Optimization of sulfuric acid treatment of peanut shells for cellulose contents through Box-Behnken design

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Abstract

Pretreating plant biomasses is an important step for effective lignin removal and degradation of cellulose to fermentable sugars. In this study, we optimized pretreatment conditions for peanut shells (*Arachis hypogeae*) to obtain the maximum yield of cellulose. For optimization, three parameters were used, i.e., sulfuric acid concentration (0.6%, 0.8%, and 1.0%), substrate loading (5%, 10%, and 15%), and residence time (4 h, 6 h, and 8 h) with and without steam (121°C, 15 psi, 15 min), based on Box-Behnken design of response surface methodology. The results confirmed that the maximum yield of cellulose (71.68%) was obtained under pretreatment conditions of 1% sulfuric acid, 15% substrate loading, and 6 h residence time. The ANOVA results indicated that the proposed model was highly significant having *F*-value and *P*-value of 158.63 and 0.000, respectively. Moreover, the efficiency of this pretreatment method was further analyzed using FTIR spectroscopy, indicating structural conformation in the pretreated biomass. The results indicate that the pretreated biomass can be utilized for further processes such as saccharification of lignocellulosic material for bioethanol production.

Key words: H_2SO_4 pretreatment, peanut shells, FTIR, RSM

Introduction

Burning fossil fuels such as petroleum, coal, and natural gas, which contain a high percentage of carbon, leads to the emission of CO_2, one of the most important greenhouse gases (GHGs). The usage of fossil fuels to fulfill energy requirements is threatened by the rise in the concentration of GHGs in the environment, which has also increased concern over global warming (Demirbas et al., 2004). Thus, a reduction in the consumption of fossil fuels can decrease CO_2 emission and the risk of environmental pollution (Demirbas, 2006); however, to fulfill this demand, additional research on the development of renewable energy resources is necessary. Recently, liquid fuels have received considerable interest from researchers as they are expected to replace fossil fuels (Yat et al., 2008).

Bioethanol, or ethyl alcohol (CH_3-CH_2-OH), is a volatile, flammable, and colorless liquid (Ruangmee and Sangwichien, 2013), which was used as fuel for the first time in 1987 in an internal combustion engine developed by Nikolas Otto (Rothman et al., 1983). Bioethanol is a good fuel for dedicated engines because of its high octane number, high flame speed, and high heat of vaporization. Similarly, lignocellulosic biomass has been proven as a successful alternative source for producing chemicals and fuel (ethanol) because of its renewable nature (Binder et al., 2009).

Various lignocellulosic biomasses have been exploited for producing bioethanol; however, because of the complex structure of the plant cell wall and its components, it is difficult to breakdown cellulose into a lignocellulosic biomass. Moreover, such cellulose is resistant to many physiochemical, compositional, and structural factors (Hsu et al., 1980). Therefore, cellulose needs to be pretreated before it can be used as a source of biomaterials so that lignin is removed and the compound is made less crystalline, but without any change in its structure. Such a pretreatment process is performed either via

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a physical process, chemical process, or a combined process. For chemical pretreatment, different acids, bases, and solvents have been used to expose cellulose, which indirectly also helps in the hydrolysis of cellulose and hemicellulose (Vancov et al., 2012). In this study, we have focused on identifying the optimal pretreatment conditions to obtain the maximum yield of cellulose from peanut shells (PS).

Materials and methods

Substrate collection

Arachis hpogea (peanut) was collected from Sargodha, Pakistan.

Preparation of substrate (peanut shells)

The samples (PS) were washed with water to remove dust and dirt. They were then cut into small pieces and the sun-dried substrate was placed in an oven (70 °C) till a consistent weight was achieved. Then, the oven-dried substrate was ground into a fine powder (2 mm) using a grinding mill.

Pretreatment of peanut shells

The PS were pretreated with sulfuric acid and sulfuric acid-steam based on the pretreatment methodology of Arshad et al. (2017).

Cellulose estimation

After pretreatment, the cellulose content was measured using Gopal and Ranjhan (1980)'s method. Acetic acid (80%; 15 ml) and concentrated HNO_3 (1.5 ml) were added to the substrate (1 g, W_1) and digested for 25 min. After refluxing, the material was filtered through Whatman filter paper #1, and the samples were washed with warm water and transferred into a crucible and oven-dried overnight at 105 °C and weighed (W_2). Then, the samples were subjected to charring and placed in a muffle furnace at 550 °C for 5 h to turn them to ash. Then, the samples were weighed again (W_3) and the cellulose content was measured using the following formula:

$$\text{Cellulose [\%]} = \frac{W_2 - W_3}{W_1} \times 100$$

W_1 – weight of material on dry basis; W_2 – weight of digested material; W_3 – weight of ash

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectra of PS biomass treated with sulfuric acid were obtained to verify the changes in the structural arrangement of cellulose. For obtaining the FTIR spectra, both the untreated PS and pretreated solid residues from the acid (H_2SO_4) pretreatment were analyzed using the Agilent technology Cary 630 spectrometer. The spectrum obtained was magnified between 500 and 4000 cm^{-1}.

Experimental design

A three-variable Box-Behnken design of response surface methodology (RSM) was used to study the combined effect of the above variables on the cellulose content in PS after pretreatment. The Box-Behnken design is appropriate for examining quadratic response surfaces and creates a second-degree polynomial model, which is used for improving the process using a small number of experimental runs. We performed randomized 13 experimental runs to exploit the effects of unsolved variability in the observed responses because of extraneous factors. The relation between the coded values and actual values is described in Equation (Eq. 1):

$$x_i = \frac{X_i - X_o}{\Delta X_i} \qquad \text{Eq. 1}$$

where x_i and X_i are the coded and actual values of the independent variable, respectively; X_o is the actual value of the independent variable at the center point; and ΔX_i is the step change of X_i (Jabasingh and Nachiyar, 2011). To estimate the response of the dependent variable and predict the optimal point, a second-degree polynomial model is fitted to the experimental data using the statistical package software Minitab v. 17.0. The second-degree polynomial model is expressed as Equation (eq. 2):

$$Y = \beta_0 + \Sigma_{i=l}^{k} \beta_{ix_i} + \Sigma_{i=l}^{k} \beta_{iix_i^2} + \Sigma_{i<j}^{k} \Sigma_{j}^{k} \beta_{ij} x_i x_j \qquad \text{Eq. 2}$$

where Y is the response of cellulose contents; β_0 is the constant coefficient; β_i, β_{ii}, and β_{ij} are coefficients for the linear, quadratic, and interaction effects, respectively; and x_i is the independent variable factor, representing either the H_2SO_4 concentration (g/l), the temperature (°C), or the reaction time (min).

Table 1. Coded and actual levels of three factors used in Box-Behnken design (BBD) for acid and acid steam pretreatment

Independent variable	Code	Code and actual factor level		
		−1	0	+1
H_2SO_4 concentration [% w/v]	x_1	0.6	0.8	1.0
Substrate concentration [g]	x_2	5	10	15
Reaction time [h]	x_3	4	6	8

Table 2. Experimental design showing the observed and predicted values of variables and cellulose percentage in samples pretreated with dilute sulfuric acid

Run number	X_1	X_2	X_3	Cellulose [%]		Residual value
				Observed	Predicted	
1	0.8	10	6	42.20000	42.20000	−0.000000
2	1.0	10	8	48.60000	48.31000	0.290000
3	1.0	15	6	71.68000	71.16000	0.520000
4	1.0	10	4	48.00000	48.01000	−0.010000
5	1.0	5	6	47.00000	47.80000	−0.800000
6	0.6	15	6	44.40000	43.60000	0.800000
7	0.8	5	4	41.40000	40.59000	0.810000
8	0.6	10	8	37.20000	37.19000	0.010000
9	0.8	15	8	45.40000	46.21000	−0.810000
10	0.6	10	4	38.20000	38.49000	−0.290000
11	0.6	5	6	54.20000	54.72000	−0.520000
12	0.8	5	8	36.40000	35.89000	0.510000
13	0.8	15	4	42.00000	42.51000	−0.510000

Table 3. Experimental designs showing the observed and predicted values of variables and cellulose percentage in samples pretreated with dilute sulfuric acid-steam

Run number	X_1	X_2	X_3	Cellulose [%]		Residual value
				Observed	Predicted	
1	0.8	10	6	41.20000	41.20000	−0.000000
2	1	10	8	44.00000	43.80250	0.197500
3	1	15	6	63.02000	62.46500	0.555000
4	1	10	4	48.20000	48.80250	−0.602500
5	1	5	6	38.60000	38.75000	−0.150000
6	0.6	15	6	41.60000	41.45000	0.150000
7	0.8	5	4	40.40000	39.64750	0.752500
8	0.6	10	8	49.60000	48.99750	0.602500
9	0.8	15	8	53.00000	53.75250	−0.752500
10	0.6	10	4	32.20000	32.39750	−0.197500
11	0.6	5	6	48.00000	48.55500	−0.555000
12	0.8	5	8	39.20000	39.24750	−0.047500
13	0.8	15	4	41.80000	41.75250	0.047500

Table 4. Analysis of variance for the regression equations of cellulose yield for dilute sulfuric acid pretreatment

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	9	742.987	82.554	158.63	0.000
Linear	3	268.058	89.353	171.69	0.000
H_2SO_4 conc. x_1	1	62.832	62.832	120.73	0.000
Substrate conc. x_2	1	137.946	137.946	265.07	0.000
Time x_3	1	67.280	67.280	129.28	0.000
Square	3	82.381	27.460	52.77	0.000
x_1^2	1	39.060	39.060	75.05	0.000
x_2^2	1	41.499	41.499	79.74	0.000
x_3^2	1	3.350	3.350	6.44	0.052
2-Way interaction	3	392.548	130.849	251.43	0.000
x_1x_2	1	237.468	237.468	456.30	0.000
x_1x_3	1	116.640	116.640	224.13	0.000
x_2x_3	1	38.440	38.440	73.86	0.000
Error	5	2.602	0.520		
Lack-of-fit	3	2.602	0.867		
Pure error	2	0.00	0.00		
Total	14		745.589		

Table 5. Analysis of variance for the regression equations of cellulose yield for dilute sulfuric acid-steam pretreatment

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	9	1043.18	115.908	151.65	0.000
Linear	3	288.41	96.138	125.78	0.000
H_2SO_4 conc. x_1	1	213.00	213.005	278.69	0.000
Substrate conc. x_2	1	74.91	74.909	98.01	0.000
Time x_3	1	0.50	0.500	0.65	0.455
Square	3	439.27	146.422	191.57	0.000
x_1^2	1	176.30	176.301	230.66	0.000
x_2^2	1	100.22	100.224	131.13	0.000
x_3^2	1	137.84	137.842	180.35	0.052
2-Way interaction	3	315.50	105.166	137.59	0.000
x_1x_2	1	297.22	297.218	388.87	0.000
x_1x_3	1	0.64	0.640	0.84	0.402
x_2x_3	1	17.640	17.640	23.08	0.005
Error	5	3.82	0.764		
Lack-of-fit	3	3.82	1.274		
Pure error	2	0.00	0.00		
Total	14		1047.00		

Statistical analysis

Minitab v. 17.0 Trial Version was used for plotting graphs and for the regression analysis. The ANOVA *F*-test was used for verifying the model's significance.

Results and discussion

Recently, a lot of researchers are focusing on biofuel production from lignocellulosic biomass, which is a renewable energy source (Binder et al., 2009). The pretreatment of lignocellulosic biomass is a very important step to remove hemicellulose and lignin by disturbing their structure and to improve enzymatic hydrolysis (Alvira et al., 2010). This process enhances the enzymatic conversion of cellulose to sugar units (Satyanagalakshmi et al., 2011) and reduces the need for digestion using hemicellulases (Saha, 2004). For improving the enzymatic hydrolysis of cellulose, pretreatment with dilute sulfuric acid and acetic acid was used in a number of studies (Satyanagalakshmi et al., 2011). Gajula et al. (2011) used groundnut shells for conversion to fermentable sugars with sodium sulfite being used for delignification. After enzymatic hydrolysis with commercial cellulase (50 h, 120 rpm, 50 °C), the yield obtained was 670 mg/g.

In this study, PS were pretreated using dilute acid to increase the accessibility of cellulose. Chemically, PS are composed of 40% cellulose, 26% lignin, and 14.7% hemicelluloses; therefore, they have a good amount of sugars that can be converted to biofuel (Van Dyk et al., 2012). Unfortunately, because of the high lignin content, PS require pretreatment. For this purpose, three parameters, i.e., the amount of substrate (5, 10, and 15%), sulfuric acid concentration (0.6, 0.8, and 1%), and pretreatment time (4 h, 6 h, 8 h) were studied. In this study, we determined that the raw PS contained 32.4% cellulose. Kiran and coworkers (2013) reported similar results with 33% cellulose content in acid-treated PS. The percentage of cellulose content in samples pretreated with dilute H_2SO_4 improved significantly (from 36.4 to 71.6%) (Table 2), while in acid-steam pretreated samples, the percentage ranged from 32.2 to 63.02% (Table 3). Note that sulfuric acid concentration of 1%, substrate loading of 15%, and 6 h of residence time resulted in the maximum yield of 71.6 and 63.02% of cellulose in pretreated sulfuric acid and sulfuric acid-steam pretreated PS samples, respectively. All responses toward

both treatments were calculated via second order polynomial regression equations, as shown in Eq. 3 and Eq. 4.

The regression equation for cellulose content for dilute H_2SO_4 pretreated samples is as follows:

$$\text{Cellulose [\%]} = 179.0 - 342.8X_1 - 11.712X_2 + 15.31X_3 + 172.8 + 0.2084 - 1.528 + 8.620X_1X_2 + 1.00X_1X_3 + 0.2100X_2X_3$$

<div align="right">Eq. 3</div>

The regression equation for cellulose content of dilute H_2SO_4-steam pretreated samples is as follows:

$$\text{Cellulose [\%]} = 85.30 - 112.1X_1 - 9.876X_2 + 12.01X_3 + 81.31 + 0.1341 - 0.2381 + 7.705X_1X_2 - 13.500X_1X_3 + 0.3100X_2X_3$$

<div align="right">Eq. 4</div>
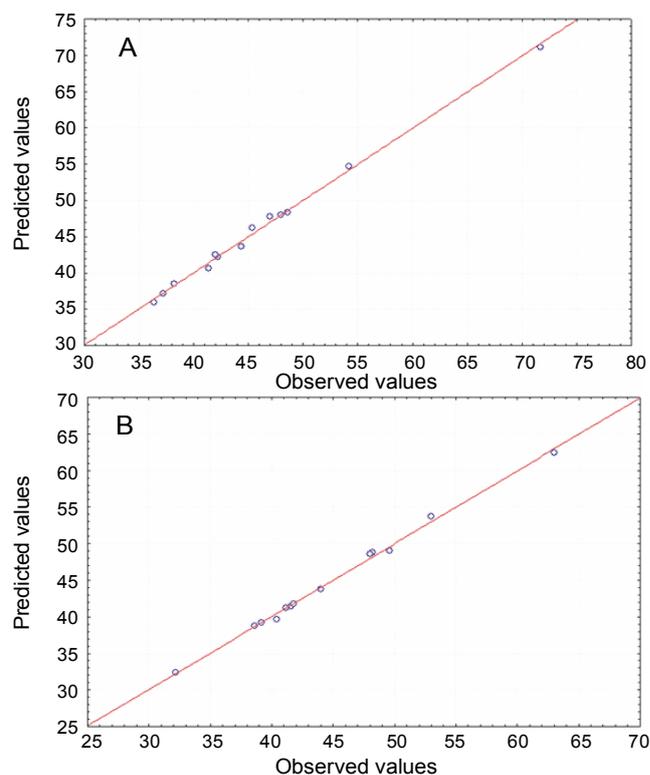
Fig. 1. Correlation between the observed versus A) the predicted values of cellulosic content of acid treated and B) acid--steam pretreated peanut shell samples

Tables 4 and Table 5 show the analysis of variance of cellulose yield for dilute sulfuric acid and dilute sulfuric acid–steam pretreatments. The large *F*-values of 158.63 (dilute sulfuric acid) and 151.65 (dilute sulfuric acid-steam pretreatment) and the corresponding value of pro-

A

Contour plot of cellulose [%] vs time [h], substrate conc. [%]

B

Contour plot of cellulose [%] vs time [h], substrate conc. [%]

Contour plot of cellulose [%] vs time [h], H₂SO₄ conc. [%]

Contour plot of cellulose [%] vs time [h], H₂SO₄ conc. [%]

Contour plot of cellulose [%] vs substrate conc. [%], H₂SO₄ conc. [%]

Contour plot of cellulose [%] vs substrate conc. [%], H₂SO₄ conc. [%]
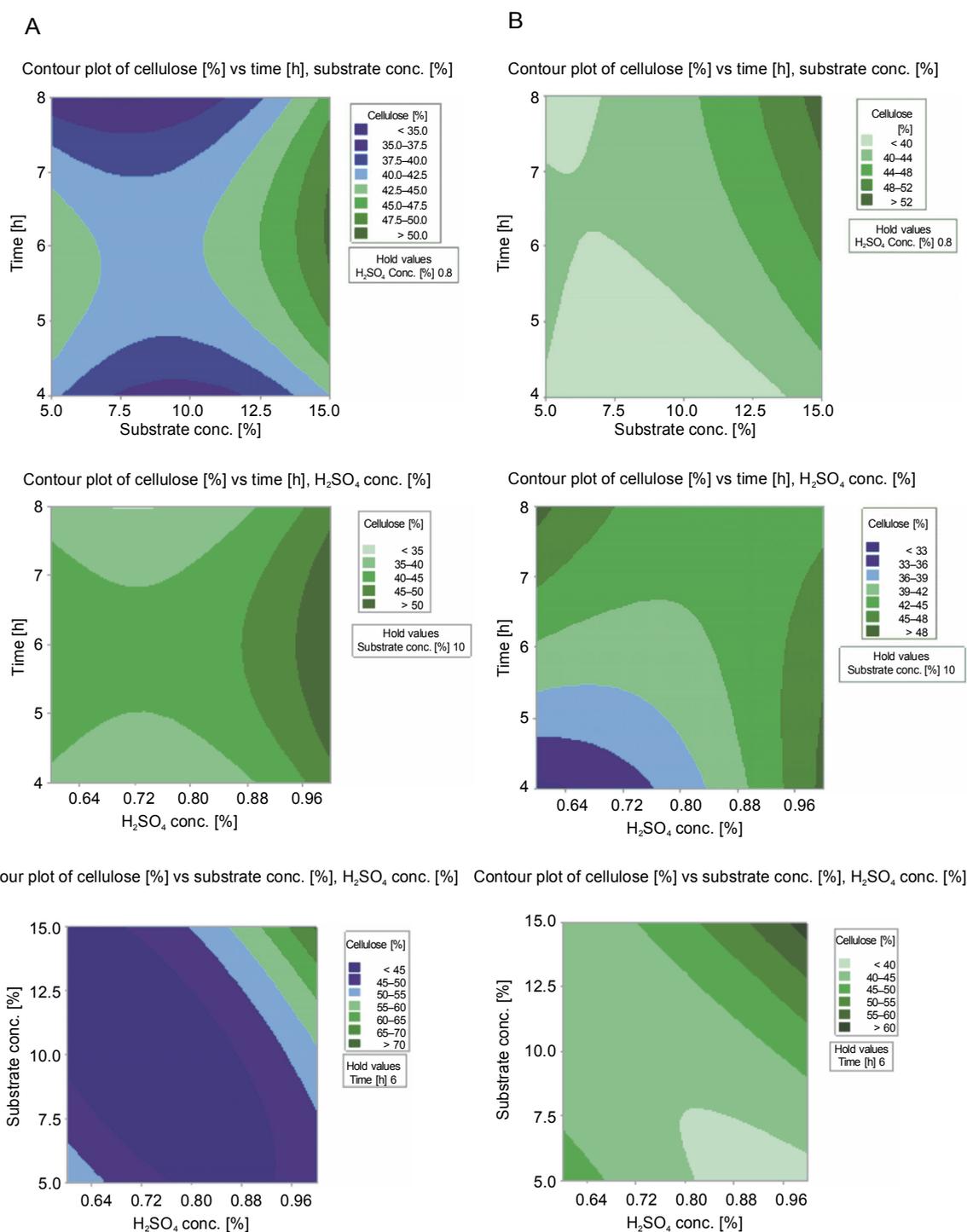
Fig. 2. Contour plots showing cellulose contents in A) acid and B) acid-steam treated peanut shells; various colors indicated the different range of cellulose content at different variables

Profiles for predicted values and desirability

Profiles for predicted values and desirability
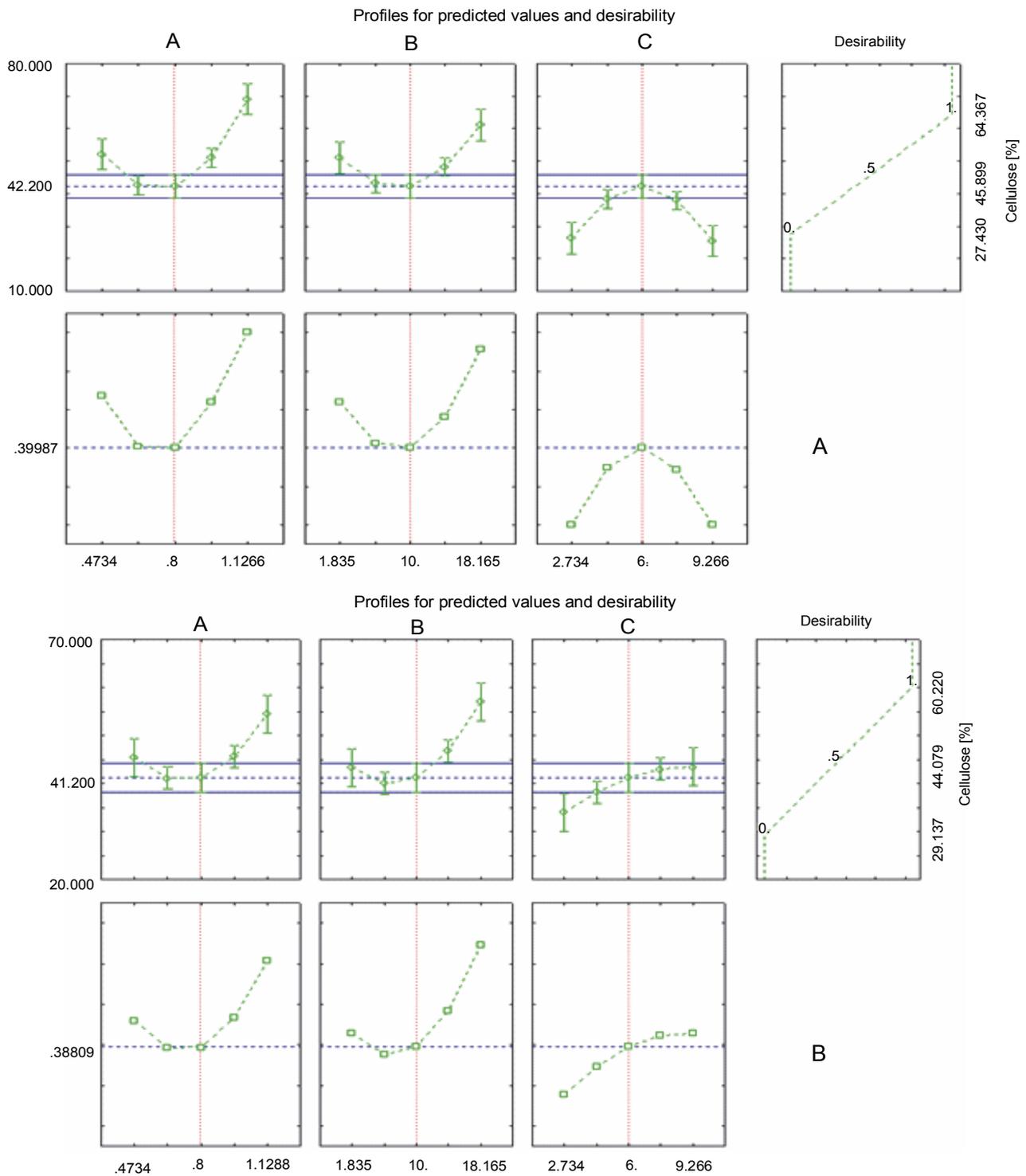
Fig. 3. Desirability chart of percentage cellulose content for A) dilute sulfuric acid pretreatment and B) dilute sulfuric acid-steam pretreatment of peanut shells
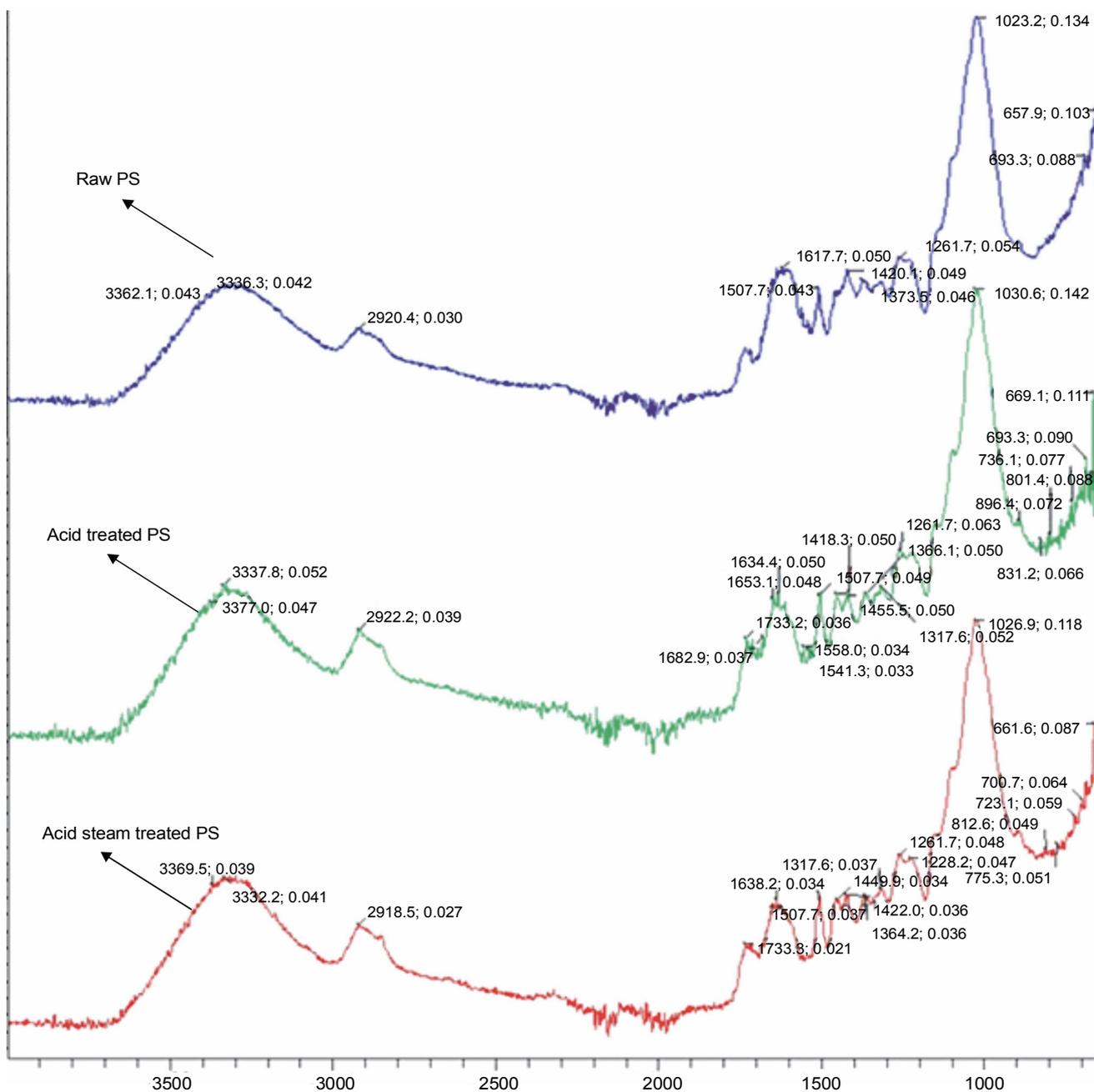
Fig 4. FTIR spectra of raw, acid pretreated, and acid-steam pretreated PS samples optimized through response surface methodology

bability $P > F < 0.001$ show that the model was significant; moreover, the probability values of 0.00 and 0.00 ($P < 0.05$) showed the significance of the models. The higher R^2 value in this model was 0.9963 and 0.9965, and its corresponding values of adjusted R^2 was 0.9898 and 0.9902, which indicated the accuracy of the proposed model (Fig. 1). The model also successfully explains variations up to 99.63 and 99.65% in acid and acid-steam pretreatment conditions, respectively. These re-

sults agree with those obtained by Bhatt and Shilpa (2014) who reported that dilute acid hydrolysis (hydrochloric acid) was the most effective out of all the chemicals that were tested.

Figure 2 shows the contour plots depicting the effect of different variables on cellulose yield. These plots clearly show that, during the pretreatment process, each factor had a significant impact on cellulose yield. In each plot, a different color area represents the range of ce-

llulose percentage at different concentrations. Figure 3 shows the desirability chart for the percentage of cellulose yield during pretreatment. It revealed that cellulose yield could be achieved at pretreatment conditions of 0.8% sulfuric acid concentration, 10% substrate loading and a residence time of 6 h with a cellulose percentage of 61.93%; these values were later validated by repetitive experiments.

The change in the cellulose structure of untreated, sulfuric acid pretreated, and sulfuric acid–steam pretreated samples was analyzed using FTIR spectroscopy. A significant difference between the raw and pretreated samples was observed, as shown in Figure 4. The absorption band at 900 and 1100 cm^{-1} was primarily observed because of the C–O–C stretching caused by the β-1,4-glycosidic linkage (Sindhu et al., 2011). Similarly, the structural changes in cellulose and hemicelluloses were observed by bands at 1000 and 1200 cm^{-1}. C–H peaks were observed at 1281 and 1373 cm^{-1} (Binod et al., 2012). Moreover, the symmetric bending of CH$_2$ was observed as peaks at 1316 and 1431 cm^{-1} (Cao and Tan, 2004). The peak at 2850 cm^{-1} was a unique feature of cellulose, which appeared because of –CH$_2$ stretching (Sun and Cheng, 2008). Similarly, the peak at 3250 cm^{-1} corresponded to the stretching of the H-bonded OH group (Sindhu et al., 2011).

The difference in the FTIR spectrum between the raw and pretreated samples was because of differences in various peaks. The increase in cellulose contents was visible by the increase in absorption peaks at 1000 cm^{-1} and 1100 cm^{-1} (Sun and Cheng, 2008). The increased absorption in the region at 3300 cm^{-1} was because of O–H stretching, while the increased absorption at 2900 cm^{-1} was because of –CH$_2$ stretching. These two bands were characteristic features of cellulose (Binod et al., 2012). The –OH stretching of hydrogen bonds was visible in the region between 3200 and 3400 cm^{-1} (Hsu et al., 2010). In this study, the FTIR spectra confirmed the bond stretching between the untreated and pretreated samples, indicating the effectiveness of the pretreatment process, which was optimized using RSM.

Conclusions

The results confirm that optimized pretreatment conditions of 1% dilute sulfuric acid, 15% substrate loading, and 6 h of residence time yielded the maximum

cellulose content of 71.16% in peanut shells. Thus, this process could potentially be used for further saccharification process required for the production of fermentable sugars that are used in valuable products, particularly ethanol.

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