Optimized yield of fermentable sugar from chemical hydrolysis of rice straw for application in ethanol fermentation

Gupta Vikas Chandra[1]*, Singh Meenu[1], Prasad Shiv[2]* and Mishra Bhartendu Nath[3]
1. Department of Biotechnology, College of Engineering and Technology, IILM University, Greater Noida 201310, Uttar Pradesh, INDIA
2. Division of Environmental Sciences, Indian Agricultural Research Institute (IARI) 110012, New Delhi, INDIA
3. Department of Biotechnology, Institute of Engineering and Technology, Dr. APJ Abdul Kalam Technical University, Lucknow 226021, Uttar Pradesh, INDIA
*vikas.gupta@iilm.edu; shiv_drprasad@yahoo.co.in

Abstract

Lignocellulosic biomass is a rich source of carbohydrate polymers with cellulose and hemicellulose being the two primary carbohydrates made up of glucose and xylose monomers. These monomeric sugar molecules act as precursor molecules for ethanol production by the microbial fermentation process. Rice straw is a potent lignocellulosic feedstock for ethanol production, but its utilization on an industrial scale still faces significant challenges. The main obstacle lies in the chemical pretreatment process which needs to be designed optimally to enable a smooth supply of biomass-based fermentable sugar for ethanol businesses in a sustainable and cost-effective manner.

The application of response surface curve analysis was made utilizing the Minitab software-based design of experiments which have demonstrated promising results in obtaining an optimized yield of fermentable sugar from the chemical hydrolysis of rice straw. The present study aimed to increase the fermentable sugar yield from chemical pretreatment of rice straw using Minitab computer software-based design of experiments. The optimal level of pretreatment variables was determined using Minitab 17 software-based analysis of the response surface curve to achieve a maximized release of fermentable sugar at 348.20 milligrams/gram of solid pretreated biomass. This study identified the corresponding optimum operating levels for each variable as (a) biomass solid loading rate (15% w/v), (b) H_2SO_4 concentration (12% v/v), (c) pretreatment reaction time (30 minutes) and (d) temperature (100°C).

Keywords: Rice straw, pretreatment, chemical hydrolysis, fermentable sugar, response surface curve analysis, optimized yield.

Introduction

Ethanol production from lignocellulosic biomass is gaining importance worldwide due to its rich carbohydrate polymer constituents. But, it must be pretreated before performing saccharification or simultaneous saccharification and fermentation (SSF) to facilitate the enzymatic conversion process effectively to produce ethanol[1]. The dilute acid pretreatment process has been reported as the most effective method for producing fermentable sugars from lignocellulosic biomass. However, it proves to be one of the costly affairs in the process[2]. In addition, it has a significant drawback resulting in the use of acid pretreatment methods, particularly at high temperatures where various inhibitor molecules are produced, which has a negative impact on the fermentation efficiency of microorganisms along with the release of sugars in broth.

Major inhibitor molecules produced in the process include pentose-derived furfurals, hexose-derived hydroxymethyl furfural (HMF), lignin-derived phenolic compounds such as ferulic acid and vanillic acid and several other acids such as acetic acid and formic acid[3]. Ethanol production at an industrial scale is influenced mainly by the high-yielding ability of ethanologenic yeasts and bacterial strains. However, overall the production system is negatively affected by the presence of these acid-derived inhibitor molecules in the hydrolysates leading to a low ethanol yield. Thus, the removal of inhibitors molecule from hydrolysates is essential to make the process more cost-effective at a large scale[4,5].

The conventional methods of removing these inhibitors from lignocellulosic hydrolysates include physico-chemical (e.g. evaporation, solvent extraction, overlining, activated charcoal adsorption, ion exchange etc.) and biological (e.g. enzyme, adaption of fermentation microorganism, microbial degradation, etc.) methods[6]. Although using most of these methods in detoxification and retaining potent inhibitors molecule aside have many disadvantages like fermentable sugar loss, by-product generation, expensive process and complicated and lengthy process operation[7]. The methods of concentration enrichments of fermentable sugars in lignocellulosic hydrolysates comprised of heating, vacuum evaporation and membrane separation, which are uneconomic at industrial scale due to their requirement of high energy in the process[8].

Membrane-based separation and detoxification of hydrolysates mainly include the application of microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) applied widely in the bioenergy research at a global scale recently due to its

distinctive ability to separate and to purify process streams[9]. Also, energy-efficient technology for simultaneous concentration and detoxification of lignocellulosic hydrolysates novel methods needs to be developed, making the process cost and energy effective in a sustainable manner[10].

In addition, most of the previous reports evaluated the performance of pressure-driven membranes using synthetic model solutions with only one or several inhibitors whereas the application of membrane-based separation in real lignocellulosic hydrolysates was found almost unavailable. The ethanol produced from lignocellulosic biomass critically depends on free fermentable sugar in pretreated biomass hydrolysate. Thus, sugar recovery from such biomass has been paid much attention recently more sustainably and economically.

Agricultural biomass waste such as rice straw is abundantly available as cheaper feedstock with the significant components of cellulose, hemicelluloses and lignin[11]. Therefore, it is of utmost importance that the carbohydrate part of rice straw is easily accessible for the enzymatic saccharification process leading to enhanced fermentable sugar. Pretreatment is a critical step for improving enzymatic accessibility to the surface of carbohydrate contents of rice straw biomass. Various pretreatment strategies have been reported to address the desired outcome of increased fermentable sugar from rice biomass[12]. The acid-pretreated rice straw has been reported as the most suitable for the enzymatic hydrolysis process for increased production of fermentable sugars[13].

The reaction time, temperature, solid biomass loading and acid concentration are vital factors that determine the efficiency of the conversion process. Optimization of chemical pretreatment by one factor at a time (OFAT technique) is old and time-consuming in which the effect of one factor could only be observed at a time.

In contrast, the design of experiments is a powerful statistical tool in which the central composite design of Response surface methodology (RSM) is mainly used to study multi-response model in different sets of combinations of experimental trials. There are many reports that have indicated the use of RSM to optimise the pretreatment of lignocellulosic biomass and fermentable sugar conversion process[14].

The present research work studies the optimal impact of selected acid pretreatment variables on the improved release of fermentable sugar from rice straw by enzymatic preparations. In the present research, the central composite design is used to optimize various parameters of acid pretreatment of rice straw such as solid biomass loading, reaction time, acid concentration and temperature for maximum production of reducing sugars as fermentable sugar. This study also assessed the effectiveness of acid pretreatment for enhanced release of reducing sugar by enzymatic saccharification.

Material and Methods

Substrate preparation and acidic pretreatment of rice straw: The rice straw was collected from a local farmer's field near Greater Noida, Uttar Pradesh. The obtained rice straw was cut into 1 cm pieces, washed with tap water to remove any dust particles and exposed to microwave radiation at different power levels (low, medium and high) for 5 minutes. Afterwards, the substrate was dried in a hot air oven at 45°C for 15 minutes. The dried rice straw was then ground and sieved to produce a powdered raw material in 30-50 mm size.

The powdered rice straw substrate was pretreated using a combination of pretreatment variables including biomass solid loading (2.5, 10, 17.5, 25 and 32.5% w/v), H_2SO_4 concentration (2.5, 5, 12.5 and 27.5% v/v), time (5, 15, 25, 35, 45 minutes) and temperature (35, 80, 125, 170 and 215°C), according to a central composite experimental design using Minitab 17 software. Finally, the pretreated rice straw samples were washed with distilled water, kept overnight at 60°C and dried. The dried substrate was then used for further enzymatic hydrolysis processes.

Analysis of sugar release from pretreated rice straw using crude enzyme preparation from *Trichoderma reesei:* Enzymatic hydrolysis of pretreated rice straw, supplemented with 0.1 M Na-acetate buffer (pH 5.0) at the appropriate substrate to moisture content, was carried out by adding crude cellulase enzyme preparation from previously conducted research (ammonium sulfate precipitated) of *T. ressei* strain (equivalent to 19 U/g) at 45°C and 140 rpm for 5 h. The samples were then centrifuged at 10,000 rpm for 10 minutes and the supernatant was used to estimate reducing sugars using the dinitro salicylic acid (DNS) method described by Miller[12].

Statistical optimization of acidic pretreatment by using CCD and RSM: Optimization of acidic pretreatment was carried out by response surface methodology (RSM) to interpret the effects of selected operating variables i.e. Biomass solid loading in % w/v (2.5, 10, 17.5, 25 and 32.5%), H_2SO_4 in % v/v (2.5, 5, 12.5 and 27.5%), time in minutes (5, 15, 25, 35, 45) and temperature in degree centigrade (35, 80, 125, 170 and 215ºC) on the liberation of reducing sugars using the central composite design (CCD) of experiments.

The central composite design developed a mathematical correlation between four independent variables on the liberation of reducing sugars[17]. These selected variables were studied at five different levels (Table 1).

A 24-factorial design including a total number of 31 varying experiments was generated using the statistical software (Minitab 17) to study their effect on saccharification. The

following quadratic equation explains the response of experimental design:

$$Y=\beta0+\beta1A+\beta2B+\beta3C+\beta4D+\beta11A2+\beta22B2+\beta33C2+\beta44D2+\beta1\beta2AB+\beta1\beta3AC+\beta1\beta4AD+\beta2\beta3\beta4BCD$$

where Y is the response as reducing sugar (mg/gram biomass), β is the factor coefficient, A, B, C and, D are the Biomass solid loading (%w/v), H_2SO_4 (% v/v), reaction time (min) and temperature (ºC), respectively.

Results and Discussion

Optimization of acid pretreatment of rice straw by response surface method for improved release of fermentable sugar: The process of acid pretreatment of rice straw has been optimized using the central composite design and response surface method of optimization. The acid pretreatment parameters were tested at five different levels of biomass solid loading in % w/v, H_2SO_4 concentration in % v/v, time in minutes and temperature in ºC for reducing sugar release as indicated earlier. A total of 31 experimental trials in the triplicate run were completed according to the designed matrix and the observed result responses are presented in table 1.

The regression equation used to calculate the release of reducing sugar (fermentable sugar) yield in the experimental trials is given as follows:

Reducing Sugar (mg/gram biomass) = -283.2 + 22.66A + 21.72B + 5.01C + 2.601D - 0.3111A*A - 0.7536B*B - 0.1643C*C - 0.00839D*D - 0.7884A*B + 0.1215A*C - 0.04915A*D + 0.2013B*C + 0.07222B*D - 0.01547C*D (1)

Table 1
Central Composite Design for Analysis of fermentable sugar yield

Std. Order	Run Order	Pt Type	Blocks	Biomass solid loading (%w/v)	H_2SO_4 (% v/v)	Reaction Time (Min)	Temp (ºC)	Reducing Sugar (mg/gram biomass)**
11	1	1	1	10	20	35	170	326.5
27	2	0	1	17.5	12.5	25	125	273.5
24	3	-1	1	10	5	15	80	168.4
8	4	1	1	10	5	35	80	123.3
3	5	1	1	17.5	27.5	25	125	153.1
26	6	0	1	17.5	12.5	25	125	279.6
31	7	0	1	17.5	12.5	25	215	219.8
10	8	1	1	17.5	12.5	25	125	283.4
20	9	-1	1	25	5	35	170	164.5
23	10	-1	1	17.5	2.5	25	125	183
29	11	0	1	17.5	12.5	25	125	286.1
4	12	1	1	32.5	12.5	25	125	203.7
17	13	-1	1	10	20	15	170	348.2
15	14	1	1	17.5	12.5	5	125	225.2
1	15	1	1	2.5	12.5	25	125	221.3
9	16	1	1	25	20	15	80	132.8
25	17	0	1	10	20	15	80	212.7
7	18	1	1	25	20	35	170	193.6
16	19	1	1	10	5	35	170	101.2
28	20	0	1	17.5	12.5	25	125	293.4
2	21	1	1	10	20	35	80	232.8
6	22	1	1	25	5	15	80	238.5
12	23	1	1	25	5	15	170	200.5
5	24	1	1	25	20	15	170	165.4
14	25	1	1	25	20	35	80	184.5
21	26	-1	1	17.5	12.5	45	125	208.3
13	27	1	1	10	5	15	170	169.9
22	28	-1	1	25	5	35	80	225
18	29	-1	1	17.5	12.5	25	35	209.3
19	30	-1	1	17.5	12.5	25	125	301.2
30	31	0	1	17.5	12.5	25	125	306.8

**Response was measured in triplicate and the mean data are shown in the table

where equation 1 predicts the values of reducing sugar (mg/gram solid biomass) in which A, B, C and D are the coded values of biomass solid loading (%w/v), H_2SO_4 (% v/v), reaction time (min) and temperature (ºC) respectively.

The results of the response surface curve and interaction between process variables are shown in figures 1 to 6. The optimum level of pretreatment variables for maximum sugar releases i.e. 348.2 milligrams/gram of solid pretreated biomass, has been selected based on the interaction and contour plot. The optimum values of each selected variable were found to optimum operating level as (a) Biomass solid loading rate (15% w/v) (b) H_2SO_4 concentration (12 % v/v) (c) Reaction time of pretreatment (30 minutes) and (d) Temperature (100°C).

The surface plot of reducing sugar (mg/gram biomass) yield vs biomass solid loading (% w/v) vs H_2SO_4 (% v/v) is presented in fig. 1 where it is evident that the low-value for sugar release was observed in both the factor i.e. biomass solid loading and H_2SO_4 concentration. The maximum statistically significant sugar release was obtained at (a) biomass solid loading (15% w/v) and (b) H_2SO_4 concentration (12 % v/v) with a high F value of 175.26 Prob > F of less than 0.00001 as shown in table 2.

The interaction surface plot between biomass solid loading (%w/v) vs reaction time (min) is presented in fig. 2 where no significant reducing sugar release was observed at the low value of biomass solid loading and with less reaction time. In contrast, the maximum statistically significant sugar released was found at optimum values of (a) biomass solid loading 15% w/v and (b) reaction time of pretreatment 30 minutes with F value of 7.40 Prob>F of less than 0.015. A similar correlation between the (a) biomass solid loading (15% w/v) and (d) temperature (100°C) was observed as shown in fig. 3 which represents the maximum statistically significant sugar release with an F value of 24.52 Prob > F of less than 0.00001 (Table 2).

Fig. 4 has shown the positive interactional effect of (b) H_2SO_4 concentration (12% v/v), (c) reaction time of pretreatment (30 min) on maximum sugar release with F value of 20.32 Prob > F of less than 0.00001 (Table 2). Response surface 3-D plot is represented in fig. 5 showing the positive impact of (b) H_2SO_4 concentration (12% v/v) and (d) temperature (100°C) on maximum reducing sugar release by enzymatic hydrolysis with a high F value of 52.94 Prob > F of less than 0.00001 (Table 2).

In fig. 6, the interactional effect of the process variable (c) reaction time of pretreatment (30 minutes) and (d) temperature (100°C) showed a maximum sugar release with F value of 4.32 Prob > F of less than 0.054. Validation of the statistical results and the model equation were analyzed using Analysis of Variance (ANOVA) presented in table 2. The CCD model fits well and significantly shows the F value of 44.09 and Prob > F of less than 0.0002.

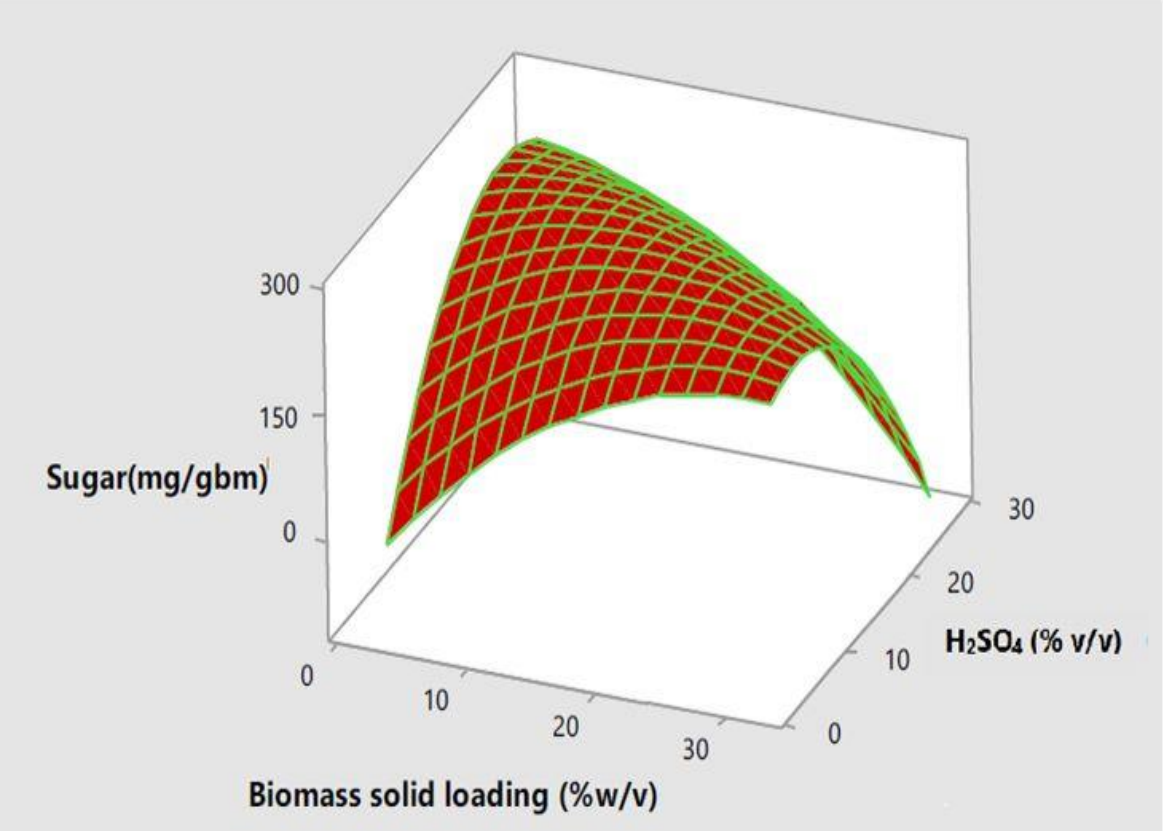

Fig. 1: Surface plot of reducing sugar (mg/gram biomass) yield vs. biomass solid loading (% w/v) vs. H_2SO_4 (% v/v); Sugar (as reducing sugar)

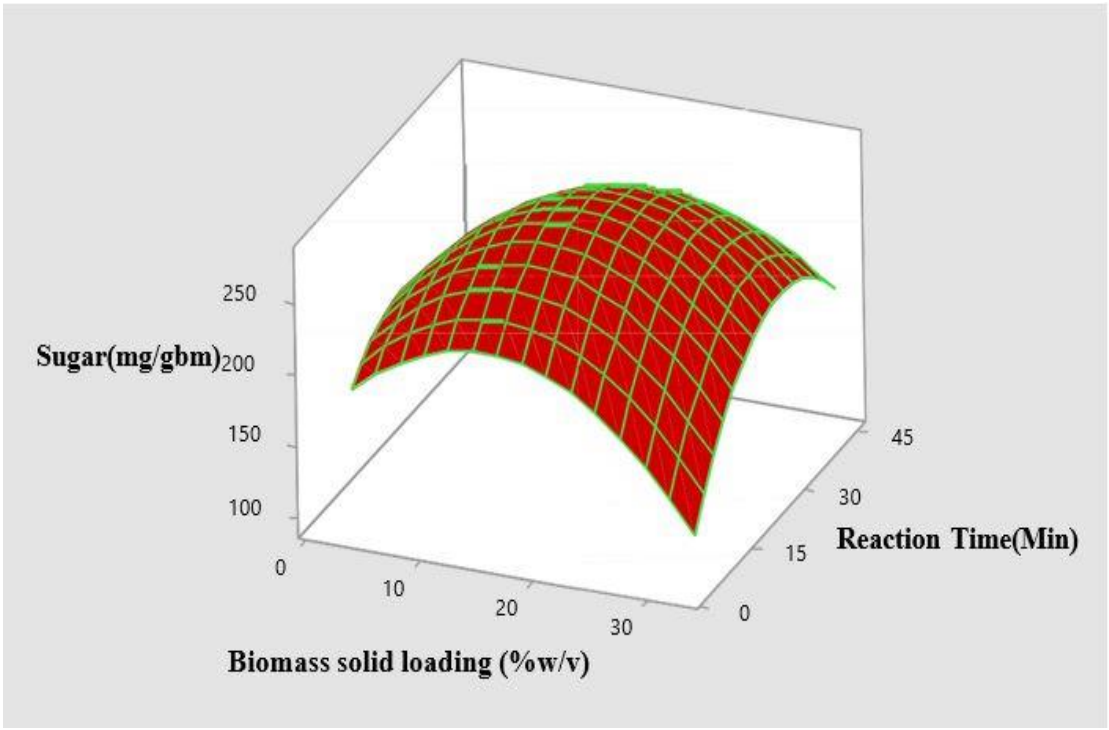

Fig. 2: Surface plot of reducing sugar (mg/gram biomass) yield vs. biomass solid loading (%w/v) vs. reaction time (min.); Sugar (as reducing sugar)

Table 2
Response Surface Regression: Reducing Sugar (mg/gram biomass) versus (a) Biomass solid loading (%w/v), (b) H_2SO_4 (% v/v, (c) Reaction time (Min) and (d) Temp (°C)

Analysis of Variance							
Source	**DF**	**Seq SS**	**Contribution**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	14	110829	97.47%	110829	7916.4	44.09	<0.002
Linear	4	8447	7.43%	26109	6527.4	36.35	0.000
A	1	1897	1.67%	4122	4122.0	22.96	0.000
B	1	4717	4.15%	15179	15179.3	84.53	0.000
C	1	588	0.52%	4521	4520.8	25.18	0.000
D	1	1244	1.09%	6543	6542.7	36.44	0.000
Square	4	51251	45.07%	51251	12812.8	71.35	0.000
A*A	1	5349	4.70%	8836	8836.2	49.21	0.000
B*B	1	31219	27.46%	33367	33366.7	185.81	0.000
C*C	1	6358	5.59%	7796	7795.6	43.41	0.000
D*D	1	8326	7.32%	8326	8326.2	46.37	0.000
2-Way Interaction	6	51132	44.97%	51132	8522.0	47.46	0.000
A*B	1	31471	27.68%	31471	31470.8	175.26	0.000
A*C	1	1329	1.17%	1329	1328.6	7.40	0.015
A*D	1	4402	3.87%	4402	4402.3	24.52	0.000
B*C	1	3648	3.21%	3648	3648.2	20.32	0.000
B*D	1	9506	8.36%	9506	9506.3	52.94	0.000
C*D	1	776	0.68%	776	775.6	4.32	0.054
Error	16	2873	2.53%	2873	179.6		
Lack-of-Fit	10	2020	1.78%	2020	202.0	1.42	0.346
Pure Error	6	853	0.75%	853	142.2		
Total	30	113703	100.00%				
Model Summary							
	S	R-sq	R-sq (adj)	PRESS	R-sq (pred)		
	13.4004	97.47%	95.26%	14102.0	87.60%		

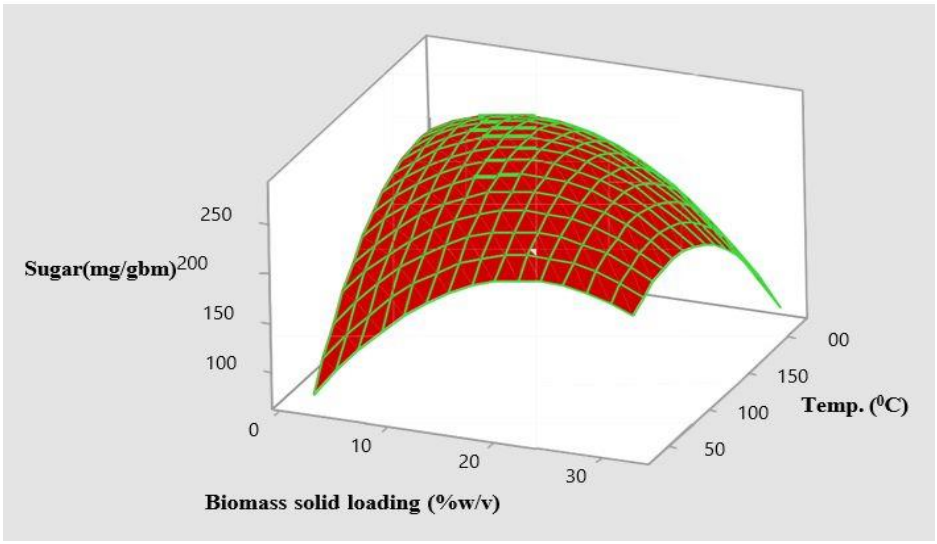

Fig. 3: Surface plot of reducing sugar (mg/gram biomass) yield vs. biomass solid loading (%w/v) vs. temperature (°C); Sugar (as reducing sugar)

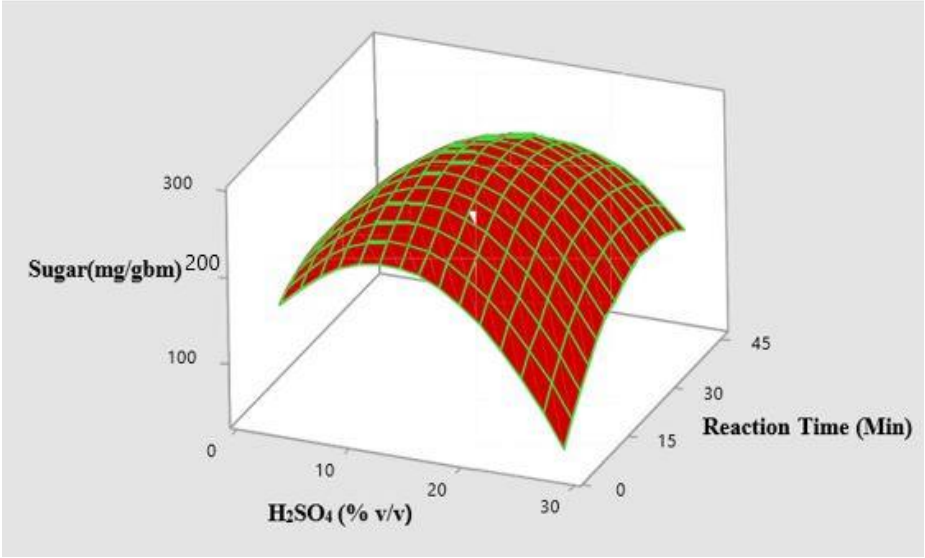

Fig. 4: Surface plot of reducing sugar (mg/gram biomass) yield vs. H_2SO_4 (% v/v) vs. Reaction time (min.); Sugar (as reducing sugar)

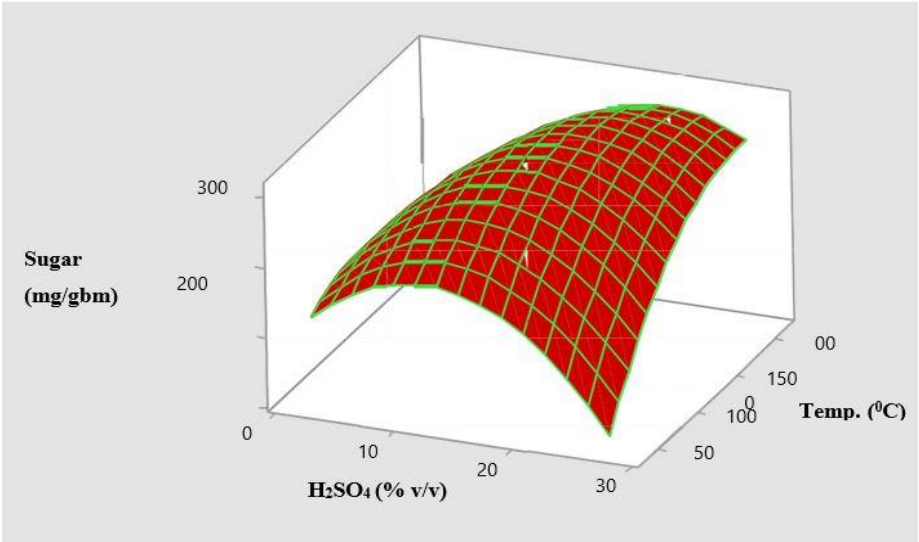

Fig. 5: Surface plot of reducing sugar (mg/gram biomass) yield vs. H_2SO_4 (% v/v) vs. temperature (°C); Sugar (as reducing sugar)

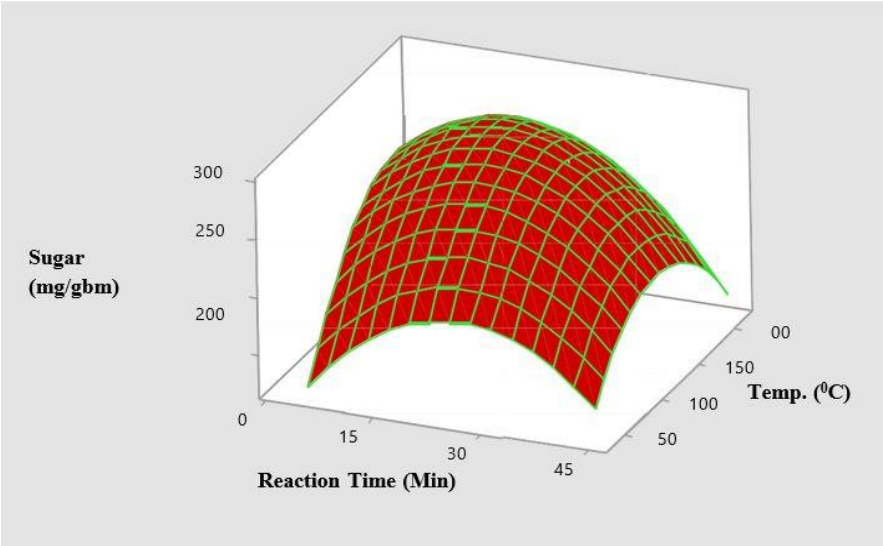

Fig. 6: Surface plot of reducing sugar (mg/gram biomass) yield vs. Reaction time (min.) vs. temperature (°C); Sugar (as reducing sugar)

Conclusion

The present study concluded that there was one and a half-fold increment in fermentable sugar yield from the selected and optimized pretreatment conditions using the response surface contour plot method. This study obtained an increased amount of reducing sugar as fermentable sugar i.e. 348.2 milligrams per gram of pretreated rice straw biomass. The response surface curve and interaction between process variables are shown in figures 1 to 6. Based on the statistical analysis of the response surface contour plot, the optimum level of pretreatment variables for maximized fermentable sugar is released from pretreated rice straw biomass effectively.

The values of each variable were found to be at the optimum operating level as follows: (a) biomass solid loading rate 15% w/v, (b) H_2SO_4 concentration 12 % v/v, (c) reaction time of pretreatment 30 minutes and (d) temperature found to be optimum at 100°C.

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