.08 | | | |
| Corrected Total | 17 | 68.19 | | | | 21.49 | | | | | | | |
| R Squared (Adjst. R Squared) | | 1.00 (0.999) | | | | 0.997 (0.995) | | | | 0.989 (0.979) | | | |

Considering parameter condition, optimal pretreatment seem to move towards higher Log $R_0$ 2.4. In these study best results in terms of raw material (cellulose content 39,48%) was obtained at temperatur 150$^o$C. These results in line with the statistical analysis using two way anova showed that third variations of temperature give different effects significant on lignin, hemicellulose and cellulose content at 95% the confidence level.

Considering the overall proccess, optimum pretreatment of bagasse sorghum were obtained with Log $R_0$ value between 2.4-2.9. High severity value in pretreatment condition resulted high in reduce lignin almost 84-86%, maximum reducing lignin content was 86% obtained at temperatur 150$^o$C for 20 minutes reaction time and cellulose increased almost two times the initial content.

## Enzymatic hydrolysis of treated substrate

Enzymatic hydrolysis is a step after pretreatment for bioethanol production. The BS substrate treated by NaOH 10% at 150$^o$C was subjected to enzymatic saccharification. This condition was selected based on the highest component of celullose of BS treated for 10, 20 and 30 minutes was 86.44-86.85% and the lowest component of lignin at 3.49-3.76%. The BS substrate was treated with commercial cellulase preparation at enzyme loading 30 FPU/g substrate. The result can be seen in Figure 1.

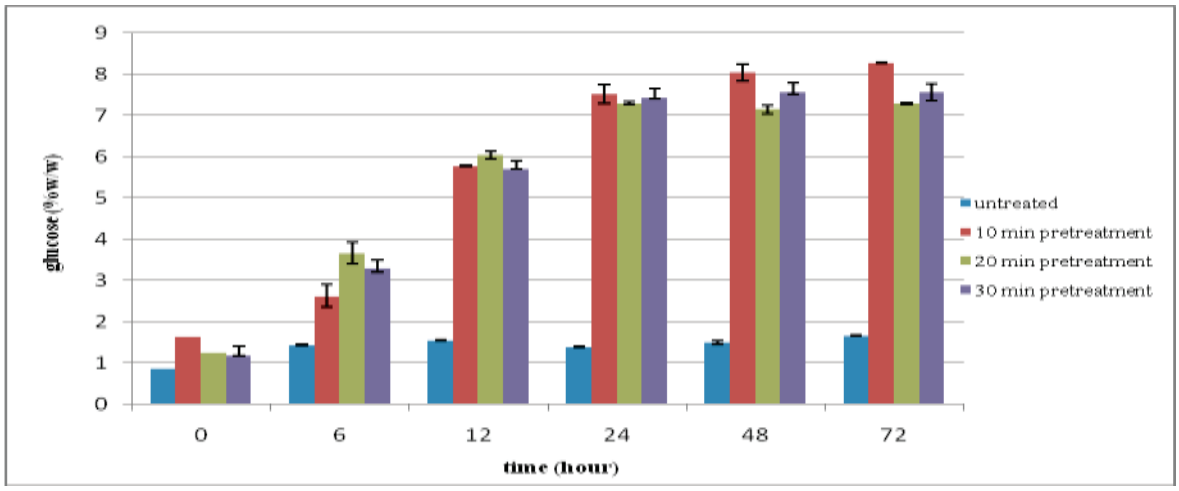


**FIGURE 1.** Glucose concentration from enzymatic hydrolysis of pretreated subtrates with alkaline pretreatment at 150°C and reaction time 10, 20 and 30 minutes

Figure 1 showed that glucose concentration from each substrates increased for 72 h of enzymatic hydrolysis. The treated substrates produced higher glucose concentration than untreated substrate. It is because of reduction of lignin content, reduction of cellulose crystallinity and increasing porosity of treated substrates. The highest concentration of glucose concentration can be obtained by pretreatment substrate at 150oC, 10 minutes. The optimum time for enzymatic hydrolysis was 48 h that produce glucose almost similar with 72 h, i.e. above 8% w/w. From the data can be conclude that alkaline pretreatment at 150oC, 10 minutes has been enough to produce high glucose concentration.

## CONCLUSIONS

Considering the overall proccess, optimum pretreatment of bagasse sorghum were obtained at Log R0 value between 2.4-2.9. Based on the results, pretreatment at 150oC gives the highest of cellulose content and the lowest of lignin content. The pretreatment condition of bagasse sorghum at 150oC for 10 minutes produces the optimal glucose concentration for bioethanol production.

## ACKNOWLEDGEMENTS

The authors are in deeply indebted to Hendris Hendarsyah Kurniawan, and Novita Ariani for their technical support and cooperation.

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