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Response Surface Methodology Optimization Of Process Parameters for Xylanase Production by *Aspergillus fumigatus* in SSF using Central Composite Design

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ABSTRACT

In this work, the production of xylanase enzyme using inexpensive substrate wheat bran by *Aspergillus fumigatus* under SSF was investigated by optimizing various process parameters such as substrate concentration, temperature, incubation time, initial moisture content and initial pH of the medium. These parameters were optimized using response surface methodology (RSM). The central composite design and RSM have been applied for designing of experiments to evaluate the interactive effects through a full 50 factorial design. The optimum conditions were substrate concentration – 10.7g, temperature – 32.7^{0}C, incubation Time – 133 hrs, initial moisture content – 83.2%, initial pH – 5.3. Higher value of the regression coefficient R^2= 0.9750 indicates excellent evaluation of experimental data by second order polynomial regression model. The Response surface methodology revealed that the maximum xylanase production of 553.17 IU/gds was obtained at the above optimum conditions. Along with xylanase, a concurrent production of a low amount of cellulase activity has also been found.

Keywords: Xylanase, central composite design, response surface methodology, regression coefficient, operating parameters

1. INTRODUCTION

Xylanases (1,4-β -D-xylan xylanohydrolase E.C. 3.2.1.8) are a family of hydrolytic enzymes that cleave internal linkages on the β -1,4-xylopyranose backbone [1]. Xylan represents the major component of hemicellulose, the second most abundant plant material in nature. Due to its complex structure several enzymes are required for its complete hydrolysis, but endo β-1,4-xylanase (β-1, 4-D-xylanohydrolase, E.C.3.2.1.8) is most crucial for depolymerization of the main backbone of xylan [2]. Xylanases have great potential in various industrial processes, including the manufacture of bread, food and drinks, improvement of nutritional properties of agricultural silage and grain feed, for processing plant fibers in the textile industry, in pharmaceutical and chemical applications, and in the cellulose pulp and paper manufacturing processes [3]. Xylanases also been studied for the production of xyloologiosaccahrides, which are used as moisturizing agents for food, sweeteners [4], in the production of several valuable products like xylitol and ethanol [5]. Other important applications are to make the bread fine and soft and extend the storage time, to purify fruit juice, wine and beer, to form xylitol glucose used in confectionery industry [6].

Enzyme productivity in solid state fermentation (SSF) is usually much higher than that of submerged fermentation [7]. Solid state fermentation has gained interest for researchers in recent years because of economic and engineering advantages [8], since this method employs agricultural residues in their natural form, thus helping to prevent the environmental impact caused by the accumulation of these residues. Agricultural residues contain 20–30% hemicellulosic materials, which can be utilized for production of xylanase by microorganisms [9]. India, being an agriculture-based economy, generates huge quantum of agro-residues which are difficult to dispose off, and their use as substrates for xylanase production will not only combat environmental pollution but will also reduce the cost of enzyme production [10].

The choice of an appropriate substrate is of great importance for the successful production of xylanases. The substrate not only serves as carbon and energy source, but also provides the necessary inducing compounds for the organism, preferentially for an extended period of time, for an increased overall productivity of the fermentation process [11]. The use of purified xylan as an inducer increases the cost of enzyme production. For this reason, different lignocellulosic residues, including wheat bran, wheat straw, corn cob and sugar cane bagasse have been used as growth substrate in cultures to produce xylanases [8]. Wheat bran is rightly known to be the gold product and finds its applications not only in fermentation industry but also in pharmaceutics and biomedical research [12]. Wheat bran remains loose even under moist conditions during the solid state fermentation (SSF) mode of culturing thereby providing a large surface area and efficient aeration [13], used as substrate for xylanase production from various organisms

including A. niveus RS2 [14], A. sydowii SBS 45 [15], A. niger DFR-5 [16]. Xylanase production on commercial scale can also be achieved by using WB as a substrate as it is an agro-economical inducer due to its high xylan content (12.65% of dry material) [17].

Most of the literature concerning xylanases dealt with their purification and characterization, and relatively fewer studies have been done regarding production, optimization of xylanases [18],[19]. Statistical optimization, allows rapid screening of a number of factors and factor interactions, and reflects the role of each component. Response surface methodology (RSM) is gaining recognition as a powerful approach for optimizing conditions for the production of industrially important products such as chemicals and enzymes [20]. In the last few years, RSM has been applied to optimize and evaluate interactive effects of independent factors in numerous chemical and biochemical processes [21], [22], [23]. The objective of the present study is to statistically optimize the process parameters such as substrate concentration, temperature, incubation time, initial moisture content & pH for production of xylanase from *Aspergillus fumigatus* under SSF using inexpensive substrate wheat bran by central composite design (CCD) in Response surface methodology.

2. MATERIALS & METHODS

2.1. Microorganism and culture media

Aspergillus fumigatus (MTCC No - 343) used in this study was purchased from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The stock culture was maintained on agar slants at 5^0C. The medium composition comprises of : *Czapek Concentrate - 10.0 ml; K_2HPO_4 - 1.0g, Yeast extract -, 5.0 g; Sucrose - 30.0 g; Agar -15.0 g; Distilled water -1.0 L. (*Czapek concentrate: $NaNO_3$ - 30.0g; Kcl - 5.0g; $MgSO_4.7H_2O$ - 5.0g; $FeSO_4.7H_2O$ - 0.1g; and Distilled water - 100.0 ml).

2.2. Solid state fermentation (SSF)

Wheat bran was used as substrate for xylanase production. Wheat bran was oven dried at 70^0C for 48 hrs, ground to 40 mesh particle size and used as a substrate. Fermentation was carried out in Erlenmeyer flasks (250 ml) with, 0.1% (v/v) of Tween-80, 0.1% (w/v) of oat spelt xylan, supplemented with nutrient concentration (% w/w) : $NaNO_3$ - 0.29, $(NH_4)_2SO_4$ - 0.14 and KH_2PO_4 - 0.24, adjusted with process parameters such as substrate (wheat bran) concentration, temperature, incubation time, % initial moisture content [24] and pH as defined by experimental design. 0.1 % of oat spelt xylan serves as an inducer for xylanase production. Each flask was covered with hydrophobic cotton and autoclaved at 121^0C for 20 min. After cooling, each flask was inoculated with 2 ml of the spore suspension containing $1x10^6$ spores/ml prepared from 6 day old slants of the culture grown at 30^0C and the inoculated flasks were incubated at 30^0C in an incubator. After fermentation 50 ml of 0.05M citrate buffer (pH – 5.3) was added to the fermented flask and the contents were agitated for 30 minutes at 200 rpm in an orbital shaker at 30^0C and filtered through a wet muslin cloth by squeezing. The extract was centrifuged at 15,000 rpm for 20 minutes and the supernatant was used for determination of enzyme activity.

2.3. Enzyme Assay

Endoxylanase activity was measured by incubating 0.5ml of 1% (w/v) oat spelt xylan in 0.05 M Na-citrate buffer (pH 5.3). And 0.5 ml of suitably diluted enzyme extract at 50°C for 30 min. The release of reducing sugar was measured by dinitro-salicylic acid (DNS) method [25] and xylose was used as the standard. One International unit (IU) of xylanase activity is defined as the amount of enzyme releasing 1 µmol of xylose per minute under the assay conditions. Xylanase production in SSF was expressed as IU/g dry substrate (IU/gds).

Cellulase activity was assayed by adding 0.5 ml of appropriately diluted enzyme to 0.5 ml of 1 % (w/v) of carboxymethyl cellulose (CMC) in 50 mM Na-citrate buffer, pH 5.3 and incubating at 50^0C for 30 min. The amount of reducing sugars released during the reaction was measured using the DNS method [25] and D-glucose was used as the standard. One International unit of cellulase activity was defined as the amount of enzyme that liberated 1 µmol of glucose equivalent under the assay conditions.

2.4. Optimization of Process Parameters

A full factorial design, is a powerful tool for understanding complex processes for relating factor interactions in multifactor systems because it includes all possible factor combinations in each of the factors. Response surface methodology (RSM) is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from designed experiments to solve multivariate equations simultaneously. A central composite experimental design with 10 star points , (2^5 = 32) axial points and eight replicates at the center point (n_0 = 8), resulting in a total of 50 experiments covers the entire range of spectrum of combinations of variables, was used for fitting a second order response surface. The experiments with substrate concentration, temperature, incubation time, initial moisture content and initial pH were employed, simultaneously covering the spectrum of variables for the production of xylanase in the central composite design, batch experiments were conducted. The coded values of the process

parameters were determined by the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad --(1)$$

Where x_i -coded value of the ith variable, X_i-uncoded value of the ith test variable and X_0-uncoded value of the ith test variable at center point. The range and levels of independent variables with coded values are shown in Table 1.

Table 1: Range and levels of independent variables

Variables		Levels						
	Code	-2.378	-2	-1	0	1	2	2.378
Substrate Concentration (g)	X1	5.2	6.0	8.0	10.0	12.0	14.0	14.8
Temperature (^{0}C)	X2	27.2	28.0	30.0	32.0	34.0	36.0	36.8
Incubation Time (hrs)	X3	62.9	72.0	96.0	120.0	144.0	168.0	177.1
Initial Moisture content (%)	X4	68.1	70.0	75.0	80.0	85.0	90.0	91.9
Initial pH	X5	3.8	4.0	4.5	5.0	5.5	6.0	6.2

Table 2: Central composite design (CCD) matrix of factors in orthogonal and real values along with enzyme activity as response

Run No.	Orthogonal and Real Values					Xylanase Activity		Cellul--ase Activity (IU/gds)
	X1- Substrate concentration	X2- Temperature	X3- Incubation Time	X4- Initial Moisture content	X5- Initial pH	Experi mental (IU/gds)	Predi - cted (IU/gds)	
1	-2.378 (5.2)	0 (32)	0 (120)	0 (80)	0 (5.0)	320.05	305.53	75.36
2	-1 (8)	1 (34)	1 (144)	1 (85)	1 (5.5)	465.18	481.77	67.65
3	-1 (8)	-1 (30)	1 (144)	1 (85)	-1 (4.5)	325.00	344.90	71.18
4	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	524.99	525.95	92.07
5	1 (12)	1 (34)	1 (144)	1 (85)	-1 (4.5)	420.00	437.18	78.60
6	-1 (8)	1 (34)	1 (144)	-1 (75)	-1 (4.5)	385.43	384.30	73.96
7	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	524.99	525.95	94.37
8	1 (!2)	1 (34)	-1 (96)	-1 (75)	-1 (4.5)	312.46	332.84	65.54
9	0 (10)	0 (32)	0 (120)	-2.378 (68.1)	0 (5.0)	385.04	368.47	69.13
10	-1 (8)	1 (34)	-1 (96)	1 (85)	-1 (4.5)	372.14	379.39	70.75
11	1 (12)	-1 (30)	1 (144)	1 (85)	1 (5.5)	478.47	489.12	74.35
12	1 (12)	1 (34)	1 (144)	1 (85)	1 (5.5)	518.34	510.31	78.87
13	0 (10)	0 (32)	-2.378(62.9)	0 (80)	0 (5.0)	290.00	273.90	62.35
14	0 (10)	-2.378 (27.2)	0 (120)	0 (80)	0 (5.0)	299.00	291.55	64.63
15	-1 (8)	-1 (30)	-1 (96)	-1 (75)	1 (5.5)	285.75	287.81	66.25
16	0 (10)	0 (32)	0 (120)	0 (80)	-2.378 (3.8)	320.06	301.74	72.45
17	-1 (8)	1 (34)	-1 (96)	1 (85)	1 (5.5)	412.02	405.15	76.07
18	2.378 (14.8)	0 (32)	0 (120)	0 (80)	0 (5.0)	418.66	407.80	71.12
19	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	524.99	525.95	82.37
20	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	524.99	525.95	80.12
21	-1 (8)	-1 (30)	1 (144)	1 (85)	1 (5.5)	418.66	392.51	77.83
22	1 (12)	1 (34)	-1 (96)	1 (85)	1 (5.5)	438.60	454.11	73.65
23	-1 (8)	1 (34)	1 (144)	-1 (75)	1 (5.5)	418.66	439.42	73.37
24	-1 (8)	-1 (30)	-1 (96)	1 (85)	1 (5.5)	250.00	276.48	62.84
25	-1 (8)	1 (34)	-1 (96)	-1 (75)	-1 (4.5)	328.98	343.14	65.57

26	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	524.99	525.95	81.37
27	0 (10)	2.378 (36.8)	0 (120)	0 (80)	0 (5.0)	445.18	425.05	78.85
28	0 (10)	0 (32)	2.378(177.1)	0 (80)	0 (5.0)	445.24	435.96	76.27
29	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	523.97	525.95	88.36
30	1 (12)	-1 (30)	1 (144)	-1 (75)	-1 (4.5)	377.41	382.95	79.46
31	-1 (8)	1 (34)	-1 (96)	-1 (75)	1 (5.5)	374.58	371.16	65.01
32	-1 (8)	-1 (30)	-1 (96)	1 (85)	-1 (4.5)	257.28	255.97	64.05
33	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	524.09	525.95	82.19
34	1 (12)	1 (34)	1 (144)	-1 (75)	1 (5.5)	428.27	428.96	66.26
35	0 (10)	0 (32)	0 (120)	0 (80)	0 (5.0)	552.09	525.95	78.67
36	0 (10)	0 (32)	0 (120)	0 (80)	2.378 (6.2)	422.43	415.37	76.43
37	-1 (8)	1 (34)	1 (144)	1 (85)	-1 (4.5)	431.64	428.92	73.76
38	0 (10)	0 (32)	0 (120)	2.378 (91.9)	0 (5.0)	462.93	454.12	75.36
39	-1 (8)	-1 (30)	1 (144)	-1 (75)	-1 (4.5)	340.75	345.60	62.12
40	1 (12)	-1 (30)	-1 (96)	1 (85)	-1 (4.5)	353.85	352.75	68.27
41	-1 (8)	-1 (30)	-1 (96)	-1 (75)	-1 (4.5)	261.60	265.05	60.57
42	1 (12)	-1 (30)	1 (144)	1 (85)	-1 (4.5)	421.70	421.24	74.43
43	1 (12)	1 (34)	-1 (96)	1 (85)	-1 (4.5)	409.15	408.08	72.76
44	1 (12)	1 (34)	1 (144)	-1 (75)	-1 (4.5)	358.49	353.56	69.07
45	1 (12)	-1 (30)	-1 (96)	1 (85)	1 (5.5)	397.38	393.52	63.12
46	1 (12)	-1 (30)	-1 (96)	-1 (75)	1 (5.5)	363.35	365.86	62.76
47	1 (12)	-1 (30)	1 (144)	-1 (75)	1 (5.5)	444.34	453.08	72.65
48	1 (12)	1 (34)	-1 (96)	-1 (75)	1 (5.5)	380.60	381.13	74.54
49	-1 (8)	-1 (30)	1 (144)	-1 (75)	1 (5.5)	393.83	395.47	73.43
50	1 (12)	-1 (30)	-1 (96)	-1 (75)	-1 (4.5)	318.27	322.83	66.23

The experimental design is shown in Table 2. A mathematical model, relating the relationships among the process dependent variable and the independent variables in a second-order equation, was developed (Equation 2). The regression analysis was performed to estimate the response function as a second order polynomial.

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

Where Y is the predicted response, βi, βj, βij are coefficients estimated from regression. They represent the linear, quadratic and interactive effects of X1, X2, X3, X4 and X5 on response. A statistical software package Design Expert 8.0.7.1.5, was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equations were validated by the statistical tests called the ANOVA analysis. Design-based experimental data were matched according to the second order polynomial equation. The independent variables were fitted to the second order model equation and examined for the goodness of fit. The quality of fit of the second order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by *F*-test. The significance of each term in the equation is to estimate the goodness of fit in each case. To establish the individual and interactive effects of the test variable on the xylanase production response surfaces were drawn. The optimal values of the test variables were obtained in coded values and transformed to uncoded values.

3. RESULTS AND DICUSSION

To examine the interactive effect of five various process parameters (independent variables), on the xylanase production, a central composite design of 2^5 = 32 , 8 centre points and 10 star points leading to a total of 50 experiments were performed. Equation (3) represents the mathematical model relating the xylanase production and the second order polynomial coefficient for each term of the equation determined through multiple regression analysis using the Design Expert 8.0.7.1.5. The experimental and predicted values of xylanase production are also given in table 2.

The results were analyzed by using ANOVA (analysis of variance) appropriate for the experimental design used and shown in Table 3. The ANOVA of the quadratic regression model indicates the model to be significant. The Model F-value of 57.54 implied the model to be significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Model P value (Prob>F) is very low [<0.0001]. This reiterates that the model is significant. The P values are used as a tool to check the significance of each of the coefficients, which in turn are necessary to understand the pattern of the mutual interactions between the test variables. The F value and the corresponding P values, along with the coefficient estimates are given in Table 3. The smaller the magnitude of the P, the more significant is the

corresponding coefficient. Values of P less than 0.0500 indicates the model terms to be significant. The coefficient estimates and the corresponding P values along with the coefficient estimate are given in table 4. The coefficients estimate and the corresponding P values suggests that, among the test variables used in the study, X1, X2, X3, X4, X5, X1X2, X1X4, X2X3, X2X4, X3X5, $X1^2$, $X2^2$, $X3^2$, $X4^2$, $X5^2$ are significant model terms. The coefficient of interaction terms X1X2, X2X4, X2X3, X1X4, X3X5 was found to be highly significant.

Table 3 : Analysis of Variance (ANOVA) for response surface quadratic model for the production of Xylanase

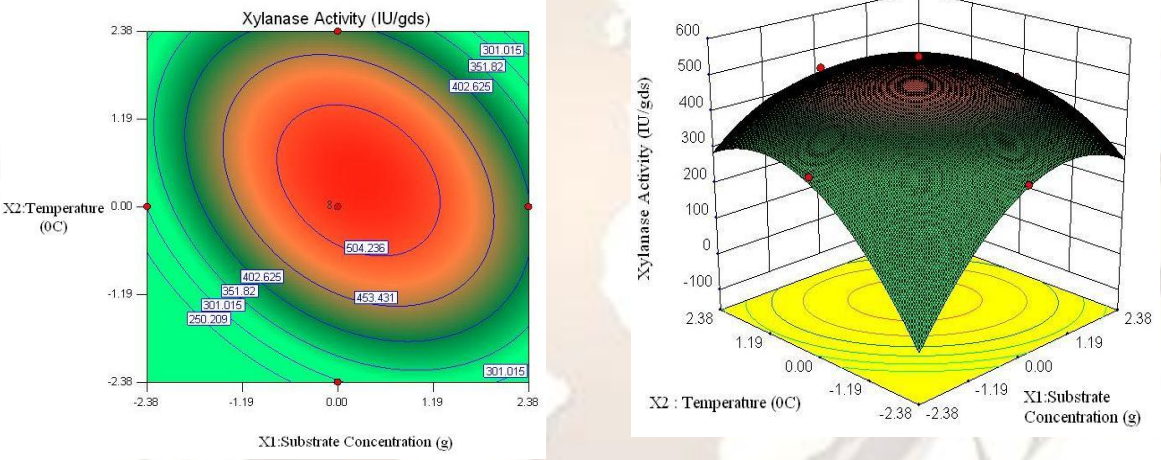
Source	Coefficient factor	Sum of Squares	DF	F	P > F	
Model	525.67	3.11E+05	20	57.54	< 0.0001	significant
X1-Substrate Concentration	21.55	20127.9	1	74.57	< 0.0001	Significant
X2-Temperature	25.73	28683	1	106.27	< 0.0001	significant
X3-Incubation Time	34.15	50527.8	1	187.2	< 0.0001	significant
X4- Initial moisture content	18.05	14114.2	1	52.29	< 0.0001	significant
X5-Initial pH	23.95	24843.7	1	92.04	< 0.0001	significant
X1X2	-17.02	9269.09	1	34.34	< 0.0001	significant
X1X3	-5.11	834.77	1	3.09	0.0892	
X1X4	9.75	3041.22	1	11.27	0.0022	
X1X5	5.07	821.75	1	3.04	0.0916	
X2X3	-9.85	3104.72	1	11.5	0.0020	
X2X4	11.33	4107.8	1	15.22	0.0005	
X2X5	1.31	55.23	1	0.2	0.6544	
X3X4	2.09	140.2	1	0.52	0.4769	
X3X5	6.77	1468.55	1	5.44	0.0268	
X4X5	-0.57	10.24	1	0.038	0.8469	
$X1^2$	-30.22	50866.3	1	188.46	< 0.0001	significant
$X2^2$	-29.74	49254	1	182.48	< 0.0001	significant
$X3^2$	-30.53	51902.6	1	192.3	< 0.0001	significant
$X4^2$	-20.58	23581.5	1	87.37	< 0.0001	significant
$X5^2$	-29.89	49749.3	1	184.32	< 0.0001	significant
Residual		7827.36	29			
Lack of Fit		7170.36	22	3.47	0.0583	insignificant
Pure Error		657.01	7			
Cor Total		3.18E+05	49			

Std. Dev - 16.43; R^2 - 97.50%; Mean - 403.52; Adj R^2- 95.85%; C.V – 4.07%;
Pred R^2- 90.77%; Adeq Precision – 25.332

Xylanase activity, Y = 525.67 + 21.55X1 + 25.73X2 + 34.15X3 + 18.05X4 + 23.95X5 - 30.22$X1^2$ - 29.74$X2^2$ - 30.53$X3^2$ - 20.58$X4^2$ - 29.89$X5^2$ - 17.02X1X2 - 5.11X1X3 + 9.75X1X4 + 5.07X1X5 - 9.85X2X3 + 11.33X2X4 + 1.31X2X5 + 2.09X3X4 + 6.77X3X5 - 0.57X4X5 ..... (3)

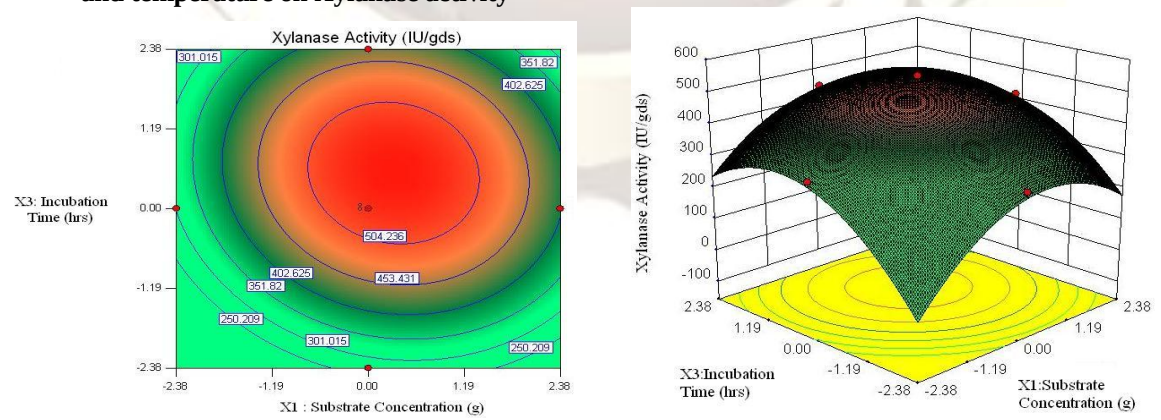
The predicted R^2 of 0.9077 is in reasonable agreement with the adjusted R^2 of 0.9585. Adequate precision measures the signal to noise ratio. A ratio of greater than 4 is desirable. This model can be used to navigate the design space. The fit of the model was also expressed by the coefficient of regression R^2, which was found to be 0.9750 indicating that 97.50% the variability in the response could be explained by the model. The closer the value of *R* (correlation coefficient) to 1, the better is the correlation between the experimental and predicted values. Here the value of R^2 (0.9750) being close to 1 indicated a close agreement between the experimental results and the theoretical values predicted by the model equation. This implies that the prediction of experimental data is quite satisfactory. The Coefficient of Variation (CV) indicates the degree of precision with which the experiments are compared. Generally, the higher the value of the CV is, the lower the reliability of the experiment. Here a lower value of CV (4.07) indicates greater reliability of the experiments performed. The response surface methodology was used with five process variables to evaluate their effect on the xylanase production. The response Eq. (3) was obtained for the xylanase production.

To study the interactive effect of two factors on the xylanase production, the response surface methodology was used and 3D surface and contour plot was drawn. Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interaction effects of these two factors. The interaction effects of the variables and optimal levels of the each variable were determined by plotting the contour & response surface graphs. The 3D response surface graphs and contour plots are shown in Figs. 1 to 10. The elliptical shape of the contour indicates good interaction of the two variables and circular shape indicates no interaction between the variables. There was a significant interaction between every two variables, and the surface confined in the smallest ellipse in the contour diagrams indicates the maximum predicted yield.



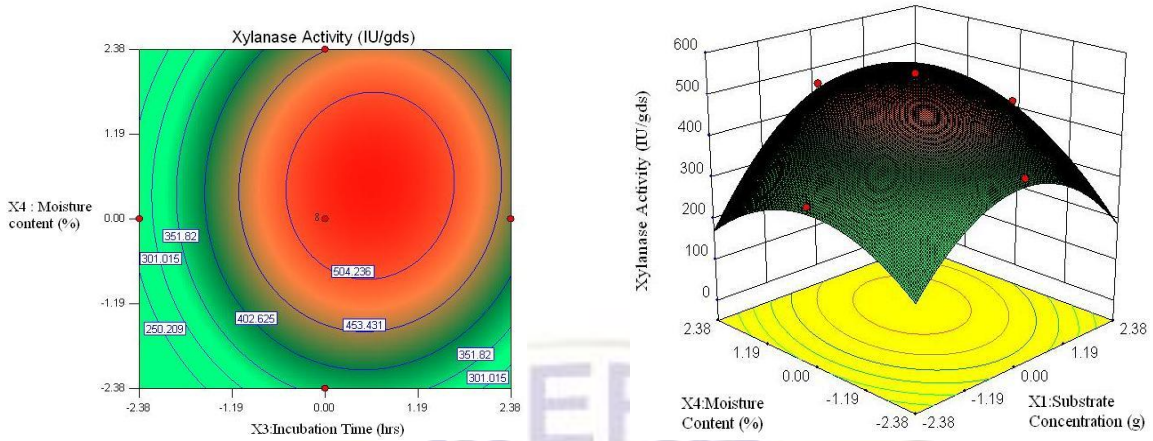
(a) **(b)**

Fig.1: (a) Contour plot (b) 3D Response surface plot showing interactive effect of substrate concentration and temperature on Xylanase activity



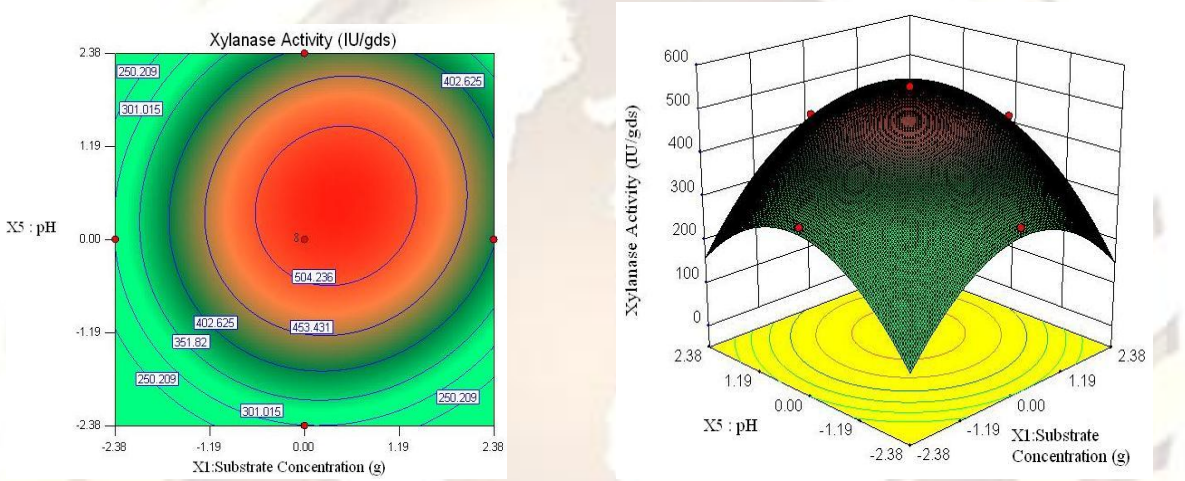
(a) **(b)**

Fig.2: (a) Contour plot (b) 3D Response surface plot showing interactive effect of substrate concentration and incubation time on Xylanase activity.



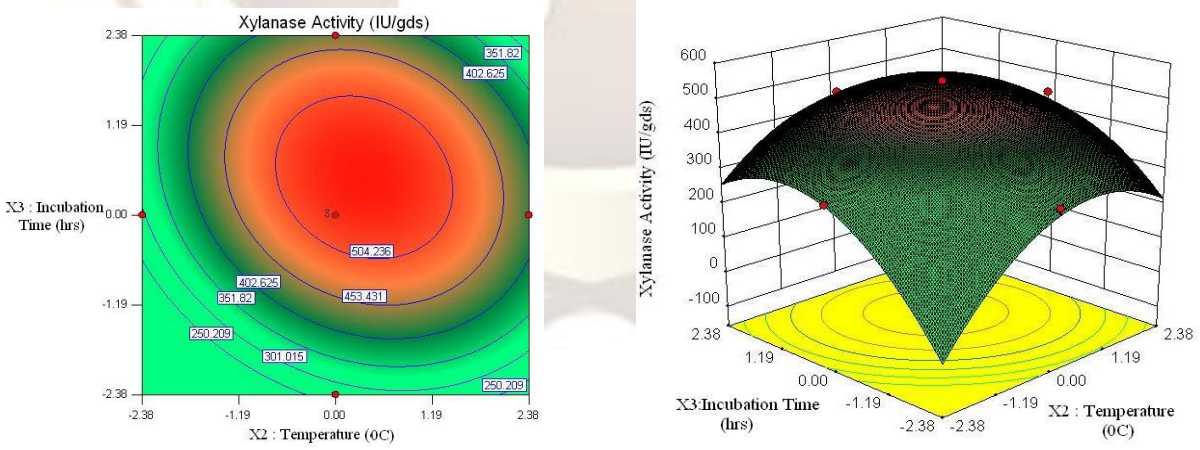
(a) **(b)**

Fig.3: (a) Contour plot (b) 3D Response surface plot showing interactive effect of substrate concentration and % moisture content on Xylanase activity



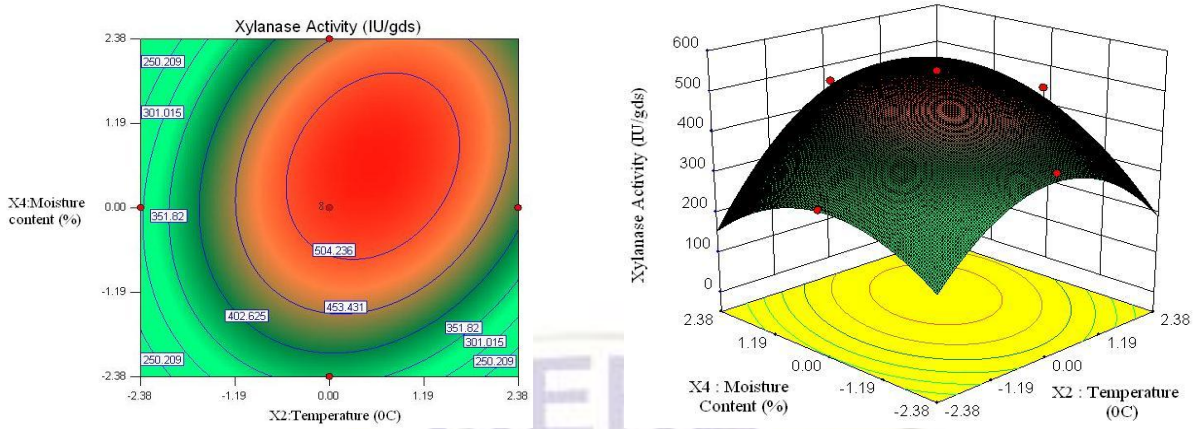
(a) **(b)**

Fig.4: (a) Contour plot (b) 3D Response surface plot showing interactive effect of substrate concentration and initial pH on Xylanase activity.



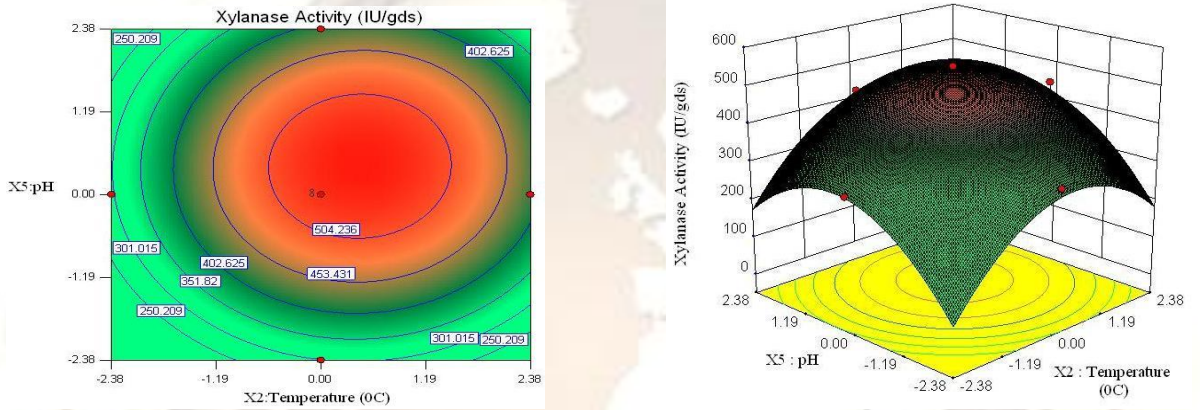
(a) **(b)**

Fig.5: (a) Contour plot (b) 3D Response surface plot showing interactive effect of Temperature and Incubation time on Xylanase activity.



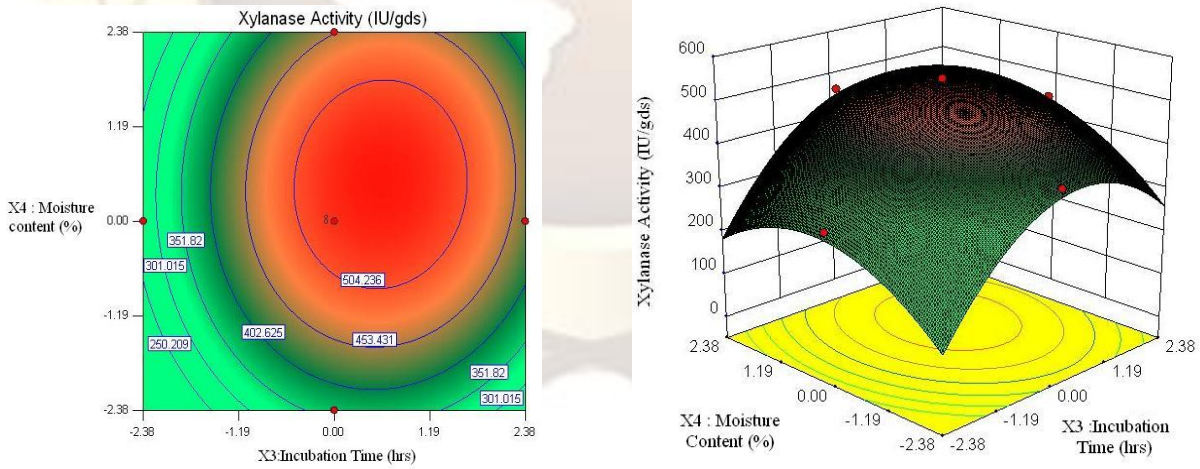
(a) **(b)**

Fig.6: (a) Contour plot (b) 3D Response surface plot showing interactive effect of Temperature and % moisture content on Xylanase activity.



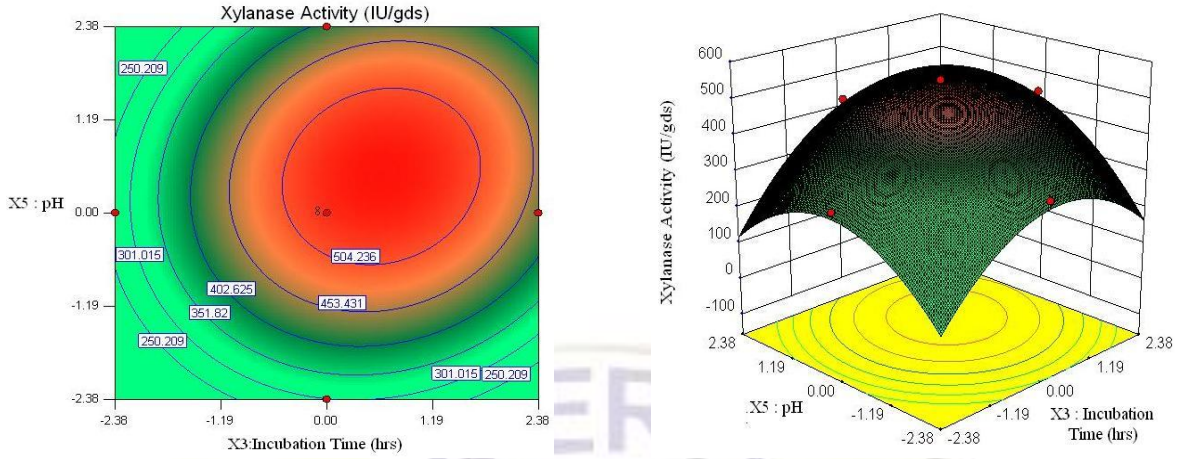
(a) **(b)**

Fig.7: (a) Contour plot (b) 3D Response surface plot showing interactive effect of Temperature and initial pH on Xylanase activity.



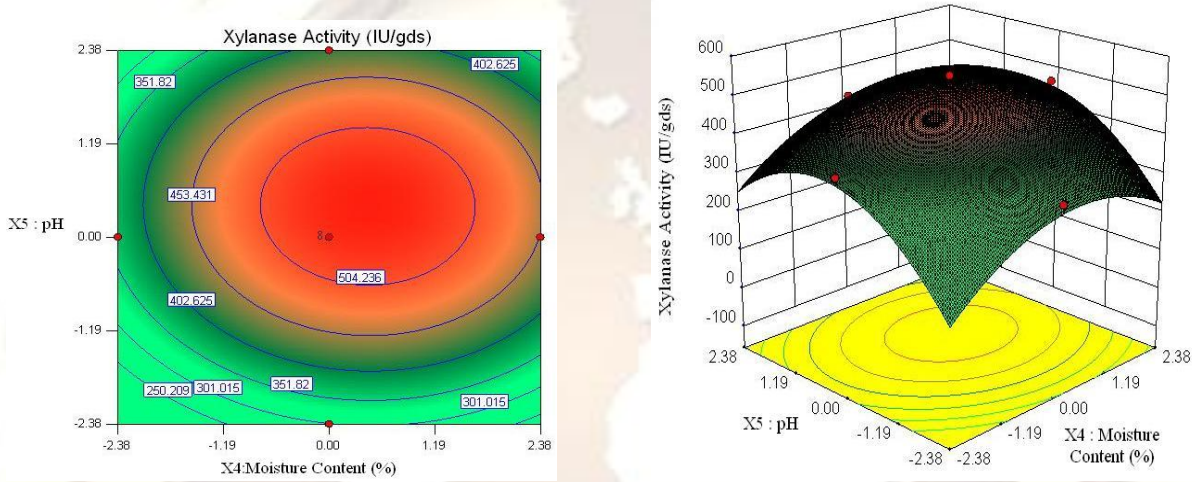
(a) **(b)**

Fig.8: (a) Contour plot (b) 3D Response surface plot showing interactive effect of Incubation time and % moisture content on Xylanase activity.



(a) **(b)**

Fig.9: (a) Contour plot (b) 3D Response surface plot showing interactive effect of Incubation time and Initial pH on Xylanase activity.



(a) **(b)**

Fig.10: (a) Contour plot (b) 3D Response surface plot showing interactive effect of % moisture content and Initial pH on Xylanase activity.

The magnitude of P and F values in (Table 3) gives the maximum positive contribution of substrate concentration, temperature, incubation time, initial moisture content and pH on xylanase activity. The interactions of substrate concentration and initial moisture content, substrate concentration and pH, temperature and initial moisture content, temperature and pH, incubation time and initial moisture content, incubation time and pH have positive effect, whereas the interactions of substrate concentration and temperature, substrate concentration and incubation time, temperature and incubation time and initial moisture content and pH have negative effect on Xylanase activity are illustrated in Figures (1-10).

So, compared with the traditional 'one-variable at- a-time' approach which is unable to detect the frequent interactions occurring between two or more factors although they often do occur, RSM has immeasurable effects and tremendous advantages. The response surfaces of mutual interactions between the variables were found to be elliptical for most cases. Optimum condition is the one at which the maximum xylanase production is attained. Such an optimum condition for Xylanase production can be obtained by solving the second order polynomial equation using RSM. The central point is the point at which the slope of the contour is zero in all directions. The coordinates of the central point within the highest contour levels in each of these figures will correspond to the optimum values of the respective constituents. The optimum values drawn from these figures are in close agreement with those obtained by optimizing the regression model Eq. (3). The sequential quadratic programming in MATLAB 7 is used to solve the second-degree polynomial regression Eq. (3). The optimum values for maximum xylanase production were: substrate concentration -10.7g, temperature - 32.7°C, fermentation time - 133hrs, % initial moisture content - 83.2% and pH - 5.3. The optimal values for the variables as predicted were found to be within the design region. This shows that the model correctly explains the influence of the chosen variables on the xylanase production.

Validation of the experimental model was carried out by conducting the batch experiment under optimal operation conditions obtained by the regression model, substrate concentration – 10.7 g, temperature – 32.7°C, incubation time - 133 hrs, initial moisture content - 83.2% and pH - 5.3. The experiments were performed in triplicates and the results are compared. The xylanase activity (553.17 IU/gds) obtained from experiments was close to the actual response (550.82 IU/gds) predicted by the regression model, which proved the validity of the model.

4. CONCLUSION

In this work, the applied Response surface methodolgy (RSM) proved to be efficient in optimizing operating parameters for xylanase enzyme production under solid-state fermentation using low cost substrate wheat bran. A small amount of cellulase production has also been obtained in all the experimental runs. From optimization studies, the optimized values of the process parameters for Xylanase production were as follows: substrate concentration – 10.7 g, temperature – 32.7^0C, incubation time – 133 hrs, initial moisture content – 83.2% and initial pH – 5.3. Using the optimized conditions the maximum xylanase production of 553.17 IU/gds was obtained. This is in reasonable agreement with the commonly used agricultural-wastes that have been reported to produce xylanase activity, in the similar ranges 488 IU/g [26], 615 IU/g [27], 280 IU/g [28], 33.9 IU/g [29].

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