Effect of Sulfuric Acid on Pretreatment of YSS-10R Variety of Sorghum and Analysis of Its Interaction with Temperature and Time

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Considering the possible threats to the oil supply due to the rapid depletion of oil reservoirs and the negative environmental impacts of petroleum use, developing an environmentally friendly biofuel such as bioethanol is needed. Pretreatment is a critical step in the production of lignocellulosic bioethanol. In this study, the effect of sulfuric acid on the pretreatment of the YSS-10R variety of sorghum was evaluated. Response Surface Methodology (RSM) was employed to develop an experimental design matrix and evaluate the effect of pretreatment parameters on the release of fermentable sugars. Sorghum straw was treated with sulfuric acid concentrations of 0.5, 1.75, and 3% (V/V) at temperatures of 70, 100, and 130 °C for reaction times of 10, 20, and 30 min. The maximum glucose yield was 7.66 g/L (0.064 g/g) and was obtained *via* pretreatment with 0.5% H_2SO_4 at 100 °C for 10 min. That of xylose was 7.62 g/L (0.064 g/g), obtained *via* pretreatment with 0.50% H_2SO_4 at 130 °C for 20 min. The pretreatment conditions for maximum xylose yield were determined to be 2% H_2SO_4, 130 °C, and 20 min. Results indicate that sulfuric acid is an efficient catalyst for pretreatment at high temperatures and relatively long reaction times.

Keywords: Sulfuric acid; Pretreatment; Sorghum; Bioethanol; Lignocellulose

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INTRODUCTION

Energy usage in the world is increasing every day. Oil is the major source of energy for many industries and for transportation (Krichene 2002; Lun *et al*. 2013). In developing countries, oil consumption is rapidly increasing due to the development of industries and increasing transportation requirements (Asif and Muneer 2007; Alam *et al*. 2013). About 55% of worldwide oil consumption is by the transportation sector, and this proportion is increasing. Due to the continuous increase in oil consumption, it is anticipated that the production of oil will soon reach its peak and decline thereafter, resulting in greater consumption than production (Koppelaar 2005; Sorrell *et al*. 2009). The burning of oil causes emissions of greenhouse gases (GHGs). These gases cause negative environmental and climatic changes that affect living creatures and the basic infrastructure of buildings and transportation. The scientists are therefore concentrating their research towards biofuels from natural sources which are believed to be eco-friendly.

Bioethanol, one such biofuel, is an environment-friendly and renewable source of energy and can be a potential substitute for petroleum-based oil and fuels. The combustion of bioethanol produces 80 to 90% less GHGs than gasoline combustion (Wang *et al*. 2007; Vancov and McIntosh 2012). It has better efficiency than gasoline due to high value of heat of vaporization and high octane number (Bailey 1996). It is less volatile, makes less smog, and has lower photochemical reactivity in the environment than gasoline (Matsakas and Christakopoulos 2013). Many different types of feedstocks can be converted to bioethanol, especially lignocellulosic biomass (*e.g.*, corn stover, sorghum straw, wheat straw, rice polish, woody residues from forestry, and others) (Farrell *et al*. 2006; Chandel *et al*. 2011). Bioethanol can be produced from lignocellulosic material *via* chemical pretreatment of biomass, involving the steps of hydrolysis of cellulose and hemicellulose to monomeric sugars, fermentation, and product recovery (Demirbas and Demirbas 2007; Verardi *et al*. 2011). Biomass pretreatment is a necessary step preceding enzymatic hydrolysis because it removes lignin, increases the porosity and surface area of the material, and decrystallizes cellulose, all of which facilitate the access of hydrolytic enzymes into lignocellulosic polymers and increase the yield of monomeric sugars, making the process more cost-effective (Balat *et al*. 2008; Zhu and Pan 2010; Kim *et al*. 2014).

Sorghum straw, due to its high sugar content and bulk production, is a particularly viable feedstock for ethanol production (Vancov and McIntosh 2012). Sorghum needs about one-third less water than sugarcane and can grow in areas with low rainfall and high temperatures. It can be harvested twice a year due to its short growing season and is suitable for crop rotation systems (Smith and Frederiksen 2001; Barcelos *et al*. 2011; Choudhary *et al*. 2013). In Pakistan, in 2010 and 2011, sorghum was cultivated on 229,000 hectares, equating to total production of 141,000 tons (Agriculture Statistics Division of Pakistan 2012).

Among agents considered for chemical pretreatment of biomass, dilute acids have been extensively used, as they are inexpensive and effective (Yang and Wyman 2008). For instance, sulfuric acid, phosphoric acid, and hydrochloric acid have been considered. In most dilute acid pretreatments, high temperatures of 160 to 220 °C are used to achieve high sugar yields, but at these high temperatures, inhibitory compounds are also readily produced by the degradation of sugars. One study reported a decrease in the ratio of total sugars-to-total degradation compounds from 50 to 10 when increasing the dilute acid pretreatment temperature from 140 to 184 °C (Banerji *et al*. 2013). Research on bioethanol from lignocellulose has mainly focused on the development of an effective method of pretreatment capable of producing a high yield of fermentable sugars. This study evaluates the interactive effects of sulfuric acid, temperature, and time on pretreatment.

EXPERIMENTAL

Preparation of Samples

Sorghum bicolor of variety YSS-10R was used in this study. Post-harvest stalks and their leaves were collected from Millet Research Station Rawalpindi. The plant material was air-dried in an open field for one month and ground. In order to assure maximum dryness, ground material was air-dried in an open field for another week. The

straw was then sieved through an 80-mesh screen to accept only particles of 178 µm in size or less. The sieved samples were stored at room temperature for further experiments.

Pretreatment

Sulfuric acid was used for biomass pretreatment. Biomass was treated with different combinations of three tested factors; acid concentration, temperature, and time. The tested acid concentrations were 0.5, 1.75, and 3% (V/V); temperatures were 70, 100, and 130 °C; and times were 10, 20, and 30 min. Samples were pretreated at a solid loading of 12% (W/V) in 100-mL blue cap bottles with temperature controlled by heating in a water bath/autoclave. Liquid samples were collected from the pretreated slurry for analysis of the monomeric sugar content.

Experimental Design and Statistical analysis

A second-order Box Behnken Design (BBD) of Response Surface Methodology (RSM) was used to develop an experimental design matrix and to evaluate the effects of pretreatment parameters on the release of fermentable sugars. Acid concentration, temperature, and reaction time were used as the independent variables and were each varied across three levels. Glucose and xylose yield were the responses, or dependent variables. Design-Expert software, Version 8.07.1 (Stat-Ease Inc., Minneapolis, MN, USA) was used for the experimental design. The following equation was used to generate the experimental model (Saini *et al*. 2013),

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{i=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where y is the response variable, x is independent variable, β_0 is the intercept, k is the number of variables, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient, and ε is the random error. Regression analysis and ANOVA were used for further data analysis (Nikzad *et al*. 2013).

Analytical Procedures

The biochemical composition of the sample was determined by the strong acid hydrolysis method as described by Mehmood *et al.* (2009). The glucose concentration was determined using the GOD-PAP enzymatic colorimetric method using a GOD-PAP glucose detection kit (Merck) on a Selectra Automatic Chemical Analyzer. The xylose concentration was determined using the method described by Ebert *et al.* (1979).

RESULTS AND DISCUSSION

Compositional Analysis

Compositional studies of biomass are an important indicator and first step during evaluation as a potential biofuel candidate. The biomass composition varies depending upon crop variety, agronomic practices used during cultivation, stage of harvest, climatic and geological condition of area from where samples are collected, and post-harvest storage conditions. The final yield of bioethanol depends upon the amount of glucose and xylose monomers released *via* the hydrolysis of cellulose and hemicellulose. Biomass

with higher concentrations of carbohydrate polymers and lower lignin contents are considered promising feedstocks for bioethanol production (Xing *et al*. 2009; Godin *et al*. 2013). Compositional analysis of YSS-10R indicated that it contained cellulose (38.15%), hemicellulose (20.28%), and lignin (16.87%). The present results were very close to those determined by the research group for other varieties of sorghum (Mehmood *et al.* 2014).

Pretreatment

The results of the sulfuric acid pretreatment of YSS-10R are shown in Table 1.

Table 1. Glucose and Xylose Yields (g/L) following H_2SO_4 Pretreatment of Sorghum (YSS-10R) with 12% Solid Loading (w/v) of Dry Mass

	Experiment			YSS-10R	
Run	Time(min)	Temp (°C)	H_2SO_4 (%)	Glucose (g/L)	Xylose (g/L)
1	30	130	1.75	5.88	6.48
2	10	130	1.75	5.93	7.32
3	20	100	1.75	6.44	5.91
4	30	100	3	6.60	5.93
5	30	70	1.75	7.26	2.46
6	10	100	3	6.79	5.41
7	20	100	1.75	6.62	6.52
8	30	100	0.5	7.55	1.60
9	20	130	0.5	6.35	7.62
10	20	70	3	6.80	3.06
11	20	130	3	5.01	7.04
12	10	70	1.75	6.87	2.36
13	20	100	1.75	6.67	5.76
14	10	100	0.5	7.66	1.96
15	20	70	0.5	7.64	1.97

Glucose

The maximum glucose yield was 7.66 g/L (0.064 g/g) following pretreatment with 0.5% H_2SO_4 at 100 °C for 10 min. The results shown here were different from those reported earlier by Téllez-Luis *et al.* (2002). The reason may be due to different concentrations of acid used. ANOVA data for the Response Surface Quadratic model is given in Table 2. The model F-value was 23.47, indicating that the model was statistically significant. There was only a 0.14% chance that such a large F-Value could occur randomly. In this case B, C, A^2, B^2, and C^2 were significant model terms (with $p<0.05$) where A is time, B is temperature, and C is acid concentration. The "Lack of Fit F-value" was 3.26, implying that the Lack of Fit was not significant relative to the pure error. There was a 24.35% chance that a "Lack of Fit F-value" this large could occur due to noise. The correlation coefficient R^2 was 0.9769, suggesting that the experimental and predicted results were well-correlated and that the results were statistically significant.

The three-dimensional surface plots in Fig. 1-1 show the interaction among the three different pretreatment factors and their correlation with the glucose yield from YSS-10R. Figure 1-1a shows the effect of temperature and time on the release of glucose from YSS-10R with acid concentration constant at 1.75%. Glucose concentration decreased significantly with increasing temperature.

Table 2. ANOVA for Response Surface Quadratic Model of Glucose

Analysis of Variance [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F Value**	**p-value Prob>F**	
Model	7.06	9	0.78	23.47	0.0014	significant
A: Time (min)	*1.620E-4*	*1*	*1.620E-4*	*4.848E-3*	*0.9472*	
B: Temp. (°C)	*3.66*	*1*	*3.66*	*109.66*	*0.0001*	
C: H_2SO_4 (%)	*2.00*	*1*	*2.00*	*59.73*	*0.0006*	
AB	*0.048*	*1*	*0.048*	*1.44*	*0.2834*	
AC	*1.866E-3*	*1*	*1.866E-3*	*0.056*	*0.8226*	
BC	*0.064*	*1*	*0.064*	*1.90*	*0.2265*	
A^2	*0.34*	*1*	*0.34*	*10.25*	*0.0240*	
B^2	*0.58*	*1*	*0.58*	*17.30*	*0.0088*	
C^2	*0.27*	*1*	*0.27*	*8.18*	*0.0354*	
Residual	0.17	5	0.033			
Lack of Fit	*0.14*	*3*	*0.046*	*3.26*	*0.2435*	*not significant*
Pure Error	*0.028*	*2*	*0.014*			
Corr. Total	7.23	14				

The maximum glucose concentration was achieved at very long and very short pretreatment times. The maximum concentration of glucose obtained was from pretreatment at 70 °C for 30 min. Figure 1-1b shows the interaction and effect of acid concentration and time on glucose yield with temperature constant at 100 °C. Increasing the acid concentration caused significant decreases in the release of glucose. The maximum glucose concentration was achieved at very long and very short pretreatment times. The maximum glucose yield with these factors was obtained with 0.5% acid concentration and 10 min pretreatment time.

Figure 1-1c shows the interaction and effects of acid concentration and temperature on glucose yield with time constant at 20 min. Glucose yield decreased significantly with increases in both acid concentration and temperature, resulting in maximum glucose yield at milder conditions. The maximum yield of glucose was obtained with 0.50% acid at 70 °C.

Xylose

The efficiency of pretreatment was judged by the yield of xylose obtained as a result of hemicellulose and lignin dissolution. Results have shown that sulfuric acid is a potential catalyst for effective pretreatment. The maximum xylose yield was 7.62 g/L (0.064 g/g), obtained after pretreatment with 0.50% H_2SO_4 at 130 °C for 20 min. The present results were different from those reported earlier by Téllez-Luis *et al.* (2002). The reason may be due to different concentrations of acid used. ANOVA data for the Response Surface Quadratic model for xylose yield is given in Table 3. The model F-value was 4.90; there was a 4.74% chance that such a large F-Value could occur due to noise. In this case, B was the significant model term with p-values less than 0.0500. The "Lack of Fit F-value" was 14.16; there is a 6.67% chance that a "Lack of Fit F-value" may be due to noise. The correlation coefficient R^2 was 0.90, suggesting that the experimental and predicted results were effectively corelated and that the results were statistically significant.

Table 3. ANOVA for Response Surface Quadratic Model of Xylose

Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	62.57	9	6.95	4.90	0.0474	significant
A: Time (min)	*0.041*	*1*	*0.041*	*0.029*	*0.8722*	
B: Temperature (°C)	*43.30*	*1*	*43.30*	*30.53*	*0.0027*	
C: H_2SO_4 (%)	*8.58*	*1*	*8.58*	*6.05*	*0.0573*	
AB	*0.22*	*1*	*0.22*	*0.16*	*0.7097*	
AC	*0.19*	*1*	*0.19*	*0.13*	*0.7293*	
BC	*0.70*	*1*	*0.70*	*0.50*	*0.5129*	
A^2	*6.28*	*1*	*6.28*	*4.42*	*0.0894*	
B^2	*0.041*	*1*	*0.041*	*0.029*	*0.8724*	
C^2	*3.96*	*1*	*3.96*	*2.79*	*0.1557*	
Residual	7.09	5	1.42			
Lack of Fit	*6.77*	*3*	*2.26*	*14.16*	*0.0667*	*not significant*
Pure Error	*0.32*	*2*	*0.16*			
Cor Total	69.66	14				

The three-dimensional surface plots in Fig. 1 (part 2) show the interaction among pretreatment factors and their correlation with xylose yield. Figure 1-2a shows the effect of temperature and time on the release of xylose from YSS-10R with acid concentration constant at 1.75%. In this case, the effect of temperature on release of xylose was significant ($p<0.05$). Increasing the temperature increased the xylose yield. Increasing time to intermediate values increased the xylose yield and then decreased the yield at higher values. The maximum xylose yield in this case was obtained at 130 °C for 10 min. Figure 1-2b shows the interaction and effects of acid concentration and time on xylose yield with temperature constant at 100 °C. Increasing the acid concentration increased the xylose yield. Time did not affect xylose yield significantly ($p>0.05$). The maximum xylose concentration occurred at an intermediate acid concentration of 1.75% and 20 min reaction time. Figure 1-2c shows the interaction and effects of acid concentration and temperature on xylose yield while keeping time constant at 20 min. Temperature had strong positive effect ($p<0.05$) on xylose yield. Increasing the acid concentration also increased the xylose concentration, but had a weaker effect than temperature ($p<0.05$). The maximum xylose yield was obtained with 0.5% acid concentration at 130 °C. Higher xylose yield was obtained with lower to intermediate values of acid concentration, at high temperatures, for longer reaction times. At higher concentrations of sulfuric acid, xylose yield dropped, likely due to its degradation into hydroxymethylfufural.

Optimization

Optimization of pretreatment parameters was done using the numerical optimization function of Response Surface Methodology (RSM). The optimized pretreatment conditions for maximum xylose yield from YSS-10R were found to be 2% H_2SO_4, 130 °C, and 20 min.

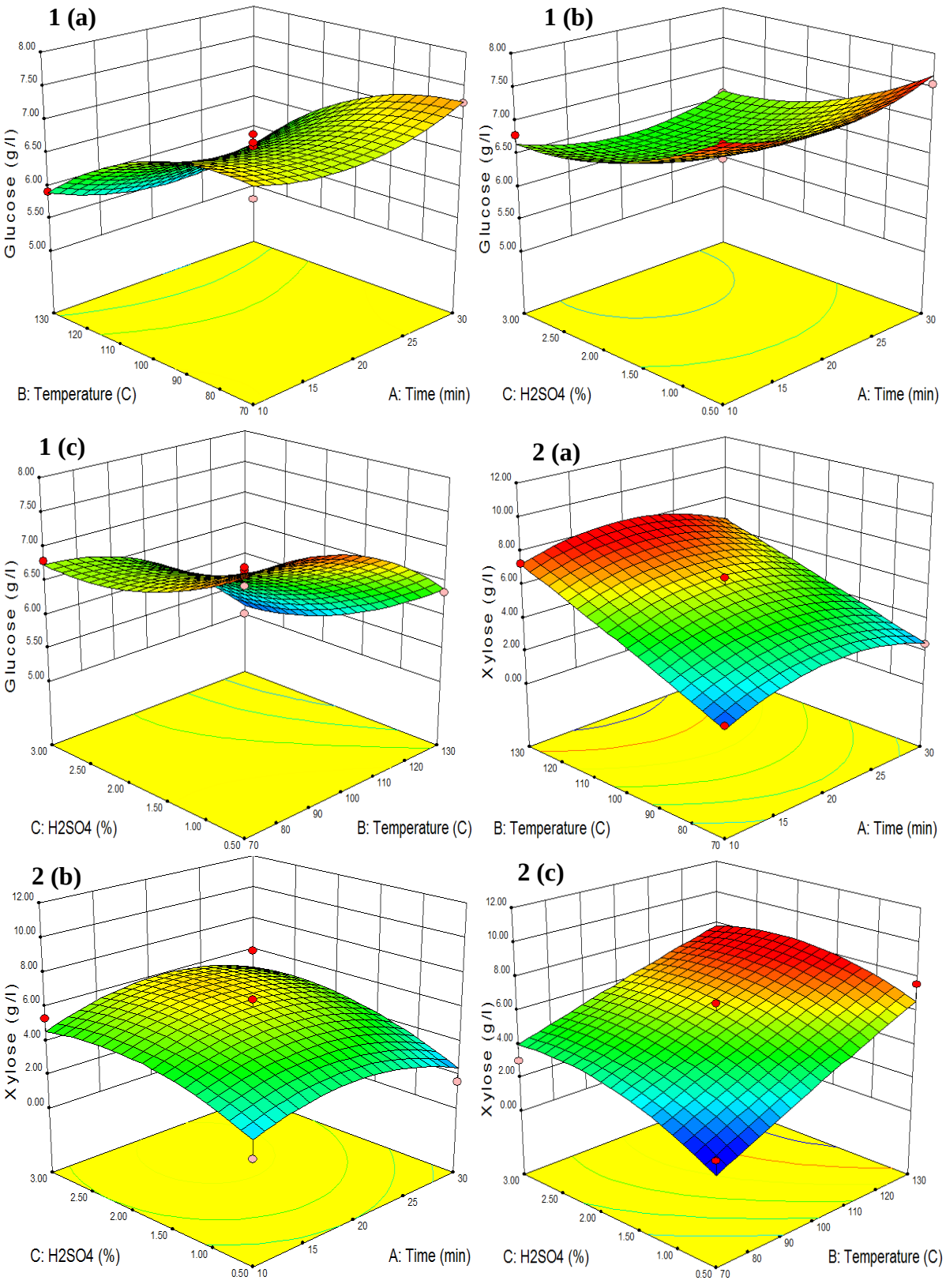

Fig. 1. Three-dimensional surface plots showing effects of pretreatment factors on yield of glucose (1) and xylose (2) from YSS-10R; (a) Temperature vs. Time; (b) H_2SO_4 vs. Time; and (c) H_2SO_4 vs. Temperature

CONCLUSIONS

1. Maximum glucose yield was 7.66 g/L (0.064 g/g) obtained with 0.5% H_2SO_4 at 100 °C for 10 min
2. Maximum yield of xylose was 7.62 g/L (0.064 g/g), obtained with 0.50% H_2SO_4 at 130 °C for 20 min
3. Dilute acid pretreatment was optimal with 2% H_2SO_4, 130 °C, and 20 min

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