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Original Article

Optimization of pretreatment conditions for increased cellulose conversion of sugarcane bagasse using peracetic acid employing central composite design

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Abstract

The present study was carried out to find out the optimum pretreatment conditions for increasing the cellulose yield in bagasse substrate. Peracetic acid (PAA), a powerful oxidizing agent that removes lignin, was used in this study. Response surface methodology was employed for the simultaneous analysis of pretreatment conditions like substrate concentration, PAA loading, pretreatment temperature and pretreatment time on cellulose, hemicellulose and lignin yield in bagasse. The experiments were carried out according to central composite design in order to establish the optimum conditions for increasing the cellulose yield in pretreated bagasse substrate. The optimized conditions to obtain maximum cellulose yield (72.6%) were; a substrate concentration of 2%, PAA loading of 20%, pretreatment temperature of 120ºC and pretreatment time of 120 min. There is a good correlation between the actual and predicted results. Saccharification results showed that the yield of reducing sugar was higher in the PAA pretreated bagasse.

Keywords: RSM, PAA pretreatment, optimization, sugarcane bagasse, cellulose

1. Introduction

Lignocellulosic biomass is an interesting and necessary enlargement of the biomass used as a potential substrate to produce ethanol which is considered as one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels (Cardona *et al*., 2007). Studies have been carried out to convert agriculture residues, such as straw, bagasse, rice hull and wood into commercial energy, providing an alternative source for conventional transportation fuel and to estimate the economic and environmental efficiency (Teramoto *et al*., 2008; Zhu *et al*., 2009). The lignocellulosic materials are formed by three structural polymers: cellulose, hemicelluloses and lignin and small quantities of other compounds (Fengel and Wegener, 1984). Among these components, carbohydrates (cellulose and hemicelluloses) can be saccharified and eventually fermented to obtain bio-ethanol.

Enzymatic hydrolysis is a promising way to obtain sugars from lignocellulosic materials, but the low enzymatic accessibility of the native cellulose is a key problem for biomass to ethanol processes. Pretreatment of biomass is always necessary to remove or modify the surrounding matrix of lignin and hemicellulose prior to the enzymatic hydrolysis of the polysaccharides (cellulose and hemicellulose) in the biomass (Zheng *et al*., 2009). Most of the pretreatment methods should be operated at high temperature resulting in high pressure, which increases the energy consumption and costs of equipments. Furthermore, these processes still leave most of the lignin in the material and limit the complete bioconversion of cellulose to sugar (Zhao *et al*., 2007).

Delignification is one of the most efficient pathways to overcome the recalcitrance of lignocellulosic biomass and increase the enzymatic digestibility of cellulose. One of the most important and available lignocellulosic biomass in tropic countries is sugarcane bagasse, the fibrous residue obtained after extracting the juice from sugarcane in the sugar production process. It has been estimated that about 5.4×10^8 dry

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tons of sugarcane are processed annually throughout the world (Cardona *et al*., 2010).

The enzymatic hydrolysis of sugarcane bagasse could be greatly enhanced by PAA pretreatment (Zhao *et al*., 2007; Mohan *et al*., 2012a), which was achieved mainly by delignification accompanying with the increase of the surface area and exposure of cellulose fibers (Zhao *et al*., 2008a). PAA is recognized as a powerful oxidizing agent and is quite selective towards the lignin structure. It oxidizes the aromatics in lignin, generating dicarboxylic acid and their lactones (Teixeira *et al*., 2000).

Statistical inference techniques can be used to assess the importance of individual factors, the appropriateness of this functional form and sensitivity of the response to each factor (Mason *et al*., 1989). Recently many statistical experimental design methods have been employed in bioprocess optimization. Among them RSM is one of the suitable methods for identifying the effect of individual variables and for seeking the optimum conditions for a multivariable system efficiently. This method has been successfully applied to optimize alcoholic fermentation and other fermentation media (Sunitha *et al*., 1998; Ratnam *et al*., 2003; Mohan *et al*., 2012b). Therefore, the objective of this research is to focus on the application of RSM and CCD to demonstrate the pretreatment conditions on cellulose, hemicellulose and lignin yield in the sugarcane bagasse substrate. Further to increase cellulose yield in pretreated sugarcane bagasse and to utilize for enzyme and ethanol production.

2. Materials and Methods

2.1 Raw material

Sugarcane bagasse was generously provided by S.V. Sugar Industries Ltd., Tirupati, India. The collected bagasse was dried in oven for 6-8 hrs at 70-80°C and dried bagasse was milled, grinded and used as powder form.

2.2 Experimental design

The experimental design and statistical analysis were performed according to the response surface analysis method using Design-Expert 8.0.6.1 (Stat-Ease Inc., Minneapolis, MN, U.S.A.) version software. Central composite design (Box and Wilson, 1951) with quadratic model was employed to study the combined effect of four independent variables namely substrate concentration (X_1, %), PAA loading (X_2, %), pretreatment temperature (X_3, °C) and pretreatment time (X_4, min). The dependent variables (Y) measured were cellulose (Y_1, %), hemicellulose (Y_2, %) and lignin (Y_3, %) in bagasse. These dependent variables were expressed individually as a function of the independent variables known as response function. In CCD, the range and the levels of the variables investigated in this study are given in the Table 1. A 2^4-factorial CCD, with eight axial points and six replications at the centre points (n_0=6) leading to a total number of 30 experiments were employed (Table 2) for optimization of the pretreatment conditions. The second degree polynomials Equation 1 were calculated with the statistical package (Stat-Ease Inc.) to estimate the response of the dependent variable. The variance for each factor assessed was partitioned into linear, quadratic and interactive components and were represented using the second order polynomial function as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 \quad (1)$$

where Y is the predicted response, X_1, X_2, X_3 and X_4 are in-dependent variables, b_0 is the offset term, b_1, b_2, b_3 and b_4 are linear effects, b_{11}, b_{22}, b_{33}, and b_{44} are squared effects and b_{12}, b_{13},b_{14}, b_{23},b_{24}, and b_{34} are interaction terms. The significance of all terms in the polynomial functions were assessed statistically using F-value at a probability (P) of 0.001, 0.01 or 0.05. The regression coefficients were then used to generate contour maps from the regression models. The three-dimensional (3D) plots were generated by keeping one variable constant at the centre point and varying the other variables within the experimental range.

The experimental design for the variables, i.e. substrate concentration (%), PAA loading (X_2, %), pretreatment temperature (X_3, ºC) and pretreatment time (X_4, min) to determine the bagasse constituents. The design was applied for selection range of each variable (minimum and maximum), total of 30 experiments were designed by the model and performed. Optimized values of four independent variables for maximum activities were determined using a numerical optimization package of Design-Expert 8.0.6.1.

2.3 Saccharification or enzymatic hydrolysis of pretreated bagasse

Saccharification or enzymatic hydrolysis experiments were done in duplicate in stoppered conical flasks (50 ml) containing low (2%) and high (20%) loading PAA pretreated bagasse (1 g), cellulase preparation (Mohan *et al.,* 2012) and surfactant Tween-80 in 0.05 M citrate buffer (pH 4.8), supplemented with 1% v/v of Penicillin-Streptomycin solution (Hi-Media, India) to prevent microbial contamination. Flasks were incubated at 50°C in a rotary shaker at 200 rpm over 60-90 hrs. Samples were withdrawn, centrifuged at 12,000 rpm for 10-15 min and the supernatant was used for sugar estimation.

2.4 Method of analysis

The lignocellulosic biomass analysis is related to plant fiber estimation. We used the method of AOAC (2005) involving multifunction process for the separation of cellulose, hemicellulose and lignin from the other constituents of lignocellulosic biomass. The method involves estimation of Neutral Detergent Fiber (NDF) which accounts for the cellu-

Table 1. Coded and actual values of the factors in central composite design.

Factor	Name	Low actual	Middle actual	High actual	Low coded	Middle coded	High coded
X_1	Substrate (%, w/v)	2	11	20	-1	0	1
X_2	PAA (%,v/v)	2	11	20	-1	0	1
X_3	Pretreatment temperature (ºC)	60	90	120	-1	0	1
X_4	Pretreatment time (min)	30	75	120	-1	0	1
Response	Name	Units	Obs[a]	Min.	Max.	Mean	Std.Dev.
Y_1	Cellulose	%	30	37.4	72.6	8.72	1.94
Y_2	Hemicellulose	%	30	31.9	56.8	7.73	1.78
Y_3	Lignin	%	30	4.3	16.2	2.53	3.76

[a] Observed run values.

Table 2. Central composite design matrix.

Std.	Substrate (%, w/v)	PAA (%, v/v)	Temp. (ºC)	Time (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3
1	-1	-1	-1	-1	39.4 (38.7)	32.6 (34.1)	15.1 (15.1)
2	1	-1	-1	-1	37.4 (40.1)	31.9 (33.8)	16.2 (14.7)
3	-1	1	-1	-1	46.3 (45.8)	34.6 (35.3)	14.6 (14.0)
4	1	1	-1	-1	39.6 (41.8)	32.8 (35.5)	15.2 (14.5)
5	-1	-1	1	-1	43.2 (40.2)	37.3(36.3)	11.8 (12.2)
6	1	-1	1	-1	40.2 (39.3)	35.8 (34.4)	12.4 (13.0)
7	-1	1	1	-1	51.4 (51.5)	39.4(40.3)	10.4 (9.88)
8	1	1	1	-1	43.6 (45.2)	37.2 (39.0)	11.8 (11.5)
9	-1	-1	-1	1	42.6 (43.1)	39.4 (40.3)	11.2 (11.2)
10	1	-1	-1	1	38.2 (38.3)	34.5 (35.9)	12.4 (12.5)
11	-1	1	-1	1	53.8 (54.9)	40.6 (44.2)	9.6 (8.55)
12	1	1	-1	1	39.4 (44.5)	36.6 (40.3)	11.4 (10.7)
13	-1	-1	1	1	58.2 (56.2)	51.2 (50.8)	9.2 (9.45)
14	1	-1	1	1	46.4 (49.0)	42.8 (44.8)	11.6 (11.9)
15	-1	1	1	1	72.6 (72.0)	56.8 (57.6)	4.3 (5.49)
16	1	1	1	1	58.6 (59.5)	51.4 (52.2)	9.2 (9.45)
17	-2	0	0	0	49.4 (53.0)	44.6 (43.4)	10.3 (10.0)
18	2	0	0	0	47.9 (41.8)	41.8 (37.7)	12.2 (13.0)
19	0	-2	0	0	42.8 (44.2)	40.7 (40.6)	12.6 (12.1)
20	0	2	0	0	65.8 (61.8)	54.2 (49.1)	6.8 (7.94)
21	0	0	-2	0	38.4 (34.2)	34.6 (28.7)	13.6 (15.4)
22	0	0	2	0	48.9 (50.6)	42.1 (42.7)	11.8 (10.6)
23	0	0	0	-2	37.6 (37.9)	34.2 (33.0)	13.8 (14.6)
24	0	0	0	2	59.4 (56.6)	56.4 (52.3)	8.2 (8.01)
25[a]	0	0	0	0	52.2 (52.2)	49.8 (49.8)	9.8 (9.8)
26[a]	0	0	0	0	52.2 (52.2)	49.8 (49.8)	9.8 (9.8)
27[a]	0	0	0	0	52.2 (52.2)	49.8 (49.8)	9.8 (9.8)
28[a]	0	0	0	0	52.2 (52.2)	49.8 (49.8)	9.8 (9.8)
29[a]	0	0	0	0	52.2 (52.2)	49.8 (49.8)	9.8 (9.8)
30[a]	0	0	0	0	52.2 (52.2)	49.8 (49.8)	9.8 (9.8)

Std: Standard run order; [a]= Central value.

lose, hemicellulose and lignin content and represents most of the fiber or cell wall fractions in biomass. Acid Detergent Fiber (ADF) was determined sequentially using the residue left from NDF determination. The hemicellulose was calculated by subtracting ADF from NDF (Jung and Vogel, 1992). The NDF and ADF treated bagasse material was then hydrolyzed with 72% H_2SO_4 to determine cellulose. Lignin was obtained by ashing of hydrolyzed residue. The reducing sugar concentration and glucose was estimated using 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The xylose and arabinose content was estimated by the method of Khabarov *et al*. (2006). The reagents (solution 1, 2 and H_2SO_4) were prepared and mixed in test tubes. It was heated on a boiling water bath, the reaction mixture was transferred into a 100 ml volumetric flask, and water was added to the mark. The absorption spectra were recorded on a Spectrophotometer. The calibration plots were constructed and the concentration of pentoses was expressed as mg/g.

3. Results

Optimization of experiments is designed to provide in depth information about a few variables identified during screening as having the greatest impact on performances. Finally, verification of experiments is used to validate the results under specific experimental conditions (Chen *et al*., 2002). The influence of substrate concentration, PAA loading, pretreatment temperature and pretreatment time on cellulose, hemicellulose and lignin yield was investigated using RSM. The results are summarized in Tables 1 and 2. The effect of each factor and their interactions were analyzed using the analysis of variance (ANOVA) and X^2 test as appropriate to the experimental design being used. The calculated regression equation for the optimization of pretreatment conditions showed that the cellulose (Y_1, %), hemicellulose (Y_2, %) and lignin yield (Y_3, %) is a function of the substrate concentration (X_1, %), PAA loading (X_2, %), pretreatment temperature (X_3, °C) and pretreatment time (X_4, min). By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found to represent the cellulose, hemicellulose and lignin yield adequately.

Cellulose (%) $$Y_1 = 52.2 - 2.8X_1 + 4.4X_2 + 4.1X_3 + 4.68X_4 - 1.19X_1^2 + 0.22X_2^2 - 2.44X_3^2 - 1.23X_4^2 - 1.36X_1X_2 - 0.57X_1X_3 - 1.57X_1X_4 + 1.04X_2X_3 + 1.14X_2X_4 + 2.88X_3X_4 \quad (2)$$

Hemicellulose (%) $$Y_2 = 49.8 - 1.44X_1 + 2.12X_2 + 3.5X_3 + 4.84X_4 - 2.3X_1^2 - 1.24X_2^2 - 3.51X_3^2 - 1.77X_4^2 + 0.13X_1X_2 - 0.38X_1X_3 - 1.03X_1X_4 + 0.72X_2X_3 + 0.69X_2X_4 + 2.08X_3X_4 \quad (3)$$

Lignin (%) $$Y_3 = 9.8 + 0.74X_1 - 1.04X_2 - 1.19X_3 - 1.66X_4 + 0.44X_1^2 + 0.05X_2^2 + 0.81X_3^2 + 0.38X_4^2 + 0.21X_1X_2 - 0.29X_1X_3 - 0.41X_1X_4 - 0.33X_2X_3 - 0.40X_2X_4 + 0.27X_3X_4 \quad (4)$$

The predicted levels of cellulose, hemicellulose and lignin yield in pretreated bagasse substrate using the above equations are given in Table 3 along with experimental data. The goodness of the model can be checked by different criteria. The R^2 values for all these response variables were higher than 0.90, indicating that the regression model explained the reaction well.

The analysis of the variance (ANOVA) of the quadratic regression model demonstrated that Equation 2 to 4 are highly statistically significant models of cellulose, hemicellulose and lignin content responses in the bagasse, as was evident from the Fisher's F-test with a very low probability value [(P model > F) = 0.0001]. The model's goodness of fit was checked by determination coefficient (R^2).

3.1 Cellulose yield

Cellulose can be hydrolytically broken down into glucose either enzymatically by cellulases or chemically by sulfuric or other acids. Hemicellulases or acids hydrolyze the hemicellulose polymer to release its component sugars (Mosier *et al*., 2005). In this study, from the regression model (Y_1) of cellulose yield, the value of the determination coefficient (R^2=0.9296) indicates that only 7.04% of the total variations were not explained by the model. The value of the adjusted determination coefficient [Adj (R^2) = 0.8584] was also high in supporting high significance of the model. Among the model terms X_2, X_3 and X_4 were significant with a probability of 99% (Table 3). The interaction between X_3 and X_4 had significant influence on increase in cellulose yield in treated bagasse.

3.2 Hemicellulose yield

Many pretreatment methods were shown to be able to remove hemicelluloses and consequently improve the enzymatic hydrolysis. But most of these processes partly remove the lignin as well (Wyman, 1996). From the experiments, the determination coefficient of hemicellulose is R^2 = 0.9113, only 8.87% of the total variations were not explained and the adjusted R^2 = 0.8285 of the model have high significance. The model terms X_3, X_4 and X_3^2 are significant with a probability of 99% and X_1, X_2, X_3X_4 and X_4^2 were significant with a probability of 95% (Table 3). The hemicellulose yield in bagasse was significantly influenced by the interaction between X_3 and X_4.

3.3 Lignin yield

The presence of lignin in the cell wall, however, impedes enzymatic hydrolysis of the carbohydrates (Mosier *et al*., 2005). In considering the lignin yield with R^2 = 0.921 has 7.9% of the total variations not explained in the model. The adjusted R^2 = 0.8473 was in significant with the model. The significant probability of 99% is with the model terms X_2, X_3, X_4, and X_3^2. The model terms X_1 and X_1^2 have 95% significant probability (Table 3). There is no significant inter-

Table 3. Analysis of variance for the experimental results of the CCD.

Source	df	F-Value			P-value		
		Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
Model	14	13.56	11.01	12.49	0.0001	0.0001	0.0001
X_1	1	17.4	4.83	13.4	0.0008$^{\#}$	0.0441*	0.0023$^{\text{“}}$
X_2	1	43.19	10.52	26.43	0.0001$^{\#}$	0.0055*	0.0001$^{\#}$
X_3	1	37.5	28.57	34.59	0.0001$^{\#}$	0.0001$^{\#}$	0.0001$^{\#}$
X_4	1	48.75	54.72	66.99	0.0001$^{\#}$	0.0001$^{\#}$	0.0001$^{\#}$
X_1X_2	1	2.73	0.027	0.73	0.1192^{á}	0.872^{á}	0.4053^{á}
X_1X_3	1	0.48	0.23	1.34	0.499^{á}	0.6409^{á}	0.2647^{á}
X_1X_4	1	3.65	1.66	2.76	0.0753^{á}	0.2174^{á}	0.1172^{á}
X_2X_3	1	1.62	0.81	1.72	0.2229^{á}	0.3837^{á}	0.21^{á}
X_2X_4	1	1.94	0.75	2.6	0.1838^{á}	0.4^{á}	0.1278^{á}
X_3X_4	1	12.32	6.75	1.23	0.0032*	0.0202*	0.2852^{á}
X_1^2	1	3.63	14.12	5.48	0.076^{á}	0.0019*	0.0334*
X_2^2	1	0.12	4.09	0.088	0.7332^{á}	0.0615^{á}	0.7707^{á}
X_3^2	1	15.21	32.95	18.1	0.0014*	0.0001$^{\#}$	0.0007$^{\#}$
X_4^2	1	3.86	8.41	4.05	0.0681^{á}	0.011*	0.0626^{á}
Residual	15						
Lack of fit	10						
Pure error	5						
Cor total	29						

Y_1=Cellulose, Y_2=Hemicellulose, Y_3=Lignin; *P<0.05–significant at 5% level,
$^{\neq}$P<0.001–significant at 1% level, $^{\#}$P<0.0001 significant at 0.1% level, $^{\alpha}$not significant.

action between individual parameters to decrease in lignin yield in bagasse.

3.4 Optimization

The response surface plots showed the effect of substrate concentration, PAA loading, pretreatment temperature and pretreatment time on cellulose, hemicellulose and lignin yield in pretreated bagasse. The results represent that the cellulose and hemicellulose response surfaces had a maximum point with lignin at limiting point. Response surface models were useful indicating the direction in which to change the variables in order to maximize the cellulose, hemicellulose and minimize the lignin yield. For cellulose, comparison of the predicted values with the experimentally obtained actual values indicated that these data are in reasonable agreement (Figure 1). The highest level of cellulose yield was achieved at higher temperature (120°C) and higher pretreatment retention time (120 min) (Figure 2). There is considerable interaction between pretreatment temperature and pretreatment time. For hemicellulose yield obtained and the results indicates that there is good correlation between the actual and predicted values (Figure 3). The surface plot of hemicellulose indicates that maximum yield of hemicellulose in pretreated bagasse was attained at the higher temperature with higher pretreatment time (Figure 4). The minimum yield of cellulose and hemicellulose along with lignin yield was obtained under the optimization of pretreatment conditions of substrate concentration (20%), PAA loading (2%), pretreatment temperature (60°C) and pretreatment time (30 min) was 37.4, 31.9, and 16.2%, respectively. For selection of the optimum conditions and range, the models were analyzed separately. Repeated experiments were performed to verify the predicted optimum. The result from three replications was coincident with the predicted value and the

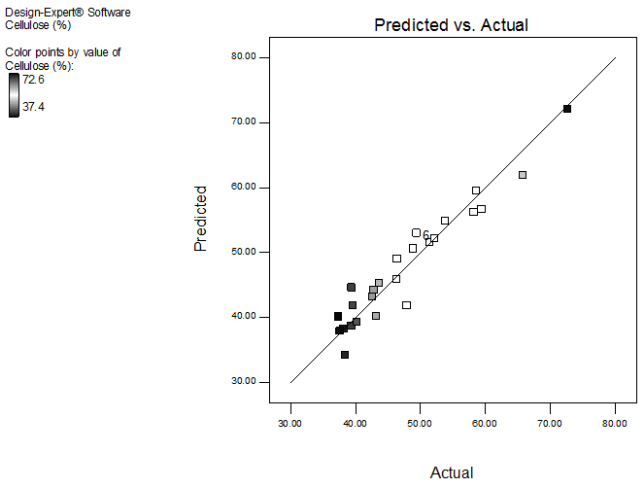

Figure 1. Predicted vs. actual observation run values for cellulose yield.

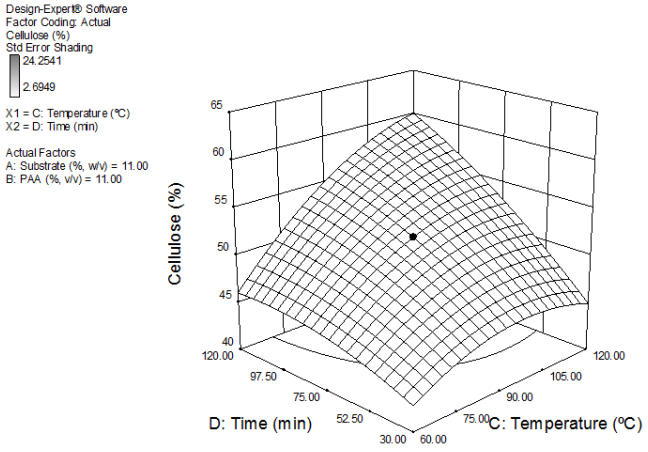

Figure 2. Response surface plot show the interactive effect of pretreatment temperature and pretreatment time on cellulose yield.

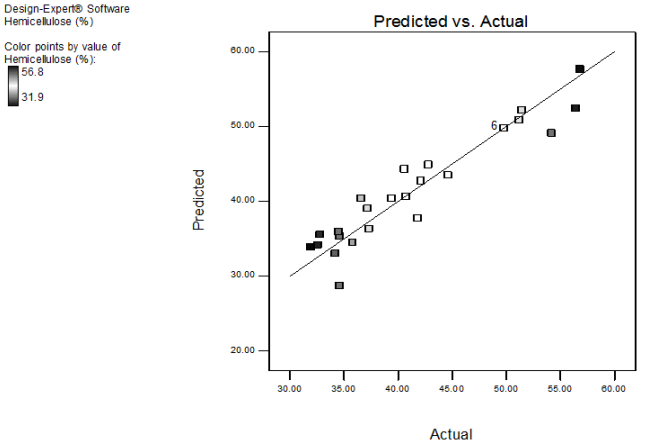

Figure 3. Predicted vs. actual observation run values for hemicellulose yield.

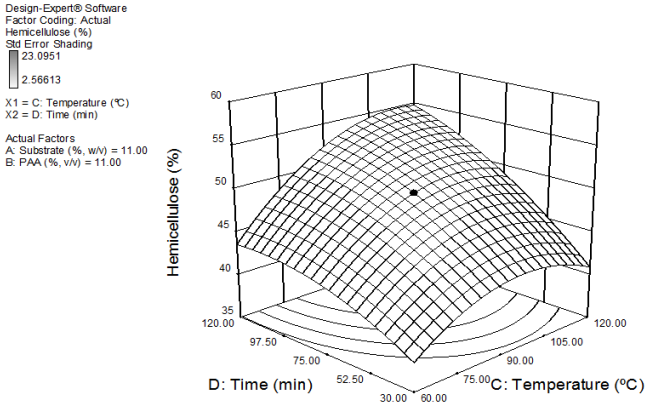

Figure 4. Response surface plot show the interactive effect of pretreatment temperature and pretreatment time on hemicellulose yield.

model was proven to be adequate. The maximum yield of cellulose and hemicellulose along with low lignin yield was obtained with the optimization of conditions of substrate concentration (2%), PAA loading (20%), pretreatment temperature (120°C) and pretreatment time (120 min) was 72.6, 56.8, and 4.3%, respectively. The maximum response predicted from the model was 72.0, 57.6, and 5.49%. By comparing the experiments the cellulose and hemicellulose yield increased from 37.4 to 72.0%, 31.9 to 56.8%, respectively, and lignin yield was decreased from 16.2 to 4.8%.

The maximum cellulose content was achieved at the temperature of 120°C at pretreatment time of 120 min. The cellulose content of 72.6% indicates that the pretreatment at 120°C effectively decrease the lignin content in pretreated bagasse. Prolonging pretreatment time led to little increase in hemicellulose content in pretreated bagasse compared to cellulose content. The adequacy of the model was validated by performing verification experiments within the experimental range. The data of the validation runs were also statistically analyzed to find out the correlation between observed actual and predicted values.

3.5 Saccharification or enzymatic hydrolysis of pretreated bagasse

Comparison of low PAA loading (2%) pretreated with higher PAA loading (20%) pretreated bagasse and the sugar yields in pretreated bagasse are presented in Table 4. Yields of reducing sugars (78.6±0.4 mg/g) were higher in the bagasse substrate pretreated at 120°C for 120 min. This pretreatment removed the hemicellulose and lignin to increase the accessibility of the cellulose. Decreasing the lignin content in pretreated substrates allows nearly complete saccharification of the polysaccharides. Thus, increased conversion of cellulose would increase the available sugar content in the hydrolyzate. The higher cellulose content of bagasse resulted in increasing the reducing sugar content and glucose content (70.1±0.1 mg/g) in higher PAA loading pretreated bagasse

Table 4. Yield of sugars (mg/g of pretreated bagasse) after pretreatment with PAA.

Substrate	Cellulose (%)	Hemicellulose (%)	Reducing sugars	Glucose	Xylose	Arabinose
Low pretreated	37.4±0.8	31.9±0.6	38.5±0.8	34.8±0.2	29.5±0.1	6.8±0.3
High pretreated	72.6±0.4	56.8±0.2	78.6±0.4	70.1±0.1	52.2±0.2	8.9±0.1

Composition of percentages calculated from values on a dry-weight basis;
Data represents the mean ± SEM, n=3.

than the other pretreated samples. The results showed that after pretreatment the availability of reducing sugars from pretreated substrates were increased from their respective carbohydrate content. The increases in cellulose and hemicellulose contents were predominantly attributed to the decreases in lignin. These results indicate that PAA pretreatment could partially disrupt the lignin structure and expose more accessible surface area of cellulose.

4. Discussion

In the present study RSM was applied to study the effect of different process variables (substrate concentration, PAA loading, pretreatment temperature and pretreatment time) on cellulose, hemicellulose and lignin yield in bagasse. Dilute acid has been shown to be a good alternative to selectively remove the hemicellulose fraction, generating a solid residue basically composed of cellulose and lignin (Mussatto and Roberto, 2005). RSM was successfully applied for xylitol production from sugarcane bagasse hemicellulosic hydrolyzate by optimizing the vacuum evaporation process variables such as pH, temperature, xylose concentration degree and treatment with activated charcoal (Rodrigues *et al*., 2003). Abraham and Kurup (1997) investigated the effects of various pretreatments on enzymatic hydrolysis of the whole water hyacinth biomass and concluded that a peracetic acid pretreatment achieved the highest reducing sugar yields. A L_{16} $(4)^5$–orthogonal experimental design was used to optimize the parameters such as temperature, time, loading of PAA and liquid to solid ratio to increase the delignification in weed stem. PAA pretreatment can remove lignin effectively and cause degradation of some hemicelluloses, which made the cellulose exposed (Zhao *et al*., 2008b).

The effectiveness of the peracetic acid pretreatment on bagasse was studied for its effect on simultaneous saccharification and fermentation and ethanol yields greater than 90% of theoretical were achieved (Teixeira et al., 2000). Zhao *et al*. (2007) reported that peracetic acid charge, reaction temperature and reaction time have been found to have significant effects on the yield of glucose from peracetic acid pretreatments. In the present study the PAA concentration was higher than the substrate concentration to attain the maximum yield of cellulose and hemicellulose in pretreated bagasse under autoclaving temperature (120°C) with prolonged pretreatment time (120 min). It was due to the fact that more lignin and carbohydrates were dissolved at higher temperature. Relatively high lignin content occurred in lower temperature and in shorter pretreatment time. Pretreatment time had a significant effect on the degree of delignification. Prolonged pretreatment time decrease the lignin content in pretreated bagasse. Low lignin content in bagasse was observed at high temperature with long retention pretreatment time (Chun *et al*., 2011). This is probably due to the fact that solubilization of lignin and hemicellulose is difficult to be carried out at a relatively short time and with less diluted medium (Teramoto *et al*., 2008).

It demonstrated that dilute acid pretreatment at 121°C or lower temperatures could not well increase the enzymatic digestibility, although it could remove much hemicellulose. A similar result was also found for acid pretreatment of corn stalks by Silverstein *et al*. (2007). Therefore, this work also verified that higher temperature was necessary for dilute acid pretreatment to break down the structure of the biomass, especially to destroy the network of hemicellulose and lignin.

This study was compared with the work of Zhao *et al*. (2009) those attain maximum yield of reducing sugars of 92.04% at 120 hrs from bagasse pre-pretreated by 10% NaOH and then pretreated by 10% peracetic acid. In the present study the pretreatment could be conducted under autoclaving conditions and was more effective for delignification with higher carbohydrates being degraded in the pretreatment process.

Temperature affected the kinetics of delignification and further affected the saccharification of substrate. In the present study the temperature at 120°C yielded more cellulose compared to hemicellulose. PAA was prepared by acetic acid and hydrogen peroxide with sulfuric acid as a catalyst. In the pretreatment these chemicals also caused the degradation of carbohydrates and lignin. Acetic acid is good solvent for lignin and used for pulping or fractionation of lignocellulosic materials (Pan and Sano, 2005). On the other hand acetic acid and sulfuric acid could catalyze hydrolysis of hemicelluloses, but sulfuric acid is the main agent leading to degradation of hemicelluloses. Hydrogen peroxide could also react with lignin, but its activity was weakened in an acid condition. Therefore, PAA plays a major role in delignification (Zhao *et al*., 2008b).

In this work the temperature and PAA pretreatment was lower than that of other pretreatment methods such as dilute sulfuric acid pretreatment at high temperature ($\geq$160°C)

and ammonia recycled percolation (ARP) process. The conventional dilute H_2SO_4 pretreatment is always conducted at 160 ºC or more (Wyman *et al*., 2005), at which hemicellulose is almost hydrolyzed but the acid-insoluble lignin still exists in the solid. For the ARP process, Kim *et al*. (2003) found that this method was highly effective in delignifying of the biomass (corn stover), reducing the lignin content by 70–85% at 170°C with 15% ammonia. Comparably, PAA pretreated bagasse contained higher cellulose yield, which demonstrated that PAA was more effective for separation of lignin and carbohydrates at autoclaving temperature with higher polysaccharides. Silverstein *et al*. (2007) studied the effectiveness of sulfuric acid, sodium hydroxide, hydrogen peroxide, and ozone pretreatments for enzymatic conversion of cotton stalks and found that sodium hydroxide pretreatment resulted in the highest level of delignification (65% with 2% NaOH in 90 min at 121°C) and cellulose conversion (60.8%). Less carbohydrate was lost under proper optimum conditions and PAA pretreatment could obtain a high recovery yield of carbohydrate as solid phase (Zhao *et al*. 2009). Ratio of PAA loading to bagasse substrate for pretreatment influence on sugar yields. High concentration of PAA results in the solubilization of more lignin and increases the cellulose content leading to higher sugar yields. The yields of different reducing sugars after the PAA pretreatment of sugarcane bagasse were shown, and corresponding yield of glucose, xylose and arabinose were increased in higher PAA loading (20%) pretreated bagasse than in lower PAA (2%) treated bagasse substrate. Studies of Xu and Tschimer (2012) confirmed that aspen showed a strong response to PAA addition rate and 9% PAA removed 14% of the original lignin and increased the rate of glucose release from 23 to 44%. This study shows that PAA is a suitable chemical oxidant for pretreatment for effective delignification in lignocellulosic substrate such as bagasse.

5. Conclusion

The results of this study have clearly indicated that RSM is an effective method for optimization of pretreatment conditions to increase the cellulose yield in sugarcane bagasse. By using response surface and contour plots, the optimum set of operating variables can be obtained graphically, in order to achieve the desired levels of cellulose, hemicellulose and lignin yield during pretreatment. The optimized pretreatment conditions for attain maximum cellulose yield were at substrate concentration of 2%, PAA loading of 20%, pretreatment temperature of 120°C and pretreatment time of 120 min. Simultaneously, the yield of reducing sugar was high in the higher PAA loading -pretreated bagasse.

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