academicJournals

Vol. 9(27), pp. 1699-1705, 8 July, 2015
DOI: 10.5897/AJMR2015.7557
Article Number: A7B79AA54168
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Response surface optimization of xylanase production by indigenous thermoalkalophillic *Bacillus* sp.

Shanthi V.* and Roymon M. G.

Department of Microbiology, St. Thomas College, Bhilai, Chhattisgarh- 490006; India.

Received 25 April, 2015; Accepted 6 July, 2015

Xylanases are an important class of hydrolytic enzymes with a wide range of industrially important applications especially in paper and pulp industry. The present study aimed to take the advantage of statistical approach of optimization to investigate the interactive effects of prominent process factors involved in xylanase production. A novel bacterial isolate *Bacillus* sp. MCC 2727 was isolated from soil possessing xylanase producing ability at alkaline pH (9.2) and optimum temperature of 50°C. Using the conventional one-factor-at-a-time method, low cost agricultural waste; wheat bran, combination of peptone and yeast extract served as best carbon and nitrogen sources, respectively. $MgSO_4$ as metal salt and xylan as additive increased the xylanase productivity. Central composite design and response surface methodology were used to optimize these significant process parameters and for evaluation of interactive factors. Maximum xylanase activity of 205.3 IU/ml was obtained with 5% wheat bran, 1% each of yeast extract, peptone, xylan and $MgSO_4$ which was in consensus with the predicted value (207.2 IU/ml) which proved the validity and the accuracy of the statistical approach of optimization.

Key words: Xylanase, response surface methodology, central composite design, optimization.

INTRODUCTION

Hemicelluloses are considered as the second most abundant polysaccharides in nature after cellulose. The most common hemicelluloses found in plants and trees are xylan. Xylan is also found in solid agricultural and agro industrial residues (Collins et al., 2005). These solid wastes can be potentially used to produce various industrially useful products like biofuels, animal feed, enzymes etc. (Abo-State et al., 2013). Xylanases are the most important xylan degrading enzymes. They have created a niche for themselves in the field of enzyme technology for the good reason that they have immense biotechnological applications. Most of the industrial applications including paper and pulp require that xylanases have a high temperature and pH optima. Although efficient producers; fungal xylanases are associated with a plethora of problems. Bacteria are more appealing compared to fungi as they are very easy to cultivate. Also bacterial xylanases have a high temperature and pH optima (Subramaniyan and Prema, 2002).

The industrial applicability of enzymes is determined by its production costs. The process economy mainly relies on the optimization of the media components leading to higher yields (Kanagasabai et al., 2013). The

*Corresponding author. E-mail: shanti_162@rediffmail.com. Tel: 9406103610.

conventional method of optimization using one- variable-at-a-time is both tedious and time consuming. One of the popular methods for optimization of different parameters affecting productivity of enzymes is response surface methodology (RSM). In recent years, RSM has found significant importance in various biochemical and biotechnological processes (Bas and Boyaci, 2007). The inability of the conventional method to explain the extent of effect of variables on the response and also the interactive effects of the process parameters can be overcome by a more satisfactory method of statistical optimization. Central Composite Design and Response Surface Methodology are efficient strategies of optimization of medium components.

MATERIALS AND METHODS

Microorganism

Several soil samples collected from local Bhilai region of Durg District, Chhattisgarh; India was screened for potent xylanase producing bacterial strains. The preliminary screening was performed on xylan agar medium (pH 9.2) and incubated at 50°C for selection of alkalophillic thermostable isolates. The secondary screening of the isolates from the preliminary screening procedures was performed by Congo red plate assay for the detection of clear zone around the colonies. Isolate showing maximum zone of xylan hydrolysis was selected and sent to National Center for Cell Science (NCCS), Pune; Maharashtra, India for identification on the basis of phenotypic and molecular characterization. The pure culture was maintained and stored on nutrient agar slants at 4°C for further use.

Xylanase production by submerged fermentation

20 ml of liquid basal medium containing 0.5% Birchwood xylan, 0.5% Peptone, 0.5% yeast extract, 0.2% K_2HPO_4 and 0.01% $MgSO_4.7H_2O$ in 100 ml Erlenmeyer flask was sterilized by autoclaving at 121ºC for 20 min and cooled to room temperature. The pH of the medium was adjusted to 9.2 by adding sterile 10% Na_2CO_3 solution after sterilization. The flask was inoculated with 1% v/v of 18 h old fresh inoculum and incubated at 50°C for 48 h on a rotary shaker at 150 rpm. After the desired interval, the contents were subjected to enzyme extraction.

Enzyme extraction and xylanase assay

Crude enzyme was extracted from the fermentation broth by centrifugation at 10,000 *g* for 10 min at 4°C (REMI, Cooling centrifuge; C-24BL, India). The supernatant obtained was used as a source of crude xylanase enzyme. The quantitative estimation of xylanase activity was done with some modifications according to the procedure of Sharma et al. (2013). A reaction mixture was prepared containing 0.5 ml supernatant and 0.5 ml of 1% Birchwood xylan (HiMedia, India) solution prepared in 50 mM Glycine-NaOH buffer (pH 9.2). The reaction was terminated by adding 3 ml DNS reagent after incubating at 55°C for 10 min. The mixture was kept in boiling water for 5 min and cooled. The amount of reducing sugar (xylose equivalents) liberated was determined according to Miller (1959). One unit (IU) of xylanase activity is defined as the amount of enzyme required to release 1 µmol of xylose per minute under the specified assay conditions. The results presented are the mean of three values obtained from experiments performed in triplicates.

Optimization of xylanase production

The optimization studies included both physico-chemical parameters and nutritional parameters. The different important parameters governing the production of xylanase were optimized by the conventional one-factor-at-a-time method (Results not shown).

The best carbon source was selected from about twelve different carbon sources which included both simple and complex forms of carbon. Nine different Nitrogen sources including both organic and inorganic forms of nitrogen were used for optimization of best nitrogen source. Optimization of additives and metal salts on production of xylanase enzyme was also optimized. Using the conventional method of optimization, the important factors which affected xylanase production were wheat bran (best carbon source), xylan (additive), $MgSO_4$ (best metal salt), peptone and yeast extract (best carbon source).

Response surface methodology (RSM)

A statistical method, Central Composite Design (CCD) was adopted to optimize five different variables: carbon source (wheat bran), nitrogen source (peptone and yeast extract) $MgSO_4$ and additive (xylan). Each variable was taken at five coded levels (-α, -1, 0, +1, +α). The variables and their coded values are shown in Figure 1. The optimization using RSM by CCD is an efficient statistical method for optimization of process variables and also helps to evaluate the interaction between the dependent variables. The statistical software package Design- Expert (version 9.0.3.1, Stat-Ease, Minneapolis; USA) was used to design the experiment and calculate the coefficients. The central coded values of all the variables were taken as '0'. The statistical significance of the linear and quadratic effects generated by the model equation was tested by applying F-test. Analysis of Variance (ANOVA) was used to estimate the various statistical parameters.

RESULTS AND DISCUSSION

Isolates from different soil samples were screened for their xylanolytic property on xylan agar medium in the preliminary screening process. Ten bacterial isolates showed good growth on the medium when grown at specified conditions indicating alkalophillic and thermostable nature of which one of the isolate produced maximum clear zone of xylan hydrolysis in the secondary screening by Congo red method. This selected isolate was motile, catalase positive, Gram positive thin rods with sub terminal ellipsoidal spores. The identification reports from NCCS, Pune; Maharashtra, India confirmed the strain belonged to Bacillus sp. and was given the accession number MCC 2727 (Table 1). The identification reports from NCCS, Pune; Maharashtra, India confirmed the strain belonged to Bacillus sp. and was given the accession number MCC 2727.

Optimization using RSM

The effect of five different variables (Wheat bran, Yeast extract, Xylan, $MgSO_4$ and Peptone) on xylanase enzyme production was evaluated by CCD and RSM. The CCD package helps to study interactive effect between the

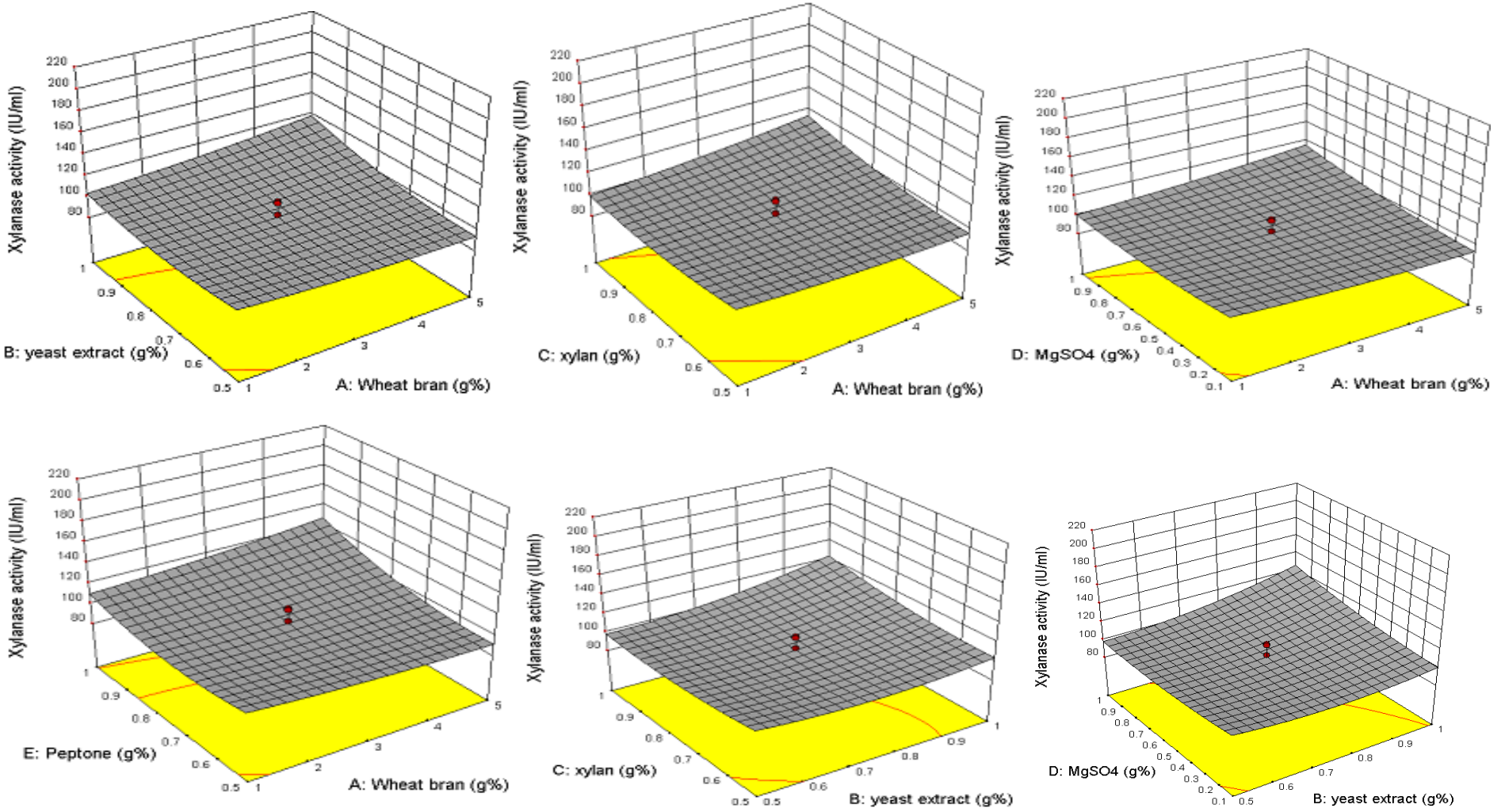

Figure 1. 3-D Response curves of xylanase production from *Bacillus* sp. MCC 2727, showing interactions between various variables.

different variables while the RSM helps to predict and evaluate the optimum variable concentrations aiding in obtaining high enzyme yields (Garai and Kumar, 2013). In the present study, the significance of coefficients of both linear and quadratic terms was tested through the p value. Analysis of variance (ANOVA) results of the CCD model are shown in Figure 2. P values < 0.05 are considered significant and p values < 0.0001 are highly significant (Zambare, 2011). The coefficients of linear model term values B (Yeast extract), C (Xylan), D ($MgSO_4$) and E (xylan) were found to

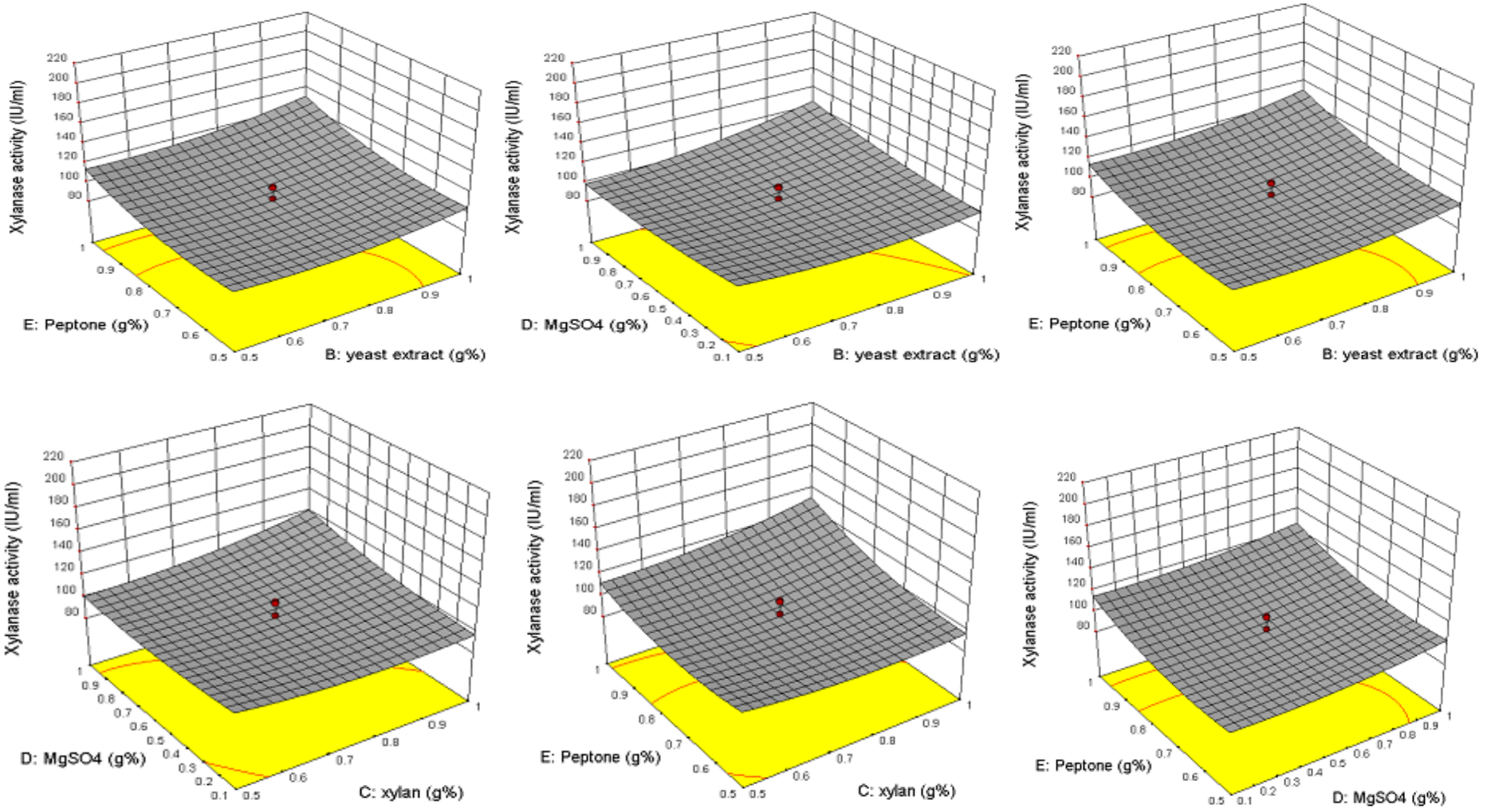

Figure 1. Contd.

significantly affect the productivity of enzyme. This implies that these variables may be acting as limiting medium components indicating that even little change in their concentrations will affect the xylanase production.

The p value of coefficients of quadratic model terms except BE and DE were found to be significant indicating interactive effect between most of the process variables.

The model F value is 32.53 implying the significance of the model. There is only 0.01% chance that F value this large could occur due to

Table 1. Variables and their coded levels for CCD.

Variable (g%)	Code	Coded level of variables				
		-α	-1	0	+1	+α
Wheat bran	A	-1.75	1.0	3.0	5	7.75
Yeast extract	B	0.15	0.5	0.75	1	1.34
Xylan	C	0.15	0.5	0.75	1	1.34
MgSO4	D	-0.52	0.1	0.55	1	1.62
Peptone	E	0.15	0.5	0.75	1	1.34

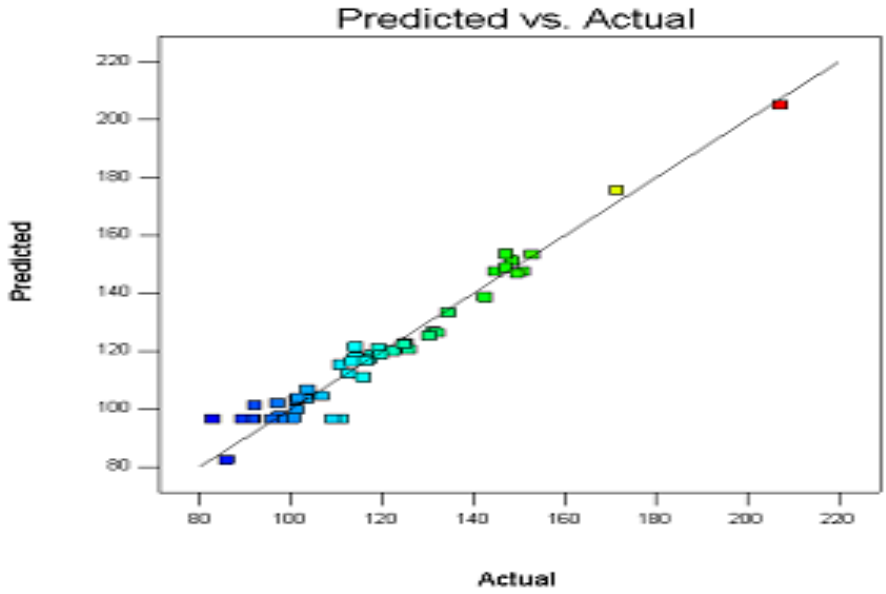

Figure 2. Parity plot of xylanase production showing correlation between predicted and experimental values.

noise.

The coefficient of determination; R^2 and Adjusted R^2 were calculated to check the Goodness of fit of the model. The values of R^2 lie in the range of 0.0-1.0 (Amani et al., 2007). The R^2 value for this model was found to be 0.9573 which is very close to 1.0 implying the accuracy of the model and better response prediction (Table 2).

The second order regression equation showing the relationship between Y (Xylanase Activity) and the five process variables in terms of coded values is given as:

$$Y= 96.3699 + 1.74523A + 6.62775B + 3.54476C + 5.60661D + 11.1619E + 5.82281AB + 5.63906AC + 5.84406AD + 6.69281AE + 6.30531BC + 7.26531BD + 2.10031BE + 6.68656CD + 6.52531CE + 0.357812DE + 2.04623A^2 + 6.1121B^2 + 5.01608C^2 + 4.15871D^2 + 9.2764E^2$$

Higher model R^2 values however always do not indicate model accuracy as inclusion of extra non-significant variables may also lead to their higher values. Adjusted R^2 values are therefore considered which manages the R^2 values according to the number of model variables (Cooman and Bahrin, 2011). The more the number of extra insignificant variables, the decreased will be the adjusted R^2 value. Ideally, for the model to be highly significant and for better response prediction, the value of R^2 should be as close to as possible to Adjusted R^2 value. The R^2 value of 0.9279 indicates that 92.79% of the variability of the response can be explained by this model. The signal to noise ratio is measured by adequate precision value which for this model is 29.441 which is greater than the desirable value of 4.0 indicating adequate signal. Simultaneously lower values of coefficient of variation (CV= 5.38%) indicates high precision and reliability of the design model.

The interactive effect between any two independent variables on xylanase production keeping the remaining variables at their central coded level can be studied from the 3D surface curves and contour plots. Elliptical contour plots indicate significant interaction between the corresponding variables while insignificant interaction by

Table 2. Analysis of variance (ANOVA) for the CCD design model.

Source	Sum of squares	Degree of freedom	Mean of squares	F Value	P Value
Model	26839.16	20	1341.96	32.53	0.0001*
A	131.93	1	131.93	3.20	0.0842
B	1902.64	1	1902.64	46.12	0.0001*
C	544.25	1	544.25	13.19	0.0011*
D	1361.53	1	1361.53	33.01	0.0001*
E	5396.39	1	5396.39	130.82	0.0001*
AB	1084.96	1	1084.96	26.30	0.0001*
AC	1017.57	1	1017.57	24.67	0.0001*
AD	1092.90	1	1092.90	26.49	0.0001*
AE	1433.40	1	1433.40	34.75	0.0001*
BC	1272.22	1	1272.22	30.84	0.0001*
BD	1689.11	1	1689.11	40.95	0.0001*
BE	141.16	1	141.16	3.42	0.0745
CD	1430.72	1	1430.72	34.68	0.0001*
CE	1362.55	1	1362.55	33.03	0.0001*
DE	4.10	1	4.10	0.099	0.7549
A2	232.67	1	232.67	5.64	0.0244*
B2	2075.93	1	2075.93	50.32	0.0001*
C2	1398.17	1	1398.17	33.89	0.0001*
D2	961.06	1	961.06	23.30	0.0001*
E2	4781.79	1	4781.79	115.92	0.0001*
Lack of fit	538.56	22	24.48	0.26	0.9929
Residual	1196.30	29	41.25		
Pure error	657.74	7	93.96		

Std Dev: 6.42, Mean: 119.42, C.V (%): 5.38, R2: 0.9573, Adj R2: 0.9279, Pred R2: 0.9010, Adeq Precision: 29.441, * - Significant terms.

circular contour plots (Narang et al., 2001). Figure 1 show the interactive responses between process variables with wheat bran as carbon source, xylan as additive, peptone and yeast extract as nitrogen source and $MgSO_4$ as metal ion. The results indicate significant increase in enzyme production when wheat bran concentrations were increased from 1 to 5%. This increased activity may be due to the fact that wheat bran consists of about 40% xylan which acts as an essential substrate for xylanase enzyme (Thiago and Kellaway, 1982). Significant interaction between wheat bran and xylan may be attributed to the gene expression pattern induced by xylan suggesting inducible nature of xylanase (Parachin et al., 2009; Hiremath and Patil, 2011).

The parity plots help to determine the correlation between the predicted and the experimental values. The parity plot in Figure 2 shows a satisfactory correlation indicated by the clustering of points around the diagonal as clustering of points around the diagonal indicate good fit of model.

Experimental validation of model

From the surface plots, it was concluded that xylanase production increased with increase in the variable concentrations. The design expert model predicted the optimum concentrations of medium components as 5, 1, 1, 1 and 1 g% for wheat bran, yeast extract, xylan, $MgSO_4$ and peptone respectively for maximum xylanase production by numerical optimization step in CCD. The maximum Xylanase activity predicted with these variables at their optimum concentrations was 207.2 U/ml. Experiment in triplicates was conducted using the predicted optimized conditions by RSM for verification of model results. The experimental xylanase activity was determined to be 205.3 IU/ml which was found very close to that of predicted value.

Conclusion

In the present study, thermoalkalophillic bacteria; *Bacillus* sp. MCC 2727 was identified as an important and potent indigenous strain possessing xylanolytic characteristics. Optimization of medium components using RSM and CCD appears to be an effective and successful tool which aims at increasing enzyme productivity using time saving statistical approach. The optimum conditions

predicted by the model were wheat bran (5 g%), yeast extract and peptone (1 g% each), $MgSO_4$ (1 g%) and xylan (1 g%) which on validation produced xylanase activity of 205.3 IU/ml. These results were in good confirmation with the predicted values thus proving the accuracy of the model. Considering these results, it can be suggested that the present organism can prove to be an important source for commercial production of xylanase enzyme for applications requiring alkaline and thermophilic conditions.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

Abo-State MAM, Ghaly MF, Abdellah EM (2013). Production of cellulases and xylanase by thermophilic and alkalophilic bacterial strains isolated from agricultural wastes. World Appl. Sci. J. 22(11):1603-1612.

Amani MD, El Ahwany, Youssef AS (2007). Xylanase production by *Bacillus pumilus*: Optimization by statistical and immobilization methods. Res. J. Agric. Biol. Sci. 3(6):727-732.

Bas D, Boyaci IH (2007). Modeling and Optimization I: Usability of response surface methodology. J. Food Eng. 78: 836-845.

Collins T, Gerday C, Feller G (2005). Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol. Rev. 29: 3- 23.

Cooman G, Bahrin G (2011). Optimization of xylanase production by *Streptomyces* sp. P12-137 using response surface methodology and central composite design. Ann. Microbiol. 61:773-779.

Garai D, Kumar V (2013). Response surface optimization for xylanase with high volumetric productivity by indigenous alkali tolerant *Aspergillus candidus* under submerged cultivation. 3 Biotechnol. 3:127-136.

Hiremath KS, Patil CS (2011). Isolation, production and characterization of alkalothermostable xylanase from newly isolated *Bacillus* sp. Int. J. Biotechnol. Appl. 3(1):48-51.

Kanagasabai V, Tangavelu V (2013). Response surface Methodological Optimization of the medium components for production of xylanase under SSF by *Aspergillus fumigatus*. J. Adv. Sci. Res. 4(2): 13-20.

Miller GL (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugars. Anal. Chem. 31:538-542.

Narang S, Sahai V, Bisaria VS (2001). Optimization of xylanase production by Melanocarpus albomyces IIS68 in solid state fermentation using response surface methodology. J. Biosci. Bioeng. 91(4):425-427.

Parachin NS, Siqueira S, Faria FP, Torres FAG, Moraes LMP (2009). Xylanase from *Crypyococcus flavus* isolate I-11: Enzymatic Profile, Isolation and heterologous expression of CFXYN1 in *Saccharomyces cerevisiae*. J. Mol. Catal. 59:52-57.

Sharma M, Mehta S, Kumar A (2013). Purification and Characterization of Alkaline Xylanase secreted from *Paenibacillus macquariensis*. Adv. Microbiol. 3:32-41.

Subramaniyan S, Prema P (2002). Biotechnology of microbial xylanases: Enzymology, molecular biology and application. Crit. Rev. Biotechnol. 22:33-46.

Thiago LRL, Kellaway RC (1982). Botanical Composition and extent of lignification affecting digestibility of wheat and oat straw and Paspalum hay. Anim. Feed Sci. Technol. 7:71.

Zambare VP (2011). Optimization of amylase production from *Bacillus* sp. using statistics based experimental design. Emir. J. Food Agric. 23(1):37-47.