Impact of NaOH Concentration and Pretreatment Time on the Lignocellulose Composition of Sweet Sorghum Bagasse for Second-Generation Bioethanol Production

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Abstract. Using lignocellulosic materials as raw materials to manufacture alternative fuels second generation (G2) is a solution for substituting fossil fuels. One source of lignocellulose is sweet sorghum bagasse. The pretreatment process is essential to increase the percentage of cellulose in the material before fermentation and hydrolysis. So, this study aims to evaluate the NaOH concentration and pretreatment time effect on the lignocellulose composition of pretreated sweet sorghum bagasse. The Factorial Completely Randomized Design (FCRD) experimental design was chosen with two independent factors, namely NaOH concentration with two levels (4% and 8%) and the pretreatment time with two levels (10 min and 30 min). The observed responses data was analyzed using a Two-Way analysis of variance (ANOVA) at a 95% confidence level, and if there were any significant differences, followed by Duncan multiple range tests (DMRT). The results showed that both independent factors affected (p-value < 0.05) the lignocellulose composition of pretreated sweet sorghum bagasse, especially in lignin reduction. The best combination in pretreated sweet sorghum bagasse was found at 8% NaOH concentration and 30 min of the pretreatment time. In this condition, the cellulose increased by 13.56%, and hemicellulose and lignin decreased by 3.09% and 10.12%, respectively, to the raw material.

Keywords: Bioethanol · Lignin · Lignocellulose Composition · NaOH concentration

1 Introduction

In the last century, the increase of industrialization in all sectors and the growth of the population worldwide was caused by the increasing consumption of energy, especially fuel-based fossils [1, 2]. The increasing utilization of fuels-based fossils is potentially

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creating an energy crisis and increasing environmental problems and global warming issues.[3] So, renewable energy from sustainable and green sources must seek fuel security and a friendly environment. One of the promising and potentially sustainable and green materials for biofuel material is lignocellulosic biomass [4, 5] because it does not compete and is a threat to food security, and usually is a form of agricultural by-product [6, 7].

The sweet sorghum bagasse (*Sorghum* bicolor L. Moench) is an agricultural product by-product from sweet sorghum juice in the sugar industry [8] and one of the potential lignocellulose materials for developing the biofuel material, especially for bioethanol production in Indonesia. It is abundant in nature, has high content, and is detached from the food chain, so it does not compete with the food sectors. Chemically, the sweet sorghum bagasse consists of 31.72 to 39.59% cellulose, 30.91 to 32.05% hemicellulose, 20.36 to 21.40% lignin [8,9], and 2.17% of ash content [9]. However, the cellulose of sweet sorghum bagasse was still bonded with hemicellulose and lignin in the lignocellulose matrix. Therefore, pretreatment is needed to degrade lignin and break down of lignocellulose structure to increase the carbohydrate accessibility of sweet sorghum bagasse and facilitate further hydrolysis and fermentation to producing bioethanol [10]. [11, 5] Alkali pretreatment was used to break down the lignocellulose structure in biomass. According to Tan et al. (2021) [5], chemical treatment, including alkalization, can change the lignocellulose structure in biomass.

The alkaline pretreatment with NaOH is the most efficient conventional method for pretreatment than other methods, such as acid and heat pretreatments, due to a few effects on the sugar product degradation and the lower inhibitor formation [12]. Furthermore, the utilization of NaOH as a solution medium in the pretreatment process can breakdown the crystalline structure of cellulose and lignin from the lignocellulose matrix by destroying the intermolecular bond between the ester and C-C bond, so increasing the cellulose and hemicellulose accessibility, as well as facilitating the further hydrolysis and fermentation [13]. Several previous studies reported the alkaline pretreatment with NaOH effectively improved the cellulose content and decreased the hemicellulose and lignin in the various lignocellulosic materials, like as cocoa pod husk [11], rubberwood sawdust [5], *Miscanthus sacchariferous* [4], wheat straw [2]¸ palm empty fruit bunch [1], *Sida acuta* [10], and rice straw [3]. [7] Based on the description and facts described in advance, this study aimed to know the effect of the NaOH concentration and pretreatment time on the lignocellulose composition of pretreated sweet sorghum bagasse.

2 Methods

2.1 Research Procedures

Material Preparation

This study used raw materials for sweet sorghum bagasse obtained from Pasuruan. Previously, sorghum bagasse had been reduced in size using a grinder of up to 5 mm. Furthermore, sorghum bagasse is measured in water content using a moisture analyzer. The sorghum bagasse used for this preliminary treatment process has a moisture content value of $\leq$ 12%. If the sorghum bagasse is above 12%, it is dried in an oven at 50 ºC for

24 h until the moisture content drops. Then, sorghum bagasse weighed as much as 250 g and was sealed into a plastic sealer.

Preliminary Treatment Process
The preliminary treatment of sweet sorghum bagasse was carried out on an explosion bench scale reactor (Changhae Engineering Co., Ltd.) Previously operating temperature, time, and pressure are determined in advance. The operating temperature was set at 130 °C. In the preliminary treatment, the ratio of biomass and solvent used is 1: 5 (250 g of biomass with 1.25 L of solvent). The solvent used is NaOH, with 4% and 8%. Part of the solvent is put into the reactor and continued with the biomass of sweet sorghum bagasse. Then the rest of the solvent is introduced. The pressure is set at 4 bar. After reaching the operating temperature, the start time is calculated according to the variations of 10 min and 30 min. The preliminary treatment results are then accommodated and put into a filter bag, which is then pressed using a hydraulic press to separate the black leachate from the substrate. Black leachate is accommodated and measured in volume. In contrast, the substrate is washed to a neutral pH and dried in an oven at 50 °C for 72 h.

2.2 Design Experiment

This study used a Factorial Complete Randomized Design (FCRD) with two independent factors: NaOH concentration and pretreatment time. The level used for each independent factor is two levels. The NaOH concentration was 4 and 8%, while the pretreatment time was 10 min and 30 min.

2.3 Research Parameters

The research parameters, including cellulose, hemicellulose, and lignin content, were analyzed using the Laboratory Analytical Procedures (LAP) established by the National Renewable Energy Laboratory (NREL) [14]. An analysis of the insoluble lignin content in acid (acid-insoluble lignin, AIL) was carried out using a furnace. Meanwhile, the analysis of the content of lignin soluble in acid (soluble acid lignin, ASL) was carried out using a UV/Vis Optizen 2120 UV-spectrometer. Total lignin is obtained from the summation of AIL and ASL. Cellulose and hemicellulose content was measured using High-Performance Liquid Chromatography (HPLC) Waters e2695.

2.4 Data Analysis

The data obtained were then analyzed using two-way ANOVA with a 5% confidence interval. If there is a noticeable difference in the ANOVA results, then a further test is carried out using DMRT (Duncan Multiple Range Test) at a confidence level of 5%.

3 Results and Discussion

3.1 Cellulose Content

Cellulose is one of the compositions in the material that needs to be considered for the process of bioethanol production. The cellulose composition is expected to increase further when the pretreatment process is carried out on sweet sorghum bagasse. The increasing cellulose content in the material will increase the glucose content produced in hydrolysis. In this study, the cellulose content of pretreated sweet sorghum bagasse was 44.60 to 57.33%, higher than the cellulose content of the raw sweet sorghum bagasse, which was 34.05%. The alkaline pretreatment increased the cellulose content of pretreated sweet sorghum bagasse by 10.55 to 23.28% more of the raw material. The ANOVA showed that the NaOH concentration and interaction of factors had a significant effect ($p < 0.05$) on the cellulose content of pretreated sweet sorghum bagasse. However, the pretreatment time had an insignificant effect. The lower cellulose content of pretreated sweet sorghum bagasse was found at the NaOH concentration of 8% and 10 min pretreatment time with 44.60%. In comparison, the combination of NaOH concentration of 4% and 10 min of pretreatment time revealed a higher cellulose content of 57.33%, as revealed in Fig. 1. The cellulose content in the pretreated sweet sorghum bagasse matrix depended on other compositions, like hemicellulose and lignin.

The lower NaOH concentration and longer pretreatment time enhanced the cellulose content in the pretreated sweet sorghum bagasse revealed in this study. It is caused by the lower NaOH concentration (4%) at all pretreatment times, disrupting the hemicellulose and the lignin in the complex matrix in the sweet sorghum bagasse. According to Mulyaningtyas and Budi (2019) [1], NaOH possessed distinct properties that increased swelling, increased the internal surface of cellulose, decreased crystallinity, and enhanced lignin disruption. Another fact is that during the pretreatment procedure, the amorphous area of cellulose would be broken down by the NaOH solution. Hence the simulant's crystallinity and cellulose content were enhanced [5]. However, the amount of cellulose in the pretreated sweet sorghum bagasse was reduced due to the extended pretreatment time and rising NaOH concentration. All lignocellulose components decreased, which may be due to the simultaneous breakdown of all lignocellulose (cellulose, hemicellulose, and lignin) in the complex matrix of sweet sorghum bagasse, so all lignocellulose components were decreased [7].

3.2 Hemicellulose Content

In a pretreatment, the decrease in hemicellulose content is one of the critical factors. The decrease in hemicellulose content is also a success factor of the pretreatment. In this study, the hemicellulose content of pretreated sweet sorghum bagasse was 11.78 to 21.76%. The alkaline pretreatment increased the hemicellulose content of pretreated sweet sorghum bagasse in NaOH concentration of 4% by 10.55 to 23.28% and decreased hemicellulose content in NaOH concentration of 8% by 2.95 to 3.05% than the raw material. The ANOVA showed that the NaOH concentration, pretreatment time, and interaction of factors had a significant effect ($p < 0.05$) on the hemicellulose content

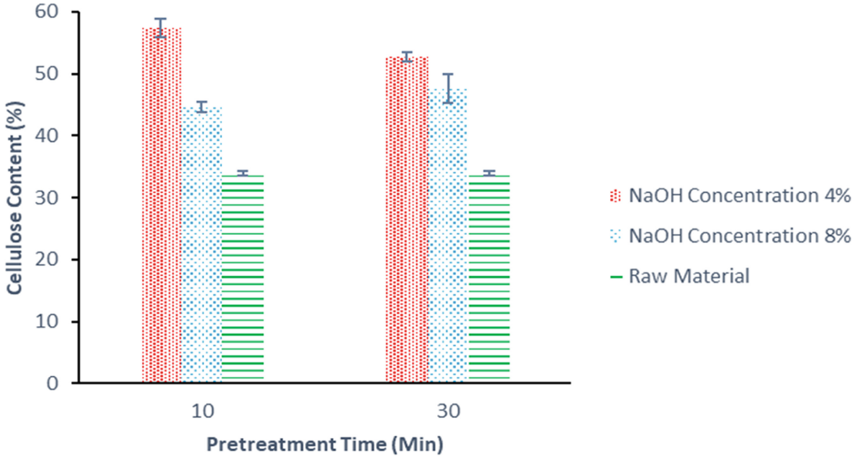

Fig. 1. The cellulose content of sweet sorghum bagasse

of pretreated sweet sorghum bagasse. Figure 2 revealed that the lower the NaOH concentration and shorter pretreatment time were produced, the higher the hemicellulose content in the pretreated sweet sorghum bagasse. The lower hemicellulose content of pretreated sweet sorghum bagasse was found at the NaOH concentration of 8% and 10 min pretreatment time with 11.93%. In comparison, the NaOH concentration of 4% and 10 min of pretreatment time revealed a higher hemicellulose content of 21.76%. In this study, using a higher NaOH concentration (8%) in all pretreatment times caused a decrease in the hemicellulose content in pretreated sweet sorghum bagasse than in raw material (14.87%). The immersion in higher NaOH concentration (8%) at a particular time increased depolymerization or degradation in hemicellulose and cellulose of sweet sorghum bagasse, so it affected decreased hemicellulose and cellulose content after pretreatment [15]. Elwin, Lutfi, and Hendrawan (2014) [16] reported that NaOH, as a strong alkaline, broke down the hemicellulose structure in materials and released it into the NaOH medium. Moreover, the more prolonged pretreatment time and higher NaOH concentration can break down the more hemicellulose in raw material to release them into an immersion medium (NaOH solution), so the hemicellulose content in the pretreated material will be decreased. Tsegaye, Balomajumder, and Roy (2019) [2] also reported increasing the NaOH concentration and pretreatment time until the optimum condition (1.5% NaOH concentration and 15 min, respectively) was increased the hemicellulose content of pretreated wheat straw for producing biofuel. Additionally, the hydrolyzable couplings of glycosidic bonds in hemicelluloses likely contributed to the hemicellulose removal with increasing NaOH concentration and pretreatment duration, leading to the partial removal of hemicellulose [17]. However, the higher hemicellulose in pretreated sweet sorghum bagasse is essential to increasing the production of fermentable sugars and bioethanol in the following stages.

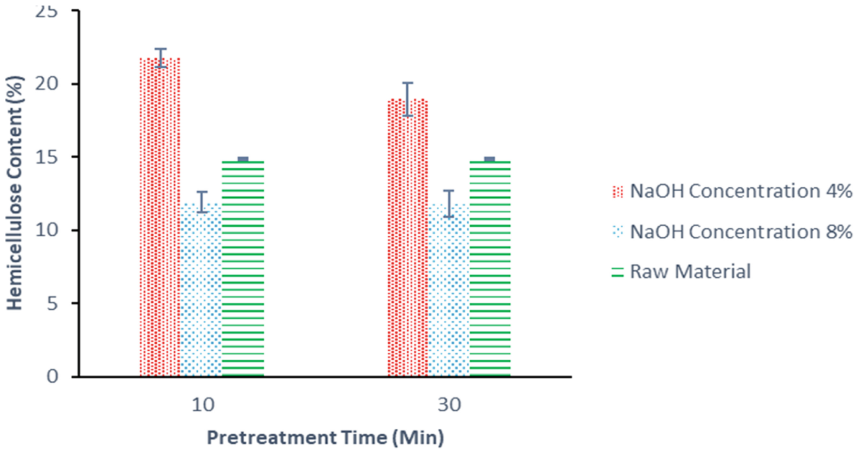

Fig. 2. The hemicellulose content of sweet sorghum bagasse

3.3 Lignin Content

Reducing the amount of lignin is one of the critical goals in pretreatment. It caused the structure of lignin to become an inhibitor to get cellulose from lignocellulosic materials. Pretreatment is successful if the lignin content decreases significantly compared to the raw material. In the present study, the lignin content of pretreated sweet sorghum bagasse was 11.50 to 17.93%, and it was lower than the raw sweet sorghum bagasse (21.61%). The alkaline pretreatment decreased the lignin content of pretreated sweet sorghum bagasse by 3.68 to 10.12% compared to the raw material. The ANOVA showed that the NaOH concentration, pretreatment time, and interaction of factors had a significant effect ($p < 0.05$) on the lignin content of pretreated sweet sorghum bagasse. The higher the NaOH concentration and longer pretreatment time were produced, the lower the hemicellulose content in the pretreated sweet sorghum bagasse, as revealed in Fig. 3. The lower lignin content of pretreated sweet sorghum bagasse was found at the NaOH concentration of 8% and 30 min pretreatment time with 11.50%. In comparison, the combination of NaOH concentration of 4% and 10 min of pretreatment time revealed a higher lignin content of 17.93%. The increased NaOH concentration and pretreatment time decreased the lignin content in sweet sorghum bagasse. It might be due to the longer pretreatment time effectivity increased the contact of the NaOH solution and the sweet sorghum bagasse, so the NaOH solution to be more ease to degradation and leaching of the lignin from the matrixes of sweet sorghum bagasse to medium solution, and it is affected by decreased lignin in the pretreated sweet sorghum bagasse.

The results were in line the Harun and Geok's study. Harun and Geok (20) [3] reported that the lignin content in pretreated rice straw, both acid-insoluble and soluble lignin decreased with the NaOH, and pretreatment time was increased. Tsegaye, Balomajumder, and Roy (2019) also reported increasing the NaOH concentration and pretreatment time until the optimum condition (1.5% NaOH concentration and 15 min, respectively) was increased the lignin removal in pretreated wheat straw for producing biofuel. The higher lignin removal is highly correlated with the depolymerization

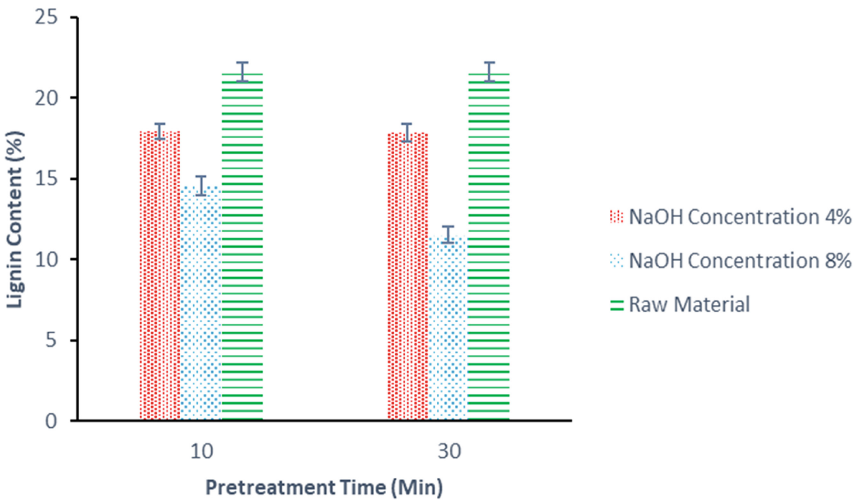

Fig. 3. The lignin content of sweet sorghum bagasse

of wheat straw. Manu and Clarkson (2022) also reported that the increasing NaOH concentration degraded the lignin in the raw material matrix and tended to change to pseudo lignin. In addition, Because of the lignin's solubilization in the NaOH solution, the pretreated sweet sorghum bagasse's lignin was removed [18]. Furthermore, the hydrolyzable connections of the a- and b-aryl ethers in lignin, which led to the partial removal of lignin, were likely the cause of the lignin removal [17].

4 Conclusion

The NaOH concentration, pretreatment time, and interaction of factors had a significant effect ($p < 0.05$) on the hemicellulose and lignin content of pretreated sweet sorghum bagasse. Moreover, the cellulose content of pretreated sweet sorghum bagasse is only affected by the NaOH concentration and interaction of factors. The best combination in pretreated sweet sorghum bagasse was found at 8% NaOH concentration and 30 min of the pretreatment time. In this condition, the cellulose increased by 13.56%, and hemicellulose and lignin decreased by 3.09% and 10.12%, respectively, to the raw material. The following research needs to optimize the NaOH concentration and pretreatment time to obtain the optimum pretreatment condition for sweet sorghum bagasse before the fermentation and hydrolysis for bioethanol production. Furthermore, the pretreatment process must be scaled to a larger scale with a low cost and economical.

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