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RESPONSE SURFACE METHODOLOGICAL OPTIMIZATION OF THE MEDIUM COMPONENTS FOR PRODUCTION OF XYLANASE UNDER SSF BY *ASPERGILLUS FUMIGATUS*

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ABSTRACT

Response surface methodology was employed for the optimization of various medium components for the production of xylanase by *Aspergillus fumigatus* in solid state fermentation. The medium components influencing the enzyme production were screeened using Plackett-Burman design. Among the twelve independent variables screened by using Plackett-Burman design, only four variables $FeSO_4.7H_2O$, Yeast extract, KH_2PO_4 and $CoSO_4$ were found to be significantly affecting variable for xylanase production. For obtaining the mutual interaction between the four variables and optimizing these variables, a 2^3 factorial central composite design (CCD) using response surface methodology was employed. The optimal values of four variables obtained for maximal production of xylanase were**:** $FeSO_4.7H_2O$ - 8.0817 mg/gds, yeast extract - 35.057 mg/gds, KH_2PO_4 - 21.700 mg/gds and $CoSO_4$ – 3.1717 mg/gds with a predicted xylanase activity of 354.33 IU/gds. These predicted optimal parameters were tested by carrying out the three repeated batch experiments in the laboratory and the maximum xylanase activity (355 IU/gds) obtained was close to the predicted value. A poor quantity of carboxy methyl cellulase activity was also obtained in all the experimental runs.

Keywords: Response surface methodology, solid state fermentation, Plackett-Burman design, central composite design

1. INTRODUCTION

Hemicellulose is the second most abundant renewable biomass and accounts for 25–35% of lignocellulosic biomass which is considered to be a promising biomass feedstock for the production of biofuels [1]. Xylan represents the major component of hemicellulose, the second most abundant plant material in nature. Due to its complex structure several enzyme 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 back bone of xylan [2]. Market trends reveal that xylanase and cellulase takes the major chunk of share amounting to 20% of the world enzyme market, together with pectinases [3]. Xylanases have commercial uses in various forms: (a) biobleaching agents in pulp and paper industry (b) enhanced utilization of biomass in the biofuel industry (c) production of xylitol (a low calorie sweetener) (d) foodstuff additive in bread, juice and wine manufacturing and (e) additive in animal feedstuff preparation [4, 5]. 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 [6]**.** Xylanases have mainly been produced from fungal and bacterial strains of microorganism. Although bacterial xylanase were reported to have higher value of optimum pH which is beneficial from the view point of applying in biobleaching process, xylanase produced from fungi usually has higher activity [7]. The Xylanase enzyme can be produced by a number of microorganisms including bacteria, yeast and filamentous fungi like Trichoderma, Aspergillus, Penicillium, Fusarium, Chaetomium, Humicola, Taloromyces, and many others [8]. In the fermentation process, xylanases produced by bacteria mostly use the liquid state fermentation while ones from fungi almost adopt the solid state fermentation [9]. Production of enzymes by solid-state fermentation (SSF) has potential advantages over submerged fermentation regarding to operation, simplicity, high productivity fermentation, less favorable for growth of contaminants and concentrated product formation [10].

SSF is an attractive method for xylanase production, and a large number of different *Aspergillus* species have been reported as good xylanases producers [11]. 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 [12, 8]. 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]. Sugarcane bagasse is abundantly and cheaply available as a byproduct from sugar industry. Sugar cane bagasse was composed of approximately 28.4% cellulose, 27.5% hemicellulose, 19.7% lignin, and 8.4% ash according to the analytical procedure recommended by Yang [13]. For industrial application, the cost of enzymes is one of the main factors determining the economics of a process. Usually, the optimizing of the fermentation medium is a basic principle for cost reduction. The optimization was traditionally done by varying one factor while keeping the other factors at a constant level. Due to the interactions among factors, this technique is time-consuming and incapable of detecting the true optimum [14]. To overcome this difficulty, Response surface methodology (RSM) was used to optimize the media composition and few process variables [15, 16]. RSM is the most commonly used statistical practice for bioprocess optimization. Response surface methodology (RSM) is a useful tool to study the interactions of two or more factors. It is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions [17]. The process consists of a low order polynomial equation in a predetermined region of independent variables. These independent variables are later analyzed to locate the optimum values of the independent variables for the best response. It usually contains three stages: (i) design of experiments, (ii) response surface modeling through regression (iii) optimization [18]. It can be used to evaluate the relationship between a set of controllable experimental variables and observed results. The interaction among the possible influencing variables can be evaluated with limited number of experiments [19]. RSM and CCD are proved to be important tools to study the effect of multiple process variables with fewer experimental trials [20, 21]. In the last few years, RSM has been applied to optimize and evaluate interactive effects of independent factors in numerous chemical and biochemical processes. In the present study, the endo β-1, 4-Xylanase was produced by *Aspergillus fumigatus (MTCC NO – 343)* in batch process, using sugarcane bagasse as an inexpensive substrate, by medium optimization. In medium optimization, screening of nutrients was done by Plackett- Burman design (PBD), the screened nutrients were further optimized by central composite design (CCD) and a regression model was established and the experimental verification of the model was validated.

2. MATERIAL AND 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°C. 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. Substrate Preparation

Sugarcane bagasse samples were obtained from the agricultural field, Cuddalore District, Tamil Nadu. The samples were sun-dried for a period of three weeks and subsequently oven-dried slowly at 50°C for 48 hours. The dried samples were chopped into small bits, made into 100 mesh particle size and used as substrate for xylanase production.

2.3. Solid state fermentation (SSF)

Fermentation was carried out in 250ml Erlenmeyer flasks (plugged with cotton) with 10 g of sugarcane bagasse, 0.1% (v/v) of Tween-80, 0.1% (w/v) of oat spelt xylan, supplemented with nutrients concentrations defined by the experimental design. 0.1 % of oat spelt xylan serves as an inducer for xylanase production. The initial moisture content was adjusted to 80% [22]. Each flask was covered with hydrophobic cotton and autoclaved at 121°C 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°C and the inoculated flasks were incubated at 30°C for 5 days in an incubator. During preliminary screening process, the experiments are carried out for 7 days and it was found that the maximum Xylanase production occurs at the 5th day. Hence experiments are carried out for 5 days. After fermentation 50 ml of 0.05M Na-citrate buffer (pH – 5.3) was added to the fermented matter and the contents were agitated for 30 minutes at 200 rpm in an orbital shaker at 30^0C and filtered through a cotton cloth by squeezing. The extract was centrifuged at 15,000 rpm for 20 minutes and the supernatant was used for determination of enzyme activity.

2.4. Enzyme Assay

Endo β-1,4-Xylanase activity was measured by incubating 0.5ml of 1% (w/v) oat spelt xylan in 0.05M 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 [23] and D-Xylose was used as the standard. One unit (IU) of xylanase activity is defined as the amount of enzyme releasing 1μmol of xylose per minute under the assay conditions. Xylanase production in SSF was expressed as IU/g dry substrate (IU/gds).

Carboxy methyl 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 [23] and D-glucose was used as the standard. One unit of carboxy methyl cellulase activity was defined as the amount of enzyme that liberated 1 µmol of glucose equivalent under the assay conditions.

2.5. Experimental Design and Statistical Analysis

Plackett-Burman experimental design 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 variable significantly affect xylanase production by *Aspergillus fumigatus*, Plackett-Burman design using statistical software package MINITAB (Release 15.1, PA, USA), was used. 12 variables (Table-1) were screened in 20 experimental runs (Table-2) and insignificant ones were eliminated in order to obtain a smaller, manageable set of factors. The low level (-1) and high level (+1) of each factor are listed in Table-1. Significant nutrient components viz. $FeSO_4.7H_2O$, Yeast extract, KH_2PO_4, $CoSO_4$ which increases the xylanase production was identified.

Table 1: Nutrient screening using a Plackett Burman design

Variables		Levels mg/gds	
Nutrient code	**Nutrient**	**Low (-1)**	**High (+1)**
A	K_2HPO_4	50.0	200.0
B	$(NH_4)_2SO_4$	100.0	200.0
C	$ZnSO_4.7H_2O$	100.0	500.0
D	KH_2PO_4	100.0	300.0
E	$CaCl_2$	20.0	30.0
F	MnSO4.7H2O	50.0	150.0
G	MgSO4.7H2O	2.0	20.0
H	Yeast extract	10.0	50.0
J	Kcl	50.0	150.0
K	CoSO4	2.0	4.0
L	$FeSO_4.7H_2O$	5.0	20.0
M	Urea	100.0	300.0

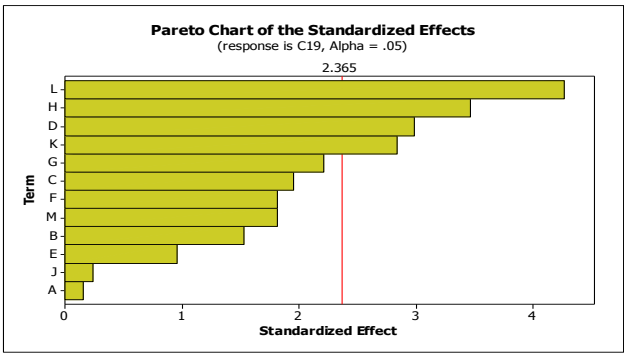

Fig. 1. Pareto chart showing the effect of media components on Xylanase activity

2.6. Response surface Methodology using Central Composite Design

Response surface methodology (RSM) using CCD was employed to optimize the selected four variables. RSM consist of a group of empirical techniques used for evaluation of relationship between cluster of controlled experimental factors and measured response. A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach. CCD was used to obtain a quadratic model, consisting of factorial trails and star points to estimate quadratic effects and central points to estimate the pure process variability with xylanase production. The central composite design is used to study the effects of variables on their responses and subsequently in the optimization studies. The four independent variables were studied at five different levels (Table-3) and sets of 30 experiments (batch experiments) were carried out (Table-4). The statistical software package

Design-Expert software (version 8.0.7.1, Stat-Ease, Inc., Minneapolis,USA) was used to analyse the experimental data. All variables were taken at a central coded value of zero. The minimum and maximum ranges of variables investigated are listed in Table-3. Upon the completion of experiments, the average maximum xylanase activities were taken as the response (Y).

This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with minimum number of experiments as well as to analyse the interaction between the parameters. In order to determine the existence of a relationship between the factors and response variables, the collected data were analysed in a statistical manner, using regression. A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables. A second order polynomial equation is,

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

Where Y is the measured response, β_0 is the intercept term, β_i are linear coefficients, β_{ii} are quadratic coefficient, β_{ij} are interaction coefficient and X_i and X_j are coded independent variables. The following equation was used for coding the actual experimental values of the factors in the range of (−1 to +1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad (2)$$

where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the value of X_i at the center point, and ΔX_i is the step change.

Table 2: Plackett–Burman experimental design matrix for screening of important variables for Xylanase production

Run No	A	B	C	D	E	F	G	H	J	K	L	M	Xylanase Activity (IU/gds)	CMCellulas Activity (IU/gds)
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	280	44.45
2	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	275	35.38
3	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	246	34.23
4	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	215	23.12
5	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	224	38.34
6	1	1	1	1	-1	-1	1	1	-1	1	1	-1	185	29.04
7	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	237	34.23
8	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	255	21.54
9	-1	1	1	1	1	-1	-1	1	1	-1	1	1	176	21.21
10	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	220	34.2
11	1	-1	1	1	1	1	-1	-1	1	1	-1	1	198	10.12
12	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	176	32.1
13	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	205	25.9
14	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	208	36.31
15	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	237	38.09
16	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	187	26.01
17	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	220	44.31
18	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	185	27.03
19	1	-1	1	-1	1	1	1	1	-1	-1	1	1	215	30.14
20	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	195	28.11

Table 3: Ranges of the independent variables used in RSM

Variables	Code	Levels (mg/g of dry substrate)				
		-2	-1	0	1	2
$FeSO_4.7H_2O$	A	5.0	8.75	12.50	16.25	20.0
Yeast extract	B	10.0	20.0	30.00	40.0	50.0
KH_2PO_4	C	100	150	200.0	250	300
$CoSO_4$	D	2.0	2.5	3.0	3.5	4.0

Statistical analysis of the data was performed by design package Design-Expert software (Version 8.0.7.1, Stat-Ease, Inc., Minneapolis,USA) to evaluate the analysis of variance (ANOVA), to determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case. The fitted polynomial equation was then expressed in the form of three-dimensional response surface plots to illustrate the main and interactive effects of the independent variables on the dependent ones. The combination of different optimized variables, which yielded the maximum response, was determined to verify the validity of the model. In order to verify the accuracy of the predicted model an experiment was conducted with initial and optimized media. The optimal concentrations of the critical variables were obtained by analyzing 3D plots. The statistical analysis of the model was represented in the form of analysis of variance (ANOVA).

Table 4: Central composite design (CCD) of factors in coded levels with Enzyme activity as response

Run No	Coded Values				Xylanase Activity		Carboxy Methyl Cellulase (CMCase) Activity (IU/gds)
	A	B	C	D	Experimental (IU/gds)	Predicted (IU/gds)	
1	1	1	-1	-1	262.45	260.84	65.02
2	-1	-1	-1	1	268.56	267.93	44.17
3	-1	-1	-1	-1	264.54	262.83	94.36
4	1	-1	1	1	245.9	245.39	90.03
5	-1	1	-1	-1	250.45	251.26	87.36
6	0	0	0	0	325.67	325.75	82.2
7	1	1	1	-1	275.85	276.78	118.34
8	-1	1	1	-1	273.54	273.23	92.76
9	0	0	0	0	325.78	325.75	91.48
10	-2	0	0	0	245.12	246.51	98.56
11	2	0	0	0	258.34	259.76	103.12
12	-1	1	-1	1	268.45	269.46	120.54
13	0	0	0	0	325.98	325.75	110.43
14	0	0	0	-2	292.32	291.48	73.96
15	0	-2	0	0	250.12	251.95	94.37
16	0	0	0	0	325.89	325.75	65.54
17	1	-1	-1	-1	274.78	278.99	69.13
18	0	2	0	0	258.09	259.07	70.75
19	0	0	0	0	325.45	325.75	77.83
20	1	-1	-1	1	280.45	277.62	73.65
21	0	0	0	2	280.23	283.87	73.37
22	1	-1	1	-1	275.34	271.20	62.84
23	-1	-1	1	1	243.24	241.72	65.57
24	0	0	0	0	325.87	325.75	81.37
25	0	0	2	0	255.34	259.04	78.85
26	-1	-1	1	-1	260.45	261.07	76.27
27	1	1	-1	1	272.9	272.58	88.36
28	1	1	1	1	265.5	264.07	79.46
29	0	0	-2	0	270.21	269.31	66.26
30	-1	1	1	1	270.89	266.98	73.76

3. RESULTS AND DISCUSSION

Plackett-Burman experiments (Table-2) showed a wide variation in Xylanase activity. This variation reflected the importance of optimization to attain higher productivity. From the Pareto chart (Fig.1), the variables, namely, $FeSO_4.7H_2O$, Yeast extract, KH_2PO_4, $CoSO_4$ were selected for further optimization to attain a maximum production of Xylanase. The levels of factors $FeSO_4.7H_2O$, Yeast extract, KH_2PO_4, $CoSO_4$ and the effect of their interactions on Xylanase production were determined by central composite design using RSM. For this study, 2^3 full factorial central composite design with sixteen star points, eight axial points and six replicates at the centre points were employed to fit the second order polynomial model which indicated that 30 experiments were required for this procedure shown in Table-4 along with the predicted and observed responses, and the results were analyzed by ANOVA.

The second-order regression equation provided the levels of Xylanase activity as the function of $FeSO_4.7H_2O$, Yeast extract, KH_2PO_4, $CoSO_4$ which can be presented in terms of coded factors as in the following equation:

$$Y = 325.77 + 3.31\,A + 39.0 + 1.78\,B - 2.57\,C - 1.90\,D - 18.16\,A^2 - 17.57\,B^2 - 15.40\,C^2 - 9.52\,D^2 - 1.64\,AB - 1.51\,AC - 1.61\,AD + 5.93\,BC + 3.28\,BD - 6.11\,CD \quad (3)$$

Where, Y is the Xylanase activity (IU/gds), A, B, C and D are $FeSO_4.7H_2O$, Yeast extract, KH_2PO_4, $CoSO_4$ respectively.

Table 5: 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	325.77	21183.97	14	208.94	< 0.0001	Significant
A	3.31	263.28	1	36.35	< 0.0001	Significant
B	1.78	76.01	1	10.50	0.0055	
C	-2.57	158.16	1	21.84	0.0003	
D	-1.90	86.98	1	12.01	0.0035	
A * B	-1.64	43.26	1	5.97	0.0274	
A * C	-1.51	36.33	1	5.02	0.0407	
A*D	-1.61	41.70	1	5.76	0.0298	
B * C	5.93	563.23	1	77.77	< 0.0001	Significant
B * D	3.28	171.68	1	23.71	0.0002	
C * D	-6.11	597.68	1	82.53	< 0.0001	Significant
A^2	-18.16	9044.82	1	1248.94	< 0.0001	Significant
B^2	-17.57	8463.02	1	1168.60	< 0.0001	Significant
C^2	-15.40	6503.29	1	897.99	< 0.0001	Significant
D^2	-9.52	*2487.44*	1	343.47	< 0.0001	Significant
Residual		*108.63*	15			
Lack of Fit		*108.45*	10	299.69	< 0.0001	Significant
Pure Error		*0.18*	5			
Cor Total		*21292.60*	29			

Std. Dev.- 2.69; R^2 – 99.49 %; Mean – 277.26; Adj R^2 – 99.01; C.V – 0.97 %; Pred R^2 – 97.07 %; Adeq Precision – 44.171

ANOVA for the response surface is shown in Table 5. The model *F* value of 208.94 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. In this case, the coefficients A, B, C , D, A^2, B^2, C^2, D^2, AB, AC, AD, BC, BD, CD were found to be highly significant. Values greater than 0.1 indicates model terms are not significant. The fit of the model was checked by the coefficient of determination R^2 for xylanase activity was calculated 0.9948, which is very close to 1, indicating that 99.48% of variability in the response could be explained by the model. The predicted R^2 value of 0.9701 was in reasonable agreement with the adjusted R^2 value of 0.9901. An adequate precision value greater than 4 is desirable. The adequate precision value of 44.171 indicates an adequate signal and suggests that the model can be to navigate the design space. In the present work, all the linear, interactive effects and square effects of *A*, B, C and D were significant for Xylanase production. The above model can be used to predict the Xylanase production within the limits of the experimental factors.

The interaction effects of variables on xylanase production were studied by plotting Contour plot & 3D surface curves against any two independent variables, while keeping another variable at its central (0) level. The 3D curves of the calculated response (Xylanase production) and contour plots from the interactions between the variables are shown in Figures.2 to 7. A circular contour plot indicates that the interactions between the corresponding variables are negligible, while an elliptical contour plot indicates that the interactions between them are significant. Fig.2 shows the dependency of Xylanase activity on $FeSO_4.7H_2O$ and yeast extract. The xylanase activity increased with increase in $FeSO_4.7H_2O$ to about 8.0817 mg/gds and thereafter Xylanase activity decreased with further increase in $FeSO_4.7H_2O$. The same trend was observed in Figure 3. Increase in yeast extract resulted in increase of Xylanase activity up to 35.057 mg/gds. This is evident from Fig. 2 and 5. Fig.3 and 5 shows the dependency of Xylanase activity on KH_2PO_4. The effect of KH_2PO_4 on Xylanase observed was similar to other variables. The maximum xylanase activity was observed at 21.70 mg/gds of KH_2PO_4. Fig 4, 6 and 7 shows the dependency of xylanase activity on $CoSO_4$.

The maximum xylanase activity was observed at 3.1717 mg/gds of $CoSO_4$. The optimum conditions for the maximum production of Xylanase were determined by response surface analysis and also estimated by regression equation. The optimum conditions are: $FeSO_4.7H_2O$ - 8.0817 mg/gds, yeast extract – 35.057 mg/gds, KH_2PO_4 - 21.700 mg/gds and $CoSO_4$ – 3.1717 mg/gds. The predicted results are shown in (Table-4). The predicted values from

the regression equation closely agreed with that obtained from experimental values. Along with nutrient optimized xylanase production, very poor carboxy methyl cellulase activity was detected in all experimental runs (Table-4). Fig.8 shows that the actual xylanase activity values agree well with the predicted response values.

Validation of the experimental model was tested by carrying out the batch experiments under optimal operation conditions. Three repeated experiments were performed, and the results are compared. The xylanase activity 355.0 IU/gds obtained from experiments was very close to the actual response 354.33 IU/gds, predicted by the regression model, which proved the validity of the model.

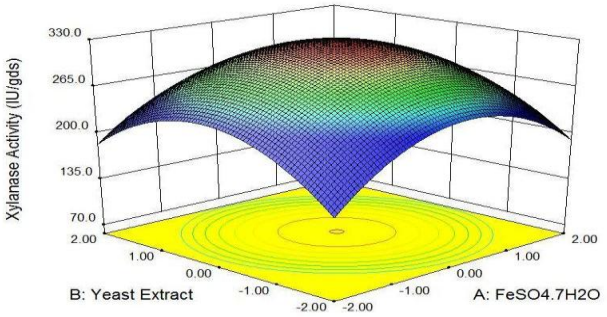

Fig. 2: Contour plot and 3D Surface plot showing the interactive effect of $FeSO_4.7H_2O$ and Yeast extract on Xylanase activity

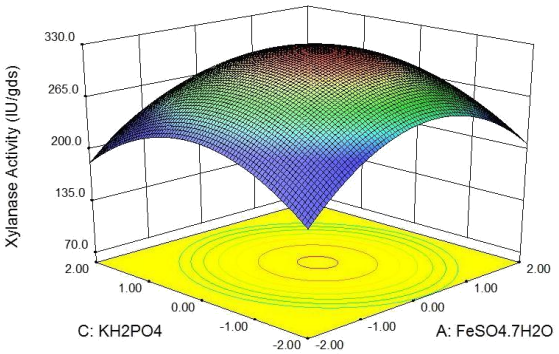

Fig. 3: Contour plot and 3D Surface plot showing the interactive effect of $FeSO_4.7H_2O$ and KH_2PO_4 on Xylanase activity

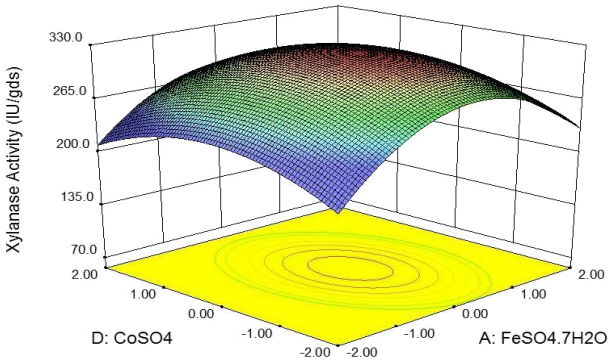

Fig. 4: Contour plot and 3D Surface plot showing the interactive effect of $FeSO_4.7H_2O$ and $CoSO_4$ on Xylanase activity

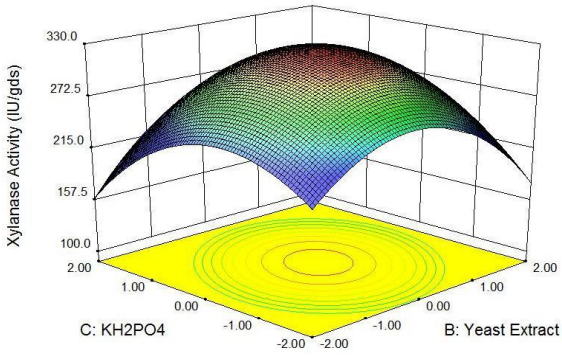

Fig. 5: Contour plot and 3D Surface plot showing the interactive effect of Yeast extract and KH_2PO_4 on Xylanase activity

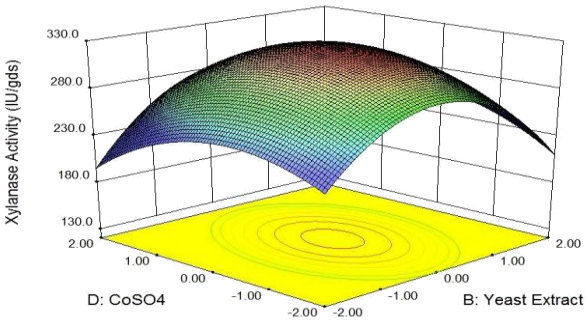

Fig. 6: Contour plot and 3D Surface plot showing the interactive effect of yeast extract and $CoSO_4$ on Xylanase activity

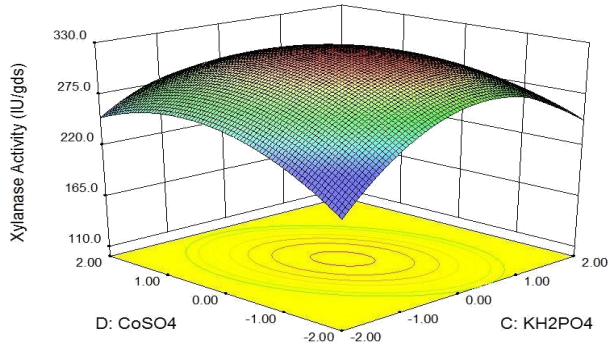

Fig. 7: Contour plot and 3D Surface plot showing the interactive effect of $KH_2PO_4$and $CoSO_4$ on Xylanase activity

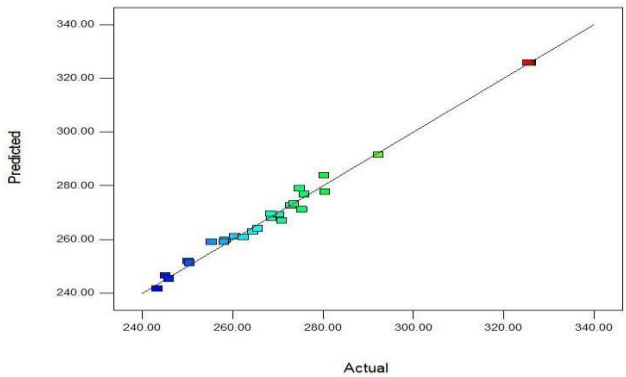

Fig. 8: Predicted Response Vs Actual value

4. CONCLUSIONS

In this work, the applied statistical methodology, a combination of Plackett–Burman design and central composite design proved to be efficient for optimizing xylanase enzyme production in solid-state fermentation. Plackett-Burman design was used to test the relative importance of medium components on Xylanase production. Among the twelve variables, $FeSO_4.7H_2O$, Yeast Extract, KH_2PO_4 and $CoSO_4$ were found to be the most significant variables. From further optimization studies, using RSM the optimized values of the variables for Xylanase production were as follows: $FeSO_4.7H_2O$ - 8.0817 mg/gds, yeast extract - 35.057 mg/gds, KH_2PO_4 - 21.700 mg/gds and $CoSO_4$ – 3.1717 mg/gds. Using the optimized conditions, the experimentally obtained Xylanase activity reaches 355.0 IU/gds. The results show a close concordance between the expected and obtained activity level. This study showed sugarcane bagasse is a cheaper and better substrate for xylanase production in solid state fermentation. Further work on optimization of process parameters such as temperature, pH, substrate concentration, innoculum size is currently underway.

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