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A Response Surface Methodology-Neural Network Approach to Optimize and Predict Xylanase Production Using Oat as Carbon Source by *Bacillus Species* 2129 Under Submerged Cultivation Conditions

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Abstract: In recent years, xylanase has become an essential option for environmental friendly industrial biotechnological applications and there is a rising demand for large scale production. In this study, a *Bacillus species* 2129 was tested for the xylanase production under submerged cultivation conditions. Maximum xylanase activities were achieved using oat as the substrate and by optimizing process conditions such as substrate concentration, pH and nitrogen source using response surface methodology (RSM). The maximum enzyme activity was 2.2 U ml^{-1} at the following test conditions: substrate concentration-1.7 %, pH -6.807 and NH_4Cl concentration-0.3%. Under optimized conditions there was an 8% increase in the enzyme activity and results from statistical approximation in the form of analysis of variance (ANOVA) shows that the squared effects of the variables were significant than both the main and interaction effects. The predictions from neural networks showed that a multilayer network (3-6-1) using the standard back propagation algorithm was able to predict enzyme activity effectively with R^2 values of 0.9995.

Key words: Oat . Xylanase production . Response surface methodology . Neural networks . Optimization . Prediction . Enzyme activity

INTRODUCTION

Xylanase is a hemi-cellulolytic polysaccharide consisting of 1,4 linked β-D-xylo pyranose residues, most commonly used for beer and juice clarification, pre-bleaching of kraft pulp, improving digestibility of animal feed, bread making and degumming of vegetable fibers such as jute ramie and hemp [1, 2]. The main constituents of microbial xylanolytic enzyme system are xylanase (endo-1,4-ß-xylanase) and β-xylosidase (β-D-xyloside xylohydrolase) [3]. Over the last few years, interest in xylanase has increased rapidly in paper and pulp industries due to their bleaching potential. Xylanases have a worldwide market of around 200 million US $ and the widespread use of xylanase in commercialized industrial applications requires extensive studies to optimize their production capability [4]. There has been extensive lab and pilot scale studies that have dealt with their production, purification, recovery and characterization [5-8]. On the other hand, very few studies have reported their product optimization [3, 9, 10]. Commercial mass production of xylanase can be quintessentially done by either submerged or solid state fermentation [2, 11], their effectiveness has been often consociated with process conditions and physico-chemical factors of prior significance. However the driving force has been to ideally produce quick and high quality xylanase from simple and inexpensive substrates. Most of the researches have been targeted on using residues/wastes from agro and food industry, thereby restricting the socio-economics related to environmental pollution. These residues contain nearly 20-30% hemicellulosic material that can be efficaciously used for the production of xylanase by microorganisms [12]. The most commonly used substrates so far are; rice bran, sugarcane baggase, wheat straw, wheat bran, corn crop, rumen, sorghum straw and cassava peel [4, 13-15].

In this study, commercially available Oat was used as the substrate for the production of xylanase under submerged conditions. Oat grains (*Avena sativa*) are high in carbohydrates and contain about 13% protein

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and 7.5% fat. Studies that have reportedly used oat as the substrate for the production of xylanase are sparse [16]. Diversified generic species of microorganisms have proven to be carriers of rich source of xylanase enzyme, especially *Bacillus species* which can secrete high levels of extra cellular xylanase. The amount of nitrogen also plays a vital role in enhancing the rate of enzyme production. NH_4NO_3, $NaNO_3$ and $(NH_4)_2SO_4$ have been used essentially as the nitrogen source [17]. Seyis and Aksoz [7] used a mixture of NH_4SO_4 and urea and found synergistic increase in xylanase activity. For successful implementation of this new substrate, process parameters such as pH, temperature, substrate concentration, cultivation and aeration time has to be optimized in appropriate reactor configurations. Though there are different optimization tools, factorial experiments and response surface methodology provides maximum information based on statistical principles by performing a minimum number of experiments [18].

An alternate predictive modeling procedure consists of a data driven approach wherein the principles of Artificial Intelligence (AI) is applied with the help of neural networks. The concept of neural network modeling has widespread applications in the field of enzymatic biotechnology. Haider *et al*. [19] optimized media constituents for enhancing lipase production by soil microbes using ANN and genetic algorithm based techniques. The results of their study showed that ANN based model was able to predict the system behavior clearly showing lipolytic activity of 7.69 U ml^{-1}. An ANN model thus developed for such systems can be used as an objective function for predicting the desired output (enzyme activity).

This study reports the optimization of substrate concentration, pH and nitrogen source for enhanced production of xylanase by a *Bacillus species* under submerged fermentation conditions. Anew, a neural network based predictive model has been formulated using substrate concentration, pH and ammonium chloride concentrations as the input variables, to predict the enzyme activity.

MATERIALS AND METHODS

Microbial strain: The microbial culture used in this study was *Bacillus sp*. 2129, obtained from National chemical laboratory (NCL), Pune, India. Stock cultures were maintained on slants of nutrient agar medium at 4°C and were periodically sub cultured to sustain microbial activity.

Media composition: The minimal medium used in this study had the following composition (per liter): beef extract 1g, peptone 1g and Sodium chloride 0.5g. Oat, obtained commercially from Quakers Company was used as the substrate (carbon source) at varying concentrations (0.52-2.87%). All other chemicals used in this study were of analytical reagent grade purchased from Sigma Laboratories (India). The values of NH_4Cl and Oat concentration are expressed in percentage, in (*weight/volume*) basis.

Experimental study: Experiments were conducted in 250 ml Erlenmeyer flasks fitted with butyl rubber stoppers having a working volume of 100 ml. The individual experimental flasks containing the media were sterilized at 15 psi, 121°C for 20 minutes prior to inoculation. *Bacillus species*, maintained on nutrient agar slants were grown for 3 days at 30±1°C. After sufficient growth, 10 ml of distilled water was aseptically added to each agar slants. Through mild scrapping with a sterilized inoculation loop and by periodic shaking, the colonies were made to suspend. For growth, 200 µl of this suspension was aseptically transferred and provided as the inoculum to the 100 ml media. The *Bacillus* strain was grown in experimental flasks kept in a rotary shaker (150 rpm) at 30±1°C and sample aliquots were withdrawn at equal intervals (12 hrs) for measuring xylanase activity.

Enzyme activity measurements: Xylanase activity was measured by monitoring the reducing sugar concentration released as xylose by the dinitrosalicylic acid (DNS) method [32]. The samples were centrifuged at 7200 rpm for 15 mins and used for analysis. 0.1 ml of this sample was mixed with 0.9 ml of birchwood xylan solution (5 g l^{-1}) in acetate buffer (0.1 M) having a pH of 5.0 at 60°C for 10 mins. The absorbance was read at 550 nm using a UV/Vis spectrophotometer (Shimadzu, Japan). A unit of xylanase activity was described as the amount of enzyme producing 1 µmol of reducing sugar equivalent to xylose per minute under standard test conditions.

The 2^3 central composite design: To investigate the effect of parameters such as substrate concentration, pH and ammonium chloride concentration on the enzyme activity, experiments were carried out according to the full factorial central composite design (CCD) as described by Montgomery [18]. The three steps of this experimental design include statically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the applicability of the model. A 2^3 CCD for three independent variables, each investigated at five levels with six star points and six replicates at the central point was experimented to fit a second order

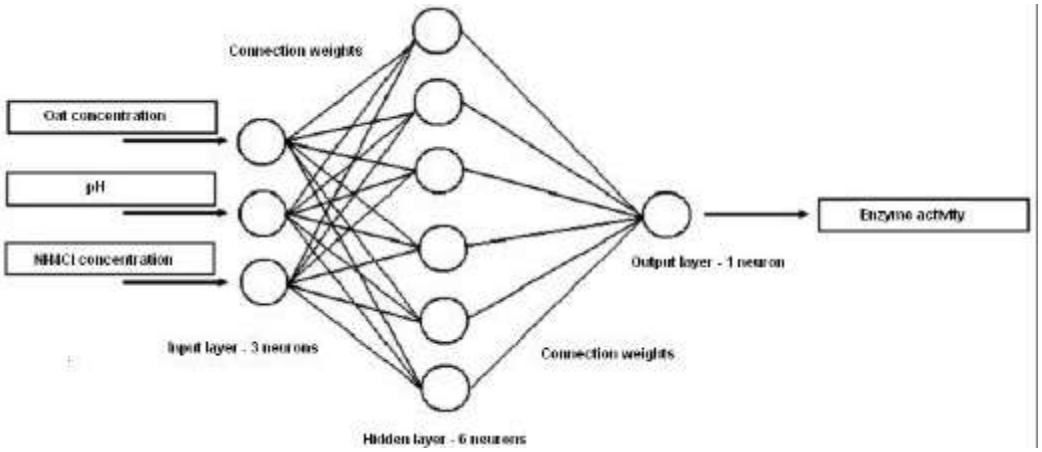

Fig. 1: Schematic of a multi layer perceptron used for predicting enzyme activity

Table 1: Range and levels of process variables

	Range and level				
Variables	-∝	-1	0	+1	+∝
X_1, Substrate concentration	0.5227	1	1.7	2.4	2.8772
X_2, pH	1.8068	3	4.75	6.5	7.6931
X_3, NH_4Cl concentration	0.3318	0.4	0.5	0.6	0.6681

polynomial model that required 20 experiments. The number of center point runs that the design specifies depends on certain inherent properties required for the design [18, 20]. The start points represent new extreme (low and high) for each factor in the design [21]. To maintain rotatability, the value of α depends on the number of experimental runs in the factorial portion of the CCD. If the factorial is a full factorial with "k" factors, then

$$\alpha = \left[2^k\right]^{1/4}$$

The dependent variable (response) selected for this study was the enzyme activity, expressed in U ml^{-1}, while the independent variables chosen were oat concentration (X_1), media pH (X_2) and ammonium chloride concentration (X_3). The range and levels of these experimental variables are given in Table 1.

According to the CCD theory, the response variable can be approximated to the process variables by a second order polynomial model of the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_{13} + b_{23}X_2X_3 + e$$

Where:

Y is the measured response, b_o the intercept term, b_{1-3} are the measures of the effects of variables (coefficients) and e is the experimental error. The test factors were coded according to the following equation.

$$x_i = \frac{X_i - X_i^x}{\Delta X_i}$$

Where:

x_i is the *coded* value, X_i is the *actual* value of the i^{th} independent variable, X_i^X is the actual value of the i^{th} independent variable at the center point and ΔX_i is the step change value. The MINITAB 14 (PA, USA) software was used for regression and graphical analysis (Response surface and contour plots) of the data obtained. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The predicted values were calculated from the regression model derived from the coefficients of the model and variations were explained by the determination coefficient (R^2 values).

The neural network predictive modeling approach: A Multi Layer Perceptron (MLP) using the back propagation algorithm [22] is the most widely used neural network for forecasting/prediction purposes [19, 23]. Neural networks acquire their name from the simple processing units in the brain called neurons which are interconnected by a network that transmits signals between them. These can be thought of as a black box device that accepts inputs and produces a desired output. MLP generally consists of three layers; an input layer, a hidden layer and an output layer (Fig. 1). Each layer consists of neurons which are connected to the neurons in the previous and flowing layers by connection weights (W_{ij}). These weights are adjusted according to the mapping capability of the trained network. An additional bias term (θ_j) is provided to introduce a threshold for the activation of

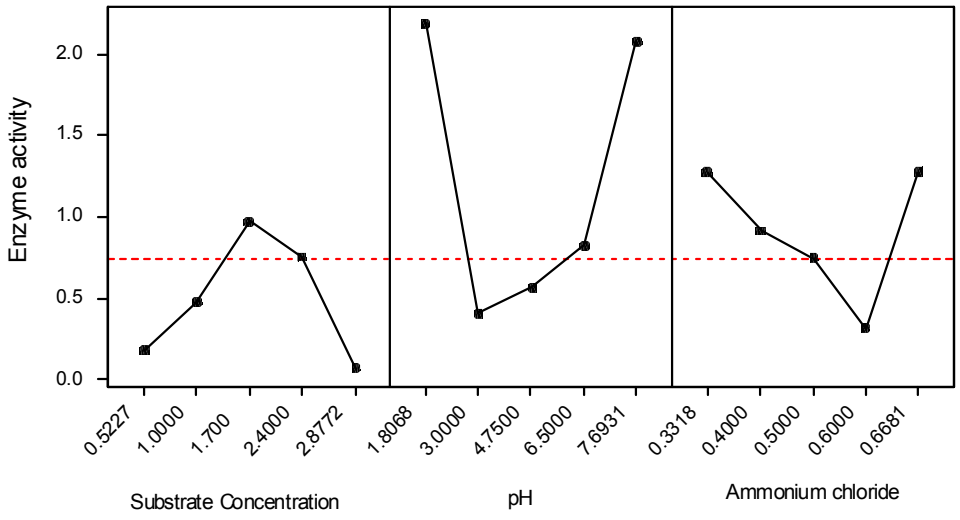

Fig. 2: Main effects plot of process parameters on enzyme activity

neurons. The input data (X_i) is presented to the network through the input layer, which is then passed to the hidden layer along with the weights. The weighted output (X_iW_{ij}) is then summed and added to a threshold to produce the neuron input (I_j) in the output layer. This is given by:

$$I_j = \Sigma W_{ij}X_i + \theta_j$$

This neuron input passes through an activation function $f(I_j)$ to produce the desired output Y_j. The most commonly used activation function is the logistic sigmoid function which takes the form;

$$f(I_j) = \frac{1}{1+e^{-I_j}}$$

ANN based predictive modeling was carried out using the shareware version of the neural network and multivariable statistical modeling software, NNMODEL (Version 1.4, Neural Fusion, NY)

RESULTS AND DISCUSSION

Optimization by response surface methodology approach: Experiments were carried out to optimize the effects of various process variables such as initial substrate (oat) concentration, pH and ammonium chloride (NH_4Cl) concentration for xylanase production according to the statistically significant 2^k full factorial central composite design (CCD). Furthermore, the results were analyzed by analysis of variance (ANOVA). This assisted in elucidating the main, squared and interaction effects among the process variables and their influence on the measured enzyme activity. All experiments were carried out in sequential order as specified by the design, in duplicate and the average values of measured enzyme activity were taken as the response variable. Figure 2 depicts the main effects of process variables on the enzyme activity, while Table 2 describes the process conditions and the

Table 2: Enzyme activity measured at different combinations of substrate concentration, ph and nh_4cl concentration

				Enzyme activity ($U\ ml^{-1}$)
Run No	Oat (%)	pH	NH_4Cl (%)	Measured
1	1.0	3.00	0.4	0.333
2	2.4	3.00	0.4	0.280
3	1.0	6.50	0.4	1.253
4	2.4	6.50	0.4	1.788
5	1.0	3.00	0.6	0.131
6	2.4	3.00	0.6	0.869
7	1.0	6.50	0.6	0.172
8	2.4	6.50	0.6	0.096
9	0.523	4.75	0.5	0.175
10	2.877	4.75	0.5	0.070
11	1.7	1.807	0.5	2.190
12	1.7	7.693	0.5	2.082
13	1.7	4.75	0.332	1.267
14	1.7	4.75	0.668	1.274
15	1.7	4.75	0.5	0.486
16	1.7	4.75	0.5	0.480
17	1.7	4.75	0.5	0.484
18	1.7	4.75	0.5	0.490
19	1.7	4.75	0.5	0.481
20	1.7	4.75	0.5	0.492

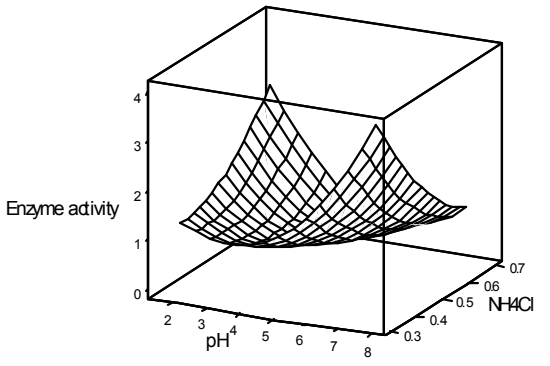

Fig. 3: Response surface plot for enzyme activity at different ranges of pH and NH_4Cl concentrations

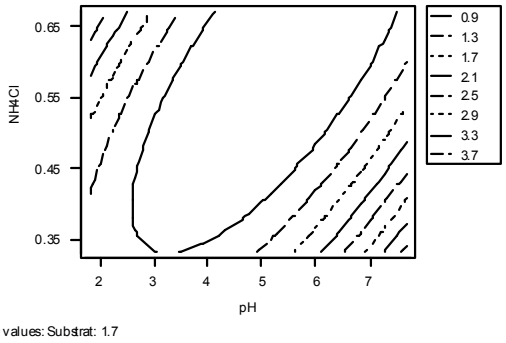

Fig. 4: Contour plot for enzyme activity at different ranges of pH and NH_4Cl concentrations

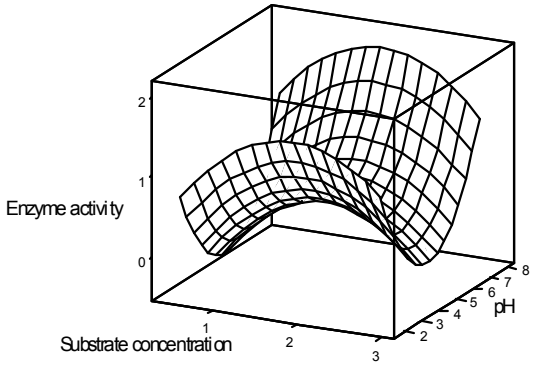

Fig. 5: Response surface plot for enzyme activity at different ranges of substrate concentration and pH

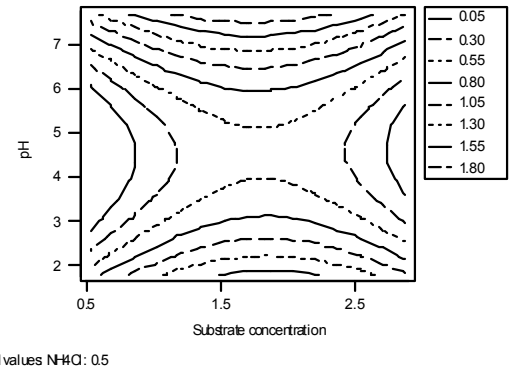

Fig. 6: Contour plot for enzyme activity at different ranges of substrate concentration and pH

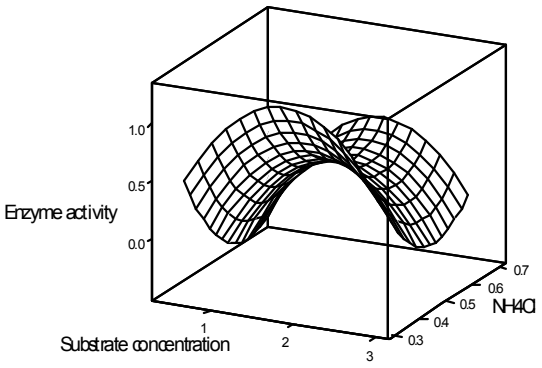

Fig. 7: Response surface plot for enzyme activity at different ranges of substrate concentration and NH_4Cl concentrations

experimentally measured enzyme activity. It was found that these profiles neither showed a single increasing or decreasing trend, but displayed a combination of both increasing and decreasing trends, suggesting the existence of an optimum condition within the range of experimental study. When the substrate concentration was increased from 0.5 to 1.7% the enzyme activity increased from a value of 0.23 to 1 U ml^{-1} and then decreased to around 0.2 U ml^{-1} at a substrate concentration of 2.8772 %. With the increase in pH concentration from low to high levels, the enzyme activity decreased from a value of 2.2 to about 0.45 U ml^{-1} and then the enzyme activity progressively increased to a value of 2.2 U ml^{-1}. Similarly, on increasing the NH_4Cl concentration, the enzyme activity first showed a declining trend and then increasing trend at a concentration of 0.6681%. The maximum enzyme activity was achieved for the substrate concentration of 1.7%, pH of 6.807 and NH_4Cl concentration of 0.5 % (run number 11). The xylanase production was between 0.48-0.49 U ml^{-1} in the medium with the three test variables at their central level. The carbon source used in this study is one of the major factors affecting the production of enzymes and their levels. The graphical representation of the interactions between process variables and their response called the response surface plots (RS plots) are presented at different levels of substrate concentration, pH and NH_4Cl concentrations in Fig. 3, 5 and 7. Each

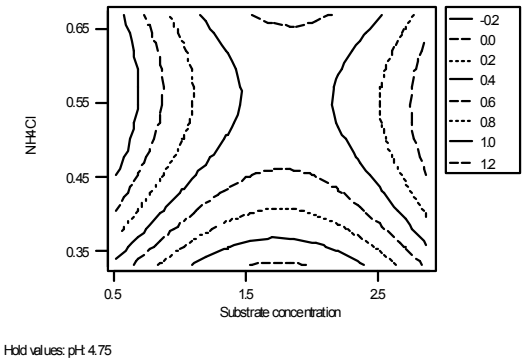

Fig. 8: Contour plot for enzyme activity at different ranges of substrate concentration and NH_4Cl concentrations

contour plot showed an infinite number of combinations of the two test variables with the other variable maintained at '0' level. The peaks and curvature indicated the maximum enzyme activity in the RS plots. The shapes of the surfaces, circular (or) elliptical indicated whether the interactions among different variables were significant or not. In general, the RS plots can be dome shaped, inverted 'U' shaped, some with a saddle point and some do not show any regular variation with increase/decrease in variables. Each RS plot is further complimented with contour diagrams (Fig. 4, 6 and 8) that reveal information on the variation of response on a XY plane. The surface confined to the smallest curve of the contour diagram suggested the location of an optimum operating condition under the experimental condition. The graphical illustrations in Fig. 3 and 4 reveal that the enzyme activity was at its maximum under the following condition: high pH and low levels of NH_4Cl, high NH_4Cl and low pH. These plots showed a shallow surface of an optimum condition at the intermediate levels and hills at extreme operating conditions. This interaction behavior causes the optimal level of one variable to change in response to changes in other variables. Mathematically, a saddle point (Fig. 5) is a point of a function with two or more variables which is at stationary point but not extremum. At such point, the surface could resemble a saddle point that curves up in one direction and curves down in one or several other directions. The saddle shaped surface does not have a unique optimum; instead it represents maximum value of the response variable in one direction, but a minimum in one or several directions. As shown in Fig. 5, the rate of increase in enzyme activity with an increase in value of pH above its saddle point is greater than the rate of increase in enzyme activity when substrate concentration is increased/decreased from the saddle point. Similarly, Fig. 7 shows that higher enzymatic activity is achieved by decreasing NH_4Cl below its saddle point value than by increasing it above its saddle point value.

Further, the contour plot (Fig. 4) depicts concentric elliptical ridges within the design boundary and this runs diagonally from the lower right to the upper left end. These types of contours passes through the steepest ascent of enzyme activity and the optimum operating conditions and in direction of maximum decline of the response with respect to increasing or decreasing values of the process variables. The interactions between substrate concentration and pH (Fig. 6) showed a rather complex behavior in comparison to those explained earlier. At low and high levels of pH, increasing the substrate concentration increased the enzyme activity up to a maximum and then decreased their values. However at intermediate levels of pH, irrespective of the values of substrate concentration, there existed a region where neither an increasing nor decreasing trend in the enzyme activity was noticed. These are represented by complex saddle type contour plot as shown in Fig. 6. Similar type of interactions was observed between NH_4Cl and substrate concentrations (Fig. 8). The non-elliptical nature of the contour plots depicts that there is no mutual interaction between the test variables. The experimental data was then analyzed statistically to obtain the following regression equation:

$$Y_{EA} = 0.5048 + 0.0707\,X_1 + 0.111\,X_2 - 0.1734\,X_3 - 0.2556\,X_1^2 + 0.4564\,X_2^2 + 0.1504\,X_3^2 - 0.0284\,X_1X_2 + 0.0227\,X_1X_3 - 0.3951\,X_2X_3$$

Where, Y_{EA}-Response variable representing enzyme activity, X_1-initial substrate concentration, X_2-pH and X_3-NH_4Cl concentration.

The determination coefficient value for this model equation was reasonably good under the experimental condition used in this study. However some of the model predictions had large standard errors (Fig. 9). The ANOVA result for the quadratic model is given in Table 3. In general the Fischer's variance ratio, the F value should be higher than the low probability, P values, for the predictions to be significant. This statistical analysis was done at the 95% confidence interval by the software, MINITAB 14. In this study among the main, squared and interaction effects of the variables on the enzyme activity, the squared effects played a major role for enzyme activity (F value of 7.61, P value of 0.006 than the main and interaction effects.

To understand the pattern of interaction and to envision synergistic and antagonistic effects between test variables, the student's t-test and P values were

Table 3: ANOVA for the quadratic regression model for enzyme activity

Source	Degrees of freedom	Seq sum of square	Adj sum of square	Adj mean square	F value	P value
Regression	9	6.483	6.483	0.720	3.59	0.029
Linear	3	0.649	0.649	0.216	1.08	0.401
Square	3	4.575	4.575	1.525	7.61	0.006
Interaction	3	1.259	1.259	0.419	2.09	0.165
Residual Error	10	2.004	2.004	0.2004		
Lack-of-fit	5	0.0001	0.0001	0.400		
Pure error	5	8.488	8.488	0.00002		
Total	19					

Table 4: Significance test for main and interaction effects of the variable on enzyme activity measured under varying operating conditions

Independent variables (parameters)	Coefficient (ß)	Standard error (ß)	't'-value	P-value
Constant	0.5048	0.1826	2.765	0.020
X_1	0.0707	0.1211	0.584	0.572
X_2	0.1110	0.1211	0.916	0.381
X_3	-0.1738	0.1211	-1.435	0.182
X_1^2	-0.2556	0.1179	-2.167	0.055
X_2^2	0.4564	0.1179	3.870	0.003
X_3^2	0.1504	0.1179	1.275	0.231
X_1X_2	-0.0284	0.1583	-0.179	0.861
X_1X_3	0.0227	0.1583	0.143	0.889
X_2X_3	-0.3951	0.1583	-2.496	0.032

tabulated using the software as shown in Table 4. The larger magnitude of t value (either ±) and smaller P value, the more significant is the corresponding coefficient [24]. Student's t-test was employed to determine the knowledge of the error mean square that is essential in testing the significance of the estimated coefficient of the regression equation. The student's t-test value can be obtained by dividing each coefficient by its standard error. A large 't' value implies that the coefficient is much greater than its standard error. The squared effects of pH and substrate concentration (P-0.003 and 0.055) had a major edge over other interaction and main effects. However the values of "t" and their sign imply the impact of their effects on the enzyme activity. The squared effects of pH increased the enzyme activity (t = 3.87), while the effects of substrate concentration (t value of-2.167) decreased the enzyme activity. On the other hand, the interaction effects between pH and NH_4Cl also showed negative effects on the enzyme activity with low P values (0.032). All the other coefficients (t values) were found to be insignificant. More precisely, the results from this study relied more on the square and interaction effects of the process variables than the main effects, while complex interactions were manifested with a statistical significance.

The optimum sets of operating conditions were obtained by solving the regression equation using the Monte Carlo simulation technique. The optimal values were first obtained in coded units (0.1523, 0.0934 and 0.6435) and then converted to the respective uncoded (real) units using the formulae described in Montgomery [18]. The optimal values of initial substrate concentration, pH and ammonium chloride concentration were 1.8066%, 4.193 and 0.5645% respectively. At these optimal conditions the enzyme activity was 2.388 U ml^{-1}, which was higher than the activity observed during regular experimentation. Nissen *et al*. [25] have suggested an optimal pH of 6-8 for increased production of xylanase. Horikoshi and Atsukawa [26] were the first to report xylanase from *Bacillus sp*., which was also active under high pH conditions. In this study, the model predicts the optimal concentration of xylanase production to be in lower pH range. Furthermore, the coded values were substituted in the regression equation to obtain the predicted enzyme activity. The standard errors observed between the measured and predicted enzyme activity is shown in Fig. 9. There appears to be a good correlation between the measured and predicted responses as seen from their R^2 value (0.8950). The findings of the present investigation compare well with

Table 5: Network training parameters for choosing the best network architecture

Training parameters	Range of values	Best value
Training cycle	1000-20000	18000
Number of neurons in input layer	3	3
Number of neurons in hidden layer	9-Mar	6
Number of neurons in output layer	1	1
Learning rate	0.3-0.8	0.7
Momentum term	0.1-0.9	0.4
Fixed parameters during training		
Error tolerance	0.0001	
Epoch size	10	
Training algorithm	Standard BEP	
Number of training data set	20	

the work of Ellaiah *et al*. [27] on response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus species*. Similarly, CCD techniques have been used to optimize maximum xylanase yields by *Schizophyllum commune* and *Thermomyces sp* with activity of 5.74 U ml^{-1} and 2.74 U ml^{-1} under submerged conditions [28, 29]. Senthil *et al*. [10] optimized xylanase yield at 1024 U/gm of wheat bran using *Aspergillus fischeri* under solid state fermentation conditions. The optimum condition predicted by the model was verified by carrying experiments in triplicate using the same procedure outlined earlier to monitor the enzyme activity. The model predicted enzyme activity value agreed well with the experimental result with an error of 7.6%.

Enzyme activity predictions by neural network approach: Artificial neural network based models requires the best combinations of network parameters such as training cycle (T_c), neurons in the input (N_I), hidden (N_H) and output layer (N_O), learning rate (η), momentum term (α) and a good algorithm for the predictions to be accurate. Determination coefficient (R^2) values between the measured and predicted outputs from the network were used as the performance indicators to determine the accuracy of the ANN predictions. The algorithm used for training in this study was the standard back error propagation (BEP) algorithm, which has potentially shown to posses' high capability in predicting process variables [19]. Table 5 shows the different network parameters used for training the network. The model was trained using different combinations of these parameters so as to achieve maximum determination coefficient values (target value = 1, i.e., 100% correlation between measured and predicted variables). This was achieved by a vigorous trial and error approach by keeping some

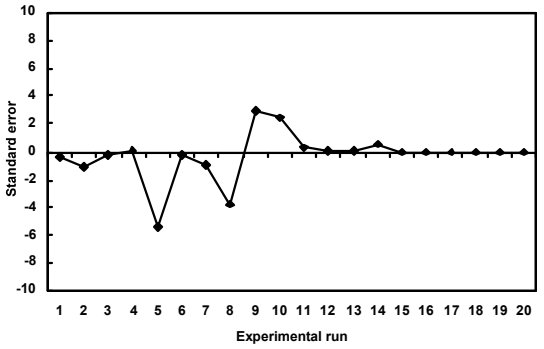

Fig. 9: Standard error values between measured and predicted enzyme activity

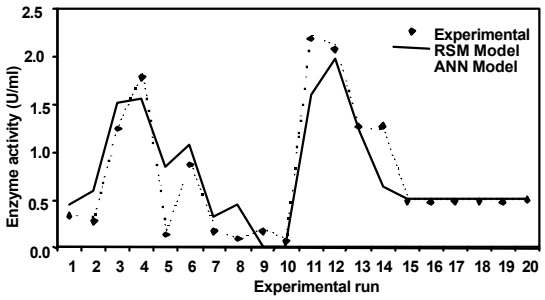

Fig. 10: Comparison of measured and predicted enzyme activity using ANN and RSM model

training parameters constant and by slowly moving the other parameters over a wide range of values. Enzyme activity, U ml^{-1} was predicted using oat concentration, pH and NH4Cl concentrations as the input variables (Fig. 10). It can be observed that, all the data points for enzyme activity were predicted accurately by the ANN model, though the predicted values after optimization using RSM was significantly lesser than the data driven ANN model (R^2 = 0.8950). Valdez-Castro *et al*. [30] reported that ANN based model was able to predict the fed-batch fermentation kinetics of *Bacillus thuringiensis*. Similarly, [19] observed that ANN predictions of lipolytic activity using oil, magnesium sulphate and ferrous sulphate as the media constituents showed R^2 value of 0.99. The weights and bias terms between the hidden layer connections obtained after network training is given in Table 6.

In order to evaluate the significant effect of the input parameters on the developed model, a sensitivity analysis was carried out by estimating the Absolute Average Sensitivity (AAS). The sensitivity is calculated by summing the changes in the output variables caused by moving the input variables by a small amount over the entire training set. The AAS is the absolute values of the change in the input [31]. These values for NH_4Cl concentration, oat concentration and pH were 0.5015,

Table 6: Weights and bias terms obtained from the trained network

	W_{11}	W_{12}	W_{13}	W_{14}	W_{15}	W_{16}
Input to hidden layer weights						
Oat concentration, mg/l	0.931	16.614	-13.469	-22.032	-29.410	-9.545
pH	6.436	-28.524	-12.853	7.608	1.926	1.246
NH_4Cl concentration, mg/l	-23.084	10.903	26.581	13.453	13.756	-1.401
Bias term	7.187	-2.015	-2.892	-2.656	4.909	-0.484

Hidden to output layer weights	Enzyme activity ($U\ ml^{-1}$)
W_{21}	15.033
W_{22}	0.127
W_{23}	3.312
W_{24}	5.956
W_{25}	-7.017
W_{26}	2.237
Bias term	-1.137

W_{11}-W_{16}-Weights between neurons in input layer and hidden layer

W_{21}-W_{26}-Weights between neurons in hidden layer and output layer

0.2904 and 0.2080 respectively. According to ANN results, NH_4Cl concentrations appear to have a significant effect in predicting the enzyme activity profiles, compared to both oat concentration and pH. This could be further supplemented by the results from ANOVA, where 't' value of-1.435 and 'P' value of 0.182 was observed for NH_4Cl concentration. This was also evident from Fig. 2, which shows a linearly declining trend in enzyme activity, as the NH_4Cl concentration increases from lower to higher levels (0.3318 to 0.6 mg/l). The results from this analysis reveal the degree of relevance of the input parameters to the outputs.

CONCLUSIONS

This research work demonstrates that response surface methodology can be a powerful and simple tool to effectively analyze the results and to determine optimal conditions for xylanase production.

Laboratory scale batch experiments were performed with commercially available oat as the substrate in a mineral salt media under controlled conditions. The results from this study showed that under optimum values of substrate concentration: 1.8066 %, pH: 4.913 and NH_4Cl: 0.5645%, the enzyme activity would be 2.388 U ml^{-1}. Further analysis with surface plots reveal that pairing factors produce saddle point response after a critical value indicating that some of the interactions were insignificant.

The enzyme activity values predicted by the ANN model showed better correlation (0.9995) with the experimental values than the RSM based predictions (0.8950). The best network architecture (3-6-1), determined by a trial and error approach showed that; learning rate, η-0.7, momentum term, α-0.4, with a training cycle of 18,000 are favorable conditions for high performance predictions. Also, results from sensitivity analysis, suggests that NH_4Cl concentrations largely affect the enzyme activity than both oat concentration and pH. The results from this study clearly establish the advantage of combining RSM for optimizing media constituents and neural networks in predicting xylanase production under submerged cultivation conditions.

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