Effect of Alkali Pretreatment on Removal of Lignin from Sugarcane Bagasse

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Lignin is complex polymer compound contained in woody plant tissue. In sugar extraction and bioethanol production from lignocellulosic materials, it is necessary to remove lignin due to its recalcitrant nature. The present work aimed to study the effect of alkali pretreatment on removal of lignin from sugarcane bagasse using sodium hydroxide. Two solid loading conditions (20 %w/v and 10 %w/v) were used in this study. For 20 % loading condition, base concentrations were varied at four levels and autoclaving time was varied at 3 levels, and the experiments were performed in 12 runs for this loading rate. For 20 % w/v solid loading, the concentrations of sodium hydroxide were 0.5 wt%, 1.0 wt%, 1.5 wt%, and 2.0 wt% at 121 °C for 10 min, 30 min and 60 min. For 10 % loading condition, the levels of each factor were increased. The base concentrations were varied at six levels and autoclaving time was varied at 5 levels, and the experiments were also performed, resulting in 30 runs for this loading rate. For 10 % w/v solid loading, concentrations were 0.5, 1.0 wt%, 1.5 wt%, 2.0 wt%, 3.0 wt% and 4.0 wt% at 121 °C for 10 min, 30 min, 60 min, 90 min and 120 min. The maximum removal of lignin for 10 % solid loading was 83.7 % under the conditions of 2.0 wt% sodium hydroxide at 121 °C for 60 min. For 10 % solid loading, 87.3 % of lignin removal was achieved in 1.5 wt% sodium hydroxide at 121 °C for 30 min. The result showed that increased solid loading may cause insufficient removal of lignin during alkaline pretreatment.

1. Introduction

Lignin can be found as deposited cell walls surrounding cellulose and hemicellulose contained in wood plant tissueIt may include 15 - 20 % of total feedstock dry matter. Lignin is an aromatic and rigid polymer composed with phenolic compounds of phenyl propionic alcohol such as cofiferyl, sinapyl and counmaryl alcohol, forming the complex matrix. This complex matrix contains the various kinds of functional group such as hydroxyl, methoxly and carbonyl. Softwoods and hardwoods contain lignin. The amount of lignin in softwoods is higher than in hardwoods, ranging from 20 % to 40 % by weight of lignin contents in both softwoods and hardwoods (Van Dyk and Pletschke, 2012). Aside from these types of wood, lignin can also be found in non-woods.
In sugar extraction and bioethanol production from lignocellulosic materials, it is needed to remove lignin due to its recalcitrant nature. It is one of the main barriers to economical production of bioethanol (Balat, 2011) and one of the challenges to lignocellulosic bioethanol production (Limayen and Ricke 2012).
The pretreatment method can be employed in various ways. These methods can be categorised into biological, physical, chemical and physicochemical pretreatments. The appropriate pretreatment method can be selected depending on the requirements of hydrolysis and fermentation that have the goals of bioethanol process such as high yields of fermentable sugar and ethanol, low production cost (Pandey et al., 2010), low toxic compounds and recovery of spent chemicals (Kumar et al., 2009). Alkali pretreatment can be used to disrupt lignin binding with hemicellulose by adding alkaline solution and can enhance the digestibility of hemicellulose and can promote enzyme access to cellulose (Bensah and Mensah, 2013). This pretreatment can also promote the internal surface and solubilise the binding wax between lignin and hemicellulose and cellulose. Removal of lignin is dependent on the uses of alkali and pretreatment conditions and content of lignin in lignocellulosic material

(Sun and Cheng, 2002). In some previous literatures, sodium hydroxide used in pretreatment of sugarcane bagasse (Ju et al., 2011) and of elephant grass (Eliana et al., 2014); potassium hydroxide in pretreatment of wheat straw (Liu et al., 2015) and calcium hydroxide in pretreatment of corn stover (Kim and Holtzapple, 2006) are extensively used for removal of lignin. Numerous studies have also been carried out on pretreatment of sugarcane bagasse with dilute mixed-acid (Moraes Rocha et al., 2011), Ultrasound-assisted alkaline (Velmurugan and Muthukumar, 2012), microwave-assisted alkali and acid (Binod et al., 2012), ozone (Trayaini et al., 2013) and wet explosion (Biswas et al., 2014).

The present work aimed to study the effect of alkali pretreatment on removal of lignin from sugarcane bagasse using sodium hydroxide. Two solid loading conditions (20 %w/v and 10 %w/v) were used in this study. Base concentration and autoclaving time were also varied and performed.

2. Materials and method

2.1 Preparation and characterisation of sample

Sugarcane bagasse was collected from juice vendors in Yangon, Myanmar. It was washed with tap water to remove debris and dried in oven at 65 °C. After drying, it was grounded into less than 0.5 mm in size by using Ultra Centrifugal Miller ZM 10, Japan. Sample was then characterised by using standard methods of National Renewable Energy Laboratory (NREL) (Sluiter et al., 2011) in term of moisture content, ash content, glucan, xylan and lignin. Required chemicals were purchased from Wako Chemicals, Tokyo, Japan.

2.2 Alkaline pretreatment

Sodium hydroxide was prepared with different concentrations. These were added into 1 g of dried sample and autoclaved at 121 °C for different residence time. For 20 % solid loading condition, base concentration was varied at four levels and autoclaving time was varied at 3 levels, and experiments were performed in 12 runs for this solid loading rate. In this loading rate, the concentrations of sodium hydroxide were 0.5 wt%, 1.0 wt%, 1.5 wt%, and 2.0 wt% at 121 °C for 10 min, 30 min and 60 min. For 10 % loading condition, the levels of each factor were increased. The base concentration was varied at six levels and autoclaving time was varied at five levels, and the experiments were also performed, resulting in 30 runs for this loading rate. For 10 % w/v solid loading, concentrations were 0.5 wt%, 1.0 wt%, 1.5 wt%, 2.0 wt%, 3.0 wt% and 4.0 wt% at 121 °C for 10 min, 30 min, 60 min, 90 min and 120 min. After autoclaving, the samples were immediately cooled down into room temperature and separated into solid and liquid portions by vacuum filtration. The remaining solid residue was washed with distilled water until sample was neutralised. Then, the solid residue was dried in oven at 65 °C for further analysis.

2.3 Analytical method

Solid residue remaining after alkali pretreatment was also analysed by NREL methods. Sample was dried at 105 °C in oven until constant weight was obtained to analyse the moisture content. The oven dried weight (ODW) sample was subjected into muffle furnace in order to measure the ash content. For analysis of glucan as cellulose and xylan as hemicellulose, 3 mL of 72 % of sulphuric acid was added into 300 mg of ODW sample and mixed at 30 °C for 1 h. Then, distilled water was added into the mixture until 4 % of sulphuric acid was achieved and autoclaved at 121 °C for 1 h. After autoclaving, the mixture was cooled down into room temperature and separated into solid and liquid fractions. Solid fraction was then dried at 105 °C again in order to measure acid insoluble residue (AIR). Acid insoluble residue was burnt in muffle furnace at 600 °C about 6 h to measure acid insoluble lignin (AIL) content. Liquid fraction was neutralised by calcium carbonate for measurement of glucose and xylose concentrations which were done in high performance liquid chromatography (HPLC) equipped with L-3300 RI monitor (Hitachi Ltd.) and Shodex SH 1011 column in 1 mL/min of 0.005 M sulphuric acid used as mobile phase at 60 °C of oven temperature. Before neutralisation, absorbance of liquid for acid soluble lignin (ASL) was also measured by UV-visible spectrophotometer (UVmini-1240, Shimizu). Percentages of glucan, xylan, lignin (Sluiter et al., 2011) and lignin removal % (Gao et al., 2014) can be calculated as follows. Lignin is the sum of acid insoluble lignin (AIL) and acid soluble lignin (ASL).

$$\text{Glucan \% wt.} = \frac{\text{Conc. of glucose}\left(\text{g/L}\right) \times \text{vol. of sample}\left(\text{L}\right)}{\text{Oven dry wt. of sample (g)} \times 1.1} \times 100 \qquad (1)$$

$$\text{Xylan \% wt.} = \frac{\text{Conc. of xylose}\left(\text{g/L}\right) \times \text{vol. of sample}\left(\text{L}\right)}{\text{Oven dry wt. of sample} \times 1.1} \times 100 \qquad (2)$$

$$\%AIL = \frac{(W_A - W_B) \times 100}{\text{Oven dry wt. of sample}} \qquad (3)$$

$$\%ASL = \frac{\text{UVabs} \times \text{Vol. of filtrated sample} \times \text{Dilution rate}}{\varepsilon \times \text{Oven dry wt. of sample} \times \text{pathlength}} \times 100 \qquad (4)$$

$$\text{Lignin removal } \% = \frac{\text{Initial lignin} - (\text{g of Sample residue} \times \text{Remaining lignin})}{\text{Initial lignin}} \times 100 \qquad (5)$$

where W_A represents weight of acid insoluble residue; W_B represents weight of acid insoluble ash; ε is absorptivity of biomass at specific wavelength; and 1.1 is the conversion factor of cellulose and hemicellulose.

3. Results and discussion

3.1 Composition of sugarcane bagasse

The composition of sugarcane bagasse in this study and previous literatures is described in Table 1. It can be seen that composition of sugarcane bagasse sample in the present study was nearly the same with previous literatures. The composition of bagasse sample can be different depending on its location, variety and agricultural practice (Cannilha et al., 2011).

Table 1: Composition (%wt.) of sugarcane bagasse of this study and previous literatures

No.	Glucan	Xylan	Lignin	Moisture	Ash	Reference
1.	40.2 ± 0.9	22.5 ± 0.5	25.4 ± 0.9	3.6 ± 0.3	2.1 ± 0.2	Present study
2.	40.7±0.2	17.2 ± 0.3	23.4 ± 0.4	-	-	(Khuong et al., 2014)
3.	41.95	21.70	23.61	-	-	(Gao et al., 2013)
4.	44.9	22.2	19.3		1.4	(Canilha et al., 2011)

3.2 Effect of alkali pretreatment on the removal of lignin

The main purpose of alkali pretreatment of lignocellulosic materials is to remove the lignin. Lignin is complex polymer and the presence of lignin in fermentation broth can prohibit the growth of microorganism in fermentation process. In this present study, alkali pretreatment of sugarcane bagasse was conducted using sodium hydroxide (NaOH) with 20 %w/v solid loading and 10 %w/v solid loading. Autoclaving temperature was kept constant at 121 °C for all the experimental runs. The conditions and effect of alkali pretreatment of sugarcane bagasse were shown in Table 2 and 3.

As can be seen in Table 2, percentage of lignin removal was increased by increasing the concentration of alkali. Xylan was also increased after the alkali pretreatment compared to the initial amount of xylan (Table 1) whereas glucan content was decreased, indicating that it could be partially decomposed during alkali pretreatment.

Khuong et al. (2014) reported that glucan was slightly decreased after pretreatment while increasing the alkali concentration at constant temperature and time. According to Gao et al. (2013), combinative pretreatments using sodium hydroxide (NaOH) and liquid hot water (LHW) methods were much more effective than one step pretreatment methods to remove lignin or hemicellulose. In their study, lignin was removed up to 84.33 % by using combinative pretreatment with NaOH and LHW methods. It can clearly be observed that autoclaving time could be more influenced on removal of lignin and increasing glucan content in sample during alkali pretreatment according to Table 2. Maximum removal of lignin for this 20 % loading rate was achieved by 87.7 % in 1.5 %wt of sodium hydroxide for 60 min of autoclaving time. By comparing with maximum content of glucan 37.3 %, the best condition was obtained in 2.0 %wt of alkali for 60 min with 83.7 % removal of lignin. It was probably due to that the chemical linkage between cellulose and lignin was still quite high at this condition (Maryana et al., 2014). For 10 % solid loading, concentration and autoclaving time were more increased to investigate their effect on removal of lignin.

Table 2: Conditions concentration of sodium hydroxide (%wt.) autoclaving time (min) and effect of alkali pretreatment of sugarcane bagasse with 20 %w/v solid loading in term of glucan, xylan, lignin and percentage of lignin removal by weight

Run	Sodium hydroxide (%wt)	Autoclaving time (min)	Glucan (%wt.)	Xylan (%wt.)	Lignin (%wt.)	Lignin removal (%wt.)
1	0.5	10	30.1	34.8	21.6	42.0
2	1.0	10	29.8	34.1	21.4	47.0
3	1.5	10	31.3	34.6	19.5	57.7
4	2.0	10	31.3	33.4	17.3	52.8
5	0.5	30	28.8	31.6	16.1	60.4
6	1.0	30	31.9	34.4	22.3	41.2
7	1.5	30	33.3	31.4	19.0	53.1
8	2.0	30	34.1	33.6	14.8	58.9
9	0.5	60	28.0	28.7	22.5	67.4
10	1.0	60	32.4	31.1	10.8	82.7
11	1.5	60	35.3	27.1	8.1	87.7
12	2.0	60	37.3	33.2	12.1	83.7

Table 3: Conditions concentration of sodium hydroxide (%wt.) autoclaving time (min) and effect of alkali pretreatment of sugarcane bagasse with 10 %w/v solid loading in term of glucan, xylan, lignin and percentage of lignin removal by weight

Run	Sodium hydroxide (%wt.)	Autoclaving time (min)	Glucan (%wt.)	Xylan (%wt.)	Lignin (%wt.)	Lignin removal (%wt.)
1	0.5	10	31.0	34.7	23.3	43.4
2	1.0	10	28.3	20.1	24.1	46.8
3	1.5	10	37.3	36.1	19.8	65.5
4	2.0	10	40.8	36.3	21.0	69.2
5	3.0	10	36.2	29.5	19.4	74.1
6	4.0	10	41.9	26.0	17.0	78.9
7	0.5	30	29.0	28.4	23.0	71.7
8	1.0	30	37.6	36.7	14.2	77.3
9	1.5	30	37.7	34.0	8.6	87.3
10	2.0	30	46.6	38.9	7.8	86.8
11	3.0	30	38.1	29.4	16.4	74.7
12	4.0	30	39.9	25.3	9.4	87.0
13	0.5	60	33.3	34.1	22.0	67.4
14	1.0	60	44.9	36.8	10.9	82.7
15	1.5	60	37.8	34.6	8.2	87.8
16	2.0	60	37.7	31.0	12.1	83.7
17	3.0	60	33.4	26.7	17.3	73.2
18	4.0	60	43.7	27.9	16.1	76.6
19	0.5	90	32.0	30.4	21.6	43.2
20	1.0	90	37.0	32.7	13.8	71.3
21	1.5	90	40.1	36.4	10.6	81.1
22	2.0	90	40.7	34.3	9.2	84.6
23	3.0	90	35.0	27.9	20.2	69.7
24	4.0	90	38.6	26.0	19.8	71.2
25	0.5	120	27.9	29.4	22.6	42.7
26	1.0	120	33.4	31.1	15.2	69.5
27	1.5	120	29.6	29.6	18.1	68.4
28	2.0	120	35.7	32.9	15.2	73.9
29	3.0	120	40.1	28.9	13.5	78.8
30	4.0	120	42.8	26.5	13.2	80.4

According to Table 3, highest amount of glucan by 46.6 wt% was obtained in 2 wt%. of alkali concentration for 30 min of autoclaving time and 87.3 % of lignin removal was achieved in 1.5 wt% sodium hydroxide at for 30 min. It was found that percentages of lignin removal and glucan were not significantly increased by increasing autoclaving time in 10 % loading rate. Amount of glucan was quite high at shorter autoclaving time by comparing with the data of 20 % solid loading. We can save the energy consumption by using 10 % solid loading. In the report of Eliana et al. (2014), alkaline pretreatment of elephant grass allowed not only the removal of lignin up to 88 % but also a high recovery of the cellulosic fraction in the solid. Optimal conditions were achieved under pretreatment conditions of 120 °C for 1 h with 2 wt% NaOH and a solid to liquid ratio of 1 : 20 (wt.). The results from present study were agreed with ones from previous literature even though different lignocellulosic materials were used. It can also be said that NaOH can be used in pretreatment of different lignocellulosic materials to efficiently remove lignin under appropriate conditions.

4. Conclusion

In bioethanol production and other biorefinery processes using lignocellulosic materials as raw material, it is necessary to remove and recover the lignin. Among various methods for removal of lignin, alkali pretreatment method is still popular due to its advantages such as low cost of chemical and high removal of lignin. Although many researchers have reported the studies regarding to alkali pretreatment of sugarcane bagasse, it is still needed to optimise the conditions because chemical composition were varied due to environment of its origin. In this study, the optimal conditions were achieved with 10 % solid loading in 2 wt%. of alkali concentration for 30 min. Lignin can significantly be removed from sugarcane bagasse by using calculated concentration of sodium hydroxide at certain temperature in this study.

Acknowledgments

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