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Alkali pretreatment of cotton stalk for bioethanol

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Abstract

Cotton stalk biomass was treated with NaOH and NaOH-steam pretreatements to get maximum cellulose content. Three factors with three levels such as biomass concentration (5, 10 and 15%), NaOH concentration (1, 3 and 5%) and residence time (4, 6 and 8 h) was performed through Box-Bhenken Design of response surface methodology. The treatment was performed with and without heating at 121°C for 15min and 15psi in an autoclave. Among these two types of treatment, maximum yield of cellulose content 87.80% was observed with 5% w/v NaOH concentration, 10g substrate loading and 4h residence time. The substrate having high cellulose content under optimized pretreatment conditions were analysed through FTIR revealing efficiency of pretreatment. The proposed model for this study was found significant in terms of lower $p<0.05$ values and findings of this study could be utilized for further processes like saccharification and fermentation to bioethanol.

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Introduction

Lignocellulose is regarded as chief renewable biomass source for biofuels production (Sun *et al*., 2013). Lignocellulosic biomass generated ethanol has diverted attention as an energy source that reduces greenhouse gas emissions up to 85% (García-Aparicio *et al*., 2011). Almost half of world biomass comes from dry lignocellulosic biomass,10–50 billion tons per year (Galbe and Zacchi, 2002). In this study cotton stalk was used as lignocellulosic biomass. Cotton stalk (CS) a renewable lignocellulosic biomass is by-product of cotton crop.Pakistan is the fourth largest cotton producer all around the world. Cotton products and cotton contribute 10% to its GDP (Muhammad *et al.,* 2009). In Pakistan, 13, 210 thousand tons of cotton stalk residues produce annually (Hanif *et al.,* 2004). Cotton stalks have high cellulose content and, are 3 times more in quantity than per acre generated cotton fibre which are frequently discarded through burning due to limited use (Reddya and Yanga, 2009). Cotton stalk biomass comprises of 58.50% cellulose, 14.40% hemicellulose and 21.50% lignin content % on dry basis (Nigam *et al*., 2009).

First step in biomass to biofuel conversion is hydrolysis which is ineffective without pretreatment due to resistance against bacterial and enzymatic attacks (Taherzadeh and Karimi, 2008). A pretreatment is therefore needed to make cellulose prone to enzymatic attack before enzymatic hydrolysis (Mosier *et al.,* 2005). The chemical pretreatment methods are fast and more effective as compared to physical and biological pretreatment methods (Mosier *et al*., 2005). Physiochemical characteristics of various lignocellulosic biomass are different, appropriate pretreatment techniques must be adopted based on properties of particular raw material. Alkaline reagents like sodium hydroxide are widely used for lignocellulosic biomass pretreatment (Alvira *et al*., 2010).

Intermolecular ester bonds (crosslinking hemicellulose, xylan and lignin) saponification describes alkaline treatment mode of action (Sun and Cheng, 2002). Cotton stalks pretreated with 2% w/v sodium hydroxide at 120 for 90min decrease lignin content up to 65% (Silverstein, 2004). Objective of this study was to find optimum pretreatment conditions, for NaOH and NaOH-steam pretreatment, for maximum cellulose content through response surface methodology and characterization of high cellulose containing substrate through Fourier Transform Infra-Red spectroscopy.

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Materials and methods

Materials

All chemicals used in this study were of analytical grade.

Biomass preparation

Cotton stalk (CS) was obtained from field of Shahkot, District Nankana Punjab, Pakistan. These cotton stalks were chopped, washed to remove redundant matters and oven-dried at 70°C till constant weight. Dried CS biomass was milled to fine powder form and stored in sealed plastic bags at room temperature for further processing.

Pre-treatment of substrate

CS was pretreated according to method described by Iqbal *et al*. (2017). This grounded CS biomass was soaked in different concentrations of NaOH for various time periods as per experimental design at room temperature. After this the material was filtered and residue was saved for further analysis. In thermo-chemical pretreatment, the material after specific soaking time was subjected to steaming at 121°C for 15min under 15psi. The pretreated samples were recovered through filtration and washed thoroughly with water until neutralized pH. These were then oven-dried for 24h at 105 and stored in sealed plastic bags at room temperature for further processing.

Cellulose estimation

Cellulose contents of pretreated CS samples were measured through a method suggested by Gopal and Ranjhan (1980). Sample weighing 0.5g (w_1) was taken in round bottom flask and added 15ml of 80% acetic acid and 1.5ml of Conc. HNO_3 and refluxed for 20 min. The material was filtered through Whatman filter paper #1 and washed with hot water. Digested material was oven dried at 105°C overnight and weighed (w_2) of digested residue. Dried matter was incinerated at 550°C for 5h in muffle furnace and weighed again (w_3) of ash. The percentage of cellulose of pretreated CS on dry matter basis was calculated using the formula.

$$\%\ \text{Cellulose} = \frac{\text{Weight of Digested Material } (w_2) - \text{Weight of Ash } (w_3)}{\text{Weight of Material on Dry Basis } (w_1)} \times 100 \quad (1)$$

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Chemical changes in cellulose structure of pretreated biomass were studied through Fourier Transform Infra-Red spectroscopy FTIR. Pretreated CS biomass samples with maximum cellulose content for NaOH pretreatment and NaOH-Steam pretreatment were analysed by Agilent technologies Cary 630 FTIR and absorption spectrum was recorded in the range of 650-4000cm^{-1} with resolution of 4.

Experimental design

Experimental designs were made using response surface methodology (RSM), employing Box-Bhenken Design (BBD) to study the combined effect of three independent variables and their response. Conditions were optimized using BBD to find the optimum NaOH and NaOH-Steam pretreatment condition for maximum cellulose content of cotton stalk. Three independent variables for BBD, NaOH concentration (1, 3, 5%), substrate loading (5, 10, 15%) and time (6, 8, 10h) were used. The BBD is appropriate for examination of quadratic response surfaces and creates a second degree polynomial model, which is used in improving a process, using a few experimental runs. Number of experiments N for BBD are designed as N = 2k (k_1) + C_0. Here k represents number of factors, C_0 is denotes number of central points. BBD with three factors k=3 and one central point C_0 = 1 total 13 experimental runs were required N = 13. According to BBD total 13 experiments were performed for each NaOH pretreatment, and NaOH-Steam pretreatment.

In Table I x_1, x_1 and x_3 are coded independent variables for NaOH concentration, substrate concentration and reaction time repectively, at three levels 1, 0 and 1. Three levels for NaOH concentration are 1, 3 and 5%w/v, substrate concentration 5, 10 and 15% and 4, 6 and 8h reaction time. Thirteen experimental runs were randomized separately for both NaOH & NaOH-Steam pretreatment of samples to exploit the effects of unsolved variability in the observed responses due to extraneous factors. The relation between observed and coded response is as follows:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (2)$$

Here x_i is coded independent variable, x_i actual value for independent variable x_o actual value at central point and Δx_i is step change for actual value.

Table I. Code and actual level of the three independent variables for the design of pretreatment experiment used in the BBD

Independent Variable	Code	Code and actual factor level		
		−1	0	+1
NaOH concentration (%w/v)	x_1	1	3	5
Substrate concentration (g)	x_2	5	10	15
Reaction time (h)	x_3	4	6	8

For dependent variable response Y and its optimal point, a second degree polynomial was fitted to observed data using the statistical package software Minitab v. 17.0. The second degree polynomial expressed as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_{ix_i} + \sum_{i=1}^{k} \beta_{iix_i^2} + \sum_{i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j \quad (3)$$

Here Y is the %cellulose response; β_o is the constant coefficient; β_i, β_{ii}, β_{ij} are coefficients for the linear, quadratic and interaction effects, respectively; and x_i is the independent variable factor, representing either NaOH concentration (g/l), temperature (°C), or reaction time (min).

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. Significance of NaOH concentration, substrate concentration and reaction time were examined through Analysis of variance ANOVA. And values differences were showed in terms of probability $p < 0.05$ values.

Results and discussion

Experimental design

Box-Bhenken design of response surface methodology (RSM) was used to analyze the effects and interaction of NaOH concentration, substrate (CS biomass) concentration and reaction time over actual response % cellulose concentration. The experimental design runs with their respective predicted values and actual responses for % cellulose of NaOH pretreated and NaOH-steam pretreated CS samples are presented in Table II. Cellulose content of untreated CS was assessed as 37.8% which increased in pretreated CS biomass. This resembles with 39.85% cellulose reported by Wang *et al.* (2016), 40.1% cellulose of dried raw cotton stalk stated by Keshav *et al.*(2016), and varied from 30% cellulose determined by Binod *et al*. (2012). Reason for dissimilarity in lignocellulosic material composition is attributable to season, geographical location, lignocellulosic biomass heterogeneity, processing techniques and analytical techniques used for composition analysis (Binod *et al*., 2012; Silverstein *et al.,* 2007).

Cellulose content in experimental response ranged from 40.8% to 87.80% in NaOH pretreated CS biomass samples. Table II shows run number 1 with minimum cellulosic yield of 40.8% and run number 4 yielded maximum cellulosic content of 87.80%.The cellulose percentage of 87.80% during NaOH pretreated CS was higher as compared to 64.20% in HPAP pretreatment reported by Wang *et al*. (2016), and 60.6% in NaOH pretreated CS estimated by Asghar *et al*. (2016). NaOH is acknowledged as an efficient chemical reagent for lignocellulosic biomass delignification (Wang *et al*., 2016).

Steam explosion of NaOH pretreated CS biomass at 121, for 15min, and 15psi results in minimum of 51.66% to maximum of 61.2% cellulose. Table II shows that run number 5 having maximum cellulose content of 61.2% and run number10 with minimum cellulose content of 51.66%. This is comparable with 64.20% cellulose of HPAP pretreatment (2g CS, 3% NaOH, at 5% S/L ratio, at 121, for 40min and 13kpa) investigated by Wang *et al.* (2016). Asghar *et al.* (2016) reported maximum 73.19% cellulose and 77.7% delignification at 2.5% NaOH, 121and 60min reaction time in physiochemical pretreatment of *Gossypiumherbaceum* (cotton stalks). Main purpose of combined alkali and steam pretreatment is lignin and hemicellulose removal which are main obstacles in efficient enzymatic hydrolysis (Ohgren *et al.,* 2007). An increase in NaOH concentration results in significantly higher $p<0.05$ lignin removal at room temperature for all reaction times (Keshav *et al.,* 2016). They estimated cellulose content of alkaline extracted steam exploded cotton stalk (SECOH) (NaOH 3% treated for 6h) as 81.641.06%. Improved cellulose content from 40.101.55 to 81.641.06% could be due to NaOH and steam collectively degradation of lignin and hemicellulose.

Increased cellulose content of pretreated CS biomass will produce enhanced reducing sugars on hydrolysis and consequently increased ethanol yields on fermentation. In this study cellulose content increased from 37.8% to 87.8% in chemical pretreatment and 61.2% in thermochemical pretreatment of CS biomass. Similar results were reported by Wang *et al*. (2016) 39.85% cellulose of raw cotton stalk increased to 64.20% in HPAP pretreated material. Upon hydrolysis with commercial cellulases this results in increased sugar yield from 55.90mg/g of raw cotton stalk to 271.70 mg/g sugars which is 4.8 folds from untreated material.

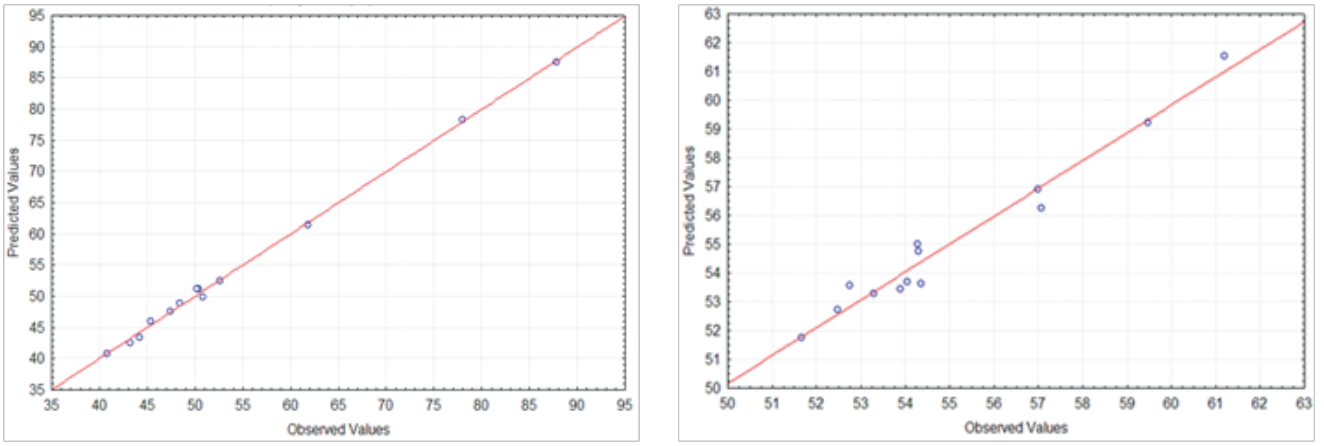
Table II. BBD with the predicted and actual values of the experimental response for cellulose content of CS biomassunder different pretreatment conditions

Run #	X_1	X_2	X_3	NaOH			NaOH-steam		
				Observed	Predicted	Residual	Observed	Predicted	Residual
1	3	10	6	40.80	40.80	0.00	53.30	53.30	0.00
2	5	10	8	47.40	47.60	0.20	57.00	56.90	0.09
3	5	15	6	43.20	42.57	0.62	54.36	53.64	0.71
4	5	10	4	87.80	87.45	0.35	54.30	54.77	0.47
5	5	5	6	50.40	51.17	0.77	61.20	61.54	0.34
6	1	15	6	44.20	43.42	0.77	54.04	53.69	0.34
7	3	5	4	61.80	61.37	0.42	57.08	56.26	0.81
8	1	10	8	78.00	78.35	0.35	53.90	53.43	0.47
9	3	15	8	48.40	48.82	0.42	52.76	53.57	0.81
10	1	10	4	52.60	52.40	0.20	51.66	51.75	0.09
11	1	5	6	45.40	46.02	0.62	54.28	54.99	0.71
12	3	5	8	50.80	49.82	0.97	59.48	59.23	0.24
13	3	15	4	50.20	51.17	0.97	52.48	52.72	0.24

The response obtained through experiments were calculated through second order polynomial regression equations (eq 4,5).These were in pragmatic relationship among test variables and % cellulose yield.

$$\text{Cellulose (\%)} = 141.09 + 12.72\,x_1 + 1.970\,x_2 - 40.68\,x_3 + 2.331\,x_1^2 - 0.1730\,x_2^2 + 4.081\,x_3^2 - 0.1500\,x_1x_2 - 4.113\,x_1x_3 + 0.2300\,x_2x_3 \text{(p-value= 0.000, Adj. } R^2 \text{ value = 0.9952)} \quad (4)$$

$$\text{Cellulose (\%)} = 56.20 + 1.220\,x_1 - 1.206\,x_2 + 0.32\,x_3 + 0.179\,x_1^2 + 0.0780\,x_2^2 + 0.050\,x_3^2 - 0.1655\,x_1x_2 + 0.0288\,x_1x_3 - 0.0530\,x_2x_3 \text{(p-value= 0.003, Adj. } R^2 \text{ value = 0.0.9690)} \quad (5)$$



(A) (B)

Fig. 1. Observed values versus mathematical model prediction of cellulosic content for NaOH pretreated CS biomass samples.

The response obtained through experiments were calculated through second order polynomial regression equations (eq 4,5).These were in pragmatic relationship among test variables and % cellulose yield.

Analysis of variance

Models significance was checked by analysis of variance (ANOVA) coefficient determination R^2. Higher R^2 values of 0.9983 and 0.9690 indicates that model explained the reaction well. The higher of Adj. R^2 values of 0.9952 and 0.9131 also support model's significance. Higher determination coefficient R^2 values and their corresponding adjusted R^2 illustrate that actual and predicted values are in agreement as shown in Figure 1 A and B. The model have F values of 321.97 and 17.35, and probability values of 0.000 and 0.003 for NaOH and NaOH-steam pretreated CS biomass samples respectively (Table III and IV). A p-value of less than 0.05 shows significance of the model. Model terms x_1, x_2 x_3 and and quadratic term interactions x_1x_2, x_2x_3, x_1x_3, x_1^2, x_2^2 and x_3^3 were also found significant. The model was found accurate as it can explain variation up to 99.83% in NaOH pretreated CS biomass and 96.90% in NaOH-steam pretreated CS biomass.

Fig. 2 and 3 depicts 3-D views for cellulose content after NaOH pretreatment and NaOH-steam pretreatments respectively. These plots indicated that each factor had significant effect on cellulose content. Increased concentration of NaOH resulted in increased cellulose content. Sukri *et al.* (2014) exhibited that an increase in NaOH conc. 0.50-5.25% and pretreatment time 15-52.50min improved cellulose content to 42.78% of oil palm fronds (OPF) biomass.

Figure 4 A and B illustrated desirability for percent cellulose content of CS biomass. This chart shows that if NaOH concentration of 3%, substrate concentration of 10% and reaction time of 6h, it results in 81.939% maximum cellulose content in NaOH pretreatment of CS biomass (Fig. 4A). Fig. 4 (B) illustrates that at 3% NaOH concentration, 10% substrate concentration and 6h reaction time it yields 60.714% maximum cellulose content in NaOH-Steam pretreatment of CS biomass.

Table III. ANOVA for the regression equation of cellulosic production for NaOH pretreated CS biomass samples

Source	DF	Adj. MS	Adj. SS	F Value	p-value
Model	9	294.28	2648.54	321.97	0.000
Linear	3	56.19	168.57	61.48	0.000
NaOH conc. x_1	1	9.25	9.25	10.11	0.025
Substrate conc. x_2	1	62.72	62.72	68.62	0.000
Time. x_3	1	96.61	96.61	105.69	0.000
Square	3	455.80	1367.40	498.69	0.000
x_1^2	1	321.07	321.07	351.28	0.000
x_2^2	1	69.07	69.07	75.57	0.000
x_3^2	1	984.02	984.02	1076.61	0.000
2-Way Interaction	3	370.86	1112.57	405.75	0.000
x_1x_2	1	9.00	9.00	9.85	0.026
x_1x_3	1	1082.41	1082.41	1184.26	0.000
x_2x_3	1	21.16	21.16	23.15	0.005
Error	5	0.91	4.57		
Lack-of-Fit	3	1.52	4.57		
Pure Error	2	0.00	0.00		
Total	14		2653.11		

Table IV. ANOVA for the regression equation of cellulose production of NaOH-steam pretreated CS biomass samples

Source	DF	Adj. MS	Adj. SS	F Value	p-value
Model	9	10.89	98.02	17.35	0.003
Linear	3	23.55	70.66	37.53	0.001
NaOH conc. x_1	1	20.99	20.99	33.45	0.002
Substrate conc. x_2	1	42.41	42.41	67.57	0.000
Time. x_3	1	7.25	7.25	11.56	0.019
Square	3	5.07	15.22	8.09	0.023
x_1^2	1	1.88	1.88	3.01	0.143
x_2^2	1	14.04	14.04	22.37	0.005
x_3^2	1	0.14	0.14	0.24	0.648
2-Way Interaction	3	4.04	12.13	6.44	0.036
x_1x_2	1	10.95	10.95	17.46	0.009
x_1x_3	1	0.05	0.05	0.08	0.0783
x_2x_3	1	1.12	1.12	1.79	0.0239
Error	5	0.62	3.13	—	—
Lack-of-Fit	3	1.04	3.13	—	—
Pure Error	2	0.00	0.00	—	—
Total	14		101.16	—	—

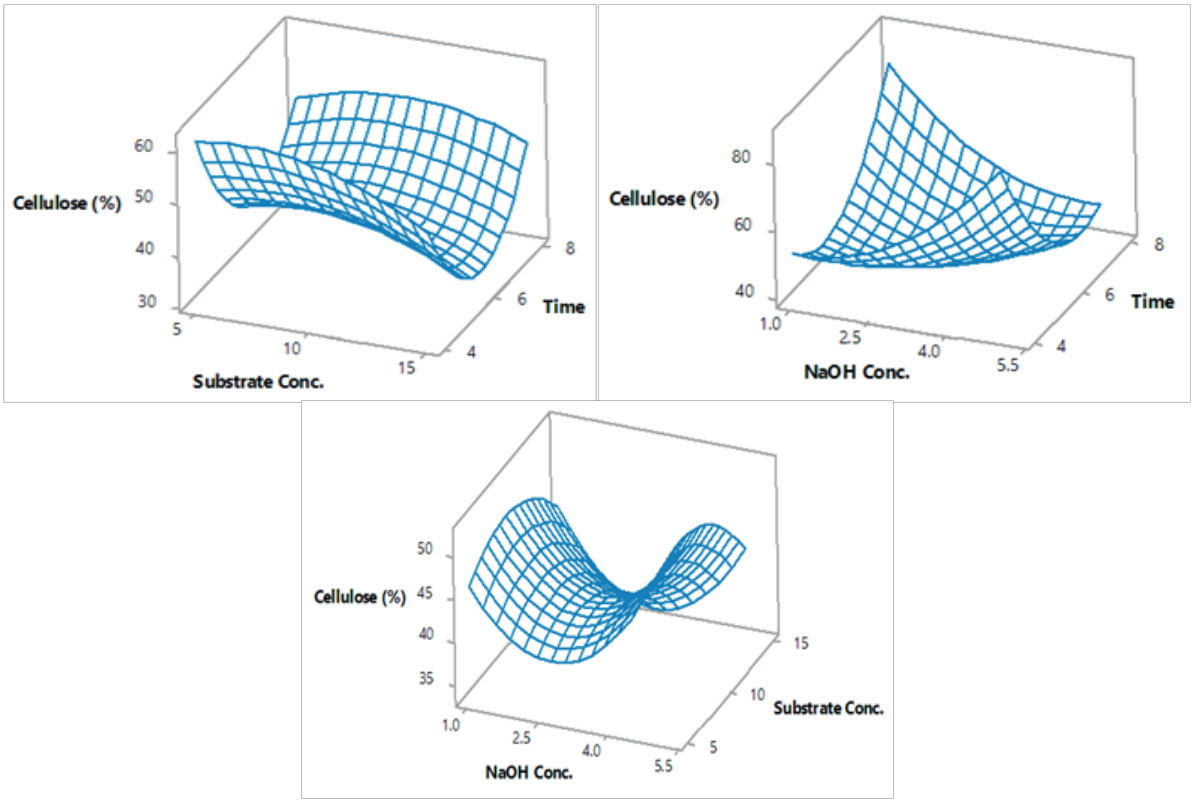


Fig. 2. Surface plot for cellulose content after NaOH pretreatment of CS biomass

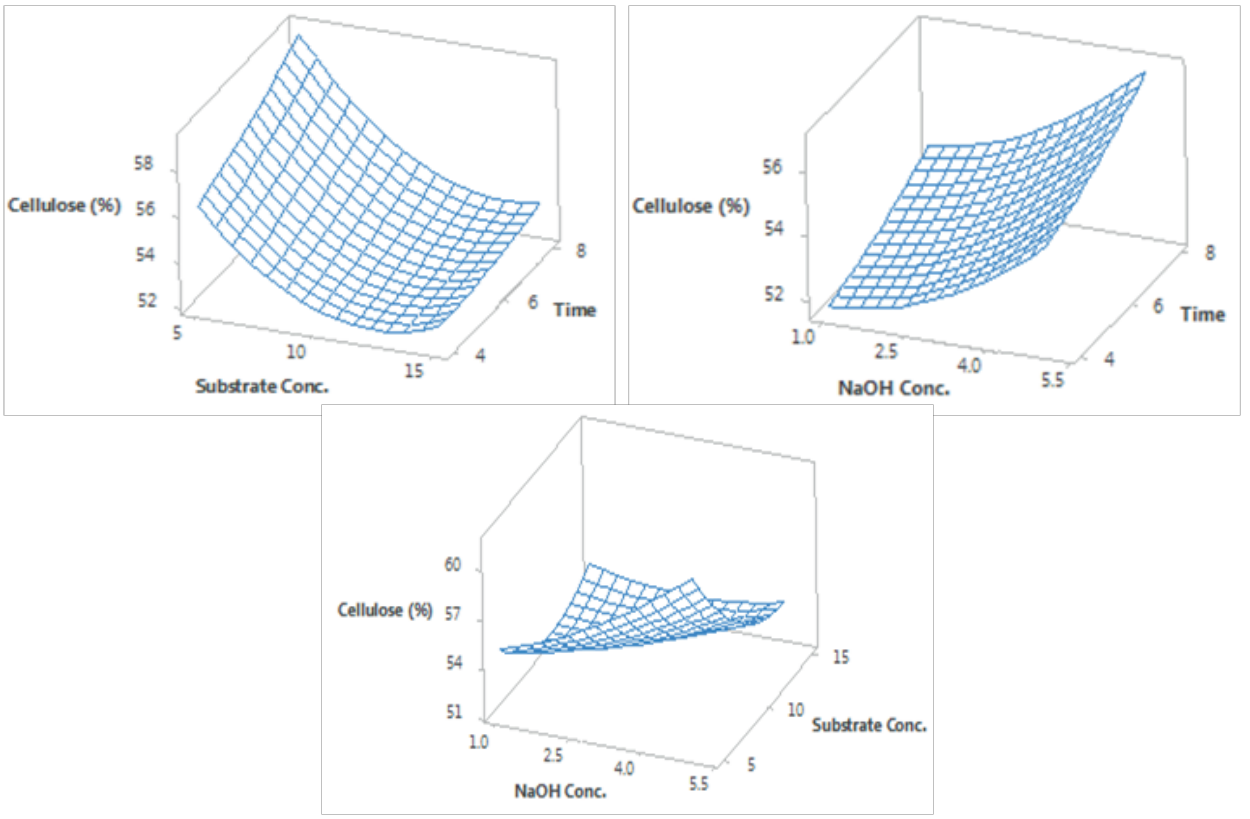
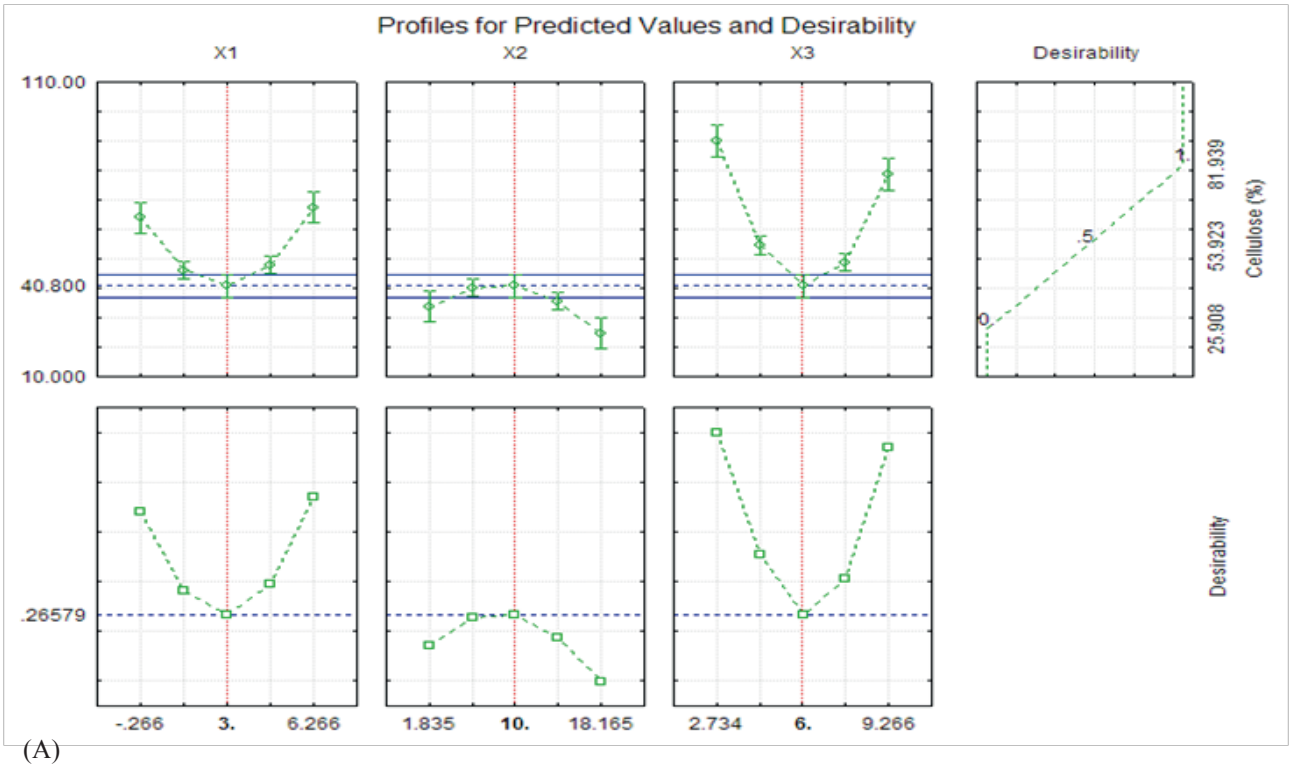


Fig. 3. Surface plot for cellulose content after NaOH-steam pretreatment of CS biomass



(A)

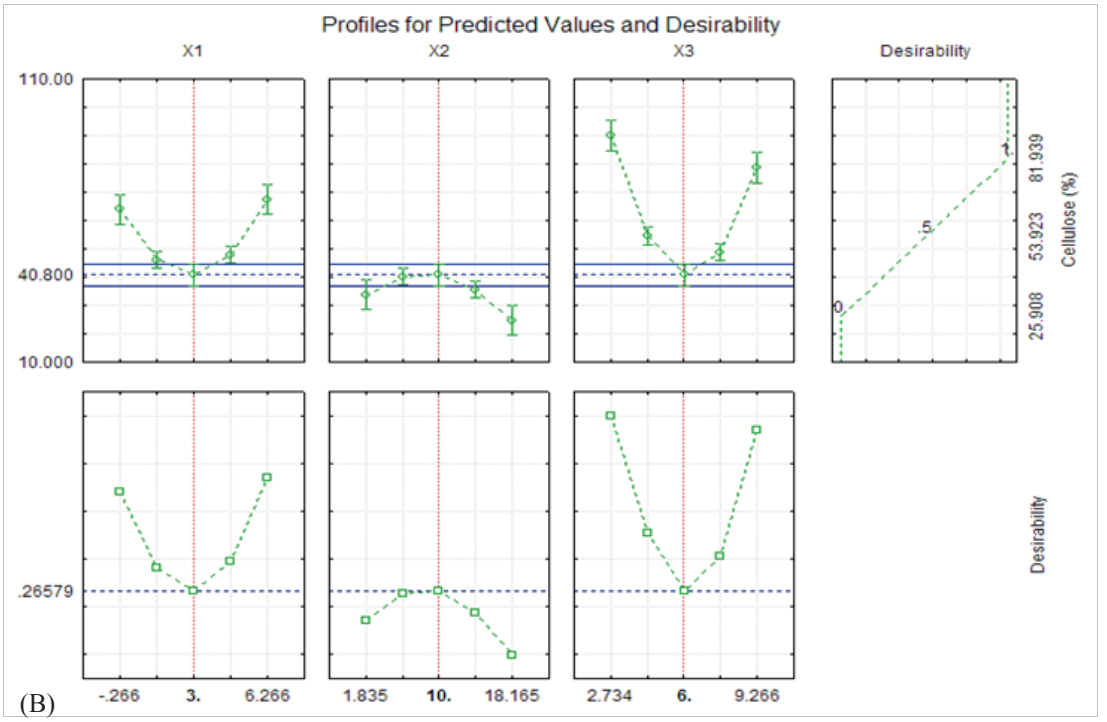


Fig. 4. Desirability chart of cellulose content for NaOH pretreatment (A) and NaOH-steam pretreatment (B) of CS biomass

FTIR Analysis

To study the physiochemical and conformational properties of pretreated CS biomass samples, these were analysed through Fourier Transform Infra-Red spectroscopy (FTIR). FTIR spectra for untreated, NaOH pretreated and NaOH-Steam pretreated CS are shown in Fig. 5. Cellulose peaks absorbance at 674.6cm^{-1}, 896.4cm^{-1} and 1026.9cm^{-1} increased in the pretreated samples. Peaks for C-O-C asymmetrical stretching appears in pretreated CS samples at 1101.4cm^{-1} and 1105.2cm^{-1} in chemical and thermochemical pretreatment respectively. Placido and Caparedo (2014) also reported rise in peaks at 898, 1030, 1050, 1090, 1150, and 1170cm^{-1} in alkali pretreated cotton gin trash (CGT). They found stronger peaks with U-NaOH 15% + HW +E pretreatment combination for CGT. Increased absorbance of cellulose peaks can be attributed to combination of various pretreatment conditions at cellulose structure. The cellulose peak found at 896cm^{-1} in untreated CS biomass representedglycosidic linkage of β-D cellulose. Sharp peaks in NaOH pretreated CS for cellulose at 896cm^{-1} wavelength, and increased intensity to 898cm^{-1} were found in NaOH-Steam pretreated CS sample. This is in accordance with the 897cm^{-1} band depicted by Wang *et al.* (2016). Degradation of β-D-cellulose makes cellulose accessible to enzymatic hydrolysis through cellulases. Strong peak for polysaccharides (cellulose, hemicellulose) was found at 1026.9 cm^{-1} in untreated CS material. Increase in intensity of peaks I025cm^{-1}, 1049.2cm^{-1}, 1030.6cm^{-1}, and 1053cm^{-1} in pretreated samples established C-O, C=C, and C-C-O stretching for polysaccharide. Increase in intensity of peaks is referred as xylan (hemicellulose) decomposition in pretreated CS samples, as decreased in transmittance signal at 1037cm^{-1} stated by Wang *et al.* (2016). NaOH-Steam pretreatment attained upsurge in peak at 1053cm^{-1} displaying increased xylan decomposition. C=O stretch for ketone aldehyde showed decreased absorbance at 1718.3cm^{-1} in NaOHpretreated samples than 1730cm^{-1} of untreated CS. Decreased C=O stretching peak describes hemicellulose solubilisation in NaOH pretreatment, and maximum hemicellulose degradation in NaOH-steam pretreatment of CS biomass. Decrease in intensity of peculiar hemicellulose band was fund at 1733cm^{-1} in H_2O_2 pretreated wheat straw signifying partial hemicellulose removal after pretreatment. Strong O-H stretching peaks at 3324.1 and 3363.9cm^{-1} presented higher lignin content in untreated CS samples. Increase in the intensity of O-H stretching at 3326.6, and

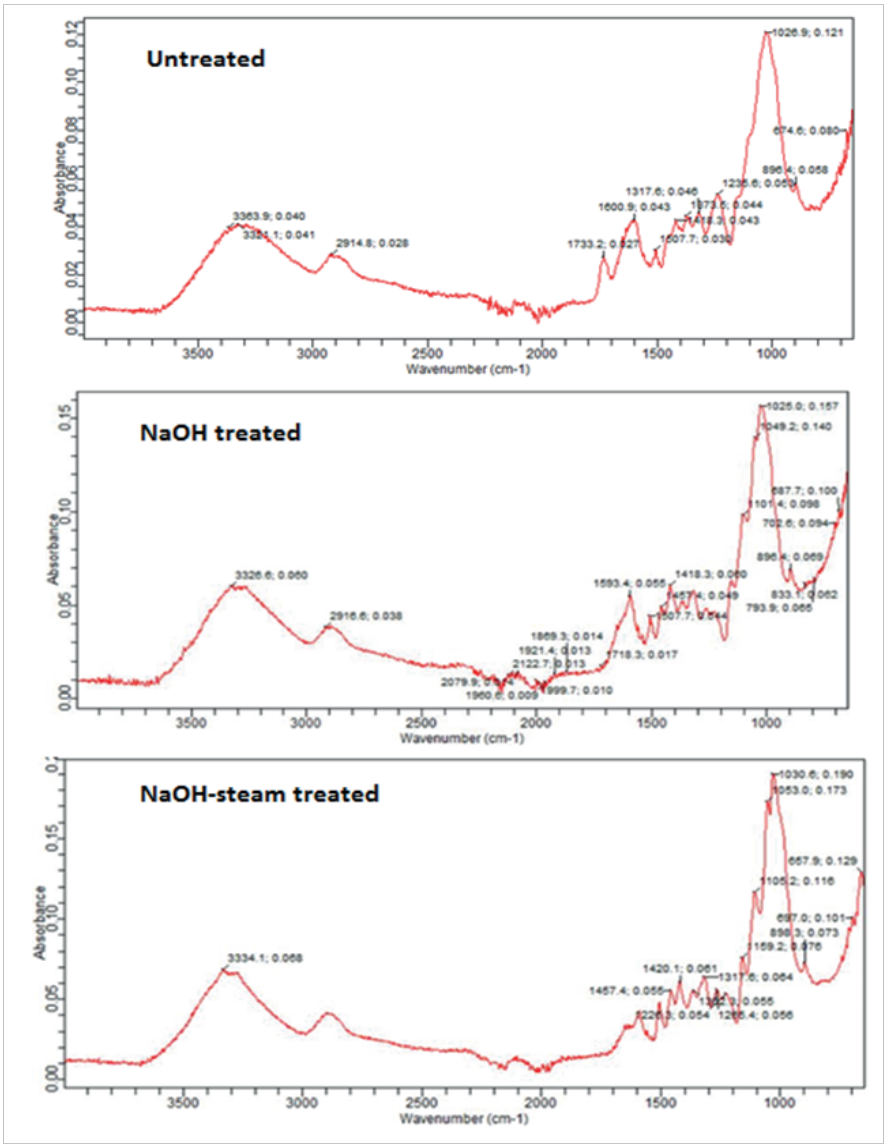


Fig. 5. FTIR spectra of CS biomass

3334.4cm^{-1} shows delignification inpretreated CS biomass. As reported by Bello *et al.* (2016), O-H absorption band intensity decreased 3434.37 to 3409.34cm^{-1} for FTIR spectra of acetylated CS. It shows decreased hydroxyl group content in pretreated CS.

Conclusion

Results concluded that among two types of treatment, chemical treatment was found best (maximum yield of cellulose content (87.80%)at 5% w/v NaOH concentration, 10g substrate loading and 4h residence time). FTIR confirmed the efficiency of chemical treatment. These results suggested that it could be potentially used in saccharification process for liberation of sugars for the production of beneficial products.

References

Alvira P, Tomás-Pejó E, Ballesteros M and Negro MJ (2010), Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review, *Bioresource Technology* **101:** 4851–4861. DOI: org/10.1016/j.biortech.2009.11.093

Asghar U, Irfan M, Nadeem M and Syed Q (2016), Effect of NaOH on pretreatment of Gossypiumherbaceum, *Enzyme Sources Part A*. **38:** 1898-1903.

Bello A, Isa MT, Aderemi BO and Mukhtar B (2016), Acetylation of Cotton Stalk for Cellulose Acetate Production, *ASRJETS*. **15:** 137-150.

Binod P, Kuttiraja M, Archana M, Janu KU, Sindhu R, Sukumaran RK and Pandey A (2012), High temperature pretreatment and hydrolysis of cotton stalk for producing sugars for bioethanol production, *Fuel* **92:** 340–345. DOI:org/10.1016/j.fuel.2011.07.044

Galbe M and Zacchi G (2002), A review of the production of ethanol from softwood, *Applied Microbiology and Biotechnology* **59:** 618–628.

García-Aparicio MP, Oliva JM, Manzanares P, Ballesteros M, Ballesteros I, González A and Negro MJ (2011), Second-generation ethanol production from steam exploded barley straw by Kluyveromycesmarxianus CECT 10875, *Fuel* **90:** 1624–1630.

Gopal K and Ranjhan SK (1980), Laboratory manual for nutrition research, Roland Press (India) Private Ltd, New Dehli, India.

Hanif M, Khan SA and Nauman FA (2004), Agricultural Perspective and Policy Ministry of Food, Agriculture and Livestock, Islamabad. 6-7.

Iqbal S, Irfan M, Shakir HA, Tabbsum F and Qazi JI (2017), Effect of alkali treatment (NaOH) of eucalyptus leaves for hypercellulase production Bacillus subtilis through submerge fermentation, *Punjab University Journal of Zoology* **32:** 25-34.

Keshav PK, Naseeruddin S and Rao LV (2016), Improved enzymatic saccharification of steam exploded cotton stalk using alkaline extraction and fermentation of cellulose sugars into ethanol, *Bioresource Technology* **214:** 363-370.

Li Y, Cui F, Liu Z, Xu Y and Zhao H (2007), Improvement of xylanase production by Penicillium oxalicum ZH-30 using response surface methodology, *Enzyme and Microbial Technology* **40:** 1381.

Mosier NS, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M and Ladisch MR (2005), Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technology* **96:** 673-686. DOI: org/10.1016/j.biortech.2004.06.025

Muhammad D, Afzal MN, Raza I and Mian MA (2009), Growth and development of cotton (Gossypium hirsutum L.) as affected by different methods of pendimethalin application, *Pakistan Journal of Weed Science& Research* **5:** 11-17.

Nigam PS, Gupta N, and Anthwal A (2009), Pre-treatment of agro-industrial residues ***In:*** Biotechnological agro-industrial residues utilization, Eds. Nigam PS, Pandey A, 1 Ed., Netherlands, Springer. 13-33.

Ohgren K, Bura R, Saddler J and Zacchi G (2007), Effect of hemicellulose and lignin removal of enzymatic hydrolysis of steam pretreated corn stover, *Bioresource Technology* **98:** 2503-2510.

Placido J and Caparedo S (2014), Analysis of alkali ultrasonication pretreatment in bioethanol production from cotton gin trash using FT-IR spectroscopy and principal component analysis, *Bioresources and Bioprocessing* **1:** 23.

Reddya N and Yanga Y (2009), Properties and potential applications of natural cellulose fibers from the bark of cotton stalks, *Bioresource Technology* **100:** 3563–3569. DOI: org/10.1016/j.biortech.2009.02.047

Silverstein R (2004), A comparison of chemical pretreatment methods for converting cotton stalks to ethanol. Unpublished MS, North Carolina State University.

Silverstein RA, Chen Y, Sharma-shivappa RR, Boyette MD and Osborne J (2007), A comparison of chemical pretreatment methods for improving saccharification of cotton stalks, *Bioresource Technology* **98:** 3000-3011. DOI: org/10.1016/j.biortech.2006.10.022

Sukri SSM, Roshanda A, Rahman, Illiasi RM and Yaakob H (2014), Optimization of alkaline pretreatment conditions of oil palm fronds in improving the lignocelluloses contents for reducing sugar production, *Romanian Biotechnological Letters* **19:** 9006-9018.

Sun JZ, Ding SY and Peterson JD (2013), Biological Conversion of Biomass for Fuels and Chemicals: exploration from natural biomass utilization systems, Royal Society of Chemistry, Oxfordshire. 83-113.

Sun Y and Cheng J (2002), Hydrolysis of lignocellulosic materials for ethanol production: A review, *Bioresource Technology* **83:** 1-11.

Taherzadeh MJ and Karimi K (2008), Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A Review, *International Journal of Molecular Science* **9:** 1621-1651. doi.org/10. 3390/ijms9091621

Wang M, Zhou D, Wang Y, Wei S, Yang W, Kuang M, Ma L, Fang D, Xu S and Du SK (2016), Bioethanol production from cotton stalk: A comparative study of various pretreatments, *Fuel* **184:** 527-532. DOI: org/10. 1016/j.fuel.2016.07.061