Original Research Article

Optimization of Medium Composition and Cultural Conditions for Enhanced Endoxylanase Production from *Aspergillus fumigatus SKF-4* using Response Surface Methodology

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A B S T R A C T

Xylanases have multiple uses in industries for obtaining value added products from lignocellulosic biomass. In this study, wheat bran concentration, pH of the medium and incubation temperature were optimized using Rotatable central composite design based Response surface methodology for enhancing the production of endoxylanase from *Aspergillus fumigatus* SKF-4. In the model that was developed, linear and quadratic effect of all the independent variables and interaction effect of wheat bran concentration and the medium pH were significant. Model predicted optimized conditions as wheat bran- 2.5 (%w/v), pH- 5.7 and temperature- 32°C with endoxylanase production as 60.3 IU/ml. Endoxylanase production under experimental condition was 60.8 IU/ml which was at par with the predicted value. Under optimized conditions endoxylanase production was 1.5 times more than the unoptimized one. Response surface methodology could help in optimization of process parameters for enhanced production of endoxylanase from *Aspergillus fumigatus* SKF-4.

Introduction

Lignocellulosic materials (LCM) as source of organic residue are available in huge quantity in the world with a production of two billons tons annually (da Silva *et al.,* 2012). Lignocelluloses are made up of cellulose (30-50%), hemicellulose (20-40%) and lignin (15-25%) (Otieno and Ahring, 2012). Cellulose is the most abundant source of organic carbon in the world and hemicelluloses is next to it of which xylan is a major component. Xylan, a heteropolymer made up of β-1,4 linked xylopyranose units and may be further substituted by arabinoses, glucouronic acid, acetyl group and ferulic acid in varying proportion depending upon the source. Xylan can be converted to various value added products like biofuel, xylitol, xylose and xylooligosaccharides using xylanases and other debranching enzymes like arabinase, glucouronidnases, esterases.

Xylanases or endoxylanases (1,4-?-D-xylan xylanohydrolase, E.C. 3.2.1.8) are produced by various microorganisms like fungi,

bacteria, actinomycetes and yeasts. Among these microorganisms fungi have been exploited the most as they produce highest titer of these enzymes which are secreted into the medium thus requiring less downstream processing. They can also be grown on cheap and readily available substrates like lignocellulosic biomasses. Cost of xylanases is an important factor that is to be considered for its industrial production. Hence there is need to minimize the cost of enzyme by using a potent microorganism, cheap substrates and different approaches for optimization of medium components. One variable at a time (OVAT) approach for selection and optimization of medium component for enhanced xylanase production is a traditional technique which involves changing a particular variable keeping all other at constant level. This approach has several shortcomings like, it is tedius as it requires large no. of experiments, does not help in the determination of optimum concentration of variables; also it misses the interaction between the variables that often do occur (Li *et al.,* 2006). Hence, recent statistical techniques like Plackett Burman Design (PBD) and Response Surface Methodology are common statistical method for optimizing the process variables.

Response surface methodology (RSM) comprises a series of statistical experimental designs which helps in identification of individual and interactive effect of independent variables and for finding out the optimum conditions in a multivariable system in a efficient way (Houng *et al.,* 1989).

Response surface methodology has been used as a successful statistical tool for optimization of medium compositions in a fermentation process for enzyme production (Dobrev *et al.,* 2007, Senthilkumar *et al.,* 2005, Kim *et al.,* 2007).

Literature survey revealed scanty information regarding the optimization of medium components for enhanced endoxylanase production with the help of response surface methodology from *Aspergillus fumigatus*. This paper aimed at determining the optimum levels of wheat bran concentration, medium pH and incubation temperature for enhanced endoxylanase production from *Aspergillus fumigatus* SKF-4 using Rotatable Central Composite Design (RCCD) based Response Surface Methodology (RSM).

Materials and Methods

Microorganism

Aspergillus fumigatus SKF-4 isolated from degraded corn cob was used in this study (Amat *et al.,* 2016) and it was maintained on PDA (Potato Dextrose Agar) slants at 4°C until further use with regular subculturing.

Production and extraction of endoxylanase

The production of endoxylanase was carried out in the modified minimal medium (Reese and Mandel, 1963) containing (gram per liter) KH_2PO_4 2.0, $MgSO_4.7H_2O$ 0.3, $CaCl_2. 2H_2O$ 0.3, $FeSO_4.7 H_2O$ 0.005, $MnSO_4. H_2O$ 0.0016, $CoCl_2. 6 H_2O$ 0.002, $ZnSO_4.7 H_2O$ 0.0014, wheat bran 10.0, proteose peptone 5.0 and pH 5.0. The optimization studies were carried out by changing the concentration of wheat bran, pH and incubation temperature as per the experimental design. For optimization, 100ml Erlenmeyer flask containing 25ml of the medium was used. Each flask was inoculated with 1ml of spore suspension containing 10^7 spores prepared from 5 days old slant of *Aspergillus fumigatus* SKF-4. The flasks were incubated at 30 °C under static condition for 6 days. After incubation, fermentation broth was filtered and the filtrate was used as crude enzyme for estimation of endoxylanase activity.

Enzyme assays

Endo-β-1, 4 -xylanase in crude extract was estimated using beechwood xylan as substrate (Ghose and Bisaria, 1987). Reducing sugars released were measured by DNSA method (Miller, 1959). One enzyme unit of endoxylanase was expressed as 1μmol xylose formed per milliliter of culture filtrate per min during hydrolysis under standard assay condition.

Optimization of process variables for enhanced endoxylanase production using rotatable central composite design based response surface methodology

Based on results of "one variable at a time" approach studied earlier (Amat *et al.,* 2016) for endoxylanase production by *Aspergillus fumigatus* SKF-4, three important independent variables i.e. wheat bran, pH and temperature were selected for optimization by response surface methodology with rotatable central composite design (RCCD). Five different coded levels (-α, -1, 0, +1, + α) of each variables were taken for optimization study and the design was formulated with the help of experimental design module of Design Expert software version 10 (Stat Ease Inc., Minneapolis, USA). Twenty experimental runs were designed which includes eight factorial points, six axial points and six replicates at the centre point. Table 1 shows the ranges and levels of independent variables i.e. (A) wheat bran, (B) pH and (C) temperature selected for the current study in coded and decoded values.

Experimental Design for the study of optimization has been given in Table 2 where values of variables at various levels along with their respective responses have been presented. Data obtained from the response surface methodology(RSM) were subjected to analysis of variance (ANOVA) to get the

regression coefficients, prediction equations and case statistics using Design Expert software. The results obtained from RSM were fitted using a polynomial quadratic equation (Eq.1) to describe the mathematical relation between the variables and the response.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{23} BC + \beta_{13} AC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \qquad (1)$$

Where Y represents the predicted response, β0 is the constant; β_1, β_2, β_3 are linear coefficients; β12, β_{23}, β_{13} are interaction coefficients and β_{11}, β_{22}, β_{33} are quadratic coefficients (Singh *et al.,* 2014). The model validation was carried out for all the variables within the design space. Quality of fit of the polynomial model equation was expressed by the co-efficient of determination (R^2); statistical significance of the model was determined by application of Fisher's F-test and level of significance was given by p-value. Contour plots and corresponding response surface plots were drawn using Design Expert software to study the effect of independent variable and their interactions on response (endoxylanase production). An experiment was carried out to confirm the predicted optimum response using the selected optimum values of three variables.

Results and Discussion

Use of beechwood xylan as substrate for endoxylanase production is highly expensive, so alternate cheap and easily available substrates mainly lignocellulosic in nature like wheat bran was used for the present study. Wheat bran, pH and temperature were taken for optimization using rotatable central composite design based response surface methodology. These variables were tested at five levels. The experimental detail with respect to their coded and actual form is given in Table 1. A total of 20 experimental runs

having different combination of wheat bran, pH and temperature were carried out. Experimental design with actual and predicted response has been given in Table 2 which showed that there existed a wide variation in the responses (β-endoxylanase) in different experimental runs depending upon the levels of three independent variables in the medium, so there was need to optimize these variables for maximum production of β-endoxylanase.

Responses (β-endoxylanase) given in Table 2 are the average of two replicates. Maximum production of β-endoxylanase (62.3 IU/ml) was observed in run no. 11 whereas minimum level of production (44.9 IU/ml) was observed in run no. 8. Runs 1, 4, 5, 7, 9, 11 were the centre point which were taken for estimation of the error in the experimental design. The overall mean and standard deviation of the runs of the experimental design were 52.72 IU/ml and 2.18 respectively.

Multiple regression analysis was carried out on the experimental results (Table 2) to get a second order polynomial equation (Eq. 2) which could explain the β-endoxylanase production.

Endoxylanase = +57.98 + 2.72 x A + 1.54 x B + 2.02 x C + 2.24 x AB + 0.46 x AC - 0.44x BC-2.2xA^2-2.61xB^2-2.89xC^2 (2)

Where A, B, C are coded value of the variables i.e. wheat bran, pH and temperature respectively.

On observation of Eq. 2, it was found that effect of one factor is being represented by that factor with its co-efficient. Further, for two factors, it has been seen that, co-efficients with two factors represents interaction among the two factors while co-efficients with second order term shows the quadratic effect of the variables. Furthermore, two types of effect for endoxylanase production can be observed from the equation i.e. synergestic effect where a positive sign is present before the terms where as antagonistic effect showed by negative sign in front of the terms. The statistical significance of the model was carried out by Fisher's test for analysis of variance (ANOVA). Results of ANOVA of the response surface quadratic model of different variables for β-endoxylanase production has been presented in Table 3.

Model of endoxylanase production as a function of (A) wheat bran, (B) pH, and (C) temperature had probability value (p-value) 0.0004. Goodness of fit of the model was tested by R2 value. In this experiment R^2 value was 0.9 and the coefficient of variation (CV) value was 4.13%. From the Table 3 it can also be found that based on p-value (p-value < 0.05) model terms i.e. A, B, C, AB, A2, B2, C2 were found to be significant and AC, BC were found non-significant. Three variables under study i.e. wheat bran, pH, and temperature showed negative quadratic effect on endoxylanase production at various levels of significance. The lack of fit test in this study was found to be non-significant (p-value > 0.05) and adequate precision value which indicates signal to noise ratio was 8.5.

The adjusted R^2 and predicted R^2 value were 0.83 and 0.74 respectively which were close to each other. The contour plot and corresponding response surface plots described by second-order polynomial equation were generated in order to investigate the interaction among the variables and optimal level of variables for xylanase production (Fig. 1a- 1f). Each figure represents the effect of two variables on production of endoxylanase while the third variable was held at middle level. The statistically optimal values of variables are obtained when moving along the major and minor axis of the contour at the centre point yields maximum endoxylanase production.

Fig. 1a and 1f represents the response surface plot and corresponding contour plot depicting the effect of medium pH and wheat bran concentration simultaneously on endoxylanase production at the middle level of incubation temperature (30°C). It was found that at low pH (4.5) when concentration of wheat bran was varied from 0.7 % (w/v) - 2.5% (w/v), the endoxylanase production was slightly affected and it was observed in the range of 52.8 IU/ml- 56.0 IU/ml respectively. Increase in pH from 4.5 to 5.5 simultaneously increasing the wheat bran concentration from 0.7 % (w/v) - 2.5%(w/v) resulted in higher endoxylanase production from 52.1 IU/ml- 59.80 IU/ml respectively which was found to be higher as compared to the enzyme produced at pH 4.5. From the Fig. 1a and 1f maximum production of endoxylanase (59.9 IU/ml) was observed in the wheat bran concentration 2.4- 2.5% (w/v) and pH 5.6-5.8. Similarly Fig. 1c and 1d depicts effect of wheat bran concentration and incubation temperature on endoxylanase production while pH of the medium was kept at middle level (5.0). At low wheat bran concentration (1% w/v), there was gradual increase in endoxylanase production from 50.7 IU/ml at 25°C to 54.1 IU/ml at 35°C whereas increase was more significant at high wheat bran concentration (2.2% w/v) from 53.6 IU/ml at 25°C to 58.3 IU/ml 35°C. From Fig. 1 c and 1d, optimum temperature and wheat bran concentration for maximum endoxylanase production (59.3 IU/ml) was observed in the range 2.1-2.3% (w/v) to 31.5°C -32.5°C respectively.

Next, Fig. 1e and 1f depicts the effect of pH and incubation temperature on endoxylanase production while wheat bran concentration was kept at middle level (1.6% w/v). It was observed that at low temperature (27°C) increase in pH from 4.0- 6.0 resulted in increase in endoxylanase production from 51.3 IU/ml to 54.9 IU/ml respectively which

was more than when pH was increased 4.0-6.0 at high temperature (33°C) resulting in endoxylanase production from 54.3 IU/ml to 56.7 IU/ml respectively. From Fig. 1e and 1f, optimal region of pH and temperature for maximum endoxylanase production (58.5 IU/ml) was found to be between 5.1-5.4 and 31°C -32.4°C respectively.

Optimization and validation of model equation

Process variables were numerically optimized using Design-Expert® software version 10 for β-endoxylanase production (Table 4). In the validation or confirmation of the model under the optimization step in rotatable central composite design, the variables were set according to specific requirements of the model. For the maximum yield of endoxylanase production, the goal of the response was set as 'maximize' and the level of variables i.e. wheat bran, pH and temperature were set as 'in range', means from -1 to +1 level. The software provided two solutions, one with medium comprising wheat bran - 2.5% (w/v), pH- 5.7 and temperature- 32°C predicted maximum endoxylanase production of 60.3 IU/ml and this solution had the maximum desirability of 0.9 and was taken for validation of the model. The model was validated by carrying out production of endoxylanase under optimized conditions and it was found that endoxylanase production of 60.8 IU/ml was achieved which was close to the predicted value of 60.3 IU/ml.

The cost of endoxylanase is an important factor for its industrial applications. So to make the enzyme cost effective, there is need to use cheap substrates like lignocellulosic biomass based agri-residue, optimization of medium components (C-source, N- sources) and cultural conditions using different statistical approaches. Keeping these things

into view, in this study, instead of using pure substrate like beechwood xylan, wheat bran, a cheap lignocellulosic substrate as carbon source was used. Along with wheat bran, pH and temperature which are important cultural conditions affecting the enzyme production were studied. One variable at a time (OVAT) approach for selection and optimization of medium component for enhanced xylanase production involves changing a particular

variable keeping all other at constant level. This approach does not help in the determination of optimum concentration of variables; also it misses the interaction between the variables that often do occur (Li *et al.,* 2006). Hence, recent statistical techniques like Response Surface Methodology (RSM) are commonly used for optimization of process variables.

Table.1 Minimum and maximum levels of independent variables used for rotatable central composite design matrix

Independent Variables	Symbols			Coded levels		
		-α	-1	0	+α	+1
Wheat bran (%w/v)	A	0.1	0.7	1.6	2.5	3.1
pH	B	3.3	4	5	6	6.7
Temperature (°C)	C	21.5	25	30	35	38.4

Table.2 Rotatable Central Composite Design experimental design matrix and the respective endoxylanase activity by *Aspergillus fumigatus* SKF-4

Run	Independent variable			Response(IU/ml)	
	A: Wheat bran (%w/v)	B: pH	C: Temperature (°C)	β-endoxylanase	
				Actual	Predicted
1	1.6	5	30	57.7	58.0
2	0.7	4	25	45.3	46.3
3	1.6	6.7	30	52.3	53.2
4	1.6	5	30	54.3	58.0
5	1.6	5	30	58.7	58.0
6	3.1	5	30	57.2	56.3
7	1.6	5	30	58.5	58.0
8	2.5	4	25	44.9	46.3
9	1.6	5	30	56.2	58.0
10	2.5	4	35	51.3	52.1
11	1.6	5	30	62.3	58.0
12	1.6	3.3	30	50.0	48.0
13	2.5	6	25	54.7	54.7
14	2.5	6	35	59.0	58.8
15	0.7	4	35	49.5	50.2
16	1.6	5	21.6	47.4	46.4
17	0.1	5	30	47.4	47.2
18	0.7	6	25	45.8	45.7
19	0.7	6	35	48.6	48.0
20	1.6	5	38.4	53.3	53.2

Table.3 Analysis of variance (ANOVA) for response surface quadratic model of different factors for β-endoxylanase production

Source	Sum of squares	Degree of freedom	Mean square	F-Value	p-Value (Prob > F)
Model	474.38	9	52.71	11.13	0.0004
A-Wheat Bran	101.23	1	101.23	21.37	0.0009
B- pH	32.19	1	32.19	6.80	0.0262
C- Temperature	55.87	1	55.87	11.79	0.0064
AB	40.05	1	40.05	8.45	0.0156
AC	1.71	1	1.71	0.36	0.5612
BC	1.53	1	1.53	0.32	0.5822
A^2	69.94	1	69.94	14.76	0.0033
B^2	98.14	1	98.14	20.72	0.0011
C^2	120.57	1	120.57	25.45	0.0005
Residual	47.38	10	4.74		
Lack of Fit	11.14	5	2.23	0.31	0.8893
Pure Error	36.23	5	7.25		
Total	521.75	19			
Mean	52.72		**R^2**	0.90	
SD^a	2.18		**Adjusted R^2**	0.83	
CV%^{b}	4.13		**Predicted R^2**	0.74	

[a] Standard Deviation, [b] Co-efficent of Variation

Table.4 Optimum value of variables for endoxylanse production by *Aspergillus fumigatus* SKF-4

Variables	Goal	Optimum level	Desirability
Wheat bran (%w/v)	In range	2.5	0.9
pH	In range	5.7	-do-
Temperature (°C)	In range	31.9	-do-
Response	**Goal**	**Predicted value**	**Observed value**
Endoxylanase (IU/ml)*	Maximize	60.8	60.3

* International Unit/milliliter

Fig.1 (a) Contour plot of combined effect of wheat bran and pH on endoxylanase (IU/ml) production by *Aspergillus fumigatus* SKF-4 with constant temperature
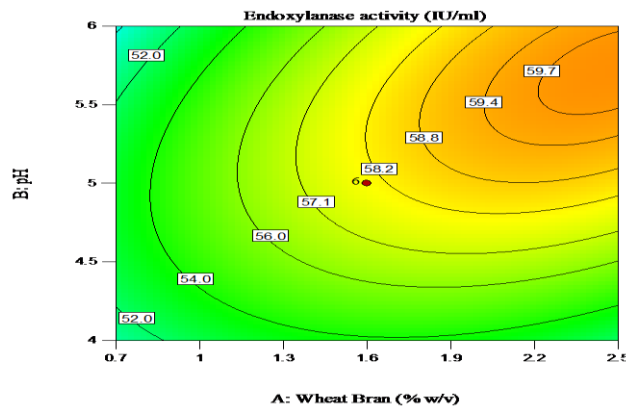
Fig.1 (b) Response surface plot of combined effect of wheat bran and pH on endoxylanase (IU/ml) production by *Aspergillus fumigatus* SKF-4 with constant temperature
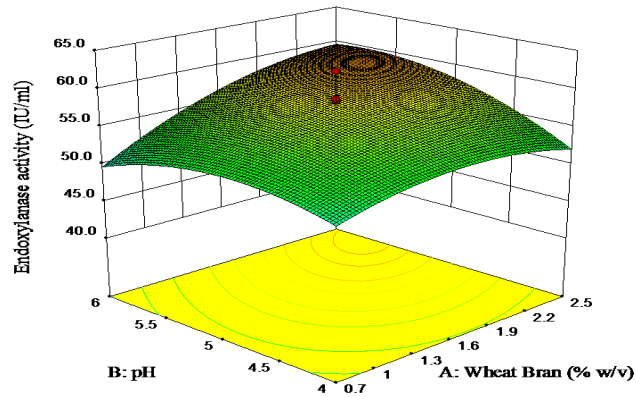

Fig.1 (c) Contour plot of combined effect of wheat bran and temperature on endoxylanase (IU/ml) production by *Aspergillus fumigatus* SKF-4 with constant pH
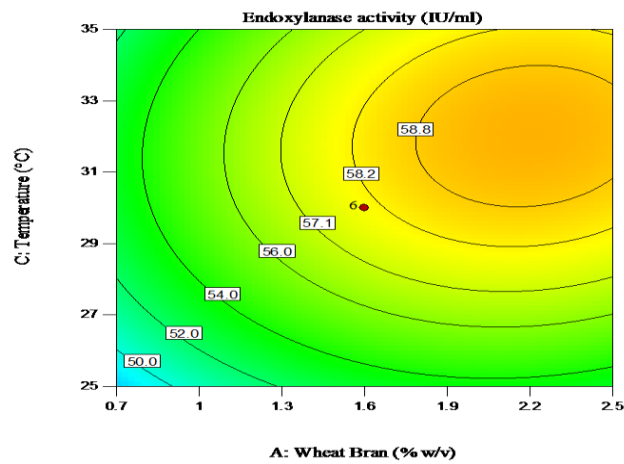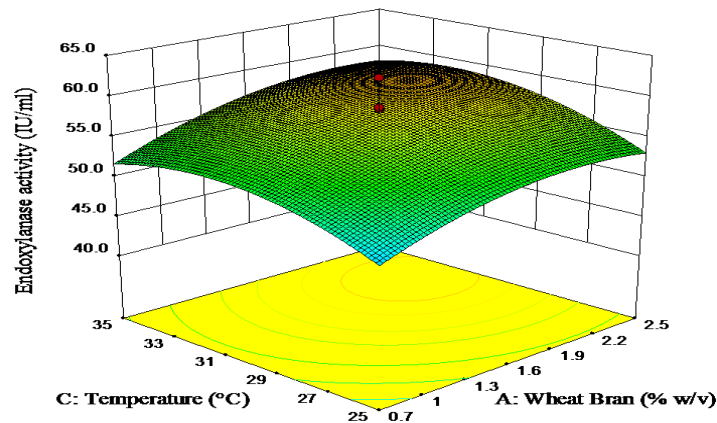

Fig.1 (d) Response surface plot of combined effect of wheat bran and temperature on endoxylanase (IU/ml) production by *Aspergillus fumigatus* SKF-4 with constant pH

Fig.1 (e) Contour plot of combined effect of pH and temperature on endoxylanase (IU/ml) production by *Aspergillus fumigatus* SKF-4 with constant wheat bran concentration
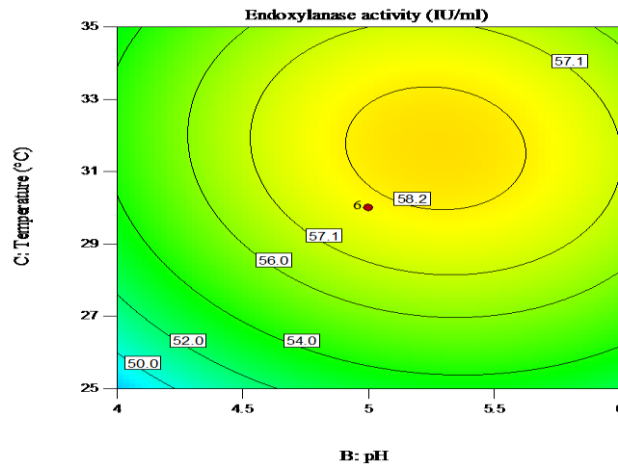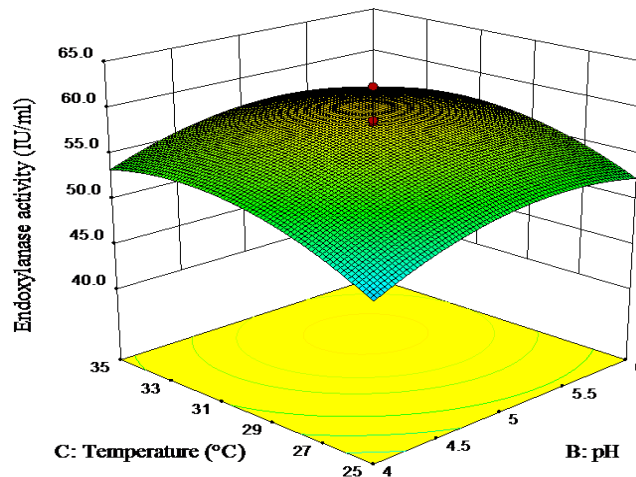

Fig.1 (f) Response surface plot of combined effect of pH and temperature on endoxylanase (IU/ml) production by *Aspergillus fumigatus* SKF-4 with constant wheat bran concentration

Wheat bran, pH and temperature were optimized using rotatable central composite design based response surface methodology. RSM is a collection of statistical and mathematical techniques, normally used for modeling and analyzing problems in which several variables influencing the response of interest may be tested and aim is to optimize the response (Myers and Montgomery, 2009). Rotatable Central Composite Design (RCCD) has been the design of choice for optimization studies in biochemical processes due to its obvious advantages of ratability and the

ability to analyze the interaction effects over mixture design (Ferreira *et al.,* 2009).

Ranges of the three variables were decided for RCCD design based on one factor at a time approach. Wheat bran concentration range was determined by taking 0.1 to 3% (w/v) wheat bran in Reese minimal medium. In the range of wheat bran concentration from 0.7%-2.5%, higher production of endoxylanase was observed (date not shown). Hence this range was selected for RCCD design. Similar range i.e. 1 to 2% (w/v) of wheat bran concentration

was selected by Saha and Ghosh (2014) for optimization using RCCD based response surface methodology.

Similarly for the RCCD design, pH range 4.0-6.0 and temperature of 25-35°C was selected, as in this range higher production of endoxylanase was observed (date not shown). Saha and Ghosh (2014) also selected pH range 3.0-6.0 for optimization by using RCCD by response surface methodology. Li *et al* (2007) selected 25-35°C for optimization of temperature using central composite design. From Table 3, it was found that model of endoxylanase production as a function of (A) wheat bran, (B) pH and (C) temperature was found to be significant (p-value< 0.05). Goodness of fit of the model is tested by R^2 (co-efficient of determination) value. R^2 value generally exist in the range of 0- 1.0. The closer the value to 1.0, the more fit is the experimental data to the model (Singh *et al.,* 2014). In this study R^2 value of 0.9 indicated that 90 % variability of response (β-endoxylanase) is attributed to the independent variables and only 10% variation could not be explained by the model. The coefficient of variation (CV) indicates the degree of precision with which the treatments are compared and the higher the value of CV, the lower the reliability of the experiment performed. The lower value of the co-efficient of variation (CV% = 4.13) in this case showed that the experiments conducted were precise and reliable. The predicted R^2 and adjusted R^2 value were close to each other which indicate that actual value and predicted values are close to each other in this experiment. From the Table 3, it can also be found that based on p- value (p-value<0.05) model terms i.e. A, B, C, AB, A^2, B^2, C^2 were found to be significant and AC, BC were found non-significant. Wheat bran, pH and temperature had significant (p-value <0.05) linear effect on endoxylanase production with trend, wheat bran > pH >temperature. Wheat bran (A)

showed significant interaction with medium pH (p-value <0.05), however, interaction between wheat bran and temperature and between pH and temperature was not significant (p-value>0.05). All the three variables showed significant negative quadratic effect on endoxylanase production. Similar findings were reported by other researcher where Rotatable Central Composite Design (RCCD) was used to optimize the concentration of wheat bran, $NaNO_3$, Tween-80 and Yeast extract in the fermentation medium for enhanced xylanase production from *Aspergillus flavus* DFR-6 in submerged cultivation and found that wheat bran showed significant linear effect and significant negative quadratic effect on endoxylanase production (Pal and Khanum, 2011). While using RCCD based response surface methodology for optimization of wheat bran, pH of the medium and temperature for enhanced endoxylanase production by *Penicillum citrinum* xym2, it was found that wheat bran, pH of the medium and temperature showed significant negative quadratic effect on endoxylanase production and wheat bran and pH of the medium had significant interaction and there was no significant interaction between pH of the medium and incubation temperature on endoxylanase production (Saha and Ghosh, 2014). Optimization of different variables i.e. wheat bran, pH and cultivation time using central composite rotary design based Response surface methodology revealed that wheat bran and pH had significant negative quadratic effect, wheat bran and pH of the medium had significant linear effect on production of endoxylanase by *Aspergillus awamori* ZH-26 (Li *et al.,* 2006). The lack of fit test in this study was found to be insignificant (p-value> 0.05) which indicated the absence of block effect in the experiment. Adequate precision value indicates signal to noise ratio and a ratio greater than 4 is desirable. Hence in this model adequate

precision value of 8.505 acknowledged an adequate signal.

Contour plot and corresponding response surface plots described by second-order polynomial equation were generated in order to investigate the interaction among the variables and optimal level of variables for xylanase production (Fig. 1a- 1f). From Fig. 1a and 1b, it can be observed that at fixed temperature, at both high and low pH; increase in wheat bran concentration within the experimental region was beneficial for increasing the endoxylanase production. These results agree well with the report that wheat bran could effectively induce higher xylanase production by *Streptomyces* sp (Beg *et al.,* 2000). Also the shape of the corresponding contour plots indicates whether the mutual interactions between the independent variables are significant or not. An elliptical nature of the contour plots indicates that the interactions between the independent variables are significant (Li *et al.,* 2007). In this case contour plot is elliptical hence by visual observation it can be observed that there was significant interactions between pH and wheat bran concentration. Similarly from Fig. 1c and 1d, it can be inferred that near optimum wheat bran concentration, increase in temperature within the experimental range has positive effect on endoxylanase production and Fig. 1e and 1f depict that at optimum pH and temperature wheat bran will have positive effect in endoxylanase production indicating organism can utilize the substrate more efficiently for production of endoxylanase under optimum pH and temperature.

In the optimization and validation step, the experimental response (endoxylanase production) of 60.8IU/ml was in close agreement with the predicted response of 60.3IU/ml, hence the model was successfully validated and therefore the model developed

was reliable for predicting the optimal conditions for variables influencing endoxylanase production by *Aspergillus fumigatus* SKF-4. The optimization of process variables for endoxylanase production using RCCD based Response surface methodology resulted in 1.5 times increase in endoxylanase production from *Aspergillus fumigatus* SKF-4 than the unoptimized one. These results from the study also indicate that at optimum temperature and pH, there was increase in endoxylanase production which could be due to better growth of the microorganism and production of enzyme for utilization of substrate and also accounted for the fact that enzyme involved in substrate degradation generally inducible in nature and are produced in presence of the substrate (Schlegel, 1989). Similar optimal temperature and pH were obtained by central composite design as 32.5°C and 5.5 respectively for enhanced production of endoxylanase from *Aspergillus niger* ITCC 7678 (Singh *et al.,* 2013). The endoxylanase produced by *Aspergillus fumigatus* SKF-4 was four times more than *Penicillium oxalicum* ZH-30 (Li *et al.,* 2007) using the RSM optimized medium containing wheat bran.

Acknowledgements

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