Madras Agric. J., 101 (4-6): 172-175, June 2014
https://doi.org/10.29321/MAJ.10.001176

Optimization of Acid Pretreatment Conditions for Enhancing Glucose Yield of Rice Straw Using Response Surface Methodology (RSM)

V. Dharmaraj*, P. Venkatachalam, M. Singaravelu and N.O. Gopal
Department of Bioenergy, Agricultural Engineering College and Research Institute,
Tamil Nadu Agricultural University, Coimbatore-641 003

Response Surface Methodology (RSM) was employed to optimize acid pretreatment condition for maximum glucose yield of rice straw with respect to H_2SO_4 concentration (1.0-5.0%), reaction temperature (60-100°C) and pretreatment time (30-90 min). The maximum glucose yield of 259.40 g kg^{-1} of biomass was obtained at the desirable pretreatment condition of H_2SO_4 3 % at 80°C for 60 min. The ANOVA test revealed that the model and all independent parameters were statistically significant at 95% confidence level.

Key words: Rice straw, Acid pretreatment, RSM, Glucose yield.

Ethanol from renewable resources has been of interest in recent decades as an alternative fuel to the current fossil fuels. Lignocellulose biomass like wood and agricultural crops residue, e.g., straw and sugar beet pulp are potential raw materials for producing several high-value products like fuel ethanol and biodiesel. In contrast to traditional fuels, fermentation ethanol does not contribute to the greenhouse effect, being a CO_2 neutral resource.

Annual production of rice straw is about 731 MT and can potentially produce 205 billion liters of bioethanol per year, which is about 5% of total consumption of petrol. Rice straw predominantly contains cellulose (32-47%), hemicelluloses (19-27%), lignin (5-24%) and ashes 18.8%. The pentoses are dominant in hemicelluloses in which xylose is the most important sugar followed by arabinose and hexoses. Its structural complexity, along with the existence of lignin, makes it recalcitrant to enzymatic and microbial attacks and thus serves a serious obstacle to the commercialization of biomass based fuels (Goh *et al*., 2010). To make cellulose content more susceptible to subsequent enzymatic hydrolysis, raw biomass feedstocks must go through a pretreatment process. Thus far, a substantial number of such pretreatment techniques have been extensively examined to process various biomass feedstocks on scale of both laboratory and pilot plants. These include alkaline, flow-through, ammonia fiber explosion (AFEX), ammonia recycle percolation (ARP), lime, steam explosion, and organosolv (OS) pretreatment (Szczodrak and Fiedurek 2010). Nevertheless, none of those options has demonstrated to be fully satisfactory, because each method has its intrinsic benefits and drawbacks. Therefore, a suitable pretreatment process is critical to make bioconversion processes more efficient.

Among the chemical hydrolysis methods, acid hydrolysis is probably the most commonly applied. It is a method that can be used either as a pretreatment preceding enzymatic hydrolysis, or as the actual method of hydrolyzing lignocelluloses to the sugars. Therefore, this study aims at assessing the effects of these important parameters on the pretreatment ability and thus finding optimal treatment conditions by means of a statistical approach called response surface methodology (RSM). RSM allows setting up a proper experimental design so that multivariate equations may be determined and simultaneously solved. The main advantage of RSM is to reduce the number of experimental trials needed to evaluate multiple parameters and their interactions (Maache-Rezzoug, 2011).In this study rice straw has been used as lignocellulosic material.

Materials and Methods

Rice straw (*Oryza sativa L.),* was collected in October 2011, from the wet land of in Tamil Nadu Agricultural University, Coimbatore. At first, the rice straw including stems and leaves was crushed to small particles by a shatter, and screened for the size of about 3 mm. Then, it was air-dried at 45°C and stored in a polyethylene black bag at room temperature.

Acid pretreatment

About 10g powdered rice straw was suspended in acid solution (1-5% sulphuric acid) with a ratio of 1:10(w/v) rice straw and sulphuric acid. The mixture was autoclaved at 121°C at 15 psi for 30, 60 and 90 min. After the pretreatment, the slurry was extensively washed with deionized water until pH decreased down between 6 and 7. The neutralized solid residue was collected by filtration and subsequently, hydrolyzed by a cellulase mixture.

*Corresponding author email: ve.dharmaraj@gmail.com

Enzymatic hydrolysis

The enzymatic hydrolysis of a pretreated sample was conducted in 0.05 mol m^{-3} citrate buffer (pH 4.80) containing 40 mg mL^{-1} tetracycline and 30 mg mL^{-1} cycloheximide according to the National Renewable Energy Laboratory (NREL) standard procedures. A mixture of cellulase from *Trichoderma reesei* (Celluclast 1.5L, Sigma) with an activity loading of 60 FPU g^{-1} cellulose and b-glucosidase from *Aspergillus niger* (Novozyme 188, Sigma) with an activity loading of 30 CBU g^{-1} cellulose was used for the enzymatic hydrolysis. The reaction mixture (100 mL) was incubated at 50 °C and 2.83 Hz for 72 h in a rotary shaker. Hydrolysates were sampled periodically for sugar analysis. All experiments were performed in triplicate and each data point was an averaged value.

Analysis

Components of the untreated and pretreated rice straw were identified according to Laboratory Analytical Procedures from NREL. Sugar contents in the treated sample were determined by HPLC at 85 °C and water as a mobile phase in the column at a flow rate of 0.6 mL min^{-1}. The remaining solid residue was dried at 105°C overnight and further placed in the muffle furnace at 575 °C for 24 h for the analysis of lignin.

Experimental design

Response Surface Methodology (RSM) was employed to obtain an optimal pretreatment condition to exhibit a highest glucose yield (Y). Three factors *viz.,* H_2SO_4 concentration (X_1), reaction temperature (X_2) and pretreatment time (X_3) were considered in this study. Tested conditions were varied with H_2SO_4 concentrations of 1.0-5.0%, temperatures of 60-100°C and reaction times of 30-90 min at fixed Solid to Liquid (S:L) ratio of 1:10. A total of 15 experimental runs with varied values of the three variables were carried out by Box-Behnken Design (BBD), and a centre point was tested in triplicate (Box GEP, 1960). Data analysis was carried out via Design-Expert software (version 8.0, Stat-Ease, Inc.,USA).

Results and Discussion

Compositional changes of pretreated rice straw

The composition of solid residue was determined after the acid pretreatment and the subsequent enzymatic hydrolysis (Table 1). The recovery of solid residue varied from 40.6 to 62.4% according to the severity of the pretreatment condition. Low recovery values were attributed to the removal of lignin and the degradation of hemicellulose. Acid pretreatment mainly removes lignin from cellulosic biomass, allowing cellulases to get easier access to cellulose molecules during subsequent hydrolysis. The amount of removed lignin ranged from 33.7 to 56.4% of the initial content of lignin in the untreated rice straw. According to previous studies (Silverstein, 2007) lignin reduction increased linearly with the increase in pretreatment severity concerning acid concentration, reaction temperature and time.

Table 1. Overall solid recovery, composition of pretreated solids and enzymatic hydrolysis.

Run	Experimental factors			Solid recovery (%)	Composition content (%)			Lignin removal (%)	Enzymatic hydrolysis (%)
	H_2SO_4 Conc. %	Temp. (°C)	Time (min)		Glucan	Xylan	Lignin		
1	1	100	60	56.2	57.9	21.1	5.0	49.3	82.89
2	3	60	90	54.4	57.0	19.5	5.8	44.4	81.31
3	3	60	30	60.0	54.7	19.8	7.5	33.7	79.82
4	3	80	60	53.2	62.9	16.4	4.4	53.3	86.48
5	3	100	90	40.8	65.4	17.9	4.4	53.1	87.86
6	5	80	90	44.2	65.3	15.8	3.8	57.1	87.95
7	5	60	60	50.4	63.7	17.1	5.4	46.9	82.68
8	5	100	60	40.6	66.5	17.2	3.9	56.4	89.39
9	1	80	30	61.0	51.6	19.8	6.8	38.0	78.21
10	3	80	60	53.4	62.1	20.6	4.5	52.3	85.85
11	3	80	60	54.3	61.4	17.7	4.4	52.8	86.08
12	3	100	30	50.2	63.7	19.8	5.5	46.1	85.67
13	1	80	90	60.0	50.1	17.6	6.3	41.5	81.8
14	1	60	60	62.4	54.2	18.1	7.4	34.3	76.59
15	5	80	30	46.8	65.2	16.0	4.1	55.2	86.73

In this study, enzymatic digestibility was also well correlated to the degree of lignin removal (R^2= 0.9886); the more lignin removed, the more cellulose digested. Similar observations were reported by Ko *et al.* (2009). In the study, the pretreatment of rice straw was done with aqueous-ammonia soaking and the lignin removal showed a high dependence on the enzymatic digestibility with the R^2 value of 0.93. The highest cellulose digestibility of 85.49% was achieved from pretreated rice straw at 100°C for 60 min using 5.0% H_2SO_4 concentration. This harsh pretreatment condition, exhibiting high cellulose digestibility, resulted in much reduced solid recovery yield, which in turn caused a significant amount of glucan loss. As a key indicator parameter for pretreatment efficacy, therefore, it is determined to employ the yield of glucose recovery from untreated biomass instead of the enzymatic hydrolysis from the solid fraction of pretreated glucan content.

Optimization of pretreatment conditions for maximum glucose yield

The Box-Behnken based prediction of glucose yield with different combinations of three independent variables and their experimental data are summarized in Table 2. The maximum glucose yield of 259.40 g kg^{-1} biomass was obtained at the optimized condition of H_2SO_4 3%, at 80°C for 60 min. Other operational conditions were used to determine the experimental error showed relatively higher glucose yields. The resulted second-order polynomial equation through BBD described the glucose yield in terms of actual parameters is shown in the following equation.

$$\text{Glucose yield}(Y) = -106.89062 +25.39X_1+6.18X_2+2.31 X_3-0.097 X_1X_2-0.045 X_1X_3-2.208\text{-}003 X_2X_3-2.05^*-0.0348^*-0.0173^*$$

Where, Y is glucose yield, X_1 is H_2SO_4 concentration, X_2 is reaction temperature, and X_3 is pretreatment time. To examine the adequacy of the developed model, the analysis of variance (ANOVA) for the quadratic model was carried out and the results are presented in Table 3. A model is regarded significant, if its p-value (also known as the ‘prob >

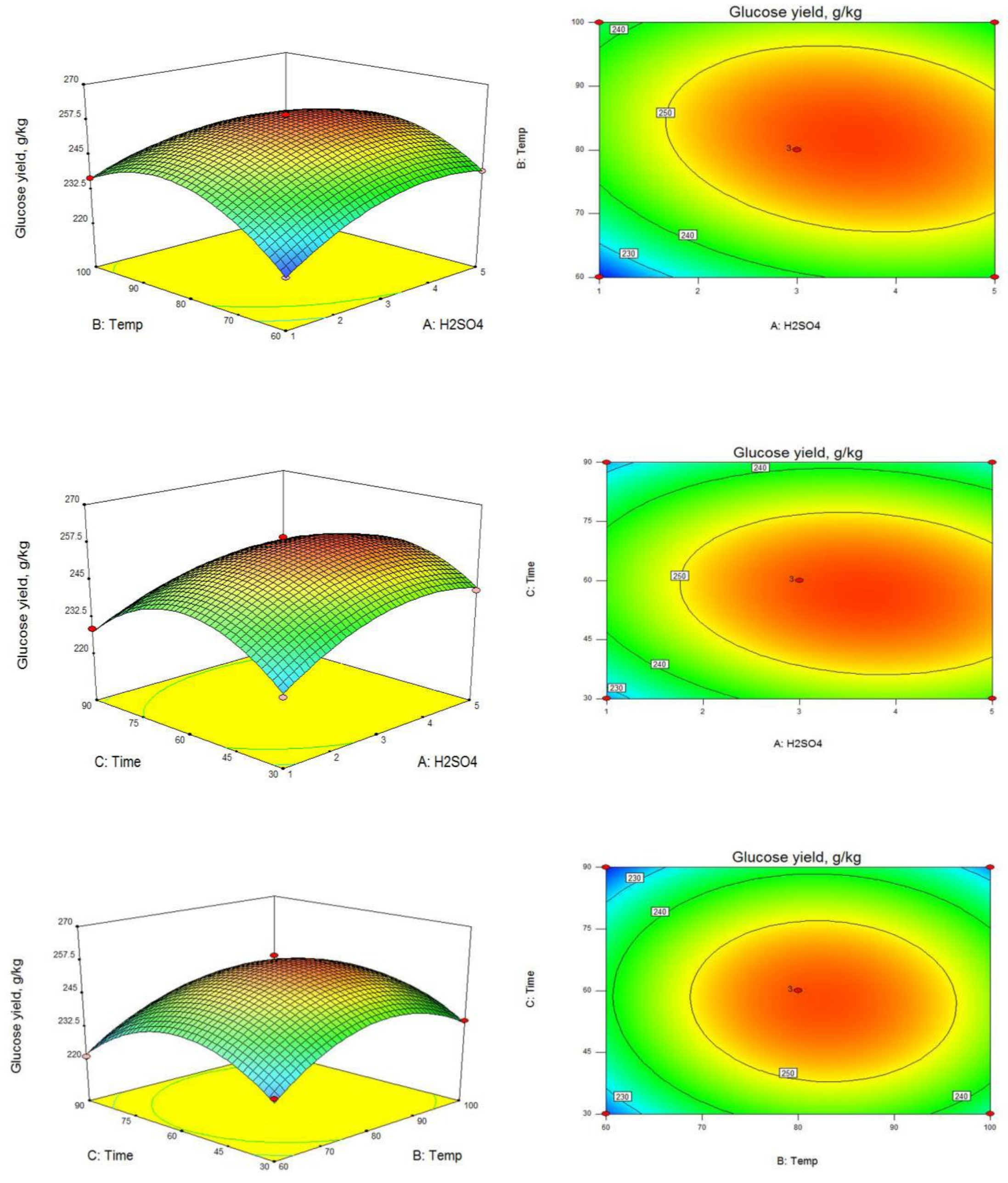

Fig. 1. The surface response plot of the effect of H_2SO_4 concentration, temperature and time on the glucose yield: (a) H_2SO_4 concentration at 60 min ;(b) Temperature at 80 °C; (c) Fixed H_2SO_4 concentration at 3 %

F' value) is lower than 0.01 indicating only a 0.03% chance that a 'model F-value' could occur because of noise (Tan *et al*., 2011). The model F-value of 48.07 with a low p-value ($p < 0.05$) implies that this model was statistically significant at 95% confidence level. In addition, the model terms X_1, X_2, X_3, X_1, X_2 had significant effect on the glucose yield, since each p-value was less than 0.05. Although X_1X_3 and X_2X_3 were insignificant ($p>0.05$), these factors could not be excluded to support the hierarchy of the model. The experimental values showed high correlation (R^2= 0.93) with predicted values.

The optimal pretreatment conditions for maximum glucose yield considering efficiency were X_1 = 3%, X_2 = 80°C and X_3 = 60 min, which would result in a predicted glucose yield of 259.40 g kg^{-1} of biomass. To confirm the result of the predicted value, experiments were conducted at the optimal conditions, displaying the glucose yield of 259.40g kg^{-1} of biomass.

Effects of variables on glucose yield

The experimental results were visualized in three dimensional response surface plots indicating the correlation between two variables with one variable being kept constant at its optimal condition. In the design boundary, each response surface plot had a clear peak, implying that the maximum glucose yield could be obtained inside the design boundary. The interactions of acid concentration, reaction temperature and pretreatment time for glucose yield are depicted in Fig. 1. The interaction of acid loading with temperature showed that a maximum glucose yield could be obtained (around 3%) at 80°C (Fig. 1(a)). From the ANOVA test, it could be concluded that

Table 2. The Box-Behnken experimental data and corresponding glucose yield with different combinations of three independent variables

Run	Coded variable level			Experimental factors			Glucose yield(g kg^{-1})	
	X_1	X_2	X_3	H_2SO_4 conc.(%)	Temp. (°C)	Time (min)	Experimental	Predicted
1	-1	1	0	1	100	60	236.8	232.0
2	0	-1	1	3	60	90	221.1	216.3
3	0	-1	-1	3	60	30	225.7	220.9
4	0	0	0	3	80	60	259.4	254.6
5	0	1	1	3	100	90	225.1	220.3
6	1	0	1	5	80	90	233.5	228.7
7	1	-1	0	5	60	60	239.3	234.5
8	1	1	0	5	100	60	239.0	234.2
9	-1	0	-1	1	80	30	226.0	221.2
10	0	0	0	3	80	60	254.4	249.6
11	0	0	0	3	80	60	255.2	250.4
12	0	1	-1	3	100	30	235.0	230.2
13	-1	0	1	1	80	90	228.5	223.7
14	-1	-1	0	1	60	60	221.5	216.7
15	1	0	-1	5	80	30	241.8	237.0

the interaction of acid loading and temperature was more significant ($p < 0.05$) than reaction time which revealed that the acid concentration and temperature had a strong influence on the treatment process.

Table 3. ANOVA for the quadratic model

Source	Sum of square	df	Mean square	F value	p-value pro> F	
Model	2104.91	9	233.88	48.07	0.0003	significant
X_1-H_2SO_4	208.08	1	208.08	42.76	0.0013	
X_2-Temp	100.11	1	100.11	20.57	0.0062	
X_3-Time	51.51	1	51.51	10.59	0.0226	
X_1X_2	60.84	1	60.84	12.50	0.0166	
X_1X_3	29.16	1	29.16	5.99	0.0581	
X_2X_3	7.02	1	7.02	1.44	0.2834	
	250.04	1	250.04	51.39	0.0008	
	718.96	1	718.96	147.76	< 0.0001	
	904.81	1	904.81	185.95	< 0.0001	
Residual	24.33	5	4.87			
Lack of Fit	9.90	3	3.30	0.46	0.7403	not significant
Pure Error	14.43	2	7.21			

As shown in Fig. 1(b), glucose yield increased with increasing acid loading from 1- 3 per cent at 60 min reaction time, but it decreased with further increase. These two contour plots present an elliptic characteristic, indicating that acid concentration was more influential for glucose yield than temperature and time. As noted from Table 3, it is also confirmed that the effect of acid loading was higher compared to the pretreatment temperature and time. On the other hand, Fig. 1(c) shows a rounded ridge running diagonally on the plot, implying that temperature and time was slightly interdependent (Pan, 2008). This result is proved by p-value more than 0.1 (Table 3), which revealed that the interaction between temperature and time might not affect the glucose yield significantly

Conclusion

In this study, the influence of acid loading, reaction temperature and pretreatment time on glucose yield was examined. From the statistical analyses, it was revealed that all the independent variables had significantly affected the yield of glucose. The maximum glucose yield of 259.40g $kg^{-1}$of biomass was obtained from rice straw at the optimal conditions viz., acid concentration of 3%, reaction temperature of 80°C and pretreatment time of 60 min.

Acknowledgement

The authors gratefully acknowledge the University Grand Commission (UGC) for funding through Rajiv Gandhi National Fellowship (RGNF), New Delhi, and Department of Bioenergy, Agricultural Engineering College and Research Institute, Tamil Nadu Agricultural University, for laboratory support.

References

Box GEP. and Behnken, D.W. 1960. Some new three-level designs for the study of quantitative variables technometrics. **2:** 455-75

Design expert version 8.0 software for windows. Stat-Ease, Inc.; E. Hennepin Avenue, Suite 480, Minneapolis, MN 55413-2726, USA. *http://www.statease.com.*

Goh, C.S., Tan K.T., Lee K.T. and Bhatia, S. 2010. Bio-ethanol from lignocellulose: status, perspectives and challenges in Malaysia. *Bioresour. Technol*., **101:** 4834-41.

Ko, J.K., Bak, J.S., Jung, M.W., Lee, H.J., Choi, I.G. and Kim, T.H. 2009. Ethanol production from rice straw using optimized aqueousammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresour. Technol*. **100:** 4374-80.

Maache-Rezzoug, Z., Pierre, G, Nouviaire, A., Maugard, T. and Rezzoug, S.A. 2011.Optimizing thermomechanical pretreatment conditions to enhance enzymatic hydrolysis of wheat straw by response surface methodology. *Biomass Bioenerg*. **35:** 3129-38.

Pan CM, Fan YT, Xing Y, Hou HW, Zhang ML. Statistical optimization of process parameters on biohydrogen production form glucose by Clostridium sp. Fanp2. *Bioresour. Technol.,* 2008. **99:** 3146-54.

Silverstein, RA., Chen, Y., Sharma-Shivappa, R.R., Boyette, M.D. and Osborne, J. 2007.A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresour. Technol.,* **98:** 3000-3011.

SluSzczodrak, J. and Fiedurek, J. 2010. Technology for conversion of lignocellulosic biomass to ethanol. *Biomass Bioenergy*.**10:** 367-75.

Tan, H.T., Lee, K.T. and Mohamed. A.R. 2011. Pretreatment of lignocellulosic palm biomass using a solvent-ionic liquid (BMIM)Cl for glucose recovery: an optimization study using response surface methodology. *Carbohyd Polym*; **83:** 1862-1868.

Received: June 01, 2013; Accepted: July 30, 2013