



Optimization for acid pre-treatment of Sweet Sorghum Bagasse to obtain fermentable sugars using Response Surface Methodology

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Abstract:

Sweet Sorghum Bagasse (SSB), one of the most abundant lignocellulosic residues represents an attractive renewable resource for bioconversion of energy. The present study was focused on utilization of SSB to obtain higher yield of fermentable sugars so as to take it as a substrate in ethanol production. As lignin is found to hinder the overall availability of fermentable sugars, chemical pre-treatment was carried out using variable acid concentration. Results shown that the maximum fermentable sugar production was observed within the range of 0.5- 2 % of sulphuric acid with the provided autoclave temperature of 125°C for 30 min. Response Surface Methodology (RSM) was used to optimize acid pre-treatment. Central Composite Design (CCD) for three variables at five levels was chosen. RSM predicted response for optimal production of glucose 14.41 mg/ml can be carried out at 0.65% acid concentration for 19.83 min at 128.93°C. Validation Results showed the glucose concentration to be 16.40 mg/ml at predicted response conditions.

Keywords: Sweet Sorghum Bagasse, acid pre-treatment, response surface methodology

Introduction:

Sweet Sorghum is a renewable, cheap and widely available resource that can be used for bioethanol production. It contains soluble and insoluble carbohydrates & lignin along with various intermediate and secondary metabolites [1]. Major fraction of carbohydrates can be extracted by juice form, and can be easily fermented to bioethanol. The solid residue (bagasse) remained as by-product during processing represents about 30% of whole plant fresh weight. Lignin is physical seal of hemicelluloses and cellulose, which forms an impenetrable barrier in the plant cell wall [2]. The lignin content, crystallinity and degree of polymerization of cellulose decrease the surface area for the enzymatic attack of cellulose fibers towards the production of fermentable sugars which are the precursors for bioethanol production [3]. Therefore

Production of fermentable sugars from lignocellulosic materials demands a pre-treatment, which can be carried out by different techniques i.e. using alkalis, acids, heat pressure, solvents, etc [4]. The chemical pre-treatment with diluted sulphuric acid solutions followed by enzymatic hydrolysis is considered to be promising method for the production of fermentable sugars [5]. RSM is a statistical technique, which is used to optimize the process parameters to get the higher yield. It is applied in the present study to optimize the acid pre-treatment conditions to make more concentrations of cellulose available for conversion to fermentable sugars during hydrolysis.

Materials and Methods:

A. Substrate preparation

Sweet sorghum bagasse was procured from local paddy fields located in Ludhiana, Punjab, India. The sun-dried bagasse was chopped into small pieces with electric toka and further dried in an oven at 70°C to constant weight. The dried bagasse was cut with chaff cutters, yielding small pieces of about 1.5 inches each. These pieces were subsequently ground to a fine powder with electrical mill (Pertin Instruments AB, Sweden), and the powder was passed through an electrically operated sieve shaker to ensure uniformity of the sample.

B. Optimization of Acid Pre-treatment of SSB by RSM

1. RSM Design

In the experimental plan, response surface methodology is used for modelling and analyzing systems to optimize response of interest considering several variables [6]. Central Composite Design (CCD) was used in this study by taking three variables including i) Acid Concentration, ii) Autoclave Temperature & iii) Autoclave Time Setting at five levels.

Table 1: Levels of variables used in CCD

Individual variable	- α	-1	0	+1	+ α
Acid (%) Concentration	0.16	0.5	1.0	1.5	1.84
Autoclave Temperature (°c)	119.89	125	132.5	140	145.11
Auto clave time (Min)	4.89	10	17.5	25	30.11

The relationship of the independent variables and the response was calculated by second-order polynomial. The behaviour of the system was explaining need by the following quadratic equation (eq1):

Where Y is the predicted response, β_0 the intercept term, β_i the linear effect, β_{ii} the square coefficient, and β_{ij} is the interaction coefficient; k is the number of factors [7].

2. RSM response analysis:

After the each experimental run enzymatic hydrolysis was carried out using cellulase (Celluclast 1.5, Novozyme) with 2.0 ml of sodium phosphate buffer (pH 6.0) incubated at 50°C in a rotary shaker set at 150 rpm for 7 days. After 7 days samples are cooled & centrifuged for 10 min at 10,000 rpm and supernatant was saved for analysis of total reducing sugars by DNS method. Quantification of monomeric sugar is done by HPLC.

Results and Discussion:

A. Acid pre-treatment:

In previous work, pre-treatment was carried out with dilute sulphuric acid (0.5 to 1.5%) at a temperature of 160°C for 40 min on Sorghum which resulted in a maximum glucose yield (80.3%) at 1% of dilute sulphuric acid [8]. Where as in our investigation maximum yield of 30.46 g/l glucose was obtained from SSB pre-treated with 2% H₂SO₄ in 15 min at 121°C temperature. From this it can be inferred that higher concentration of acid is required to carry out the process with reduced time. So as to avoid harsh condition temperature was kept at 121°C to achieve better economy compared with earlier work [9]. Further optimization was carried out by employing statistical design taken with acid conc., autoclave temperature & residence time.

Table 3: a) Solid Fraction analysis after Acid pre-treatment

S no.	Acid conc.	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)
1.	0.5%	8.94	0.67	0.22
2.	1%	17.71	1.31	0.65
3.	1.5%	29.15	3.17	0.97
4.	2%	30.46	3.09	0.92

3.2. Optimization of Acid Pre-treatment by response surface methodology:

The purpose of the pre-treatment is to remove hemicelluloses and lignin, reduce cellulose crystallinity, and increases the porosity of the materials prior to enzymatic hydrolysis. Sweet sorghum bagasse was pre-treated by acid treatment with dil. H₂SO₄. There were three factors namely Acid concentration, Autoclave temperature and Autoclaving time and three levels of each parameter were varied as shown in Table 4. The software analyzed the data and suggested both linear and quadratic models as significant for optimal condition of acid pre-treatment. The RSM was used to investigate the effects of the assigned parameters on the sugar production.

The results from the central composite design were examined using statistical analysis in order to identify the significant parameters that affect acid pre-treatment of sweet sorghum bagasse. Analysis of variance (ANOVA) was performed using Design expert 7.0 software and the results are shown in Table 5. From the ANOVA analysis, it was found that the values of the determination coefficient ($R^2 = 0.9892$) and the adjusted determination (Adj. $R^2 = 0.9795$) were very high, which indicated a high significance of the model. It shows a good correlation between the independent variables and the glucose response. Based on the analysis, Glucose concentration (g/L) can be evaluated from the following equation that was obtained from the polynomial model:

$$\text{Glucose Reponses} = +15.15 - 0.45 * A + 0.53 * B + 0.77 * C - 0.59 * A * B + 0.92 * A * C - 0.37 * B * C - 1.79 * A^2 + 1.31 * B^2 - 0.95 * C^2 \dots (2)$$

Table 4- R.S.M. Design layout

Ru n	Acid Conc. % (V/V)	Autoclave Temperatur e(°C)	Autoclave Time (min)	Glucose (mg/ml)	Xylose (mg/ml)
1	1.00	132.50	17.50	14.85	0.617
2	1.50	125.00	10.00	11.47	0.72
3	1.50	140.00	25.00	15	0.21
4	0.50	140.00	10.00	16	0.929
5	1.00	132.50	17.50	15.65	0.685
6	1.00	132.50	4.89	11.18	1.18
7	0.50	125.00	25.00	13.5	0.685
8	1.50	125.00	25.00	15.45	0.574
9	0.50	140.00	25.00	14.84	0.684
10	1.50	140.00	10.00	11.9	0.336
11	1.00	132.50	17.50	15.09	0.608
12	1.00	132.50	17.50	14.92	0.625
13	0.50	125.00	10.00	12.6	0.89
14	1.84	132.50	17.50	9.0	0.141
15	0.16	132.50	17.50	10.8	0.745
16	1.00	119.89	17.50	17.94	0.307
17	1.00	145.11	17.50	19.4	0.001
18	1.00	132.50	17.50	15.44	0.592
19	1.00	132.50	17.50	15.01	0.617
20	1.00	132.50	30.11	13.41	0.935

Where A, B, and C are the Pre-treatment Acid Concentration, Autoclave Temperature, and Autoclave Time. The most significant parameter that was found in this study is the acid concentration followed by the autoclave temperature and the autoclave time ($P < 0.05$).

The second-order interaction between Acid conc. and Autoclave Temperature, Acid Conc. and Autoclave Time, Autoclave Temperature and Autoclave Time and Square of Acid conc., Autoclave Temperature and Autoclave Time were significant at a 95% confidence level (Table 5). The significance of data is judged by its p- value being closer to 0. The p-value should be less than 0.1, 0.05 and 0.01 for a 90%, 95%, and 99% confidence level, respectively for the factors to be termed as significant. Ballesteros et al.^[10] reported temperature as the most significance parameter when treating cardoon using dilute sulphuric acid whilst acid concentration and residence time mostly affected the acid pre-treatment of rye straw.

Table 5- Analysis of Variance table (Response Glucose)

Source	Sum of squares	df	Mean square	F Value	p-value prob>F	
Model	115.77	9	12.86	101.82	<0.0001	Significant
A-Acid Conc.	2.77	1	2.77	21.90	0.0009	
B-Auto clave Temperature	3.77	1	3.77	29.84	0.0003	
C-Autoclave time	8.18	1	8.18	64.76	<0.0001	
AB	2.83	1	2.83	22.42	0.0008	
AC	6.73	1	6.73	53.31	<0.0001	
BC	1.08	1	1.08	8.55	0.0152	
A ²	46.32	1	46.32	366.69	<0.0001	
B ²	24.65	1	24.65	195.11	<0.0001	
C ²	12.90	1	12.90	102.11	<0.0001	
Residual	1.26	10	0.13			
Lack of Fit	0.76	5	0.15	1.53	0.3264	not significant
Pure Error	0.50	5	0.100			
Cor Total	117.03	19				

R²- 0.9892, Adjusted R²- 0.9795

Fig 1- Model Graphs- 3D-Graph

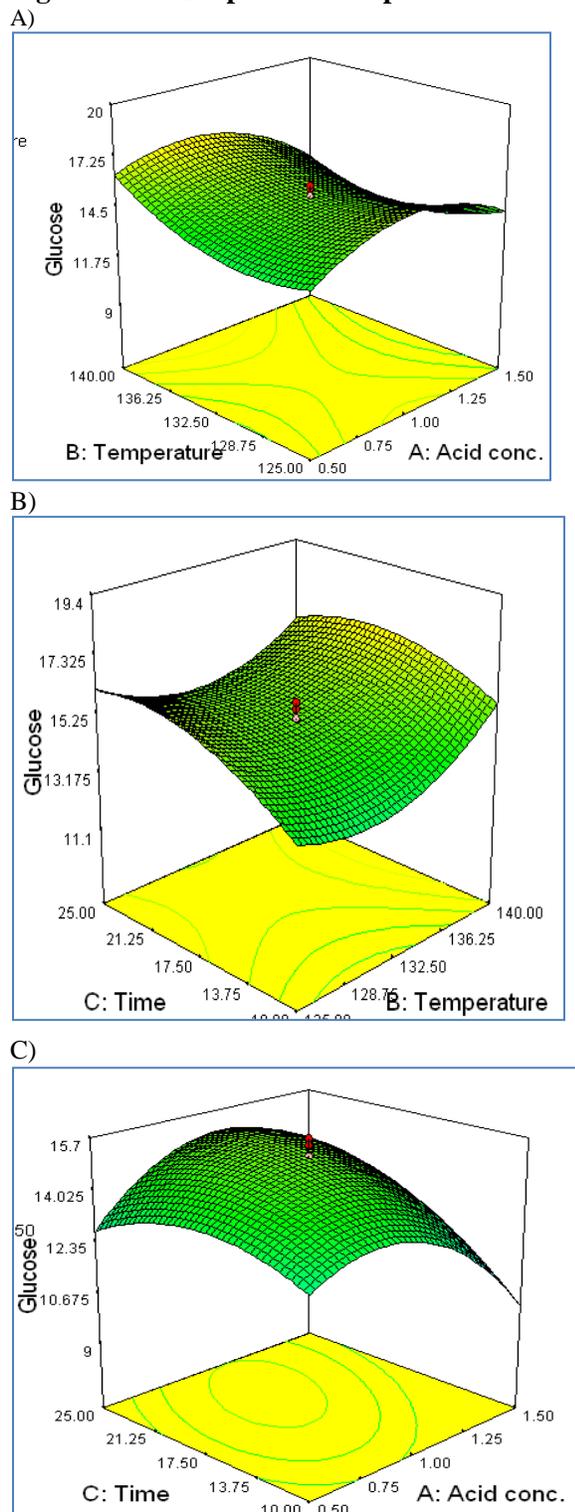


Fig.1- Response surface plots of Glucose production by acid pre-treatment. A) Interaction of temperature with Acid conc. B) Interaction of time with Acid conc. C) Interaction of time with temperature

The response surfaces shown in Fig.1 were based on the final model in which one variable was kept constant at their optimum values and the other two were varied within their experimental range. It is clear from the model graph responses that the interaction of Acid conc. with autoclave temperature, acid conc. with autoclave time and time with temperature had a significant effect on glucose production. Increasing the concentration of both the variables resulted in an increase in glucose concentration. Numerical optimization of the parameters was done to maximize glucose concentration in least possible time. The independent variables using numerical optimization were fixed in the range selected. The range for glucose concentration was kept between 9.0 and 19.4 g/l (lowest and highest responses observed during RSM trial). The numerical optimization package suggested 30 different combinations with desirability of 1.0. Thus, after evaluating the model graphs and the combinations suggested by the numerical optimization package, validation experiment was done at acid concentration 0.65 %, autoclave temperature 129.83°C and autoclave time 19.83 min respectively.

3.3. Validation experiment using Optimized conditions:

Results of validation experiment indicate that use of optimized parameters not only helped increase glucose concentration, but also improved process productivity because higher glucose concentration was obtained in less time. Neither glucose nor Xylose concentrations increased after 36 h of pre-treatment and significantly decreased during further (Fig 2). The rate of increase in glucose, Xylose and Arabinose concentrations declined after 36 h, and an appreciable increase in glucose and Xylose concentrations was seen during 24-36 h of pre-treatment (Fig.2). The Glucose, Xylose and Arabinose concentrations during validation run were 17.12, 0.454, 0.245 g/l, respectively, in 36 h, indicating that the models can mathematically represent glucose and Xylose concentrations during acid pre-treatment of SSB. The graph shows that Glucose, Xylose and Arabinose concentrations profile were significantly increased in between 24 to 36 h. Use of the optimized parameters at acid conc. 0.65 %, temperature 128.93°C, and Time 19.83 min

resulted in an increase in glucose and Xylose concentrations and substantial savings in pre-treatment period.

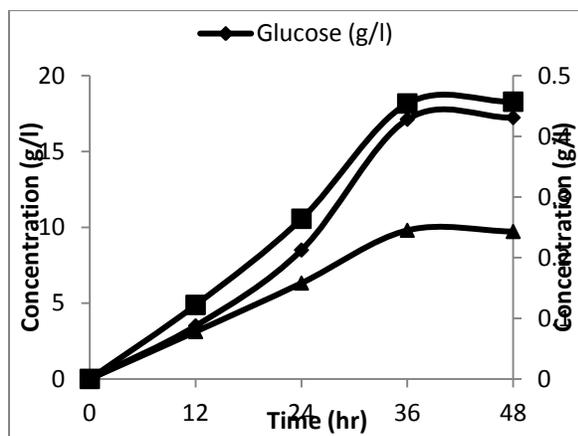


Fig 2: Experimental validation of optimized pre-treatment conditions at acid conc. of 0.65 %, autoclave temperature 128.93°C and autoclave time 19.83 min respectively

Conclusion:

The main objective of acid pre-treatment is to remove the lignin and hemicelluloses structures, preserving more cellulose to make accessible to enzymatic hydrolysis for producing reducing sugars. Thus the optimal production of glucose of 16.40 mg/ml was achieved at 0.65% acid concentration for 20 min at 129°C. These results showed that three variables effected on yield of glucose.

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References:

1. Balat, Mustafa, and Havva Balat. "Recent trends in global production and utilization of bio-ethanol fuel." *Applied Energy* 86.11 (2009): 2273-2282.
2. Saxena, R. C., D. K. Adhikari, and H. B. Goyal. "Biomass-based energy fuel through biochemical routes: A review." *Renewable and Sustainable Energy Reviews* 13.1 (2009): 167-178.
3. Zhang, Yi-Heng Percival, and Lee R. Lynd. "Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase

systems." *Biotechnology and bioengineering* 88.7 (2004): 797-824.

4. Ahring, Birgitte, and Peter Westermann. "Coproduction of bioethanol with other biofuels." *Biofuels* (2007): 289-302.

5. Hendricks AT Zeeman Pre-treatment to enhance the digestibility of lignocellulosic biomass. *Bioresource Technol* (2009): 100:10-8

6. Myers, Raymond H., Douglas C. Montgomery, and Christine M. Anderson-Cook. *Response surface methodology: process and product optimization using designed experiments*. Vol. 705. Wiley, (2009).

7. Liu, Jian-Zhong, et al. "Optimization of glucose oxidase production by *Aspergillus niger* in a benchtop bioreactor using response surface methodology." *World Journal of Microbiology and Biotechnology* 19.3 (2003): 317-323.

8. Xu, Feng, et al. "Sulfuric acid pre-treatment and enzymatic hydrolysis of photoperiod sensitive sorghum for ethanol production." *Bioprocess and biosystems engineering* 34.4 (2011): 485-492

9. Cara, Cristobal, et al. "Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification." *Process Biochemistry* 41.2 (2006): 423-429.

10. Ballesteros I, Ballesteros M, Manzanares P, Negro MJ, Oliva JM, Saiez F. Dilute sulfuric acid pre-treatment of cardoon for ethanol production. *Biochem Eng J* (2008);42:84-91.
