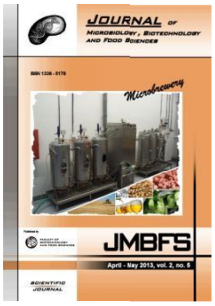

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STATISTICAL OPTIMIZATION OF MEDIUM COMPOSITION AND PROCESS VARIABLES FOR XYLITOL PRODUCTION FROM RICE STRAW HEMICELLULOSE HYDROLYSATE BY *DEBARYOMYCES HANSENII VAR HANSENII*

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ABSTRACT

Optimization of the culture medium and process variables in xylitol production was carried out using *Debaryomyces hansenii var hansenii*. The optimization of xylitol production using rice straw hemicelluloses hydrolysate as substrate was performed with statistical methodology based on experimental designs. The screening of nine nutrients for their influence on xylitol production was achieved using a Plackett-Burman design. $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, peptone and yeast extract were selected based on their positive influence on xylitol production. The selected components were optimized with Box-Behnken design using response surface methodology (RSM). The optimum level (g/L) is: $MgSO_4.7H_2O$ – 1.28, $(NH_4)_2SO_4$ – 4.30, peptone – 4.98 and yeast extract – 4.58. Then the influence of various process variables on the xylitol production was evaluated. The optimal levels of these variables were quantified by the central composite design using RSM, which permitted the establishment of a significant mathematical model with a co-efficient determination of R^2= 0.92. The interactive effects of process variables were determined to be significant. The optimum level of process variables are: temperature (30 ^oC), substrate concentration (3.26 g/L), pH (7.28), agitation speed (170.4 rpm), inoculum size (3.36 ml). These conditions were validated experimentally which revealed an enhanced xylitol yield of 0.72 g/g.

Keywords: Xylitol, Rice straw, *Debaryomyces hansenii var hansenii*, Optimization, RSM

INTRODUCTION

Lignocellulosic biomass is the most abundant and in expensive material for xylitol production. Moreover, it accumulation in the solid form in the nature causes serious problems of environmental contamination **(Molwitz et al., 1996; Pimentel, 2002; Sun et al., 2004)**. The development of fermentation process using carbohydrates from lignocellulosic materials has generated a great deal of interest on a worldwide level for several decades. Rice straw is a renewable, widespread and cheap lignocellulosic material largely available in India, accounting for about 22% of world wide rice production **(FAO, 2008)**. Rice straw contains about 25% of hemicellulose. A heteropolymer compressed mainly of xylose **(El-Marsy, 1983; Kuhad and Singh, 1993)**. The hemicellulose fraction of rice straw can easily be hydrolysed using acids to yield a xylose solution. Many studies have demonstrated that the obtained xylose solution can be used to produce xylitol by fermentation **(Roberto et al., 1995; Roberto et al., 2003; Mussatto and Roberto, 2004; Faveri et al., 2004)**.

Xylitol is a five-carbon sugar alcohol obtained from xylose reduction. The annual xylitol market is estimated to be $340 million, priced at $4-5 kg^{-1} **(Kadam et al., 2008)**. It is a natural functional sweetener, has sweetness equal to that of sucrose and can replace sucrose on a weight to weight basis **(Mäkinen, 1979)**. It promotes oral health and caries prevention **(Emidi, 1978)**. Xylitol has found increasing use in the food industry due to these properties. Xylitol is also a sugar substitute for diabetics **(Pepper and Olinger, 1988)** because insulin is not needed to regulate its metabolism.

On an industrial scale, xylitol is produced by the catalytic hydrogeneation of D-xylose from hemicellulose hydrolysates. A high-cost process that requires extensive xylose purification steps and yields 50-60 % xylitol **(Parajo et al., 1998)**. A good alternates to this process would be a method based on the use of microorganisms **(Converti and Dominguez, 2001; Converti et al., 2002)**, that requires very little xylose purification and is, therefore, more economical. Among microorganisms, yeast have been shown to possess some desirable properties has a potential xylitol producer **(Dominguez et al., 1997; Gırio et al., 1994)**. Therefore in the present study, yeast strain of species *Dabaryomyces hansenii var hansenii* was selected for xylitol production.

Very often hydrolysate containing toxic substances have to be purified before they can be used as fermentation media. To overcome the inhibitory effect of thee toxic compound during fermentation by yeasts, several types of treatment have been employed, including evaporation **(Converti et al., 2000)**, overliming **(Roberto et al., 1991; Martinez et al., 2001)**, and activated charcoal treatment **(Silva et al., 1998; Mussatto et al., 1998)**. Combinations of those treatments are also reported in the literature **(Alves et al., 1998; Converti et al., 1999)**. Response surface methodology (RSM) is a mathematical and statistical analysis, which is useful for the modelling and analysis problems that the response of interest is influenced by several variables **(Montgomery, 2001)**. RSM was utilized extensively for optimizing different biotechnological process **(Li et al., 2007; Naveena et al., 2005)**.

In the present study, the screening and the optimization of medium composition and process variables for xylitol production by *Debaryomyces hansenii var hansenii* using Plackett-Burman and RSM are reported. The Plackett-Burman screening design is applied for knowing the most significant nutrients enhancing xylitol production. Then, Box-Behnken design and central composite design (CCD) was applied to determine the optimum level of each of the significant nutrients and process variables respectively.

MATERIAL AND METHODS

Microorganisms and maintenance

The yeast strain *Debaryomyces hansenii var hansenii* (MTCC 3034) was collected from Microbial Type Culture Collection & Gene bank, Chandigarh. The lyophilized stock cultures were maintained at 4 ^oC on Culture medium supplemented with 20 g agar. The medium composition (g/L) was compressed of the following: Malt extract - 3.0; Yeast extract - 3.0; Peptone - 5.0; Glucose -10.0 and pH - 7. It is sub-cultured every thirty days to maintain viability.

Size reduction

Rice straw was collected from agricultural farms at Cuddalore, Tamilnadu, India. The collected raw material are dried in sunlight for 2 days, crushed and sieved for different mesh size ranging from 0.45 mm to 0.9 mm (20–40 mesh) and used for further studies. The rice straw containing (% w/w): cellulose- 43.5, hemicellulose- 22.0, lignin- 17.2, ash- 11.4 which used for xylitol production.

Acid hydrolysis

The pretreatment were carried out in 500 ml glass flasks. 2 g of rice straw in solid loading of 10% (w/w) mixed with 1% dilute sulfuric acid (w/w) and pretreated in an autoclave at 120 °C with residence time of 1 hour. The liquid fraction was separated by filtration and unhydrolysed solid residue was washed with warm water at 60 °C. The filtrate and wash liquid were pooled together.

Detoxification

Hemicellulose acid hydrolysate was heated at100 °C, and maintained for 15 min to reduce the volatile components. The hydrolysate were overlimed with solid $Ca(OH)_2$ up to pH 10, in combination with 0.1% sodium sulfite and filtered to remove the insoluble materials. The filtrate was adjusted to pH 7 with H_2SO_4. The water phase was treated with activated charcoal.

Activated charcoal treatment

Activated charcoal treatment is an efficient and economic method of reduction in the amount of phenolic compounds, acetic acid, aromatic compounds, furfural and hydroxymethylfurfural normally found in hemicellulosic hydrolysates. After centrifugation, the solutions were mixed with powdered charcoal at 5% (w/v) for 30 min and stirred (100 rpm) at 30 °C. The liquor was recovered by filtration, chemically characterized and used for culture media.

Fermentation Conditions

Fermentation was carried out in 250 ml Erlenmeyer flasks with 100 ml of pretreated rice straw hemicelluloses hydrolysate at pH 7. This is supplemented with different nutrients concentration for tests according to the selected factorial design and sterilized at 120 °C for 20 min. After cooling the flasks to room temperature, the flasks were inoculated with 1 ml of grown culture broth. The flasks were maintained at 30 °C under agitated at 200 rpm for 48 hours.
After the optimization of medium composition, the fermentation was carried out with different parameter levels (Table 5) with the optimized media for tests according to the selected factorial design. During the preliminary screening process, the experiments are carried out for 5 days and it was found that the maximum production was obtained in 48 hours. Hence experiments were carried out for 48 hours.

Analytical Methods

Sugar and sugar alcohols in the culture broth were measured by high performance liquid chromatography (HPLC), model LC-10-AD (Shimadzu, Tokyo, Japan) equipped with a refractive index (RI) detector. The chromatography column used a Aminex HPX-87H (300 x 7.8 mm) column at 80 °C with 5 mM H_2SO_4 as mobile phase at a flow rate of 0.4 ml/min, and the injected sample volume is 20 µL.

Optimization of Xylitol production

Design of Experiment (DOE)

The RSM has several classes of designs, with its own properties and characteristics. Central composite design (CCD), Box–Behnken design and three-level factorial design are the most popular designs applied by the researchers. A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach.

Plackett–Burman experimental design

It assumes that there are no interactions between the different variables in the range under consideration. A linear approach is considered to be sufficient for screening. Plackett–Burman experimental design is a fractional factorial design and the main effects of such a design may be simply calculated as the difference between the average of measurements made at the high level (+1) of the factor and the average of measurements at the low level (–1).
To determine which variables significantly affect xylitol production, Plackett–Burman design is used. Nine variables are screened in 12 experimental runs (Table 1) and insignificant ones are eliminated in order to obtain a smaller, manageable set of factors. The low level (-1) and high level (+1) of each factor (-1, +1) were listed as follows (g/L): K_2HPO_4 (6.6, 7), yeast extract (1.5, 5), peptone (2, 5), KH_2PO_4 (1.2, 3.6), xylose (9.8, 10.2), $(NH_4)_2SO_4$ (1, 4), $MgSO_4.7H_2O$ (0.7, 1.3), malt (2.8, 3.2) and glucose (9.8, 10.2) and they were coded with A, B, C, D, E, F, G, H, I respectively. The statistical software package 'Minitab 16', is used for analyzing the experimental data. Once the critical factors are identified through the screening, the Box–Behnken design is used to obtain a quadratic model. After the central composite design (CCD) was used to optimize the process variables and obtain a quadratic model.

Table 1 Plackett – Burman Experimental Design for nine variables

Run Order	A	B	C	D	E	F	G	H	I	Xylitol yield (g/g)
1	-1	1	-1	-1	-1	1	1	1	-1	0.17
2	1	-1	-1	-1	1	1	1	-1	1	0.33
3	1	1	-1	1	1	-1	1	-1	-1	0.35
4	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.41
5	-1	-1	-1	1	1	1	-1	1	1	0.17
6	-1	-1	1	1	1	-1	1	1	-1	0.46
7	1	1	1	-1	1	1	-1	1	-1	0.51
8	-1	1	1	-1	1	-1	-1	-1	1	0.49
9	1	-1	1	-1	-1	-1	1	1	1	0.39
10	1	1	-1	1	-1	-1	-1	1	1	0.59
11	-1	1	1	1	-1	1	1	-1	1	0.37
12	1	-1	1	1	-1	1	-1	-1	-1	0.56

The Box-Behnken design and CCD is used to study the effects of the variables towards their responses and subsequently in the optimization studies. This method are suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. In order to determine the existence of a relationship between the factors and response variables, the collected data were analyzed in a statistical manner, using regression. A regression design is normally employed to model a response as a mathematical function (either known or empirical) of a few continuous factors and good model parameter estimates are desired **(Montgomery, 2001)**.

The coded values of the process parameters are determined by the following equation:

$$x_i = \frac{X_i - X_o}{\Delta x} \quad \text{.... (1)}$$

Where x_i – coded value of the i^{th} variable, X_i – uncoded value of the i^{th} test variable and X_0 – uncoded value of the i^{th} test variable at center point. The regression analysis is performed to estimate the response function as a second order polynomial

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1,i<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad \text{.... (2)}$$

Where Y is the predicted response, β_0 constant, β_i, β_j, β_{ij} are coefficients estimated from regression. They represent the linear, quadratic and cross products of X_i and X_j on response.

Table 2 Ranges of variables used in Box-Behnken design

S.No	Variables	Code	Levels (g/L)		
			-1	0	1
1	$MgSO_4.7H_2O$	A	0.6	1.2	1.8
2	$(NH_4)_2SO_4$	B	2	4	6
3	peptone	C	3	5	7
4	Yeast Extract	D	2	4	6

Model Fitting and Statistical Analysis

The regression and graphical analysis with statistical significance are carried out using Design-Expert software (version 7.1.5, Stat-Ease, Inc., Minneapolis, USA). The optimum values of the process variables are obtained from the regression equation. The adequacy of the models is further justified through analysis of variance (ANOVA). Lack-of-fit is a special diagnostic test for adequacy of a model and compares the pure error, based on the replicate measurements to the other lack of fit, based on the model performance **(Noordin et al., 2004)**. *F*-value, calculated as the ratio between the lack-of-fit mean square and the pure error mean square, this statistic parameters are used to determine whether the lack-of-fit is significant or not, at a significance level.

RESULTS AND DISCUSSION

Plackett-Burman experiments (Table 1) showed a wide variation in xylitol production. This variation reflected the importance of optimization to attain higher productivity. From the pareto chart shown in Figure 1 the variables, viz., $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, peptone and yeast extract were selected for further optimization to attain a maximum response.

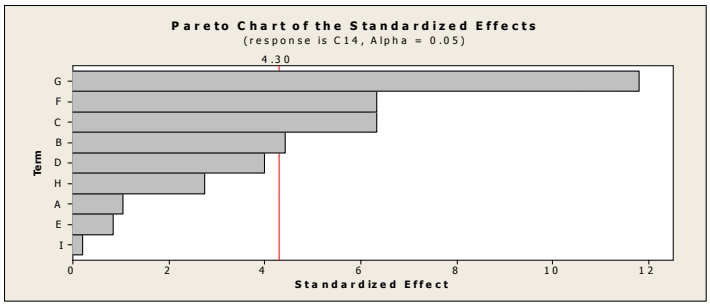

Figure 1 Pareto chart showing the effect of media components on xylitol production

Table 3 Box-Behnken design in coded levels with xylitol yield as response

Runs	A	B	C	D	Xylitol Yield (g/g)	
					Experimental	predicted
1	0	-1	1	0	0.43	0.44
2	0	1	0	-1	0.29	0.30
3	0	0	1	1	0.55	0.51
4	1	0	1	0	0.37	0.43
5	0	0	0	0	0.69	0.69
6	0	1	0	1	0.56	0.57
7	-1	0	0	1	0.50	0.53
8	-1	1	0	0	0.32	0.30
9	0	0	1	-1	0.50	0.46
10	0	-1	-1	0	0.37	0.38
11	-1	0	0	-1	0.33	0.37
12	0	0	0	0	0.69	0.69
13	0	0	-1	-1	0.40	0.38
14	-1	-1	0	0	0.50	0.50
15	-1	0	1	0	0.55	0.53
16	-1	0	-1	0	0.40	0.36
17	0	0	-1	1	0.54	0.53
18	1	0	0	1	0.53	0.52
19	1	0	0	-1	0.48	0.48
20	1	1	0	0	0.61	0.56
21	0	0	0	0	0.69	0.69
22	1	-1	0	0	0.36	0.33
23	0	1	1	0	0.40	0.42
24	1	0	-1	0	0.51	0.55
25	0	-1	0	1	0.38	0.39
26	0	1	-1	0	0.41	0.43
27	0	-1	0	-1	0.45	0.45
28	0	0	0	0	0.69	0.69
29	0	0	0	0	0.66	0.69

The levels of factors and the effect of their interactions on xylitol production were determined by Box-Behnken design of RSM. The design matrix of experimental results by tests planned according to the 29 full factorial designs. Twenty nine experiments were preferred at different combinations of the factors shown in Table 2 and the central point was repeated five times. The predicted and observed responses along with design matrix are presented in Table 3 and the results were analyzed by ANOVA. The second order regression equation provided the levels of xylitol production as a function of $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, peptone and yeast extract, which can be presented in terms of coded factors as in the following equation:

$$Y = 0.68 + 0.022A + (8.333E-003)B + 0.014C + 0.051D + 0.11AB - 0.073AC - 0.030AD - 0.017BC + 0.085BD - 0.022CD - 0.11A^2 - 0.15B^2 - 0.11C^2 - 0.10D^2 \quad \ldots..(3)$$

Where Y is the Xylitol yield (g/g) and A, B, C and D are $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, peptone and yeast extract respectively. ANOVA for the response surface is shown in Table 4. The model *F*-value of 19.77 implies the model is significant. There is only a 0.01% chance that a "Model *F*-value" this large could occur due to noise. Values of "prob > *F*" less than 0.05 indicate model terms are significant. Values greater than 0.1 indicates model terms are not significant. In the present work, linear terms of D and all the square effects of A, B, C, D and the combination of A*B, A*C and B*D were significant for xylitol production. The co-efficient of determination (R^2) for xylitol production was calculated as 0.9519, which is very close to 1 and can explain up to 95.00% variability of the response. The predicted R^2 value of 0.731 was in reasonable agreement with the adjusted R^2 value of 0.9037. An adequate precision value greater than 4 is desirable. The adequate precision value of 14.201 indicates an adequate signal and suggests that the model can be to navigate the design space.

Table 4 Analyses of variance (ANOVA) for response surface quadratic model for the production of xylitol using Box-Behnker design

Source	Sum of square	Df	Mean square value	F-value	P-value
Model	0.39	14	0.028	19.77	<0.0001
A- $MgSO_4.7H_2O$	5.633E-003	1	5.633E-003	4.01	0.0650
B- $(NH_4)_2SO_4$	8.333E-004	1	8.333E-004	0.59	0.4539
C- peptone	2.408E-003	1	2.408E-003	1.71	0.2114
D- Yeast Extract	0.031	1	0.031	22.08	0.0003
AB	0.046	1	0.046	32.91	<0.0001
AC	0.021	1	0.021	14.97	0.0017
AD	3.600E-003	1	3.600E-003	2.56	0.1317
BC	1.225E-003	1	1.225E-003	0.87	0.3662
BD	0.029	1	0.029	20.58	0.0005
CD	2.025E-003	1	2.025E-003	1.44	0.2498
A2	0.074	1	0.074	52.88	<0.0001
B2	0.15	1	0.15	110.25	<0.0001
C2	0.080	1	0.080	56.65	<0.0001
D2	0.066	1	0.066	46.88	<0.0001
Residual	0.019	14	1.404E-003		
Lack of fit	0.014	10	1.894E-003	10.52	0.0182
Pure Error	7.200E-004	4	7.200E-004		
Cor Total	0.41	28			

The above model can be used to predict the xylitol production within the limits of the experimental factors that the actual response values agree well with the predicted response values.

Table 5 Ranges of variables used in Central Composite design

S.No	Variables	Code	Levels				
			-2.37	-1	0	1	2.37
1	Temperature (°C)	A	20	25	30	35	40
2	Substrate Concentration (g/L)	B	1	2	3	4	5
3	pH	C	6	6.5	7	7.5	8
4	Agitation speed (rpm)	D	50	100	150	200	250
5	Inoculum size (ml)	E	1	2	3	4	5

Experimental conditions for optimization of the process variables for xylitol yield were determined by Central composite design. Five process variables and assessed at 5 coded levels as shown in Table 5. The design matrix of experimental results by tests planned according to the 50 full factorial designs and the central point was repeated eight times. The predicted and observed responses along with design matrix are presented in Table 6 and the results were analyzed by ANOVA.

Table 6 Central Composite design (CCD) in coded levels with Xylitol yield as response

Runs	A	B	C	D	E	Xylitol yield(g/g)	
						Experiment	Predicted
1	-2.37	0	0	0	0	0.53	0.51
2	-1	1	1	1	1	0.66	0.63
3	-1	-1	1	1	-1	0.33	0.38
4	0	0	0	0	0	0.69	0.71
5	1	1	1	1	-1	0.67	0.65
6	-1	1	1	-1	-1	0.50	0.53
7	0	0	0	0	0	0.71	0.71
8	1	1	-1	-1	-1	0.30	0.22
9	0	0	0	-2.37	0	0.43	0.36
10	-1	1	-1	1	-1	0.49	0.41
11	1	-1	1	1	1	0.62	0.61
12	1	1	1	1	1	0.58	0.62
13	0	0	-2.37	0	0	0.25	0.27
14	0	-2.37	0	0	0	0.44	0.42
15	-1	-1	-1	-1	1	0.51	0.55
16	0	0	0	0	-2.37	0.20	0.25
17	-1	1	-1	1	1	0.56	0.61
18	2.37	0	0	0	0	0.44	0.46
19	0	0	0	0	0	0.71	0.71
20	-1	-1	1	1	1	0.53	0.54
21	1	1	-1	1	1	0.57	0.51
22	-1	1	1	-1	1	0.56	0.62
23	-1	-1	-1	1	1	0.57	0.57
24	-1	1	-1	-1	-1	0.27	0.33
25	0	0	0	0	0	0.71	0.71
26	0	2.37	0	0	0	0.49	0.51
27	0	0	2.37	0	0	0.60	0.59
28	0	0	0	0	0	0.71	0.71
29	1	-1	1	-1	-1	0.47	0.48
30	-1	1	-1	-1	1	0.58	0.54
31	-1	-1	-1	1	-1	0.32	0.31
32	0	0	0	0	0	0.71	0.71
33	1	1	1	-1	1	0.54	0.49
34	0	0	0	0	0	0.71	0.71
35	0	0	0	0	2.37	0.58	0.54
36	-1	1	1	1	-1	0.60	0.54
37	0	0	0	2.37	0	0.49	0.56
38	-1	-1	1	-1	-1	0.43	0.42
39	1	-1	-1	1	-1	0.41	0.39
40	-1	-1	-1	-1	-1	0.30	0.27
41	1	-1	1	1	-1	0.64	0.58
42	1	1	-1	1	-1	0.38	0.42
43	1	1	1	-1	-1	0.50	0.51
44	1	-1	-1	1	1	0.58	0.54
45	1	-1	-1	-1	1	0.37	0.39
46	1	-1	1	-1	1	0.47	0.53
47	1	1	-1	-1	1	0.25	0.31
48	-1	-1	1	-1	1	0.60	0.58
49	0	0	0	0	0	0.71	0.71
50	1	-1	-1	-1	-1	0.21	0.23

Table 7 Analyses of variance (ANOVA) for response surface quadratic model for the production of xylitol using CCD

Source	Sum of square	Df	Mean square value	F-value	P-value
Model	0.98	20	0.049	18.56	<0.0001
A-Temperature (^{o}C)	4.965E-003	1	4.965E-003	1.88	0.1808
B-Substrate Concentration(g/L)	0.014	1	0.014	5.17	0.0305
C-pH	0.19	1	0.19	71.62	<0.0001
D-Agitation speed (rpm)	0.074	1	0.074	28.13	<0.0001
E-Inoculum size (ml)	0.16	1	0.16	60.61	<0.0001
AB	0.012	1	0.012	4.40	0.0447
AC	0.021	1	0.021	7.76	0.0093
AD	0.033	1	0.033	12.55	0.0014
AE	0.027	1	0.027	10.23	0.0033
BC	4.753E-003	1	4.753E-003	1.80	0.1901
BD	4.278E-003	1	4.278E-003	1.62	0.2132
BE	9.453E-003	1	9.453E-003	3.58	0.0685
CD	8.778E-003	1	8.778E-003	3.32	0.0786
CE	0.025	1	0.025	9.37	0.0047
DE	1.531E-004	1	1.531E-004	0.058	0.8114
A^2	0.083	1	0.083	31.37	<0.0001
B^2	0.099	1	0.099	37.39	<0.0001
C^2	0.13	1	0.13	51.00	<0.0001
D^2	0.10	1	0.10	38.97	<0.0001
E^2	0.17	1	0.17	64.65	<0.0001
Residual	0.077	29	2.641E-003		
Lack of fit	0.076	22	3.465E-003	69.30	<0.0001
Pure Error	3.500E-004	7	5.000E-005		
Cor Total	1.06	49			

The second order regression equation provided the levels of xylitol production as a function of temperature, substrate concentration, pH, agitation speed and inoculums size, which can be presented in terms of coded factors as in the following equation:

$$Y = 0.71 - 0.011A + 0.018B + 0.066C + 0.041D + 0.061E - 0.019AB + 0.025AC + 0.032AD - 0.029AE + 0.012BC + 0.012BD - 0.017BE - 0.017CD - 0.028CE - (2.188E-003)DE - 0.039A^2 - 0.042B^2 - 0.050C^2 - 0.043D^2 - 0.056E^2 \quad \ldots\ldots(4)$$

Where Y is the Xylitol yield (g/g), A, B, C, D and E are temperature, substrate concentration, pH, agitation speed and inoculums size respectively. ANOVA for the response surface is shown in Table 7. The model *F*-value of 18.56 implies the model is significant. There is only a 0.01% chance that a "Model *F*-value" this large could occur due to noise. Values of "prob > *F*" less than 0.05 indicate model terms are significant. Values greater than 0.1 indicates model terms are not significant. In the present work, linear terms of B, C, D, E and all the squares effects of A, B, C, D, E and the combination of A*B, A*C, A*D, A*E and C*E were significant for xylitol production. The co-efficient of determination (R^2) for xylitol production was calculated as 0.9275, which is very close to 1 and can explain up to 92.00% variability of the response. The predicted R^2 value of 0.7331 was in reasonable agreement with the adjusted R^2 value of 0.8775. An adequate precision value greater than 4 is desirable. The adequate precision value of 14.767 indicates an adequate signal and suggests that the model can be to navigate the design space.

Both design the interaction effects of variables on xylitol production were studied by plotting 3D surface curves against any two independent variables, while keeping another variable at its central (0) level. The 3D curves of the calculated response (xylitol yield) and contour plots from the interactions between the variables are shown in Figures 2-17. Figure 2 shows the dependency of xylitol on $MgSO_4.7H_2O$ and yeast extract. The xylitol production increased with increase in $MgSO_4.7H_2O$ to about 1.2 g/L and thereafter xylitol production decreased with further increase in $MgSO_4.7H_2O$. The same trend was observed in Figures 3-7. This is evident from above Figures shows the dependency of

$(NH_4)_2SO_4$, peptone and yeast extract on xylitol production. The optimal operation conditions of $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, peptone and yeast extract for maximum xylitol production were determined by response surface analysis and also estimated by regression equation. The predicted results are shown in Table 4. The predicted values from the regression equation closely agreed with that obtained from experimental values.

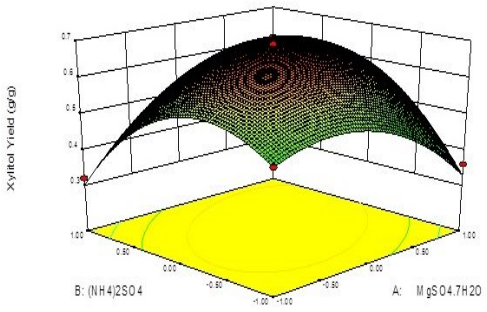

Figure 2 3D Plot showing the effect of $MgSO_4.7H_2O$ and $(NH_4)_2SO_4$ on xylitol yield

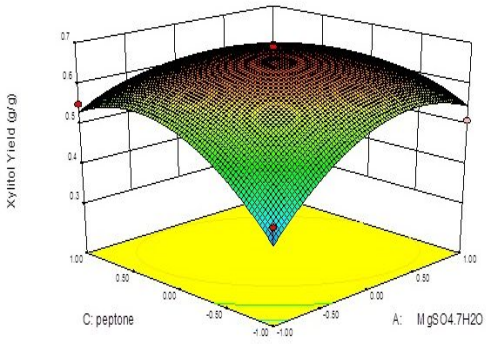

Figure 3 3D Plot showing the effect of $MgSO_4.7H_2O$ and peptone on xylitol yield

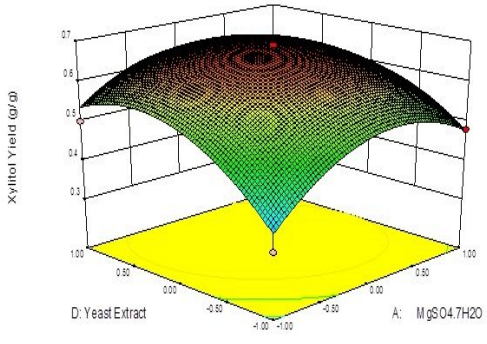

Figure 4 3D Plot showing the effect of $MgSO_4.7H_2O$ and yeast extract on xylitol yield

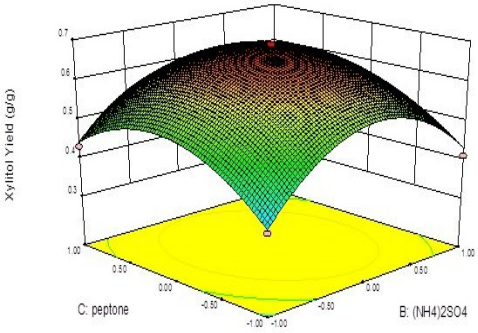

Figure 5 3D Plot showing the effect of $(NH_4)_2SO_4$ and peptone on xylitol yield

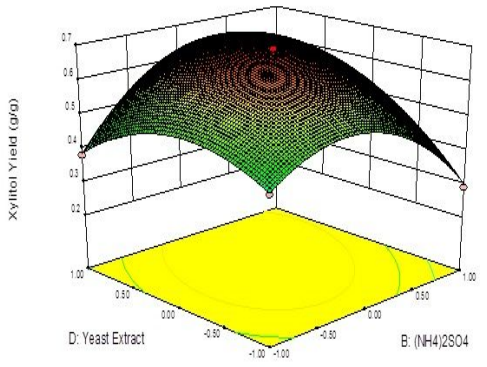

Figure 6 3D Plot showing the effect of $(NH_4)_2SO_4$ and yeast extract on xylitol yield

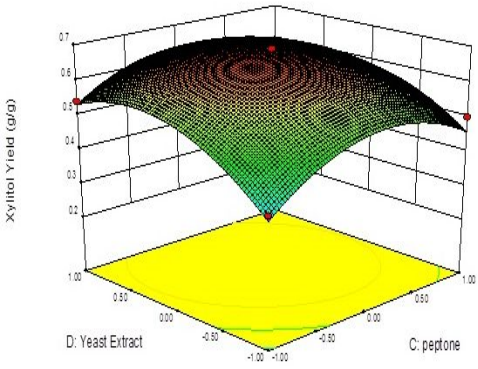

Figure 7 3D Plot showing the effect of peptone and yeast extract xylitol yield

In CCD the Figure 8 shows the dependency of xylitol on temperature and substrate concentration. The xylitol production increased with increase in temperature to about 30°C and thereafter xylitol production decreased with further increase in temperature. The same trend was observed in Figures 9-17. This is evident from below Figures shows the dependency of pH, substrate concentration, agitation speed, inoculum size on xylitol production. The optimal operation conditions of temperature, substrate concentration, pH, agitation speed, inoculum size for maximum xylitol production were determined by response surface analysis and also estimated by regression equation. The predicted results are shown in Table 7. The predicted values from the regression equation closely agreed with that obtained from experimental values.

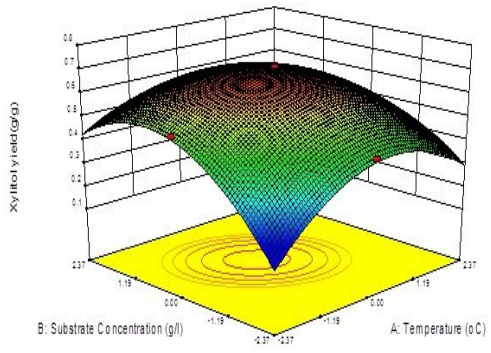

Figure 8 3D Plot showing the effect of temperature and substrate concentration on xylitol yield

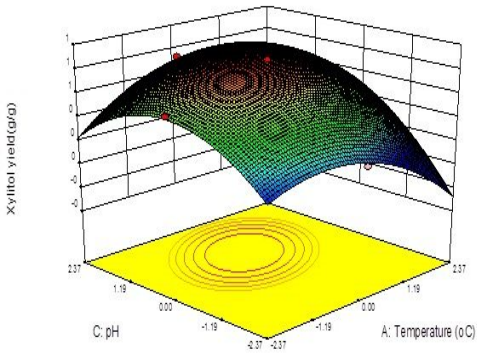

Figure 9 3D Plot showing the effect of temperature and pH on xylitol yield

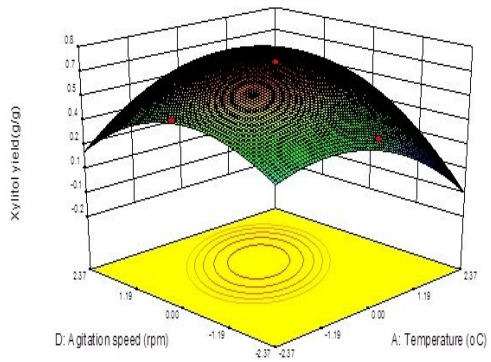

Figure 10 3D Plot showing the effect of temperature and agitation speed on xylitol yield

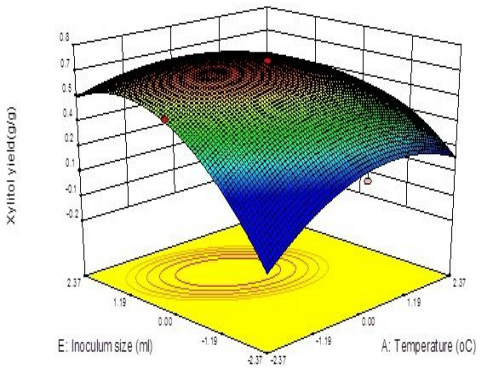

Figure 11 3D Plot showing the effect of temperature and inoculum size on xylitol yield

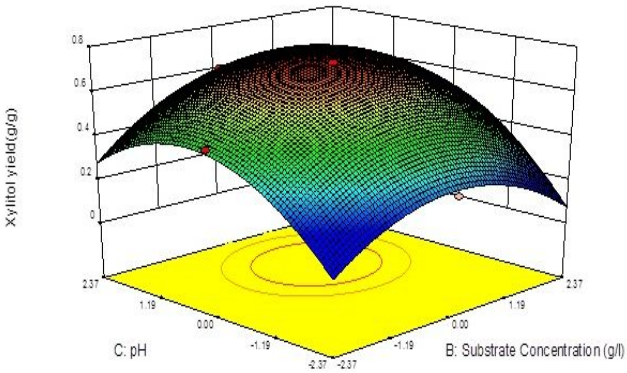

Figure 12 3D Plot showing the effect of substrate concentration and pH on xylitol yield

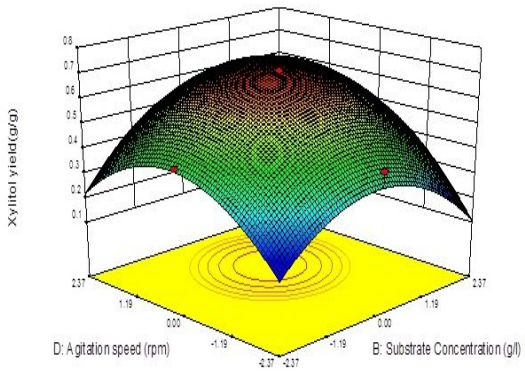

Figure 13 3D Plot showing the effect of substrate concentration and agitation speed on xylitol yield

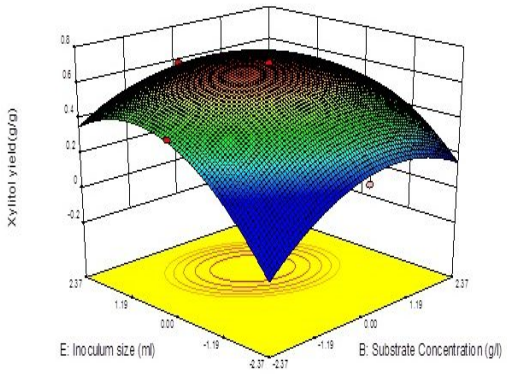

Figure 14 3D Plot showing the effect of substrate concentration and inoculum size on xylitol yield

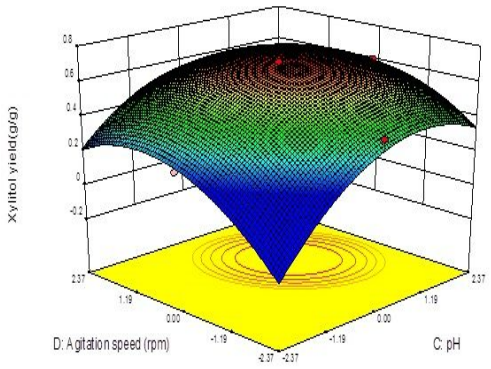

Figure 15 3D Plot showing the effect of pH and agitation speed on xylitol yield

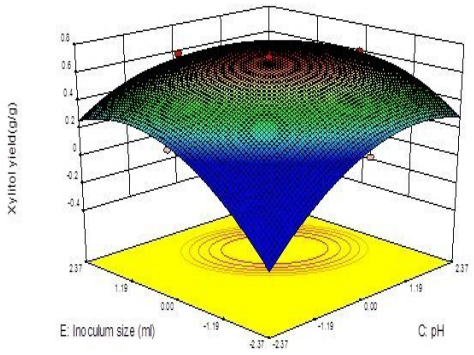

Figure 16 3D Plot showing the effect of pH and inoculum size on xylitol yield

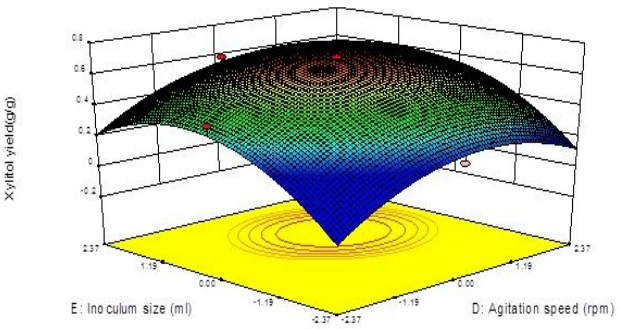

Figure 17 3D Plot showing the effect of agitation speed and inoculum size on xylitol yield

Validation of the experimental model

Validation of the experimental model was tested by carrying out the batch experiment under optimal operation conditions (g/l): $MgSO_4.7H_2O$- 1.28, $(NH_4)_2SO_4$- 4.30, peptone – 4.98 and yeast extract - 4.58 established by the regression model. Under optimal process variables levels are temperature (30 ^oC), substrate concentration (3.26 g/L), pH (7.28), agitation speed (170.4 rpm) and inoculum size (3.36 ml). Four repeated experiments were performed and the results are compared. The xylitol production (0.72 g/g) obtained from experiments as very close to the actual response (0.71 g/g) predicted by the regression model, which proved the validity of the model.

CONCLUSION

In this work, Plackett -Burman design were used to determine the relative importance of medium components on xylitol production. Among the variables, $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, peptone and yeast extract were found to be the most significant variables. From further optimization studies the optimized values of the nutrients for xylitol production were as follows (g/l): $MgSO_4.7H_2O$ - 1.28, $(NH_4)_2SO_4$ - 4.30, peptone - 4.98 and yeast extract - 4.58. Then the influence of various process variables namely temperature, pH, substrate concentration,

agitation speed and inoculum size on the xylitol production was evaluated by CCD, which permitted the establishment of a significant mathematical model with a co-efficient determination of R^2= 0.92. The interactive effects of temperature and all other variables, substrate concentration and inoculums size, pH and inoculums size were determined to be significant. The optimum levels of process variables are: temperature (30 ^{o}C), substrate concentration (3.26 g/L), pH (7.28), agitation speed (170.4 rpm), inoculum size (3.36 ml). This study showed that the rice straw is a good source for the production of xylitol. Using the optimized conditions, the xylitol yield reaches 0.72 g/g. The results show a close concordance between the expected and obtained production level.

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