ASEAN Journal of Chemical Engineering 2021, Vol. 21, No. 2, 133–142

Enhancement of Delignification and Glucan Content of Sugarcane Bagasse by Alkali Pretreatment for Bioethanol Production

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Submitted 26 August 2020 *Revised* 10 September 2021 *Accepted* 14 September 2021

Abstract. The current work aimed to enhance the delignification of sugarcane bagasse (SCB) for bioethanol production. The optimization of alkali (sodium hydroxide) pretreatment parameters such as concentration and residence time was carried out by the Taguchi method using L_{16} orthogonal array with two factors and four levels. Sugarcane bagasse powder was mixed with sodium hydroxide (NaOH) solution (0.5-2 wt.%) and heated in an autoclave at 121°C and at varied times (30-120 min). From the statistical analysis of data, it was observed that delignification and glucan increased with the increased concentration and short time. The optimum parameters of NaOH pretreatment were 2 wt.% of NaOH concentration and 30 minutes of residence time. At the optimum conditions, 86.8% delignification and 46.6% glucan content of SCB were obtained. Thus, alkali pretreatment optimized by Taguchi design is the effective method to remove lignin and to increase cellulose or glucan content in sugarcane bagasse for facilitating the further catalytic hydrolysis in bioethanol production.

Keywords: Delignification, Glucan, Lignin, Sugarcane bagasse, Alkali pretreatment

INTRODUCTION

Sugarcane bagasse (SCB) is one of the largest cheap lignocellulosic materials among various agricultural residues and it is a waste of the sugar juice extraction process (Ju et al. 2011). Current uses of SCB are energy sources in sugar mills and distillery plants, generation of electricity to be sold to the national grid (Rocha et al. 2011), pulp and paper production, phenolic compounds, polymer films, fertilizer and pesticides, ethanol, butanol, furfural, natural adhesive resin, xylitol, nanocomposites, animal feeds and other products (Ju et al. 2011). However, a major part of the bagasse in the sugar mill remained underutilized (Rocha et al. 2011).

Sugarcane bagasse is a kind of herbaceous lignocelluloses (Wang et al. 2020). SCB is mainly composed of cellulose,

DOI: 10.22146/ajche.59093

hemicellulose, and lignin. Among the compositions, carbohydrate, lignin, extractives, and ash were 61-63%, 25-28%, 5-6%, and 6-7% respectively (Jonglertjunya et al. 2014). Cellulose and hemicellulose as the major carbohydrates play vital roles in bioethanol production through hydrolysis converting into glucose and xylose (Jung et al. 2018). The carbohydrates of dried solid contain 40-50% cellulose composed of several glucose units and include a crystalline structure. The second-largest portion of SCB is hemicelluloses (25–35%) which are composed of an amorphous polymer. In hemicelluloses, xylose exists as major pentose sugar, and small units of galactose, arabinose, glucose, and mannose are also found. The remained portion is mostly lignin (10–14%) and other traces of compounds.

Of all constituents in SCB, lignin is the most difficult one to decompose and is more impervious to any biological degradation than cellulose and hemicellulose. Lignin is a biopolymer derived from plants and wood (Clavo-Flores et al. 2015). The lignin of herbaceous biomass like SCB mainly consists of the three major phenylpropane units, namely guaiacyl (G), p-hydroxyphenyl (H), and syringcyl (S) (Wang et al. 2020). The chemical arrangement of lignin bonds obtained the lignin degradation slowly at a temperature from low to high (100 – 900°C) (Jonglertjunya et al. 2014). Moreover, lignin is considered a distinctive naturally available renewable resource for the manufacture of aromatic chemicals (Long et al. 2013). Although there are several methods in the determination of lignin, the controversy remains to characterize the lignin type because of different issues such as the origin of lignin, plant species, analytical techniques, and isolation methods. The oldest and most common method is the gravimetric isolation using mineral acid which derives the solid residue lignin, also known as Klason lignin (Clavo-Flores et al. 2015).

Due to its complex structures and nonproductive binding, the pretreatment step to remove the lignin component of the biomass material is a crucial step in the bioconversion process. However, the pretreatment method is an important step and has higher costs in bioconversion of cellulosic biomass for their applications in biorefinery concepts. Several pretreatments methods such as chemical, physical and physico-chemical pretreatment for different kinds of lignocellulosic materials have been reported by several researchers (Singh et al. 2014, Rocha et al. 2011, Asgher et al. 2013, Jung et al. 2018, Wang et al, 2020). Among several pretreatment methods, pretreatment with alkali has been considered as an effective way for pretreating different lignocellulosic materials to increase the removal of lignin and to enhance the glucan content in pretreated residue, which makes the easier saccharification to obtain a high concentration of glucose (Wang et al. 2020, Kim et al. 2016, Jung et al. 2018, Gao et al. 2013). Glucose is also the starting material for many important chemicals like furfural, 5-hydroxy methyl furfural, and 2, 5-furandicarboxilic acid (Radhakumari et al. 2014) aside from ethanol production. Alkali pretreatment is also claimed to be greater potential for industrial application due to its mild processing conditions (Kim et al. 2016, Jung et al. 2018).

Main reactions of alkali pretreatment occur as decomposition of lignin and hemicellulose, and saponification of intermolecular of ester bonds, enhancing the porosity of lignocellulosic biomass (Kim et al. 2016, Hosgun et al. 2017). Alkali pretreatments are usually performed with alkali reagents such as sodium hydroxide (NaOH), potassium hydroxide (KOH), lime, or ammonium hydroxide. NaOH is considered as the most effective chemical because of its cheaper price, higher solubility, and stronger alkalinity (Kim et al. 2016, Hosgun 2017, Wang et al. 2019). While performing the pretreatment, NaOH may cause bond cleavage between carbohydrates and lignin, resulting in the dissolution of lignin in NaOH (Jung et al. 2018). Wang et al. (2020) revealed that lignin removal by NaOH pretreatment was found to be easier in herbaceous biomass than in hardwood and softwood, and NaOH pretreatment could remove 70% lignin in wheat straw, corn straw, and SCB.

However, there remains a challenge in alkali pretreatment of SCB with sodium hydroxide to refrain from the delignification of SCB during pretreatment using an appropriate design of experiment. It is important to optimize the NaOH-pretreatment parameters using the most robust design so that it is a more adaptable and practical technology for industrial application and improvement of economic feasibility. Systematical optimization of NaOH-pretreatment is performed with the design of experiments (DOE) and includes the determination of the interactions between the main parameters in the pretreatment. There are several methods of DOE in which the full factorial method is the most complete one. However, it is expensive, a large number of experiments, and time-consuming. Instead, fractional factorial designs are being used because they minimize the size of experiments, time, and costs of the experimental run. The Taguchi method is one of the simple and robust designs used for DOE (Darvishi and Moghaddami 2019). The main purpose of the Taguchi DOE approach is to reduce the cost and experimental run without altering the quality of products with improved process performance showing its higher robustness (Das et al. 2016).

The present work was to focus on the sodium hydroxide pretreatment of SCB to highly remove the lignin, and enhance the glucan content from SCB for subsequent use in the fermentation process of bioethanol. In this treatment, concentrations of sodium hydroxide (NaOH) and residence time at constant temperature (121°C) were varied to determine their effect on the removal of lignin and enhancement of glucan from SCB using L_{16} orthogonal array of Taguchi DOE method that would contribute to more feasible and applicable pretreatment method.

MATERIALS AND METHOD

Preparation and Characterization of Sample

SCB was obtained from markets in Yangon, Myanmar. All chemicals including NaOH, glucose and sulfuric acid used in this research were analytical grade and purchased from Wako Chemicals, Japan. The sample was cleaned with running water and oven-dried at 65°C. The dried sample was then milled in Ultra Centrifugal Miller ZM 10 (Japan) to obtain less than 0.5mm in size and characterizations of raw SCB were carried out as described in our previous studies (Wunna et al. 2017). The moisture content of the dried SCB was 3.6% and the lignin, glucan, and xylan content were 25.4, 40.2, and 22.5% respectively.

Alkali Pretreatment of SCB

Sodium hydroxide pretreatment was involved with different concentrations (0.5-2.0 wt.%) of NaOH solution. The solution was added into 1 g of the dried sample as 10 wt.%

solid-liquid ratios and heated in an autoclave at 121°C and at a varied time (30-120 min) according to Table 1. After heating, rapid cooling of the sample was done to reach room temperature and vacuum filtration was carried out to obtain solid residue and liquid. The solid residue was then neutralized with distilled water and dried as mentioned before.

Analytical Method

Analysis of dried pretreated solid was conducted based on NREL methods (Sluiter et al. 2011) as mentioned in our previous study (Wunna et al. 2017) and glucose, xylose, and lignin were measured. Percentages of glucan, lignin, and delignification were calculated by Eq. (1) to Eq. (4) based on previous literature (Sluiter et al. 2011, Gao et al. 2013). Lignin is the component combining acid-insoluble lignin (AIL) and acid-soluble lignin (ASL).

$$\mathrm{G} = \frac{A \times V}{W \times 1.1} \times 100 \tag{1}$$

$$\%\mathrm{AIL} = \frac{W_A - W_B}{W} \times 100 \tag{2}$$

$$\%\mathrm{ASL} = \frac{UV_{Abs} \times V \times DR}{\varepsilon \times W \times p} \times 100 \tag{3}$$

$$\mathrm{DL} = \frac{L - [W \times (AIL + ASL)]}{W} \times 100 \tag{4}$$

Optimization Using Taguchi Method

The Taguchi method is based on orthogonal arrays, which is a robust design of the experiment method. It gives a set of the minimum number of experimental runs along with finite data about the significance of factors involved in the process parameter. In this study, the Taguchi method was carried out according to Table 1 where X_1 is alkali concentration (wt.%), X_2 is residence time, Y_1 is delignification (wt.%) and Y_2 is glucan (wt.%). In the optimization of the pretreatment parameter, the signal-to-noise ratio (S/N), termed as the logarithmic transformation of results of desired performance, is used as the desired function of the optimization process. There are three criteria to be considered in assigning the S/N ratio to determine the optimum response parameter: (1) larger is the best, (2) nominal is the best and (3) smaller is the best (Radhakumari et al. 2014).

Table 1. Design Matrix for Taguchi method to optimize the parameters of delignification process of SCB

Run No.	Variables		Response	
	X_1	X_2	Y_1	Y_2
1	0.5	30	71.7	29.0
2	0.5	60	67.4	33.3
3	0.5	90	43.2	32.0
4	0.5	120	42.7	27.9
5	1.0	30	77.3	37.6
6	1.0	60	82.7	44.9
7	1.0	90	71.3	37.0
8	1.0	120	69.5	33.4
9	1.5	30	87.3	37.7
10	1.5	60	87.8	37.8
11	1.5	90	81.1	40.1
12	1.5	120	68.4	29.6
13	2.0	30	86.8	46.6
14	2.0	60	83.7	37.7
15	2.0	90	84.6	40.7
16	2.0	120	73.9	35.7

Our main goals are to maximize the delignification and glucan content in SCB. Therefore, the ‘larger is the best’ criterion was chosen to optimize the pretreatment parameters and S/N ratio was calculated as Eq. (5):

$$\frac{S}{N} = 10log_{10}\left[\frac{1}{n_j}\sum_{j=1}^{n}\frac{1}{Y_i^2}\right] \quad (5)$$

where i is the experiment number, n is the number of replication 'i', j is the number of replicates and Y is the performance parameter. Analysis of variance (ANOVA) and S/N ratio analysis was done in Minitab17© software.

RESULTS AND DISCUSSION

Pretreatment plays a crucial role in bioethanol production from lignocellulosic materials. In this work, optimization of alkali pretreatment was approached not only to reduce the lignin but to improve glucan in SCB according to L_{16} orthogonal array of Taguchi method since it could reduce the number of experiments, costs of experimental run and time consuming compared to the other full factorial designs (Darvishi and Moghaddami 2019). The design matrix and observed data were listed in Table 1.

Effect of parameters – S/N ratios

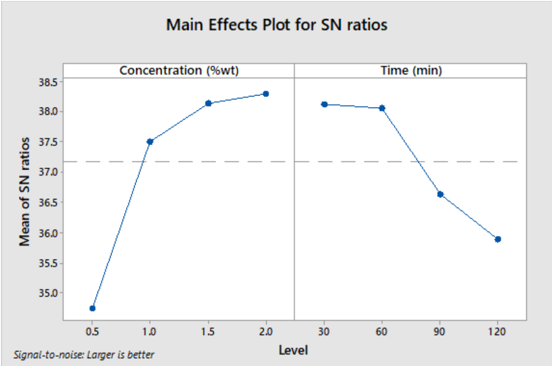
In optimization of pretreatment parameters, the signal to noise ratio (S/N) i.e., the logarithmic transformation of mean squared deviation (MSD) of the desired response, is the better way to accommodate the analysis of result data. Among three criteria, the "larger is best" criterion was chosen to calculate S/N ratio for both responses Y_1 and Y_2 as the aim is to increase delignification and to enhance glucan of SCB. S/N ratios at each level for all the factors are shown in Table 2.

The highest value of S/N ratio indicates both maximum delignification and glucan in SCB and their main effect plots are shown in figures 1 and 2. Ranks were assigned based on the delta values of each factor subtracting the lowest value of S/N ratio from the highest one. The higher the difference in S/N ratio of each factor, the higher is its influence on delignification and glucan.

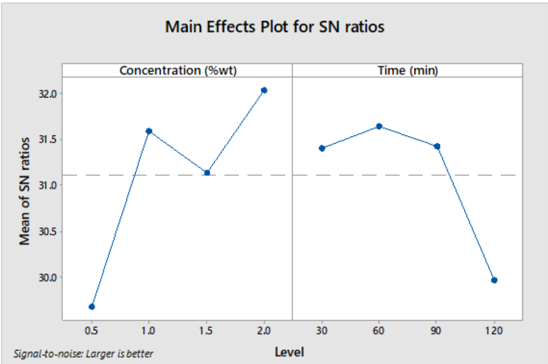
Table 2. Response table for S/N ratios corresponding to Y_1 and Y_2

Level	Y_1		Y_2	
	X_1	X_2	X_1	X_2
1	34.75	38.12	29.68	31.41
2	37.50	38.06	31.60	31.64
3	38.14	36.62	31.14	31.43
4	38.29	35.88	32.03	29.97
Delta	3.54	2.24	2.36	29.97
Rank	1	2	1	2

From Table 2, it is clear that the factor concentration was presenting a positive effect on Y_1 indicating that the higher the concentration of alkali, the better was its performance. In contrast, reaction time was showing a lower effect on Y_1 leading to that decrease in time resulting in increased performance. Therefore higher alkali concentration enhanced the delignification of SCB with a short duration. It is possible that strong alkali (NaOH) disrupts the cross-ester linkages between lignin and structural carbohydrates especially hemicellulose, resulting in the degradation of lignin and hemicellulose and saponification of ester bonds (Kim et al. 2016, Hosgun et al. 2017, Jung et al. 2018). This further contributes to higher glucan content in pretreated solid residue. Jung et al. (2018) reported that higher delignification and glucan content of switchgrass was achieved with higher NaOH concentration and longer time using full factorial design. Therefore, this study could save processing time, leading to less energy consumption. In addition, NaOH pretreated SCB gave higher glucan content and lignin removal than other pretreatment methods like liquid hot water (LHW), which has high power consumption, and HCl (Gao et al. 2013).



(a)



(b)

Fig. 1: Main effect plots for S/N ratio of (a) delignification (Y_1) and (b) glucan (Y_2)

An unusual trend was observed in the performance of Y_2 with respect to the concentration factor. As can be seen in Figure 1(b), S/N ratio of concentration increased from level 1 to 2 and decreased to level 3, and then increased to level 4. This could be due to the fact that a long time with a certain temperature further degraded the glucan into undesirable products (Palmqvist and Hahn-Hagerdal 2000). However, the concentration of alkali has a larger effect on the yield of glucan followed by time according to rank values. The highest S/N ratio corresponding to delignification and glucan of SCB illustrated that the best combination of parameters was level 4 of alkali concentration and level 1 of time (i.e 2.0 %wt. and 30 min) when the temperature was fixed at 121°C for all experiments.

Effect of parameters – the analysis of variance (ANOVA)

The analysis of variance (ANOVA) was performed using observed data and is presented in Tables 3 (a) and (b). It revealed that all the factors were a statistically significant effect on both Y_1 and Y_2 except from time in the case of Y_2 as 95% confidence interval and ($p<0.05$) were chosen. It was also observed that the F-value of alkali concentration was higher than that of time, confirming its highest influence on the delignification process as well as enhancement of glucan in SCB.

Table 3(a). Analysis of variance corresponding to Y1

Source	Y1				
	DF	Adj SS	Adj MS	F-Value	P-Value
X_1	3	1741.4	580.5	15.9	0.001
X_2	3	839.1	279.7	7.7	0.008
Residual Error	9	328.9	36.5		
Total	15				

Table 3(b). Analysis of variance corresponding to Y_2

Source	Y_2				
	DF	Adj SS	Adj MS	F-Value	P-Value
X_1	3	207.1	69.0	6.1	0.015
X_2	3	118.0	39.3	3.5	0.064
Residual Error	9	102.1	11.3		
Total	15				

Regression analysis was done based on the observed data on delignification as well as glucan and summarized in Tables 4 (a) and (b).

Table 4(a). Regression analysis corresponding to Y1

Source	Y1				
	DF	Adj SS	Adj MS	F-Value	P-Value
R	2	2173	1087	19.2	0.000
X_1	1	1410	1410	24.9	0.000
X_2	1	763.8	763.8	13.5	0.003
Error	13	736	56.6		
Total	15	2909			

Table 4(b). Regression analysis corresponding to Y1

Source	Y1				
	DF	Adj SS	Adj MS	F-Value	P-Value
R	2	219	109.5	6.8	0.009
X_1	1	145.3	145.3	9.1	0.010
X_2	1	73.7	73.7	4.6	0.051
Error	13	208.2	16.02		
Total	15	427.2			

The equations resulted are as follows:

$$Y_1 = 68.17 + 16.79X_1 - 0.2060X_2 \tag{6}$$

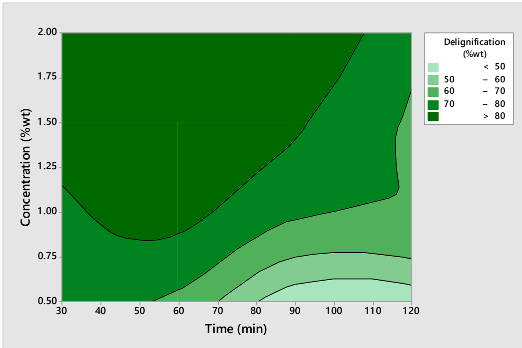
R^2 = 74.70%

$$Y_2 = 34.38 + 5.39X_1 - 0.64X_2 \tag{7}$$

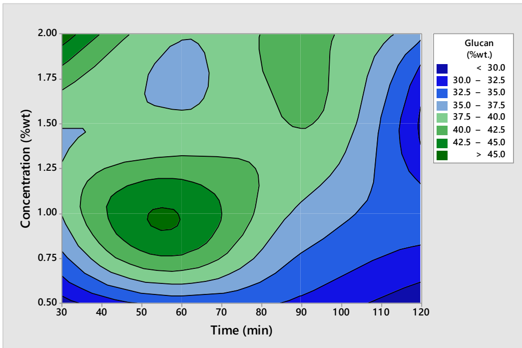
R^2 = 51.26%

The model equations fitted with experimental data are plotted as shown in figures 2(a) and 2(b). From the contour plots, it can be concluded that maximum delignification appeared at 1-2% alkali concentration and at 30-90 min, and the maximum glucan percentage at 1% alkali concentration and 50-60 min.

From the statistical analysis of the observed data, it was concluded that the delignification of SCB increased with the increase of alkali concentration and decreased time. It indicates that delignification and glucan do not vary with prolonged time. This result demonstrates that alkali concentration plays important role in the removal of more lignin from SCB than processing time. It can be explained that alkaline conditions make the solubilization of some phenolic compounds attached to cell wall components (i.e polysaccharides and lignin), resulting from the decomposition of α-ester bonds in lignin-polysaccharide complexes due to the nature of ester linkages (Ju et al. 2011).



(a)



(b)

Fig. 2: Contour plots for (a) delignification (Y_1) and (b) glucan (Y_2)

Furthermore, cellulose has less potential for solubilization on pretreatment. On the other hand, cellulose cannot be easily hydrolyzed by alkali pretreatment because it is known to be a much more recalcitrant and surface-governed reaction mechanism (Canilha et al. 2011). Xu et al. (2012) and Ahgher et al. (2013) stated that mild alkali treatment conditions completely converted cellulose I into cellulose II, indicating that it is a more stable form with antiparallel chain structure in NaOH solution. However, processing time should be enough to break down bonds present in lignin-polysaccharide complexes. Moreover, the achievement of alkali pretreatment lies in the lignin content in the substrate (Ju et al. 2011).

According to overall data analysis, the optimum conditions of NaOH pretreatment were 2 wt.% of NaOH concentration (level 4) and at 30 min (level 1). At optimum conditions, 86.8% delignification and 46.6% glucan content of SCB were obtained. Asgher et al. (2013) reported maximum delignification (48.7%) of SCB was obtained by 5% NaOH treatment for 30 min at 121°C. Liu et al. (2016) investigated the pretreatment of corn stover using high concentration (12 wt.%) at 140°C for 20 min, and lignin could be reduced by 18.5% to 7.4% and glucose increased 36.7 to 65.0%, respectively. Low consumption of NaOH in alkali pretreatment could probably minimize the use of wash water and generation of wastewater after pretreatment which are the current bottleneck problems and are considered as another factor in further research. On the other hand, black liquor should be recycled in another pretreatment. In another recent study (Wang et al. 2020), 72.1% delignification and 56.3% glucan content of the pretreated SCB were achieved by 2% (m/v) for 120 min at 80°C with a 10% (m/v) solid-liquid ratio. Compared to previous literature, the NaOH pretreatment of SCB using the Taguchi method shows the best performance in terms of delignification and the comparable performance in terms of glucan content.

CONCLUSIONS

The alkali (NaOH) pretreatment could enhance the delignification and glucan content of SCB by using the Taguchi method. The optimum parameters of alkali pretreatment of SCB were NaOH concentration of 2 wt.% and residence time of 30 min. It was found that NaOH concentration was the most influenced parameter in the alkali pretreatment of SCB. Enhanced delignification and glucan content can help the further catalytic hydrolysis and make the easier saccharification to obtain high sugar concentration as well as high ethanol concentration through the fermentation process. Additionally, the use of the Taguchi method in the optimization of alkali pretreatment provides an effective procedure to find the optimum condition. However, to more understand the alkali delignification process in bioethanol production, the changes of lignin chemistry in solid residue and liquid fraction should be further studied.

ACKNOWLEDGEMENT

The authors would like to express their sincere thanks to AUN/SEED-Net and JICA for their financial support of this work.

NOMENCLATURE

G	:	Glucan [wt.%]
DL	:	Delignification [wt.%]
L	:	Initial lignin [wt.%]
W_A	:	Weight of acid-insoluble sample [g]
W_B	:	Weight of acid-insoluble ash [g]
W	:	Weight of dried sample [g]
AIL	:	Acid insoluble lignin [wt.%]
ASL	:	Acid soluble lignin [wt.%]
V	:	Volume of sample solution [L]
DR		Dilution rate
ε	:	Absorptivity of biomass at a specific wavelength
p	:	Path length [mm]

UV_{Abs}	:	UV absorbance
$S/_N$	:	Signal to Noise ratio
X_1	:	Concentration of NaOH [wt.%]
X_2	:	Time [min]
Y_1, Y_2	:	Response [Delignification and glucan, respectively]
DF	:	Degree of freedom
$AdjSS$	:	Adjusted Sum of Square
$AdjMS$	:	Adjusted Mean Square
F	:	Variance Ratio
P	:	Level of Significance

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