OPTIMIZATION OF DILUTE SULPHURIC ACID PRETREATMENT OF COTTON STALK THROUGH BOX BEHNKEN DESIGN OF RESPONSE SURFACE METHODOLOGY

MUHAMMAD IRFAN,[*] RAAZIA KIRAN,[*] NOOR FATIMA,[**] MISBAH GHAZANFAR,[*] HAFIZ ABDULLAH SHAKIR,[**] MUHAMMAD KHAN,[**] IRFAN AHMAD[***] and MARCELO FRANCO[****]

[*]*Department of Biotechnology, University of Sargodha, Sargodha, Pakistan*
[**]*Microbial Biotechnology Laboratory, Institute of Zoology, University of the Punjab, Lahore, Pakistan*
[***]*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha, Saudi Arabia*
[****]*Department of Exact Science, State University of Santa Cruz, Ilheus, Brazil*
✉ *Corresponding author: M. Irfan, irfan.ashraf@uos.edu.pk*

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The purpose of the current study was to use diluted sulfuric acid to optimize the pretreatment conditions for cotton stalk. Different quantities (w/v) of cotton stalk (5%, 10%, 15%) were pretreated with different concentrations of H_2SO_4 (0.6%, 0.8%, 1%) for 4, 6 and 8 hours to degrade the crystalline structure of cellulose and to facilitate the hydrolysis of the cellulosic component. Dilute acidic pretreatment was also conducted in steam conditions at 121 °C, 15 psi. A statistical model was created using a three-level Box Behnken design (BBD) to optimize the process variables. Maximum results regarding cellulose exposure (85%) were recorded with 15% substrate loading, 0.8% acid concentration and time period of 8 hours followed by steam. Maximum total phenolic compounds (8.17 mg/mL) were observed under the same conditions, except steam. The effectiveness of the pretreatment was also analyzed by FTIR and XRD techniques. The results were analysed using ANOVA with a second order polynomial equation. The P value < 0.05 showed the significance of the model. The pretreatment conditions that allowed obtaining maximum cellulose content can be used for enzymatic hydrolysis to produce maximum sugars.

Keywords: cellulose, cotton stalk, pretreatment, acid, H_2SO_4, substrate

INTRODUCTION

The extensive manipulation of fossil fuels and the associated environmental dangers, such as the emission of greenhouse gases and climate change, have prompted researchers to look into alternative and sustainable energy sources. With its high sugar content and abundance, lignocellulosic biomass is a capable feedstock for bioethanol production. Natural resources for lignocellulosic biomass are widely available; these include softwood, hardwood, plants, grasses, and agricultural wastes. The three primary constituents of lignocellulosic material are cellulose, hemicelluloses and lignin, with amounts of 35–50%, 20–35% and 10–25%, respectively. The kind of substrate and the processing both affect the composition. These components have strong chemical connections, making it challenging to break down into sugars that can subsequently be transformed into biofuels.[1-4] Cellulose can be hydrolyzed into glucose, which is then fermented to produce ethanol. Ethanol produced from lignocellulose biomass can be utilized in place of or in addition to fossil fuels, due to its minimal impact on the environment.[5]

A most important challenge in the field of biotechnology is the low yield at which lignocellulosic biomass can be bio-converted into sugars. Because of the crystalline assembly of the cellulose and the presence of lignin, raw lignocellulosic biomass is hard to degrade.[6]

Phenolic chemicals are found in lignin in considerable amounts.[7] Cellulolytic enzyme cannot reach cellulose because of lignin. Pretreatment is essential to alter the structural and chemical composition of lignocellulosic biomass to enable the swift and active conversion of carbohydrates into sugars.[2,8] Enzymes have been made more accessible to cellulosic fibers using a wide range of physical (hydrothermolysis and comminution), biological pretreatment, chemical (ozone, solvents, acid and alkali) and physicochemical (ammonia fiber explosion and steam explosion) procedures.[9,10]

Lignocellulosic material is cheap and is available in surplus for the production of bioethanol. Cotton, also known as white gold, is a chief marketable crop globally and playing an important part in social, economic and political affairs,[11,12] accounting for about 40% of the fiber produced worldwide.[13] One type of lignocellulosic agricultural waste is the cotton stalk, which includes the branches and stems that are left over after the cotton is harvested. The world's cultivable cotton acreage is estimated to be 32 million hectares. Two metric tons of cotton stalk are thought to be produced for each hectare of cotton produced.[14,15] Lignin (31%) and holocellulose (46%) make up the majority of cotton stalks, however this varies depending on the area.[16,17]

A statistical and mathematical modeling tool called response surface methodology (RSM) is used to look at how various factors and how they interact affect productivity. With this approach, several biotechnological processes are optimized.[18,19] The popular one element at a time method of condition optimization is laborious and time-consuming, and it may lead to erroneous conclusions in the end. Whereas response surface methodology delivers a faster interaction of several factors on the answer. As a result, scientists are already employing this method to maximize production by optimizing many process parameters. The Box-Behnken design (BBD) was used in this investigation at three distinct levels to improve cotton stalk pretreatment conditions for the production of bioethanol at various H_2SO_4 concentrations.

EXPERIMENTAL

Substrate preparation

Cotton stalk were picked from the fields of Shahkot, District Nankana, Punjab, Pakistan. After washing, these were dried and milled to powder form (approximately 2 mm) and saved for later use.[20]

Pretreatment of cotton stalk

The substrate (10 g) was soaked in dilute sulphuric acid solution (100 mL) at room temperature for two hours, followed by a steam pretreatment, according to the experimental design. Then, the substrate was filtered and washed up to neutrality.[21]

Fourier transform infrared spectroscopy (FTIR) analysis

The chemical modifications in treated samples, compared to the untreated one, were examined using FTIR. The sample (without any preparation) was placed in the sample holder of the FTIR spectrometer (Cary 630 FTIR Spectrometer, Agilent Technologies, USA). At a resolution of 4 cm^{-1}, the spectra were captured in the frequency range of 4000–400 cm^{-1}.[21]

Analytical methods

Using the approach described by Gopal and Ranjhan, the content of cellulose in the residue was determined.[22] The technique of Dubois *et al*. was used to assess the levels of total sugars (TS).[23] Total phenols (TP) in the filtrate were measured according to Carralero *et al*.[24]

Design of experiment

In this work, the pretreatment conditions were optimized using BBD, which has three components and three levels. Table 1 lists the independent variables that were used: time (X3), substrate concentration (X2), and NaOH concentration (X1). This architecture yields a second-order polynomial regression model and is best suited for the quadratic response surface. The equation that follows describes the relationship between real and coded values:

$$x_i = \frac{X_i - X_o}{\Delta X_i} \quad (1)$$

where X_o is the independent variable's real value at the center point, ΔX_i is the change of x_i, and x_i and X_i are the independent variable's coded and actual values. Y is the response, X_1, X_2, and X_3 are independent variables, $ß_0$ is the intercept, $ß_1$, $ß_2$, and $ß_3$ are linear coefficients, $ß_{11}$, $ß_{22}$, and $ß_{33}$ are square coefficients, and $ß_{12}$, $ß_{13}$, and $ß_{23}$ are interaction coefficients. The response was computed from the following equation using Minitab software (17th version):[2]

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

Table 1
BBD levels and codes of the variables

Independent variable	Codes	Coded and actual values		
		-1	0	+1
H_2SO_4 Conc. (%)	X_1	0.6	0.8	1
Temperature (°C)	X_2	5	10	15
Time (h)	X_3	4	6	8

RESULTS AND DISCUSSION

This investigation involved treating powdered dry cotton stalk at various sulphuric acid concentrations. Pretreatment was done using both chemical and thermochemical techniques. The substrate in the chemical pretreatment approach was merely treated with H_2SO_4 solution, in the thermochemical procedure, sulphuric acid pretreatment was followed by steaming at 15 psi, 121 °C, and 15 minutes. It was assumed that the highest release of TP reflects the biggest degradation of lignin, since phenol is formed during the degradation of lignin and the TS represents the breakdown of the cellulose and hemicelluloses content of cotton stalk biomass. We measured the amount of cellulose together with TP and TS since the goal was to reveal the largest amount of cellulose and eliminate the maximum amount of lignin. So, we employed BBD of RSM with three variables and three stages to optimize the pretreatment conditions. Concentration of H_2SO_4 solution (X1), substrate concentration (X2), and residence duration (X3) were the three pretreatment variables that were employed. Three analyses were performed: the residue was used to quantify cellulose, and the filtrate of pretreated biomass was used to test total phenols (mg/mL) and total sugar (mg/mL). Equations (3-8), which are 2nd order polynomial regression equations, were used to determine the response (Tables 2 and 3).

Maximum cellulose (85%) was recorded in thermochemical pretreatment at 15% substrate loading, 0.8% acid concentration and time period of 8 hours. Meanwhile, 47.2% cellulose was observed in chemical pretreatment alone using 0.8% sulphuric acid at 5% substrate loading for 4 h. After 0.8% acid content, 15% biomass loading, and an 8-hour soaking period without steam pretreatment, the maximum total phenolic value was 8.17 mg/mL; after thermochemical pretreatment, it was 5.27 mg/mL. Maximum TS released was 124.7 mg/mL during H_2SO_4 pretreatment, when followed by autoclaving at 0.8% H_2SO_4 concentration, 10% biomass loading and 6 h residence time, while maximum TS observed during chemical pretreatment was 63.46 mg/mL. Compared to the chemical treatment, there was a greater generation of sugar during the thermochemical treatment. Additionally, the cellulose content was greater than it was with just chemical treatment. This indicates that lignin, hemicelluloses, and cellulose were all very well solubilized using thermochemical treatment.

Regression equations for chemical treatments:

$$\text{Cellulose (\%)} = 1.1+107.6X_1 - 0.325X_2 + 3.53X_3 - 45.0X_1^2 + 0.0400X_2^2 - 0.113X_3^2 - 2.000X_1X_2 - 5.13X_1X_3 + 0.1250X_2X_3 \quad (3)$$

$$\text{Total sugars (mg/mL)} = 141.8 - 219X_1 - 0.64X_2 - 13.4X_3 + 110.6X_1^2 + 0.122X_2^2 + 0.719X_3^2 + 2.02X_1X_2 + 5.03X_1X_3 + 0.017X_2X_3 \quad (4)$$

$$\text{Total phenols (mg/mL)} = 23.9 - 12.9X_1 - 0.97X_2 + 3.90X_3 + 7.1X_1^2 + 0.0103X_2^2 + 0.335X_3^2 + 0.796X_1X_2 - 1.03X_1X_3 + 0.0643X_2X_3 \quad (5)$$

Regression equations for chemical treatments followed by steam:

$$\text{Cellulose (\%)} = -24.4 + 177.6X_1 + 3.43X_2 - 3.41X_3 - 101.2X_1^2 + 0.3240X_2^2 + 1.087X_3^2 - 1.750X_1X_2 - 0.00X_1X_3 - 1.2650X_2X_3 \quad (6)$$

$$\text{Total sugars (mg/mL)} = -564.8 + 985.3X_1 + 19.20X_2 + 590.8X_1^2 - 0.7573X_2^2 - 4.090X_3^2 + 2.71X_1X_2 - 6.12X_1X_3 + 0.310X_2X_3 \quad (7)$$

$$\text{Total phenols (mg/mL)} = 16.28 - 1.91X_1 + 0.117X_2 - 4.565X_3 + 0.72X_1^2 - 0.00114X_2^2 + 0.3684X_3^2 - 0.064X_1X_2 + 0.032X_1X_3 + 0.0172X_2X_3 \quad (8)$$

Table 2
BBD table for yield of cellulose, TS and TP after pretreatment with H_2SO_4

Run no.	X_1	X_2	X_3	Cellulose (%)			Total phenols (mg/mL)			Total sugars (mg/mL)		
				Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual
1	0.8	10	6	43.2	43.2	0.00	3.30	3.30	0.00	30.1	30.10	0.00
2	1	10	8	34.0	34.5	-0.52	4.45	4.41	0.04	36.46	39.93	-3.47
3	1	15	6	35.0	35.5	-0.50	4.45	5.97	-1.51	63.46	58.87	4.59
4	1	10	4	41.8	41.3	0.47	6.49	5.51	0.97	41.71	38.14	3.56
5	1	5	6	43.8	43.2	0.55	2.29	1.79	0.49	14.87	19.57	-4.69
6	0.6	15	6	45.0	45.5	-0.55	3.81	4.31	-0.49	56.24	51.55	4.69
7	0.8	5	4	47.2	48.2	-1.02	2.92	4.39	-1.47	20.81	19.68	1.12
8	0.6	10	8	44.2	44.6	-0.47	4.20	5.17	-0.97	29.06	32.63	-3.56
9	0.8	15	8	42.8	41.7	1.02	8.17	6.70	1.47	51.6	52.72	-1.12
10	0.6	10	4	43.8	43.2	0.52	4.58	4.62	-0.04	42.35	38.88	3.47
11	0.6	5	6	45.8	45.3	0.50	4.83	3.32	1.51	15.73	20.33	-4.59
12	0.8	5	8	43.0	43.0	-0.02	2.29	2.83	-0.54	25.28	17.11	8.16
13	0.8	15	4	42.0	41.9	0.02	6.23	5.69	0.54	46.44	54.60	-8.16

Table 3
BBD table for yield of cellulose, TS and TP after steam pretreatment with H_2SO_4

Run no.	X_1	X_2	X_3	Cellulose (%)			Total phenols (mg/mL)			Total sugars (mg/mL)		
				Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual
1	0.8	10	6	48.4	48.4	0.00	2.80	2.80	0.00	124.7	124.70	0.00
2	1	10	8	43.0	42.3	0.70	4.20	4.17	0.02	85.31	86.38	-1.07
3	1	15	6	53.2	54.9	-1.72	3.18	3.22	-0.04	113.62	112.76	0.85
4	1	10	4	55.2	54.3	0.85	4.20	3.94	0.25	92.88	95.19	-2.31
5	1	5	6	49.4	49.2	0.17	1.65	1.89	-0.23	66.22	63.68	2.53
6	0.6	15	6	59.0	59.1	-0.17	4.07	3.83	0.23	92.622	95.16	-2.53
7	0.8	5	4	48.6	49.6	-1.02	3.56	3.58	0.01	66.22	66.43	-0.21
8	0.6	10	8	42.2	43.0	-0.85	4.37	4.63	-0.25	81.42	79.10	2.31
9	0.8	15	8	47.8	46.7	1.02	5.27	5.25	0.01	106.39	106.17	0.21
10	0.6	10	4	54.4	55.1	-0.70	4.43	4.45	-0.02	79.20	78.12	1.07
11	0.6	5	6	48.2	46.4	1.72	2.29	2.25	0.04	56.07	56.92	-0.85
12	0.8	5	8	62.0	62.8	-0.87	3.66	3.44	0.21	67.25	68.71	-1.46
13	0.8	15	4	85.0	84.1	0.87	4.48	4.69	-0.21	117.75	116.28	1.46

Table 4
Analysis of variance of cellulose, TS and TP after H_2SO_4 pretreatment

	Source	DF	Adj SS	Adj MS	F value	P value
Cellulose (%)	Model	9	172.323	19.1417	22.74	0.002
	Linear	3	115.910	38.6367	45.89	0.000
	X_1	1	73.205	73.2050	86.94	0.000
	X_2	1	28.125	28.1250	33.40	0.002
	X_3	1	14.580	14.5800	17.32	0.009
	Square	3	17.353	5.7844	6.87	0.032
	X_1^2	1	11.963	11.9631	14.21	0.013
	X_2^2	1	3.692	3.6923	4.39	0.090
	X_3^2	1	0.784	0.7477	0.89	0.389
	2-way interaction	3	39.060	13.0200	15.46	0.006
	X_1*X_2	1	16.000	16.0000	19.00	0.007
	X_1*X_3	1	16.810	16.8100	19.96	0.007
	X_2*X_3	1	6.250	6.2500	7.42	0.042
	Error	5	4.210	0.8420		
	Lack of fit	3	4.210	1.4033		
	Pure error	2	0.000	0.000		
	Total	14	176.533			
Total sugars (mg/mL)	Model	9	2670.72	296.75	5.46	0.03
	X_1	1	21.50	21.50	0.40	0.55
	X_2	1	2486.54	2486.54	45.76	0.00
	X_3	1	9.90	9.90	0.18	0.68
	X_1^2	1	72.25	72.25	1.33	0.30
	X_2^2	1	34.54	34.54	0.64	0.46
	X_3^2	1	30.53	30.53	0.56	0.4
	X_1*X_2	1	16.34	16.34	0.30	0.60
	X_1*X_3	1	16.16	16.16	0.30	0.60
	X_2*X_3	1	0.12	0.12	0.00	0.96
	Error	5	271.71	54.34		
	Lack of fit	3	271.71	90.57		
	Pure error	2	0.00	0.00		
	Total	14	2942.43			
Total phenols (mg/mL)	Model	9	25.21	2.80	1.18	0.45
	X_1	1	0.00	0.00	0.00	0.95
	X_2	1	13.35	13.35	5.62	0.06
	X_3	1	0.15	0.15	0.07	0.80
	X_1^2	1	0.29	0.24	0.12	0.73
	X_2^2	1	0.24	0.24	0.10	0.76
	X_3^2	1	6.62	6.62	2.79	0.15
	X_1*X_2	1	2.53	2.53	1.06	0..34
	X_1*X_3	1	0.68	0.68	0.29	0.61
	X_2*X_3	1	1.65	1.65	0.70	0..44
	Error	5	11.89	2.37		
	Lack of fit	3	11.89	3.96		
	Pure error	2	0.00	0.00		
	Total	14	37.10			

Table 5
Analysis of variance of cellulose, TS and TP after H_2SO_4 steam pretreatment

	Sources	DF	Adj SS	Adj MS	F value	P value
Cellulose (%)	Model	9	1650.18	183.353	8.00	0.017
	Linear	3	460.81	153.603	6.70	0.033
	X_1	1	1.13	1.125	0.05	0.833
	X_2	1	169.28	169.280	7.39	0.042
	X_3	1	290.41	290.405	12.67	0.016
	Square	3	537.03	179.010	7.81	0.025
	X_1^2	1	14.52	14.524	0.63	0.462
	X_2^2	1	381.64	381.64	16.65	0.010
	X_3^2	1	152.26	152.26	6.63	0.050
	2-way interaction	3	652.34	217.447	9.49	0.017
	X_1*X_2	1	12.25	12.250	0.53	0.497
	X_1*X_3	1	0.00	0.000	0.00	1.000
	X_2*X_3	1	640.09	640.090	27.93	0.003
	Error	5	114.58	22.915		
	Lack of fit	3	12.07	4.023	0.08	
	Pure error	2	102.51	51.253		
	Total	14	1764.76			
Total sugars (mg/mL)	Model	9	8043.71	893.75	140.43	0.00
	X_1	1	296.59	296.59	46.60	0.00
	X_2	1	3812.02	3812.02	598.97	0.00
	X_3	1	30.69	30.69	4.82	0.07
	X_1^2	1	2062.19	2062.19	324.03	0.00
	X_2^2	1	1323.53	1323.53	207.96	0.00
	X_3^2	1	988.43	988.43	155.31	0.00
	X_1*X_2	3	91.73	30.58	4.63	0.08
	X_1*X_3	1	29.45	29.45	3.76	0.11
	X_2*X_3	1	23.95	23.95	6.02	0.05
	Error	5	31.82	6.36		
	Lack of fit	3	31.82	10.61		
	Pure error	2	0.00	0.00		
	Total	14	8075.53			
Total phenols (mg/mL)	Model	9	13.05	1.45	20.97	0.00
	X_1	1	0.46	0.46	6.77	0.04
	X_2	1	4.24	4.24	61.44	0.00
	X_3	1	0.08	0.08	1.28	0.31
	X_1^2	1	0.00	0.00	0.04	0.84
	X_2^2	1	0.00	0.00	0.04	0.84
	X_3^2	1	8.01	8.01	115.91	0.00
	X_1*X_2	1	0.01	0.01	0.23	0.64
	X_1*X_3	1	0.00	0.00	0.01	0.92
	X_2*X_3	1	0.11	0.11	1.71	0.24
	Error	5	0.34	0.06		
	Lack of fit	3	0.34	0.11		
	Pure error	2	0.00	0.00		
	Total	14	13.39			

After statistical analysis of all the data, regression equations were used to explain the noteworthy findings. The contour plots of cellulose, TS, and TP released under various pretreatment conditions are displayed in Figures 1 and 2. More sugars were liberated as a result of the greater hemicelluloses content breakdown caused by the H_2SO_4 and steam treatment under pressure. Table 4 displays the Fisher's F-test values for cellulose, TS, and TP for the chemical pretreatment approach, which are 22.74, 5.46, and 1.18, respectively. The F-test results for cellulose,

TS, and TP after thermochemical treatment were 8.00, 140.43, and 20.97, respectively (Table 5). The coefficient of determination (R^2) values for cellulose, total sugars, and total phenols of acid pretreatments were 97.62%, 90.77%, and 67.95%, respectively. In contrast, R^2 values for cellulose, total sugars, and total phenols in the case of acid steam pretreatment were 93.51%, 99.61%, and 97.42%, respectively. These values were used to assess the model's fitness. Thus, the model is appropriate for analyzing and optimizing the concentrations of cellulose, total sugars, and total phenols in accordance with sulfuric acid pretreatment conditions. The corrected R^2 value for cellulose, total sugars, and total phenols was 93.32%, 98.90%, and 92.77% for the acid pretreatment, and 81.82%, 98.90%, and 92.77% for the acid steam pretreatment, respectively. These values were used to further verify the model's correctness.

Acidic pretreatment was found efficient in breaking down the hemicelluloses and lignin, especially when followed by steam, because significant amounts of TS and TP were recorded. It means that chemical treatment, followed by steam, was more efficient in solubilizing lignin, hemicelluloses, and cellulose. In correspondence to our results, Sindhu *et al*.[25] also found better pretreatment of bamboo biomass with H_2SO_4, among various organic and mineral acids used. It was discovered that pretreatment with acid works better than pretreatment with alkali. The banana stem's lignin content decreased to 15.92% and 16.34%, respectively, and its cellulose content increased to 52.11% and 50.6%, following acidic and basic treatments.[26] Waste from palm tree trunks was pretreated in a separate research using varying HNO_3 and NH_4OH concentrations. The highest outcomes were obtained with a 10% HNO_3 pretreatment.[27]

A previous study on H_2SO_4 pretreatment of poplar biomass used three-factor BBD with three levels and achieved maximum liberation of TP of 57.39 mg/mL at H_2SO_4 concentration of 0.8%, biomass loading of 15% and 4 h soaking time. The TS and reducing sugars (RS) released under these conditions were of 161.20 and 5.24 mg/mL, respectively. The F-value of 48.39 supported their model.[28]

It had been reported that 1% H_2SO_4 concentration was best for maximum release of RS (33.35 g/L) during pretreatment of water hyacinth.[29] Shi and coworkers also recommended 1% H_2SO_4 pretreatment for maximum production of sugars from switch grass.[30] A recent study reported maximum sugars (0.16 g) released at 3% H_2SO_4 and 70 min incubation at 90 °C using the central composite design of RSM.[31]

Following the pretreatment, the mass balance and % degradation was computed. The greatest degradation (43.6%) was observed for hydrolysis using 0.8% H_2SO_4, 5% biomass loading, and 8 hours after steaming (Fig. 3). In contrast, the maximum degradation index in the chemical pretreatment was 35.14% obtained at 0.8% acid, 15% substrate loading, and 4 hours of steaming. In previous research, maximum breakdown (80%) was seen at 0.6% sulphuric acid solution with a 6-hour residence duration at room temperature. Maximum deterioration of 66.5% and minimum breakdown of 14.7% were observed when the same pretreatment was followed by steam exposure (121 °C, 15 min).[28] After pretreating poplar hybrids for five minutes at 200-220 °C, several studies observed a decent recovery rate of 74.9–67.3 g/100 g dry weight.[32]

As shown in Figure 4, a solid peak for polysaccharides (cellulose, hemicelluloses and lignin) was found at 1026.9 cm^{-1} in untreated cotton stalk. The increase in height of the peak up to 1030.6 cm^{-1} and 1030.6 cm^{-1} in treated biomass from 1026.9 cm^{-1} in the untreated one illustrates broadening of C-O, C=C, and C-C-O for polysaccharides. The peak at 674.6 cm^{-1} in untreated biomass became sharper in acid pretreated biomass at 697.0 cm^{-1}, depicting hemicelluloses' glycosidic bonding, while for the thermochemically pretreated substrate, the peak shifted to 667.2 cm^{-1}. The OH-stretching (3363.9 cm^{-1}) in raw biomass shifted to 3334.1 cm^{-1} in both pretreated samples. The peak at 1735.1 cm^{-1} became sharp, indicating ketone/aldehyde C=O stretch for hemicelluloses in both acid and acid steam pretreated biomass. CH_2 wagging was observed in raw, acid and acid steam pretreated samples at 1317.6 cm^{-1}. The peak at 896 cm^{-1} in all three plots is ascribed to the β-glycosidic bonds among the monomeric sugars of the cotton stem.[33,34] In raw cotton stalks, the C-O stretch peak is located at 1028 cm^{-1}. For both pretreatments, this peak migrated to 1030 cm^{-1}. The band at 1105 cm^{-1} was identified as the result of anti-symmetric stretching in planes.[35] The peak at 1157 cm^{-1} displays the C-O-C antisymmetric stretching of the bridge,[36,37] especially in the methoxy vibrations of cellulose and lignin; as well as the aromatic CCH bend.[38-41]

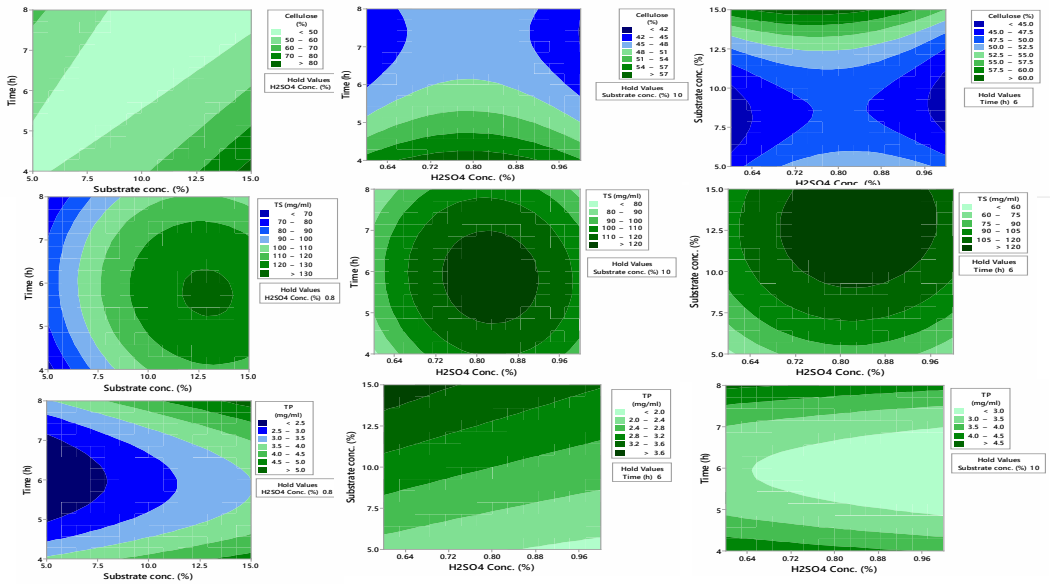

Figure 1: Contour plots of cellulose, TS and TP after H_2SO_4 pretreatment

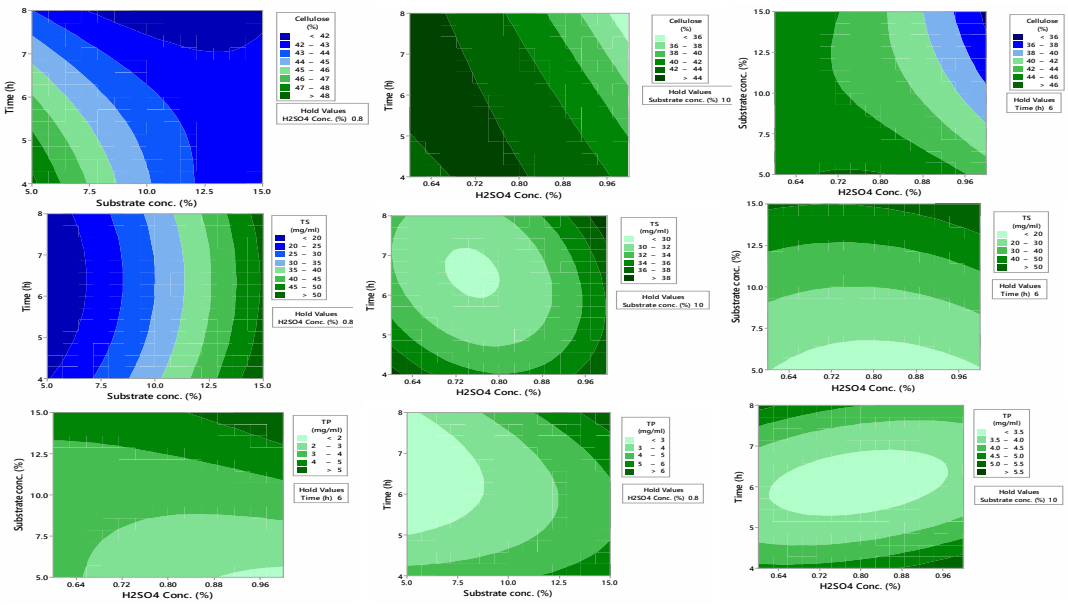

Figure 2: Contour plots of cellulose, TS and TP after H_2SO_4 steam pretreatment

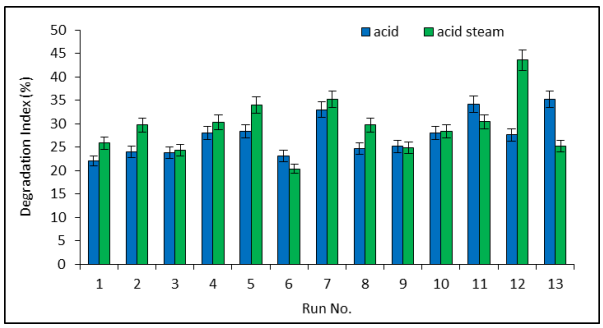

Figure 3: Degradation index of acid pretreatment and acid steam pretreatment of cotton stalks

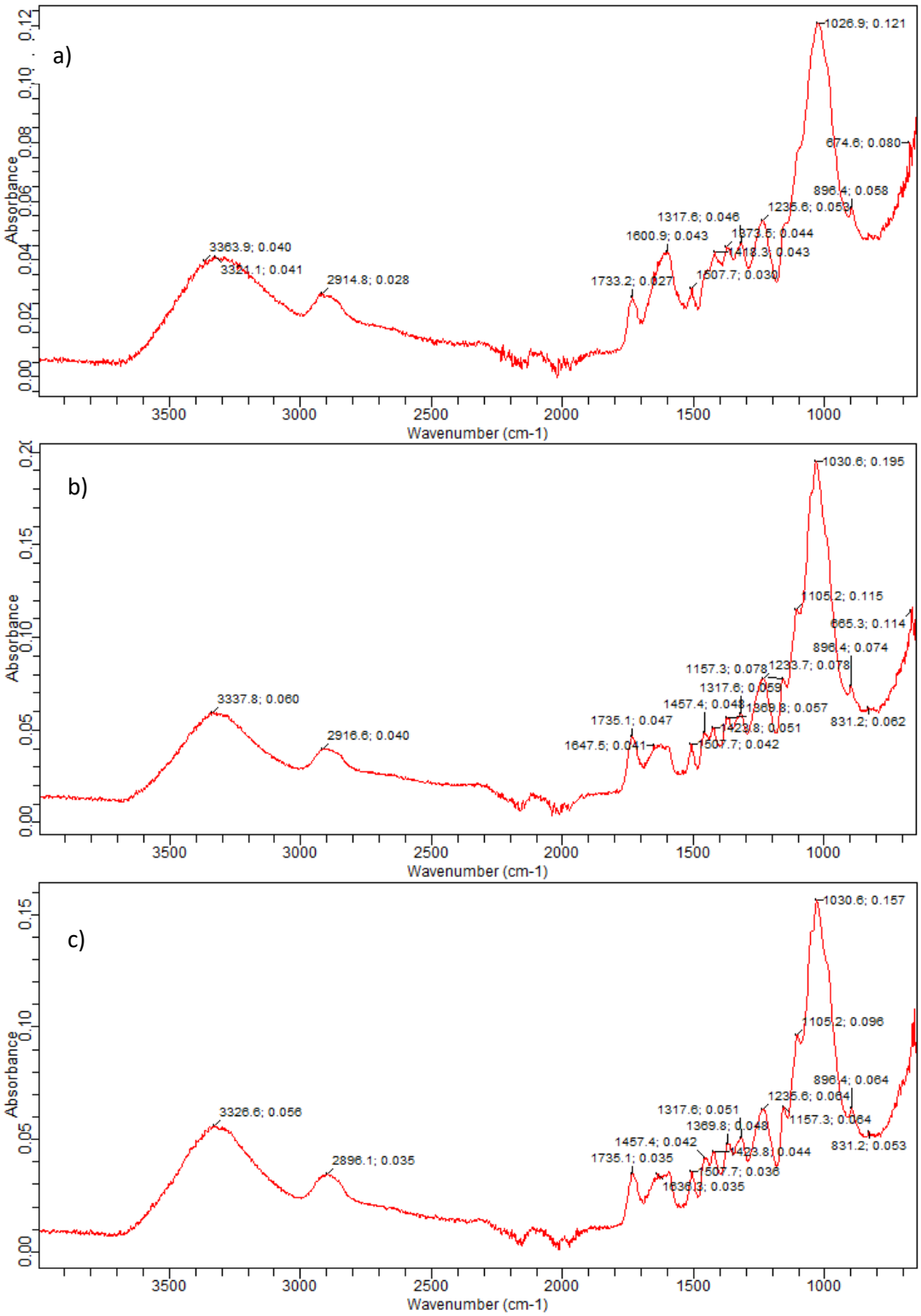

Figure 4: FTIR analysis of (a) raw, (b) H_2SO_4 treated and (c) H_2SO_4 steam treated cotton stalk

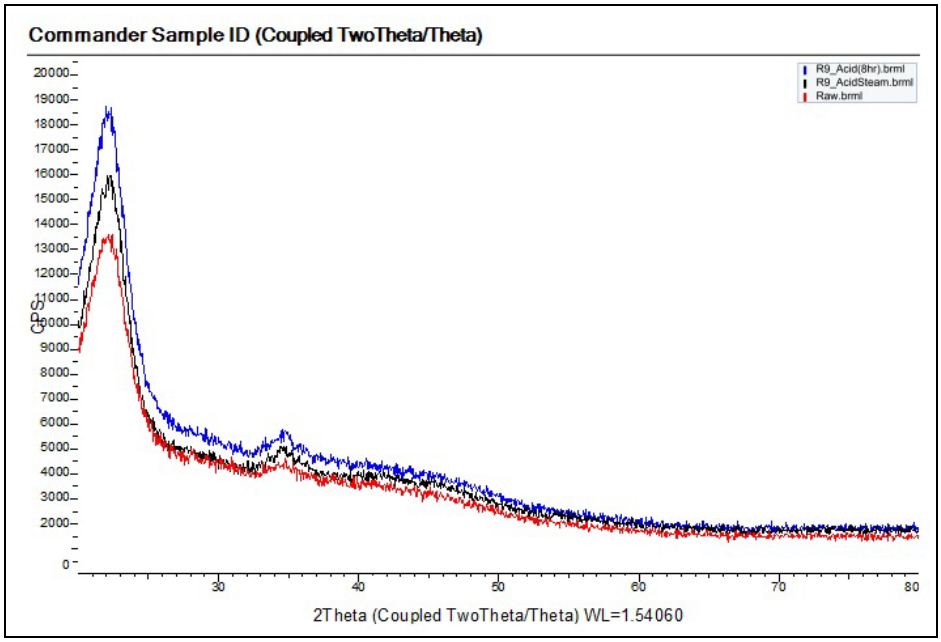

Figure 5: XRD patterns of raw and pretreated cotton stalks

The bands in the 2896-3337 cm^{-1} range indicate O-H stretching in the OH functional group, whereas the bands in the 2914-2916 cm^{-1} range are thought to represent C-H bending and stretching that may exist in the materials' methyl groups and methylene.[42] This demonstrates that H_2SO_4 pretreatment efficiently reduced the lignin content.

To measure the crystallinity of the cotton stalk, the X-ray diffraction analysis was conducted for untreated and pretreated samples. Figure 5 shows the X-ray diffraction patterns of the untreated and acid treated cotton stalk. The crystallinity of the material has been observed as the negative factor on the enzymatic digestibility of the biomass.[43-45] The crystalline value in the lignocellulosic material represents the amount of crystalline cellulose in the substrate. Lower crystalline value indicated the more amorphous part in the biomass.[46] The graph shows that raw cotton stalk has higher crystallinity 73.1%; the crystallinity decreases to 72.4% after acid pretreatment and to 70.3% after acid steam pretreatment. This indicates some alterations in the crystalline assembly of cellulose, along with elimination of hemicelluloses and lignin, which are amorphous structures.[47] The reduction in the substrate crystallinity may be related to the decrease in the particle size and the enhancement in the available surface area.[48-50]

CONCLUSION

The present study was conducted to obtain maximum cellulose from cotton stalk, and sulphuric acid pretreatment was used for this purpose. Maximum results (85% cellulose) were recorded after 0.8% acid pretreatment under steam conditions. The findings suggest that this pretreatment enables cotton stalk to become a potential feedstock for bioethanol production and could be efficiently used on an industrial scale.

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