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Statistical Optimization of Alkali Pretreatment on Cellulose Content of *Saccharum* through Response Surface Methodology

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Research Article

Abstract

The aim of this study is to optimize pretreatment conditions for maximum cellulose content from *Saccharum* biomass. Three independent variables like NaOH concentration, substrate concentration and residence time with three levels were optimized using response surface methodology with and without steam condition. Results showed that cellulose content was enhanced from 40.2% of untreated *Saccharum* biomass to maximum 83.80% of NaOH-Steam pretreated *Saccharum* biomass. Optimized pretreatment conditions for maximum cellulose content achieved were 3% NaOH, 10% substrate and 6 h for NaOH-steam pre-treatment. FTIR (Fourier Transform Infra-red Spectroscopy) analysis revealed the effectiveness of pretreatment process. The proposed model was found statistically significant as revealed by corresponding F and p values.

Keywords: NaOH; pretreatment; cellulose; *Saccharum*; RSM.

1. Introduction

In the 21st century, energy demand is increasing day by day for transportation, heating and industrial use. Biomass fuel is a renewable source but still it is incomplete, natural biomass is a renewable energy source. Great efforts have been carried out to produce bio-fuels because it is believed that biofuels do not contribute to the greenhouse effect [1]. Because of less production of biofuels and increasing demand of energy needs another sources of energy that's fulfill the energy demands, it is considered that lingo-cellulosic materials has been the important sources for fuels production [2,3]. A common biopolymer that is considered as the most significant resource for the production of biofuels is lingo-cellulosic biomass [4]. Biomass raw materials are commonly present in softwood, grasses, hardwoods, plants and agricultural residues. The composition of lignocellulosic material is hemi-cellulose (20%-35%), cellulose (35%-50%), and lignin (10%-25%), composition is different in all substrates and substrates processing [5].

Saccharum spontaneum biomass is considered as important biomass with affinity of ethanol production. It is crop of short rotational time with qualities of large scale production and needs less amount for starting all over the year. It is C4 plant having the quality to maintain photo-synthesis rate and good capacity for use of water in similar situation [6]. Cell wall of *Saccharum* composed of 68% carbohydrate which includes 43.78% cellulose and 24.22% hemi-cellulose for the production of bio-fuels [7]. It is widely present in zone of East, zone of central point and many countries causing discarding of fields [8,9].

Pretreatment is an important part in the conversions of bio-processes, because all its resulting products like sugar estimation (saccharification), ethanol production and other processes (downstream procedures) are the outcomes of pretreatment. The process of pre-treatment enhance the enzymatic hydrolysis, feedback, minimizes inhibitory compounds and include reasonable and efficient prices with less energy input and less amount of unwanted material [10, 11].

The analysis (mathematical and statistical) of lingo-cellulosic material used for the modeling problems is Response Surface Methodology (RSM) that is affected by many variables of interest. Response surface methodology has been mostly used for evaluating various stages of biotechnological methods [12,13] and also used to analyze enzymatic hydrolysis. This study was designed to find optimum pretreatment conditions for *Saccharum* biomass through response surface methodology.

2. Materials and Methods

2.1 Biomass preparation

The *Saccharum* biomass was obtained from field of Shahkot, District Nankana Punjab, Pakistan. These *Saccharum* were cut down into small pieces, washed to remove redundant matters and oven-dried at 70°C until constant weight. Dried biomass was chopped and ground to fine powder with grinder and kept at room temperature in plastic bags.

2.2 Chemical pre-treatment

Sodium hydroxide solution with different concentrations (1%, 3% and 5% w/v) were mixed with different concentrations (5%, 10% and 15%) of biomass and placed at room temperature for different time periods (4, 6 and 8 h). After that the slurry was filtered and solid biomass was washed with distilled water until the pH become neutral. The solid residues were placed in an oven at 70°C till constant weight [14]. The fully dried material was packed in polythene bags and further used for cellulose analysis.

2.3 Thermo-chemical pre-treatment

Sodium hydroxide solution with different concentrations (1%, 3% and 5% w/v) were mixed with different concentrations (5%, 10% and 15%) of biomass and placed at room temperature for different time periods (4, 6 and 8h). After completing time, the samples were placed in an autoclave with conditions of 121°C for 15 min at 15 ibs pressure. After that the slurry was filtered and solid biomass was washed with distilled water until the pH become neutral. The solid residues were placed in an oven at 70°C till constant weight [14]. The fully dried material was packed in polythene bags and further used for cellulose analysis.

2.4 Cellulose estimation

Cellulose content was measured as described in Asghar et al. [15].

2.5 Fourier transform infrared spectroscopy (FTIR) Analysis

Agilent technologies Cary 630 FTIR was used to analyze the treated and untreated biomass and absorption spectrum was recorded in the range of 650-4000 cm^{-1} with resolution of 4.

2.6 Experimental design

Box-Bhenken design (BBD) of response surface methodology was used to conduct experiments. The variables used, their codes and levels are mentioned in Table 1.

2.7 Statistical analysis

Minitab v. 17.0 Trial Version of Statistical software package was used to plot the response surfaces and regression analysis of experimental data. Statistical parameters were examined through Analysis of variance ANOVA. And values differences were showed in terms of probability $p<0.05$ values.

3. Results

Box-bhenken design of response surface methodology was used to analyze the effects and interaction of NaOH concentration, substrate concentration and reaction time over actual response of % cellulose content. The actual responses with their respective predicted responses for % cellulose of NaOH pretreated and NaOH-steam pretreated *Saccharum* samples are presented in Tables 2 and 3 respectively. The response obtained was calculated through second order polynomial regression equations as shown in equation 1 and equation 2 for NaOH and NaOH-steam pretreated substrate respectively. The cellulose content was increased from 40.2% to 83.8% in untreated and NaOH-steam treated *Saccharum* respectively. Among two types of pretreatments, NaOH with steam treatment was found very effective in achieving maximum cellulose content under conditions of 3% NaOH conc. and 10% substrate concentration with residence time of 6 h.

Regression equation for % cellulose content of NaOH pretreated samples.

$$\text{Celllulose (\%)} = -17.31 + 10.073a + 0.717b + 16.46c - 0.5975a^2 + 0.0344b^2 - 1.0225c^2 - 0.1750ab - 0.6750ac - 0.2150bc \quad (1)$$

Regression equation for % cellulose content of NaOH-steam pretreated samples.

$$\text{Cellulose (\%)} = -46.99 + 14.32a + 0.806b + 37.83c - 2.561a^2 - 0.2962b^2 - 3.602c^2 + 0.3450ab - 0.362ac + 0.5212bc$$

Significance of data was evaluated by analysis of variance and it was found that the model was very significant having F values of 166.93 and 218.47, and probability values of 0.000 and 0.000 for NaOH and NaOH-steam pretreated *Saccharum* biomass respectively (Tables 4 and 5). The model's large F values and their corresponding p-values shows model's accuracy (Figure 1). The coefficient of determination R^2 values of 0.9905, 0.9974 and the adjusted R^2 of 0.9734, 0.9927 indicates that actual results are in accordance with the values predicted by the model (Figure 1). The models can explain variations in responses up to 99.05% and 99.74%.

Table 1. Coded and actual level of the three independent variables for the pretreatment.

Independent Variable	Code	Code and actual factor level		
		-1	0	+1
NaOH concentration (%w/v)	*a*	1	3	5
Substrate concentration (g)	*b*	5	10	15
Reaction time (h)	*c*	4	6	8

Table 2. BBD with the predicted and actual values of the experimental response for % cellulose of NaOH pretreatment of *Saccharum* biomass.

Run #	a	b	c	Cellulose (%)		
				Observed	Predicted	Residue
1	3	10	6	49.7800	49.7800	-0.00000
2	5	10	8	41.4000	42.0000	-0.60000
3	5	15	6	46.0000	45.8250	0.175000
4	5	10	4	47.2000	47.3500	-0.15000
5	5	5	6	54.0000	53.4250	0.575000
6	1	15	6	46.0000	46.5750	-0.57500
7	3	5	4	46.0000	46.4250	-0.42500
8	1	10	8	44.8000	44.6500	0.150000
9	3	15	8	42.8000	42.3750	0.425000
10	1	10	4	39.8000	39.2000	0.600000
11	1	5	6	47.0000	47.1750	-0.17500
12	3	5	8	50.8000	50.7750	0.025000
13	3	15	4	46.6000	46.6250	-0.02500

Table 3. BBD with the predicted and actual values of the experimental response for % cellulose of NaOH-steam pretreatment of *Saccharum* biomass.

Run #	a	b	c	Cellulose (%)		
				Observed	Predicted	Residue
1	3	10	6	83.80000	83.80000	-0.00000
2	5	10	8	56.00000	55.63125	0.368750
3	5	15	6	65.00000	65.26875	-0.26875
4	5	10	4	64.40000	63.56875	0.831250
5	5	5	6	67.00000	67.93125	-0.93125
6	1	15	6	58.40000	57.46875	0.931250
7	3	5	4	74.60000	74.50000	0.100000
8	1	10	8	56.80000	57.63125	-0.83125
9	3	15	8	59.80000	59.90000	-0.10000
10	1	10	4	59.40000	59.76875	-0.36875
11	1	5	6	74.20000	73.93125	0.268750
12	3	5	8	59.60000	59.03750	0.562500
13	3	15	4	53.95000	54.51250	-0.56250

Table 4. ANOVA for the regression equation of cellulosic content of NaOH pretreated *Saccharum* biomass.

Source	DF	Adj.MS	Adj.SS	F-Value	p-Value
Model	9	192.947	21.4385	57.94	0.000
Linear	3	48.750	16.2500	43.92	0.001
NaOH conc. (a)	1	15.125	15.1250	40.88	0.001
Substrate conc. (b)	1	33.620	33.6200	90.86	0.000
Time (c)	1	0.005	0.0050	0.01	0.912
Square	3	84.297	28.0989	75.94	0.000
a^2	1	21.091	21.0908	57.00	0.001
b^2	1	2.731	2.7308	7.38	0.042
c^3	1	61.765	61.7653	166.93	0.000
2-way Interaction	3	59.900	19.9667	53.96	0.000
ab	1	12.250	12.2500	33.11	0.002
ac	1	29.160	29.1600	78.81	0.000
bc	1	18.490	18.4900	49.97	0.001
Error	5	1.850	0.3700		
Lack-of-fit	3	1.850	0.6167		
Pure Error	2	0.000	0.0000		
Total	14		194.797		

Table 5. ANOVA for the regression equation of cellulosic content for NaOH-steam pretreated *Saccharum* biomass.

Source	DF	Adj SS	Adj MS	F-Value	p-Value
Model	9	1595.24	177.249	211.73	0.000
Linear	3	235.26	78.419	93.68	0.000
NaOH conc. (a)	1	1.65	1.620	1.94	0.223
Substrate conc. (b)	1	182.88	182.883	218.47	0.000
Time (c)	1	50.75	50.753	60.63	0.001
Square	3	1195.28	398.427	475.95	0.000
a^2	1	387.45	387.450	462.83	0.000
b^2	1	202.53	202.532	241.94	0.000
c^3	1	766.30	766.302	915.40	0.000
2-way Interaction	3	164.70	54.900	65.58	0.000
ab	1	47.61	47.610	56.87	0.001
ac	1	8.41	8.410	10.05	0.025
bc	1	108.68	108.681	129.83	0.000
Error	5	4.19	0.837		
Lack-of-fit	3	4.19	1.395		
Pure Error	2	0.00	0.000		
Total	14		1599.42		

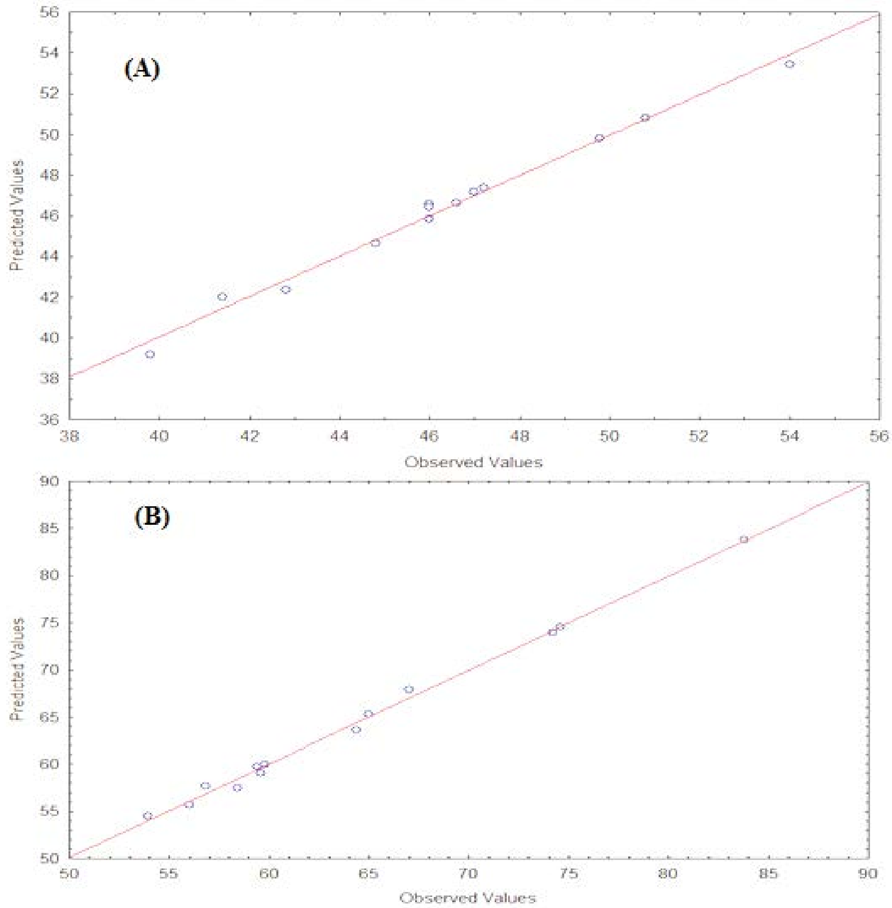

Figure 1. Observed values ver sus mathematical model prediction of cellulosic content of NaOH (A) and NaOH-steam (B) pretreated *Saccharum* biomass samples.

Figure 2 shows 2-D contour plots for NaOH (A) and NaOH-steam (B) pretreatment. These graphs were plotted between three variables keeping one variable constant. These plots indicated that each variable

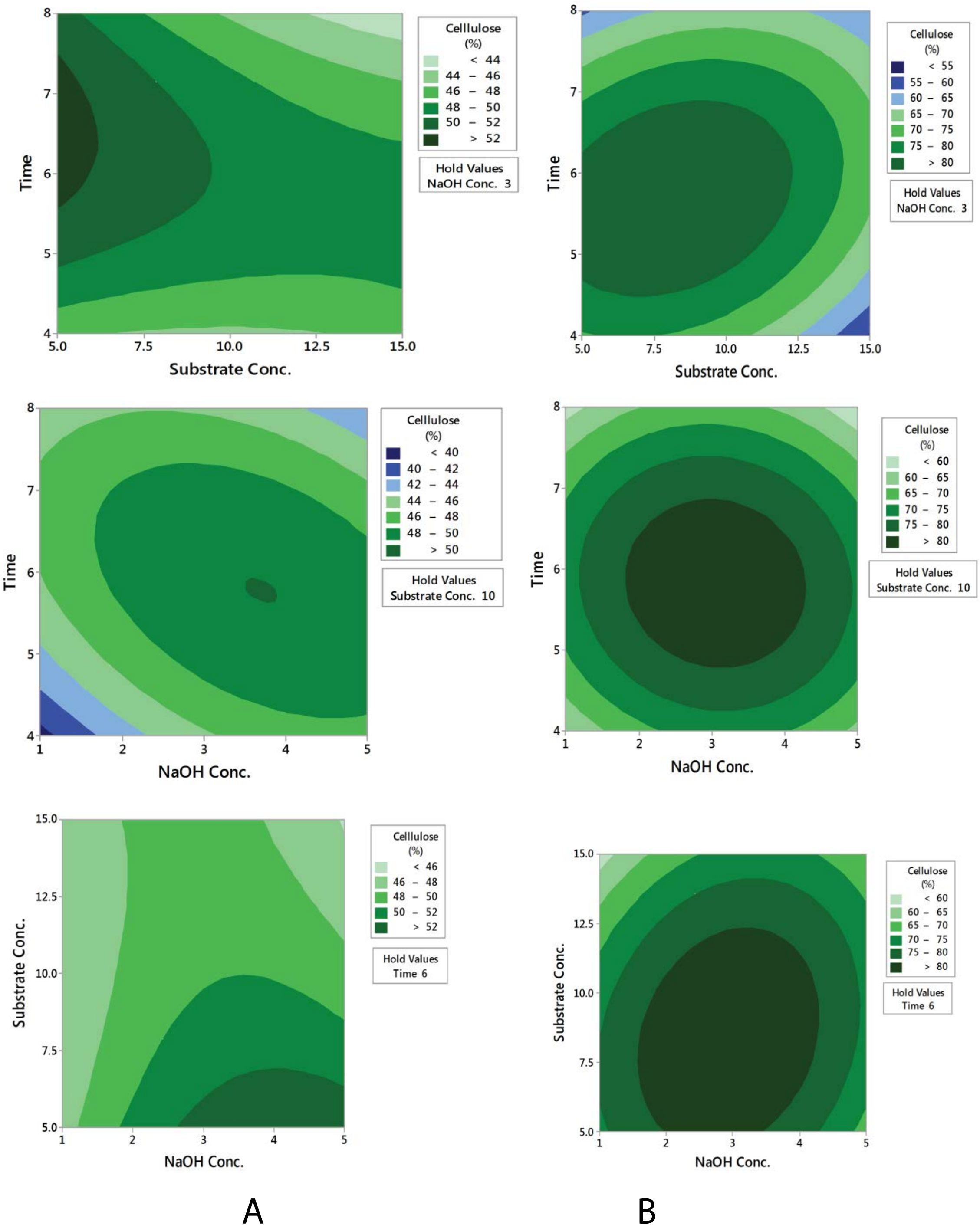

Figure 2. Contour plots for cellulose content after NaOH (A) and NaOH-steam (B) pretreatment of *Saccharum*.

had significant effect on cellulose content. Different color bands in these plots indicated different range of cellulose content at different conditions.

FTIR spectroscopy was used to examine pretreatment effect on functional group of *Saccharum* biomass through chemical modifications. FTIR spectra for untreated, NaOH pretreated, and NaOH-Steam pretreated *Saccharum* are shown in Figure 3. Results indicated that some peaks decreased their intensity in pretreated samples as compared to untreated which showed degradation of hemicellulose or lignin as a result of pretreatment. Peak at 1599.1cm^{-1} in raw, indicating aromatic vibrations C=O stretch for lignin in raw which was changed to 1507.7 cm^{-1} in NaOH and 1537.2 cm^{-1} in NaOH steam treatment. Strong peak for cellulose and hemi-cellulose was found at wavenumber 1034.3 cm^{-1} in untreated *Saccharum* material. Decrease in intensity of peak from 1034.3 cm^{-1} to 1030.6 cm^{-1} and 1030.6 cm^{-1} in pre-treated samples shows stretching of C-O, C=C, and C-C-O. At 667.2 cm^{-1} in raw the peak became sharper in base pre-treated at 670.9 cm^{-1} showing glycosidic linkage for hemicelluloses. While for base steam the peak was at 661.6 cm^{-1}.

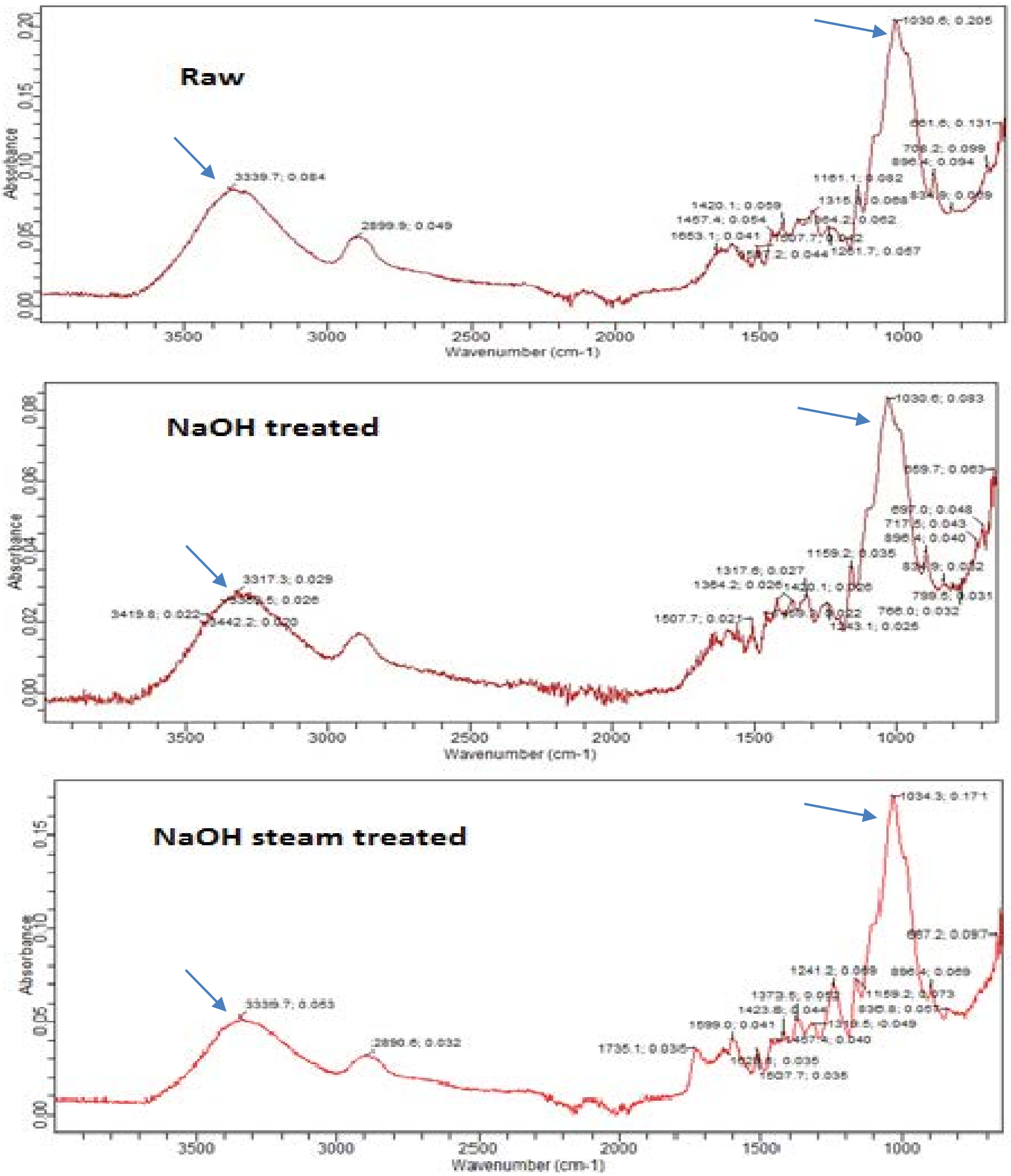

Figure 3. FTIR spectra of *Saccharum* biomass under different conditions. Arrows indicate change in peaks.

4. Discussion

Saccharum spontaneum (Kans grass) is considered as good source for the production of biofuels [6]. Kans grass cell wall consists of 68% carbohydrate on dry mass, contains 43.78% cellulose and 24.22% hemicelluloses [7]. Lignocellulosic biomass pretreatment causes lignin degradation and disrupt cellulose crystalline structure and makes it susceptible to enzymes and acid attack for hydrolysis [16]. Alkaline pretreatment removes lignin while other components integrity is maintained, and uses lower temperature and pressure then other pretreatment techniques [17]. NaOH is considered as an efficient chemical reagent for ligno-cellulosic biomass delignification [1]. In this study, sodium hydroxide (NaOH) was used for *Saccharum* biomass pretreatment. Alkaline pretreatment is observed as an effective pretreatment technique for delignification of lignocellulosic material. *Saccharum* biomass was pretreated with 1%, 3% and 5 % concentrations of NaOH with 5%, 10% and 15% biomass concentration for 4, 6 and 8 h of residence time. The sodium hydroxide effectively degraded the biomass resulting increased cellulose content up to 83.80% after pretreatment. Sodium hydroxide is best for the pretreatment process for various substrates like grass and straws [15]. It was previously reported that when soaking time was 24 h, maximum cellulose (47%) was noted in kallar grass (*Leptochloa fusca*), although a decline was observed when soaking time was increased to 48 h [18]. At 121°C for 90 min, substrate *Saccharum* was used at 2% NaOH and 65% delignification was noticed [19].

Sun and Cheng [20] stated that many changes occur when lingo-cellulosic material was treated with dilute NaOH. Ligno-cellulosic material swells, which enhancing the surface area, decreases the amount of polymerization, crystallinity reduction and structural association break up between carbohydrates and lignin. Ruangmee and Sangwichien [21] also reported similar findings with increased cellulose content at optimized pretreatment conditions of 5% NaOH concentration and 120 min reaction time at 100°C of narrow-leaf cattail. Increased concentrations of NaOH and time could increase the cellulose content of oil palm fronds biomass [22].

In this study BBD of response surface methodology was applied to optimize pretreatment conditions for *Saccharum* biomass. Most of the research work on pretreatment conditions optimization was done through response surface methodology and most of their results are widely accepted as reported previously [21,23]. The proposed model in this study was found significant as revealed by their probability values.

FTIR was used to study the physiochemical and conformational properties of pretreated *Saccharum* biomass samples. Previous studies indicated that FTIR spectrum is very helpful in studying the effects of different pretreatment on biomasses showing degradation of lignin, hemicellulose and cellulose in terms of bond vibrations between different functional groups [24-25].

5. Conclusion

RSM was used for evaluation of independent variables and their response. Pretreated Saccharum biomass showed enhanced cellulose content from 40.2% of untreated Saccharum biomass to maximum 83.80% of NaOH-Steam pretreated Saccharum biomass. Optimized pretreatment conditions for maximum cellulose content achieved were 3% NaOH, 10% substrate and 6 h for NaOH-steam pre-treatment.

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