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The Optimization of Dilute Acid Hydrolysis of Cotton Stalk in Xylose Production

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Abstract Cotton stalk, a lignocellulosic waste material, is composed of xylose that can be used as a raw material for production of xylitol, a high-value product. There is a growing interest in the use of lignocellulosic wastes for conversion into various chemicals because of their low cost and the fact that they are renewable and abundant. The objective of the study was to determine the effects of H_2SO_4 concentration, temperature, and reaction time on the production of sugars (xylose, glucose, and arabinose) and on the reaction by-products (furfural and acetic acid). Response surface methodology was used to optimize the hydrolysis process in order to obtain high xylose yield and selectivity. The optimum reaction temperature, reaction time, and acid concentration were 140 °C, 15 min, and 6%, respectively. Under these conditions, xylose yield and selectivity were found to be 47.88% and 2.26 g g^{-1}, respectively.

Keywords Xylose · Xylitol · Cotton stalk · Optimization

Introduction

There is an increasing interest in the utilization of lignocellulosic wastes for industrial purposes because these are inexpensive, renewable, and widely available in nature. Cotton stalk is one of the widely distributed and abundant lignocellulosic wastes found in Turkey [1]. Since cotton stalk is mainly composed of hemicellulose, cellulose, and lignin, it can be used as a renewable material for production of value-added products [2, 3] such as ethanol, glucose, xylose, and xylitol.

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The hemicellulose content of cotton stalks was reported to be between 15% and 25% [2, 4]. The main component of the hemicellulosic fraction is xylan, a heteropolysaccharide with homopolymeric backbone of xylose units [5]. This pentose sugar can be a source for production of chemicals including food-related products. One such compound is xylitol, a five-carbon sugar alcohol that is equivalent to sucrose in sweetness. It not only occurs widely in nature but is also produced in human metabolism. Unlike sucrose, this natural sweetener is anticariogenic and can be consumed by diabetics because it is metabolized by an insulin-independent pathway. Since it has a high negative heat of solution, it gives a pleasant cool and fresh sensation [6]. It has been used in various food products such as chewing gum, candy, soft drinks, and ice cream [7, 8].

The hemicellulosic fraction of lignocellulosic materials can be easily hydrolysed by mild acid treatment due to its amorphous structure, whereas cellulosic and lignin fractions remain unaltered. Although controlled acid hydrolysis of lignocellulosic biomass mainly produces xylose from hemicellulose, other by-products such as glucose, acetic acid, and furfural are produced in low amounts [9]. Hemicellulose hydrolysis of different lignocellulosic materials using dilute acid has been studied by many researchers [9–14]. The results showed that the amount of sugar released during hydrolysis is dependent on the type of material and operating conditions of the experiment including temperature reaction time and acid concentration [9]. Hydrolysis of hemicellulosic fraction involves the production of sugar and degradation of sugars to various products [14]. Acid concentration is the most important parameter that affects the sugar yield, while temperature is mainly responsible for the degradation of sugars to various by-products such as furfural [9] which strongly affects the microbial metabolism during xylitol production. To overcome this problem, it is necessary to run the hydrolysis reaction at less severe conditions to keep the degradation products at low concentrations.

As there has been no study on the use of acid hydrolysis of cotton stalks to produce xylose, this was the objective of the present investigation. For this purpose, the present study deals with the effect of acid concentration, reaction temperature, and reaction time on the yield of sugars (xylose, glucose, and arabinose) and the reaction by-products (furfural and acetic acid). Response surface methodology was used as a statistical design to optimize the formation of xylose in the hydrolysate.

Materials and Methods

Materials

Cotton stalks were collected from local farmers in Turkey. These were air-dried and milled into particles with 1–5 mm long and 1 mm thick. Aminex HPX 87H column (dimension 300×7.8 mm; average particle size 25 μm) and cation H cartridge were purchased from Bio-Rad Laboratories, Hercules, CA, USA. All the chemicals were analytical grade obtained either from Sigma Chemical Company, MO, USA, or Merck KGaA, Germany.

Cotton Stalks Composition

The stalks were analyzed following standard methods for the determination of moisture, ash, and lignin [15]. Moisture and ash were determined gravimetrically by desiccation of the samples at 105 °C and by ignition in an oven at 600 °C, respectively. Klason lignin (acid insoluble lignin) was gravimetrically measured as the insoluble fraction after digestion

with 72% sulfuric acid. Acid soluble lignin was determined by measuring the UV absorption at 205 nm using an extinction coefficient of 1,101 g^{-1} cm^{-1} [11]. Uronic acid was determined spectrophotometrically using glucoronic acid as a standard for quantification [16]. The protein content of the cotton stalk was measured by the Kjeldahl method (Protein=6.25×N).

The polysaccharides in the stalk were hydrolyzed according to Browning [17], and the monosaccharides composition was determined. Ground cotton stalk (300 mg) was mixed with 72% (*w*/*w*) sulfuric acid (3 mL), and the mixture was held at 30 °C for 1 h with stirring. The concentration of acid in the mixture was adjusted to 4.0% by adding water, and the mixture was refluxed for 2 h. The sugars in the aliquot of the hydrolysate were assayed by HPLC as described below. The monosaccharide presents in the hydrolysate were converted to percent monosaccharides: D-glucose to glucan, D-xylose to xylan, and D-arabinose to arabinan.

Acid Hydrolysis

Hydrolysis experiments were performed in a 100-mL stainless-steel pressure batch reactor. The reactor was loaded with 2 g of cotton stalk and 20 mL of sulfuric acid solution. The reactions were carried in the range of 100–140 °C under different sulfuric acid concentrations (2–6% *w*/*v* H_2SO_4) and residence times (15–45 min). After the reaction was completed, the solid material was separated with filtration, and the filtrate was analyzed for xylose, glucose, acetic acid, and furfural.

Analytical Methods

Hydrolysate samples were analyzed by HPLC system (Perkin Elmer) equipped with a refractive index detector (Perkin Elmer Series 200) and column oven (Perkin Elmer Series 200) on Aminex HPX 87H (300×7.8 mm), which was preceded by its complementary cation H cartridge. Before injection, samples were filtered through 0.22-μm filter. Aliquots of filtered sample (20 μL) were injected to the HPLC system. Sugars and acetic acid were eluted with 5 mmol L^{-1} H_2SO_4 as the mobile phase. Operational conditions were 45 °C and a flow rate of 0.5 mL min^{-1} [11]. A complete analysis was carried out in 70 min. Concentrations were determined by comparing the average peak areas of samples and standards (xylose, glucose, arabinose, acetic acid) and expressed as milligram per milliliter of sugar.

Furfural was analyzed by absorbance at 277 nm using a standard curve derived from a known concentration of furfural [18].

Experimental Design and Response Surface Methodology

A 2^3 rotatable central composite design was used to fit a second-order model and the design consisted of 20 sets of experiments. The quadratic model was selected for predicting the optimal point and is expressed as

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where Y represents response variables (xylose yield and selectivity), b_0 is the interception coefficient, b_1, b_2, and b_3 are the linear terms, b_{11}, b_{22}, and b_{33} are the quadratic terms, and X_1, X_2, and X_3 represent the variables studied.

Table 1 Composition of the raw material, expressed as percent of dry weight

Components	Content (g/100 g cotton stalk)
Glucan	33
Xylan	17
Arabinan	0.8
Acetyl groups	2.0
Uronic acid	7.5
Klason lignin	28
Acid soluble lignin	1.8
Proteins	1.7
Ash	3.8
Others (by diff.)	4.4

The Design Expert v. 7 (Stat-Ease Inc., Minneapolis, MN) was used for regression and graphical analyses of the data obtained. Fischer's test was used to determine the type of model equation, while the Student's *t* test was performed to determine the statistical significance of regression coefficients.

Results and Discussıon

Composition of Cotton Stalk

The chemical compositions of lignocellulosic biomass vary depending on the growing location, season, harvesting methods, as well as analysis process. The structures of these materials are very complex, but a detailed knowledge of their composition is necessary to

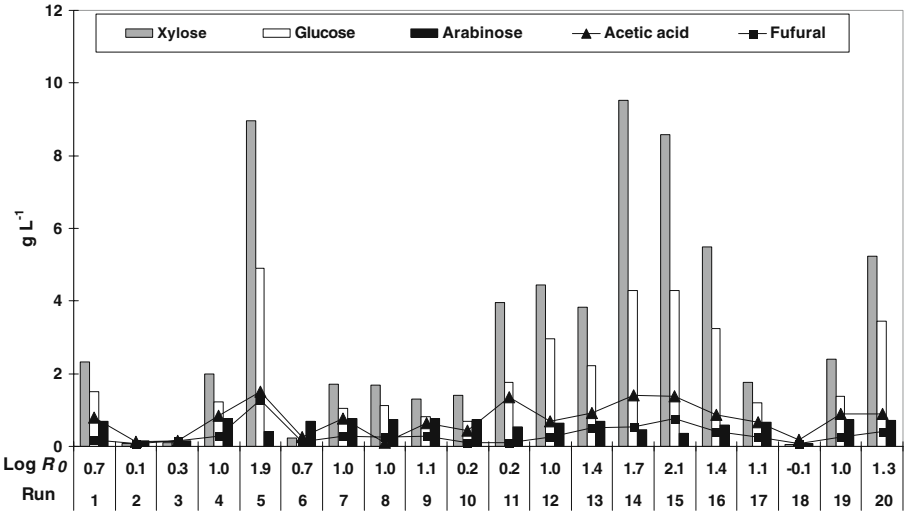

Fig. 1 Formation of xylose, glucose, arabinose, acetic acid, and furfural under selected hydrolysis conditions and severity factors (R_0=[10^{-pH}×*t*×exp(T-100)/14.75]) (*t* time; *T* temperature) of the pretreatments calculated from pH of hydrolysate

Table 2 Experimental range and levels of independent process variables

Independent variable	Symbol	Range and levels				
		$-\alpha$	−1	0	+1	$+\alpha$
Temperature (°C)	X_1	86	100	120	140	154
Reaction time (min)	X_2	5	15	30	45	55
Acid concentratin (%)	X_3	1	2	4	6	7

calculate the theoretical yields of xylose. Table 1 shows the chemical composition of cotton stalk. Although a different method was used for the quantification of xylan and cellulose, the main polymeric components of the cotton stalk based on dry weight are glucan (33%), xylan (17%), and klason lignin (28%), which is in agreement by previous studies [4]. Other components (acid soluble lignin, protein, and ash) have also been determined. The rest of the components (extractives such as hot water, cold water, or ethanol extractives) have minor importance in this study and are reported as others. According to Table 1, the potential maximum concentration of xylose produced in the hydrolysate was 19.66 g L^{-1}.

Table 3 Experimental design and results obtained by hydrolysis of cotton stalk

Runs	Variables			Responses	
	X_1	X_2	X_3	Y_1 (%)	Y_2 (g/g)
1	−1	1	1	11.74	1.55
2	−1	−1	−1	0.36	1.53
3	0	0	−1.68	0.54	1.16
4	0	0	0	10.18	1.62
5	1.68	0	0	45.23	1.83
6	−1	1	−1	1.17	1.53
7	0	0	0	8.57	1.63
8	0	0	0	8.51	1.49
9	0	0	0	6.58	1.61
10	−1	−1	1	7.09	1.99
11	0	−1.68	0	20.07	2.23
12	1	−1	−1	22.30	1.50
13	0	0	1.68	19.30	1.72
14	1	−1	1	48.34	2.22
15	1	1	1	43.59	1.99
16	1	1	−1	27.89	1.70
17	0	0	0	8.86	1.46
18	−1.68	0	0	0.24	1.95
19	0	0	0	12.07	1.74
20	0	1.68	0	26.62	1.52

Y_1 (xylose yield)=100×(Xyl/Xyl_{max}); Y_2 (selectivity)=Xyl/Glc, Xyl=xylose concentration obtained in the hydrolysate Xyl_{max}=maximum xylose concentration (19.66 g l^{-1}) based on the xylan concentration, Glc=glucose concentration obtained in the hydrolysate

Table 4 Analysis of variance for xylose yield ad selectivity

Source	Sum of squares		Degrees of freedom		Mean square		*F* value		*P* value	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Model	4261.83	1.18	9	9	473.54	0.13	71.24	6.63	<0.0001	0.0033
Residual	66.47	0.20	10	10	6.65	0.020				
Lack of fit	49.45	0.14	5	5	9.89	0.29	2.91	2.76	0.1333	0.1443
Pure error	17.02	0.052	5	5	3.40	0.01				
Total	4237.54	1.37	19	19						
R^2	0.98	0.86								

Sugar and By-Product Formation

The xylose and glucose concentrations showed a dependence on the experimental operating conditions, which were also explained by severity factor (R_0; Fig. 1). The severity factor combines the experimental effects of temperature, reaction time, and acid concentration for easy comparison of results [19]. The highest xylose concentration was 9.52 g L^{-1}, achieved at 140 °C for 15 min with 6% of acid concentration (LogR_0=1.7). During the hydrolysis of cotton stalks, other sugars were released, mainly glucose. These originated from cellulosic fraction or some heteropolymers of hemicellulosic fraction. With the increase in the severity factor, the glucose concentration also increased. The high concentration of glucose in the fermentation media adversely affects microbial conversion of xylose to xylitol [20]. Therefore, it is necessary to optimize the hydrolysis parameter to keep the glucose level low in the hydrolysate. Because plant xylans are partially acetylated, the concentration of acetic acid coming from the hydrolysis of the acetyl groups also increased with the severity factor. During the production of xylose, another reaction is taking place—dehydration of xylose to furfural. It is necessary to monitor temperature, acid concentration, and time to control this reaction. When the operating temperature and the reaction time were 154 °C and 30 min, respectively, and the acid concentration was maintained at 4% (LogR_0=1.9), glucose (4.90 g L^{-1}), acetic acid (1.51 g L^{-1}), and furfural (1.27 g L^{-1}) concentrations were at a maximum. In all experiments, arabinose concentration remained between 0.1 and 0.8 g L^{-1}.

Statistical Modeling

The experimental range and levels of independent variables investigated are given in Table 2. The design of this research including the dependent variables or responses, xylose yield (Y_1) and selectivity (Y_2), are given in Table 3. The quadratic models with coded variables are shown in Eqs. 2 and 3, which represent the xylose yield (Y_1) and selectivity (Y_2) as a function of temperature (X_1), time (X_2), and acid concentration (X_3).

$$Y_1 = 9.06 + 14.38X_1 + 1.27X_2 + 0.67X_3 + 4.90{X_1}^2 + 5.33{X_2}^2 + 0.66{X_3}^2 - 0.58X_1X_2 + 3.06X_1X_3 - 0.81X_2X_3 \quad (2)$$

$$Y_2 = 1.59 + 0.043X_1 - 0.12X_2 + 0.19X_3 + 0.11{X_1}^2 + 0.11{X_2}^2 - 0.061{X_3}^2 + 0.052X_1X_2 + 0.068X_1X_3 - 0.11X_2X_3 \quad (3)$$

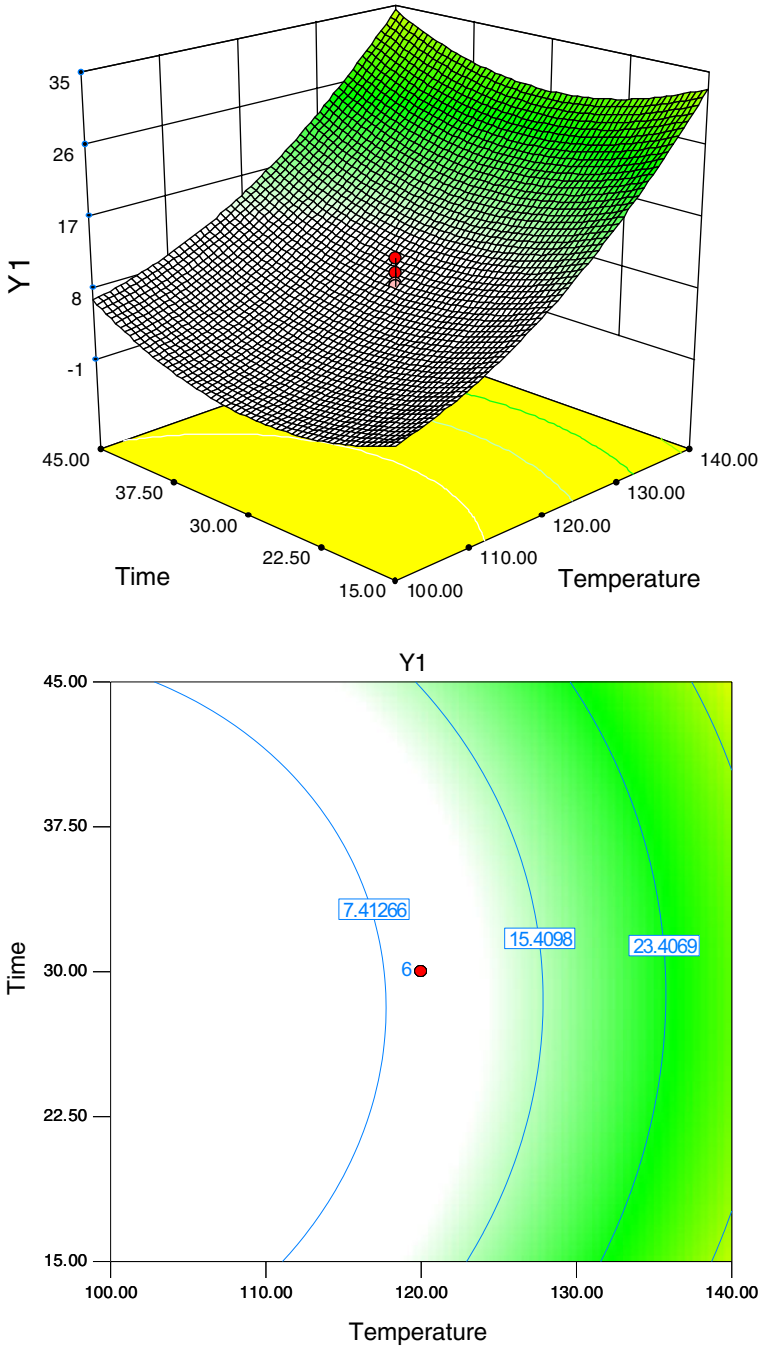

Fig. 2 Effect of reaction temperature and time on xylose yield when acid concentration was set at 4% as the center point

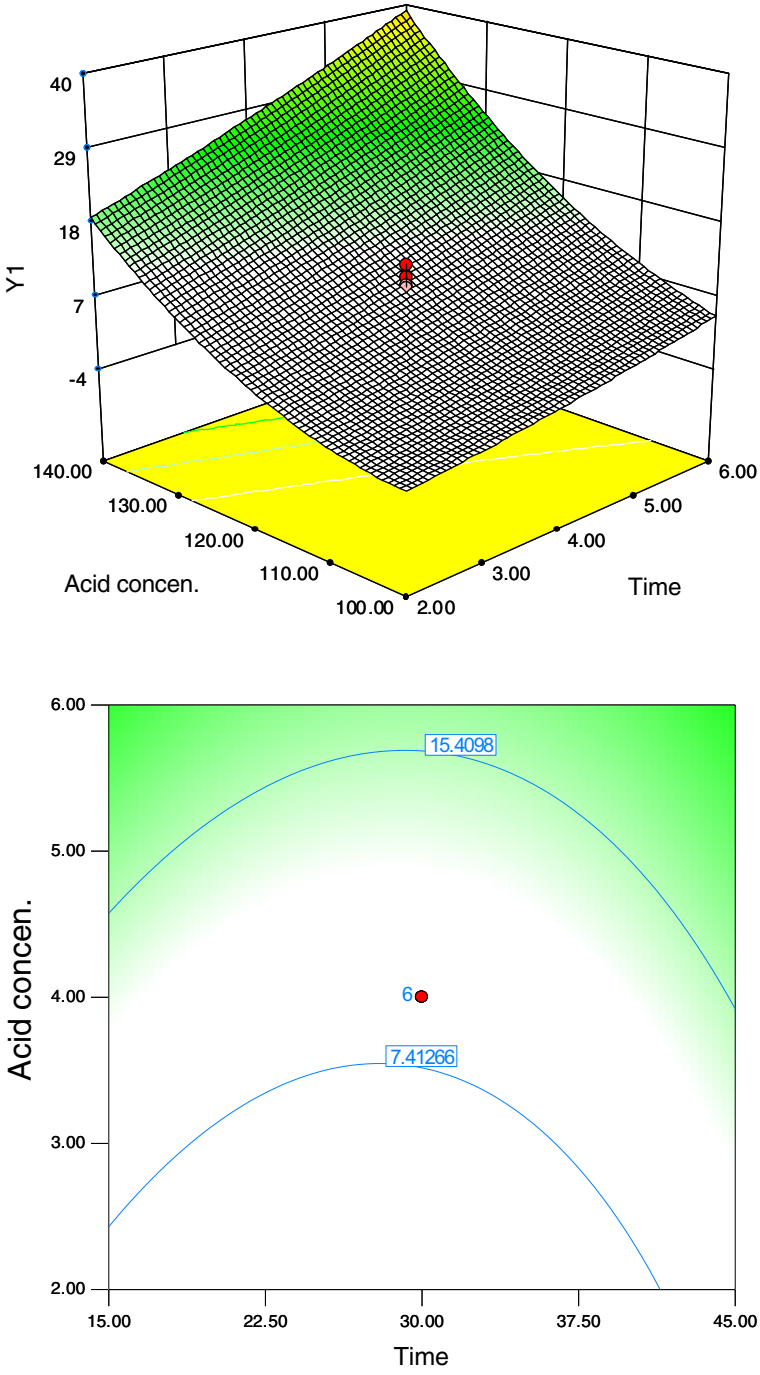

Fig. 3 Effect of H_2SO_4 conentration and reaction time on xylose yield when temperature was set at 120 °C as the center point

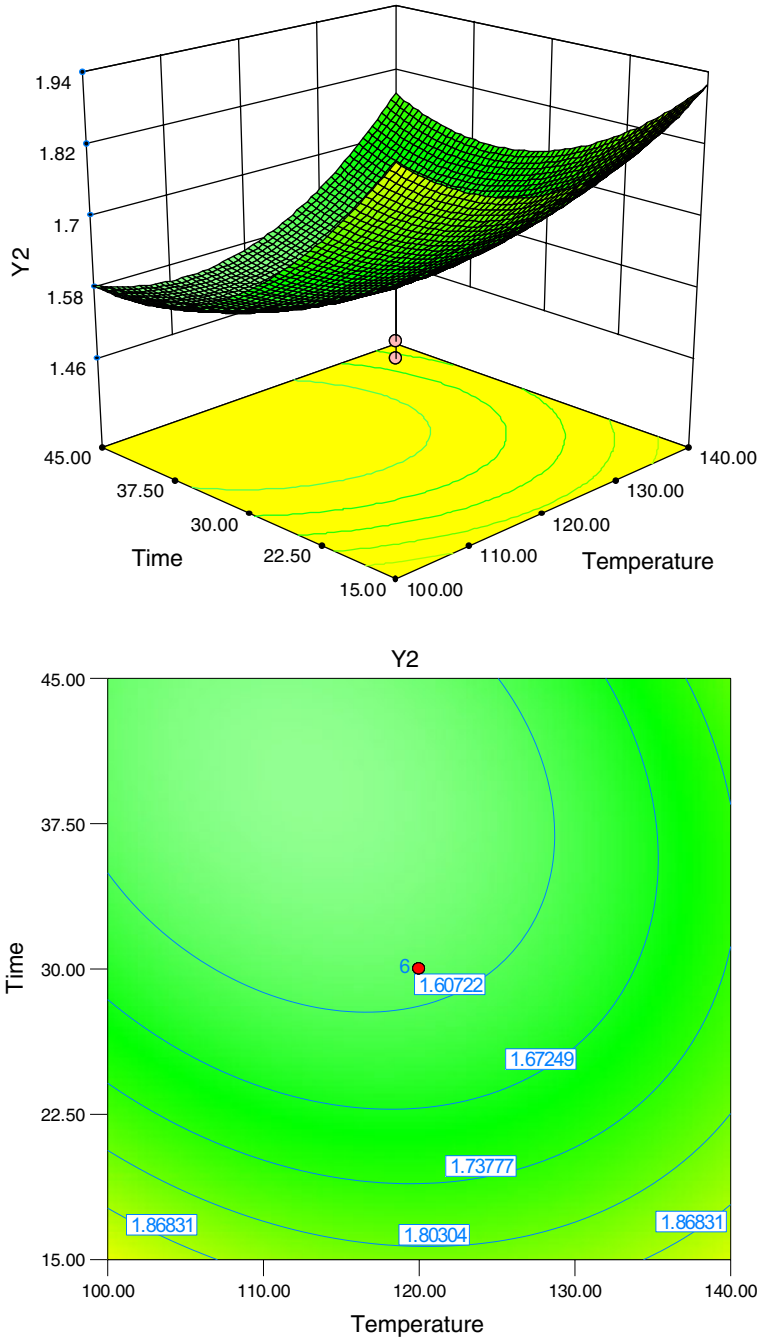

Fig. 4 Effect of reaction temperature and time on selectivity when acid concentration was set at 4% as the center point

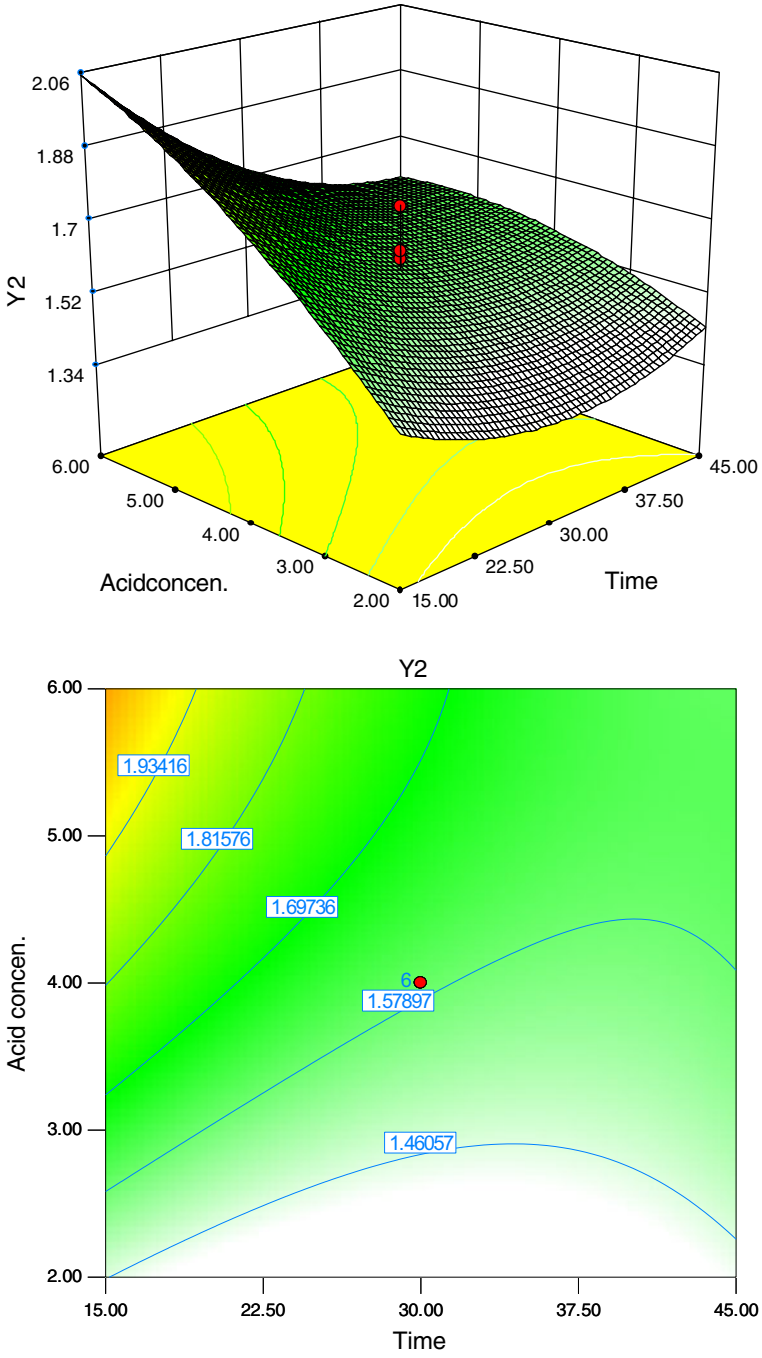

Fig. 5 Effect of H_2SO_4 conentration and reaction time on selectivity when temperature was set at 120 °C as the center point

Regression analysis was performed to fit the response function and experimental data. The second-order model for xylose yield and selectivity was evaluated by ANOVA, which is shown in Table 4. For both responses, the regression was statistically significant at the 95% confidence level. The model for both responses did not show lack of fit. The determination coefficient (R^2) for the first (Y_1) and the second responses (Y_2) was 0.98 and 0.86, respectively, explaining 98% and 86% of the variability in the responses.

Figures 2, 3, 4, and 5 show the response surfaces to estimate the xylose yield and selectivity over the independent variables of temperature (X_1), time (X_2), and acid concentration (X_3). Figure 2 shows the effect of temperature and time on xylose yield when acid concentration was set at 4% as the center point. The maximum xylose yield (34%) was obtained at 140 °C and 45 min reaction time. When the reaction temperature was set at 120 °C as a center point, as shown in Fig. 3, it was interpreted that the maximum xylose yield (22%) was obtained with a 6% acid concentration and 45 min reaction time. Figure 4 shows the effect of temperature and time on selectivity. When the acid concentration was set at 4% as the center point, the maximum selectivity (1.8 g g^{-1}) was obtained at 140 °C and 45 min of reaction time. When the reaction temperature was set at 120 °C as the center point, maximum selectivity (2.1%) was obtained with a 6% acid concentration and 15 min reaction time (Fig. 5).

When the overall xylose yield and selectivity of this study were compared with the previous studies done with wheat straw [12], rice straw [14], and oil palm empty fruit bunch fiber [9], it was found that xylose yield and selectivity were lower. Generally, in lignocellulosic biomass, xylan exists in xylan–lignin complex and becomes resistant to hydrolysis. Therefore, the higher amount of lignin in the cotton stalk (Table 1) limited the xylan hydrolysis and decreased xylose yield and selectivity.

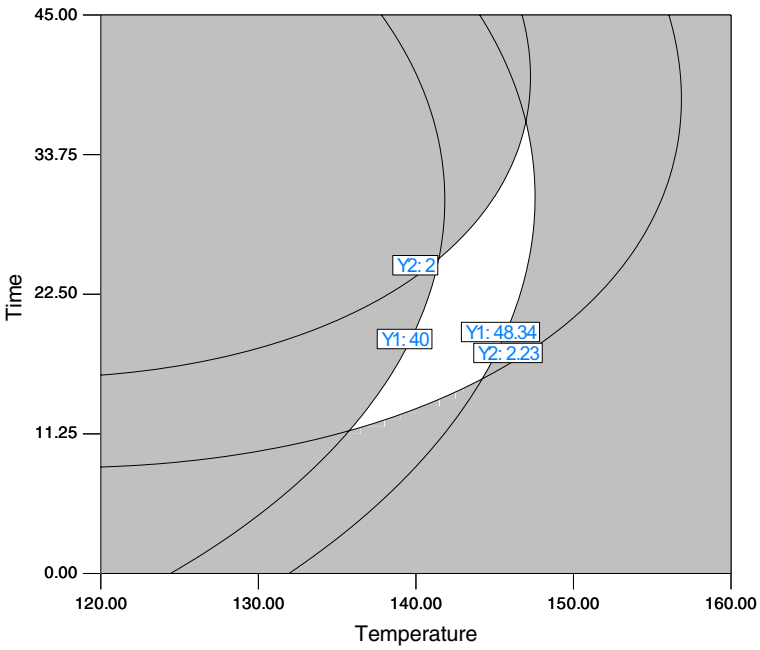

Fig. 6 Overlaying plots of xylose yield and selectivity

Based on the two models, a graphical optimization was conducted with the help of the "Design-expert" program. Optimization method consists of overlaying the contour plots of both models. The optimal working conditions, based on high level of xylose yield and selectivity, were chosen using the following criteria: xylose yield >40 and selectivity >2 g g^{-1}. In the overlaying plot (Fig. 6), regions with a shaded area do not fit the optimization criteria, while the non-shaded area meets the optimization criteria. As an optimum point, 6% acid concentration, 140 °C, and 15 min were selected. Under this condition, the severity factor of this treatment, calculated as LogR_0, was found to be 1.7. To confirm these results, hydrolysis runs were conducted in triplicate at optimized conditions, and the xylose yield and selectivity were obtained as 47.88% and 2.26 g g^{-1}, respectively. Under these conditions, acetic acid and furfural level were calculated as 1.39 and 0.43 g L^{-1}, respectively.

Conclusion

Dilute-acid hydrolysis is a suitable process to produce sugars from cotton stalk for further processing to different value added products. Hydrolysis of cotton stalk was carried out with dilute sulfuric acid under different operating conditions to produce xylose. A 2^3 rotatable central composite design was used in designing the experiments, and response surface methodology was used to optimize the hydrolysis conditions. The optimum yield of xylose and selectivity were 47.88% and 2.26 g g^{-1}, respectively, at a temperature of 140 °C, an acid concentration of 6%, and a reaction time of 15 min with low generation of by products. Under selected hydrolysis conditions, cotton stalk demonstrated to be a promising source of xylose which could be used for the production of different chemicals, mainly xylitol.

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