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ARTICLE

Avermectin B1b Production Optimization from *Streptomyces avermitilis* 41445 UV 45(m)3 Using Response Surface Methodology and Artificial Neural Network

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Abstract Present study was conducted to optimize avermectin B1b production from *S.avermitilis* 41445 UV45(m)3 using artificial neural network and response surface methodology. Three variables NaCl, KCl, and pH were used for optimization. Coefficient of determination and adjusted coefficient of determination have very poor values for RSM. Values predicted by RSM for experiments were also much different from the observed avermectin production. Comparatively predicted avermectin levels by ANN were very close to observed values with much higher R^2 and adjusted R^2. Optimum levels of NaCl, KCl, and pH predicted by ANN were 1.0 g/L, 0.5 g/L, and 7.46 respectively. Sensitivity analysis predicted highest effect being shown was by pH followed by NaCl and KCl. About 37.89 folds increase in avermectin B1b production was observed at optimum levels of three variables envisage by ANN. Optimum levels, ranking order of variables, and the predicted avermectin on the optimum levels by the RSM was much different from ANN values. Results revealed that ANN is a better optimization tool for given strain than RSM.

Keywords artificial neural network · avermectin B1b · cultural conditions · optimization · response surface methodology · *Streptomyces avermitilis* 41445 UV 45(m)3

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Introduction

Avermectins, a complex polyketide lacking significant antibacterial and antifungal activities, is the secondary metabolite and produced as fermentation product of *Streptomyces avermitilis* (Yong and Byeon, 2005). They exhibit broad spectrum of activities against Nemathelminthes and arthropod parasites with low level of toxicity (Burg et al., 1979; Ikeda and Omura, 1997). Secondary metabolite production is dependent on medium composition. Therefore, a proper medium should be designed for the fermentation process in order to obtain the best production. Conventional one-parameter medium optimization is laborious and a time-consuming process entailing large number of experiments to acquire optimum levels of all variables (Dey et al., 2001; Xiong et al., 2008). In addition, this method of optimization did not give the correlation between different parameters and involved sequential manipulation of one parameter; therefore it is not a convenient method for designing a medium (Rao et al., 2000; Xiong et al., 2008). Statistical and mathematical optimizations including the response surface methodology and artificial neural networks are adopted to resolve the problem that one can face during conventional optimization (Azaman et al., 2010; Rusli et al., 2010; Nelofer et al., 2011).

Response surface methodology has been proved to be a powerful and useful tool for medium optimization during secondary metabolite production (Li et al., 2008a). It has eliminated all the drawbacks of classical and conventional optimization techniques (Liu and Wang, 2007; Sayyad et al., 2007; Deepak et al., 2008). Improved secondary metabolite production reduced process variability, minimum cost and time, and more accurate confirmation of response to nominal, and target requirements can be achieved through statistics-based experimental design technology during fermentation process (Hounsa et al., 1996). Comparative importance of all factors affecting the production of the end product can be determined using RSM even in the presence of complex

interactions (Hamsaveni et al., 2001). This methodology not only reveals the effect of independent variable in the medium but also provide a mathematical design for the better understanding of the running process (Bezerra et al., 2008). Screening experiments are required to be carried out if number of variables is too large, before the selection of any optimization design (Deepak et al., 2008). The disadvantage of the RSM is that it could not be applied to the optimization process, which is not being explained by second order polynomial (Bas and Boyaci, 2007).

An artificial neural network has been found to express the non-linearities in a much simpler and better way in contrast to RSM, and has been considered as most appropriate technique for optimization (Dutta et al., 2004). The information is processed by neurons of the model; these neurons are sometimes termed as processing elements. The artificial neural networks being flexible can read the input/output relationships through training (Noorossana et al., 2009). In ANN screening experiments are not required before its application and can be applied to both the statistically designed and non designed data. For applying ANN the data must contain all operating conditions, and the model then is formed according to the variable behavior. The factors which do not significantly affect the regression analysis must not be included in the data when ANN is being applied (Dasari et al., 2009). In ANN, if one of its elements fails to perform, it could begin to perform through its other elements, and hence can perform the entire assigned task that cannot be performed through linear programming and it does not need any programming essential for other statistical programs (Hill and Lewicki, 2006).

Articles on the comparison of RSM and ANN techniques for the optimization of avermectin B1b production from *Streptomyces avermitilis* are not available in the literature. Therefore, the present study was conducted for the comparison of ANN efficiency in modeling and optimization of avermectin B1b production from *S. avermitilis* 41445 UV45(m)3 using RSM. The correlation coefficient determination (R^2), adjusted R^2, absolute average deviation (AAD), and root mean square error (RMSE) values were used for the comparative analysis of the ANN and RSM optimization and prediction capabilities.

Materials and Methods

Microorganism and Inoculum preparation. *Streptomyces avermitilis* 41445 UV 45(m)3 strain obtained from the UV irradiation mutagenesis of *S.avermitilis* DSM 41445 (provided by "Deutsche Sammlung von Mikroorganismen (DSM) and Zelkulturen GmbH") was used in the present study. A loopful culture of the mutant strain (maintained on nutrient agar slants) was used to inoculate 50 mL of Yeast extract malt extract glucose (glucose 4.0 g/L, yeast extract 4.0 g/L, malt extract 10.0 g/L, $CaCO_3$ 2.0 g/L, and 1000 mL distilled water) medium in a 250-mL shake flask. The inoculum medium was incubated in orbital shaker at 150 rpm for 16-18 h at 28°C.

Fermentation medium. All fermentations were carried out in 250-mL shake flasks with 50 mL of fermentation medium containing potato starch (140 g/L), peptone (2.0 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $CaCO_3$ (0.8 g/L), α-amylase (0.5 g/L), KCl (1.0–4.0 g/L), and NaCl (0.5–4.0 g/L). The pH of the media was adjusted to different pH ranging from 7–7.5 for obtaining the optimization level of pH. Inoculum was transferred in each medium at 5%. The flasks were incubated in orbital shaker at 150 rpm for incubation of 10 days at 28°C. The variant compositions of different media are given in Tables 1 and 2.

Extraction of Avermectin B1b. Fermentation broth from each flask was centrifuged at 4°C for 20 min at 8,000 rpm after incubation. The supernatant was discarded, and the cell biomass was taken, because avermectin is the intracellular molecule. The cell biomass in the form of pallet was then crushed with proper amount of methanol to disperse it. The mixture of crushed cell biomass and methanol was centrifuged again to collect the supernatant that was later applied to high-performance liquid chromatography (HPLC) for the quantitative determination of avermectin B1b.

HPLC Analysis of Avermectin B1b. Reverse phase HPLC was employed for the quantitative determination of avermectin B1b. About 20 μL of each sample was applied to the HPLC (LC-2080 Shimadzu, Japan). The samples were separated on C18 column (SMA C-18), detected by UV detector (UV Variable Wavelength Detector STD-M20A Shimadzu) at 246 nm and eluted by methanol : acetonitrile (98:2; v/v) at a flow rate of 0.5 mL/min (Chen et al., 2007).

Experimental design. A total of 20 experiments using KCl, NaCl, and pH variables were conducted according to the Box-Wilson (BW) 2^3 full factorial central composite design (CCD). Each variable was set at five different levels of variations (Table 1). The first eight experiments (2^3=8, factorial CCD) were at factorial points, six at axial points (α =2), and six replicates were at central points.

Response surface methodology. Second order model (Eq. 1) used for the calculation of predicted response and the optimum values are as follows.

$$Y = \beta_i + \beta_4 X_4 + \beta_5 X_5 + \beta_8 X_8 + \beta_{44} X_4^2 + \beta_{55} X_5^2 + \beta_{88} X_8^2 + B_{45} X_4 \times X_5 + \beta_{48} X_4 \times X_8 + \beta_{58} X_5 \times X_8 \quad (1)$$

Where Y is the response variable and (β_i) is the interception coefficient. B_1, β_2, and β_3 are the coefficients of linear effects. B_{44}, β_{55}, and β_{88} are the coefficients of quadratic effects. B_{45}, β_{48}, and β_{58} are coefficients of interaction effects for three independent variables (X4= KCl, X5= NaCl, and X8= pH).

Artificial neural network. The data used for the optimization of avermectin B1b production from *S. avermitilis* UV 45(m)3 by RSM was also used to be optimized by ANN for comparison of two techniques. Thirty regression-based networks, constructed by STATISTICA, were studied for determining the best network, depending on the highest correlation coefficient determination (R^2) and lowest selection error, among which the best one was a

Table 1 Box-Wilson 2^3 factorial central composite design for optimization of avermectin B1b production from *S. avermitilis* UV 45 (m) 3 for RSM and ANN

Exp. No.	KCl (g/L) (X1)	NaCl (g/L) (X2)	pH (X3)	Observed	Predicted by RSM	Predicted by ANN
1	2.2	1.9	7.2	59.293	204.234	75.885
2	2.2	1.9	7.4	2037.1109	1223.188	1999.68
3	2.2	3.3	7.2	884.255	393.520	907.051
4	2.2	3.3	7.4	784.2876	604.063	809.126
5	3.4	1.9	7.2	694.5715	125.870	715.000
6	3.4	1.9	7.4	961.8703	703.679	1002.85
7	3.4	3.3	7.2	832.3824	897.379	797.007
8	3.4	3.3	7.4	743.7067	258.308	761.712
9	4	2.6	7.3	258.8651	408.102	263.948
10	1	2.6	7.3	107.5976	374.126	117.531
11	2.8	4	7.3	501.1463	556.823	487.669
12	2.8	0.5	7.3	85.5713	393.682	65.977
13	2.8	2.6	7.5	596.2421	1039.784	608.892
14	2.8	2.6	7	152.0718	287.816	179.242
15	2.8	2.6	7.3	144.7085	458.759	159.817
16	2.8	2.6	7.3	141.1498	458.759	159.817
17	2.8	2.6	7.3	249.9209	458.759	259.817
18	2.8	2.6	7.3	170.8978	458.759	159.817
19	2.8	2.6	7.3	156.5448	458.759	169.817
20	2.8	2.6	7.3	252.4276	458.759	259.817

Italic = select bold = training normal = testing.
[a] % difference was calculated as the % difference between observed value and the corresponding predicted value over the observed value.

Table 2 Analysis of variance for optimization of avermectin B1b production from *S.avermitilis* UV 45(m)3 using Box-Wilson Design calculated by RSM data

Variables	SS	Degree of freedom	MS	F	t-value	P-value	C.L -95%	C.L +95%
Intercept	13729	1	13729.1	0.054	0.234	0.819	--	--
KCl (X1)	356316	1	356315.8	1.424	1.193	0.260	-29.463	97.434
KCl^2 ($X1^2$)	7104	1	7103.6	0.028	-0.168	0.869	-2.905	2.496
NaCl (X2)	723009	1	723008.5	2.890	1.700	0.119	-15.043	111.905
$NaCl^2$ ($X2^2$)	1852	1	1852.2	0.007	0.086	0.933	-2.041	2.2051
pH (X3)	58764	1	58764.5	0.234	-0.484	0.638	-55.443	35.631
pH^2 ($X3^2$)	165126	1	165126.2	0.540	0.734	0.479	-30.315	60.157
KCl (X1) * NaCl (X2)	15095	1	15095.3	0.060	0.245	0.810	-4.622	5.767
KCl (X1) * pH (X3)	360922	1	360921.7	1.442	-1.201	0.257	-97.626	29.235
NaCl (X2) * pH (X3)	740398	1	740398.3	2.9598	-1.720	0.116	-112.328	14.443
Error	2501489	10	250148.9					

multilayer perception network that was then selected and used for the optimization and prediction. The experiments used for testing (5), training (10), and selection (5) are shown in Table 1.

Comparison of optimization capability of RSM and ANN. Adjusted R^2, ADD, RSME, and R^2 values were determined using Eqs. 2, 3, 4, and 5, respectively, for the comparison of optimization capabilities of ANN and RSM.

$$R^2 = \frac{\Sigma_{i=1-n}(X_i - Y_i)^2}{\Sigma_{i=1-n}(\overline{Y}_i - Y_i)^2} \quad (2)$$

where X is the ANN predicted avermectin B1b concentration, Y is the observed B1b concentration, and Y is the average observed B1b concentration.

$$\text{Adjusted } R^2 = 1 - \left[(1 - R\ ) \times \frac{N-1}{N-K-1}\right] \quad (3)$$

Where, N is the total no. of observations and K is the number of input variables.

$$AAD = \{[\Sigma_{i=1}^{p}(|y_{i,exp} - y_{i,cal}|/y_{i,exp})]/P\} \times 100 \quad (4)$$

Where, $y_{i,exp}$ and $y_{i,cal}$ are the experimental and calculated responses, respectively, and P is the number of experiments.

$$RMSE=\sqrt{\frac{\Sigma(y_{i,exp}-y_{i,cal})^2}{n}} \quad (5)$$

Where, $y_{i,exp}$ and $y_{i,cal}$ are the experimental and calculated responses, respectively, and n is the number of experiments.

Statistical analysis. STATISTICA software version 7 was used for the construction of plots and analysis of data. Regression model was evaluated by calculating the R^2 and adjusted R^2. The significance of the factors and their interaction effects were calculated by the regression analysis. Desirability charts were used for the prediction of optimum levels in RSM. Intelligent problem solver function of ANN in STATISTICA software was used for the construction of networks. Optimum levels were calculated from the surface graphs of the best network, and sensitivity analysis was used to observe the significance and ranking of variables.

Results

Optimization using RSM. Values of R^2 (0.4214) and adjusted R^2 (0.338) are very small as calculated by the RSM regression analysis. The calculated p values were greater than 0.05 for all parameters, interaction effects, and the model. Thus, the RSM model is not significant according to the above mentioned statistical values (Table 2). The non-significant RSM model indicates that it is not explaining the data correctly. This can also be seen from the big differences of observed values and values predicted by RSM (Table 1). No factor and their pair wise interaction effects showed the p values less than 0.5. Maximum effect was due to the NaCl followed by KCl and pH sequentially. The optimum levels of KCl, NaCl, and pH given by RSM insignificant model were 2.14 g/L, 2.56 g/L and 7.5 respectively.

Optimization using ANN. The ANN predicted values are much closer to the observed vales as compared to the RSM predicted values. Topology of network consisted of three layers (3:7:1), an input layer consisted of three fermentation variables, a middle hidden layer of seven neurons and one output layer for avermectin B1b production (Fig. 1). Different colors represented the activation levels of neurons in ANN processing. The optimum levels of KCl, NaCl, and pH given by ANN were 1.0 g/L, 0.5 g/L and 7.46 respectively. The highest effect was from pH followed by NaCl and KCl as is shown by sensitivity analysis in Table 3. This order of effects was not in accordance to that obtained from RSM. However ANN model accuracy is best supported by better values of R^2 (0.9353) and adjusted R^2 (0.8706). The interaction effects of these parameters are shown by 3D surface plots (Figs. 2, 3, and 4). As observed from curvature of the surfaces, no interaction had strong effect on avermectin production.

Comparative analysis of RSM and ANN. The predicted avermectin B1b values obtained from selected neural network of ANN and selected model RSM for experimental runs are shown in Table 1, revealing that predicted ANN values were much closer to the experimental values as compared to the RSM predicted values. The ANN predicted levels for KCl, NaCl, and pH were 1.0 g/L, 0.5 g/L, and 7.46, respectively, with predicted 4105.76 mg/L of avermectin B1b production. The RSM predicted levels for KCl, NaCl, and pH were 2.1 g/L, 2.56 g/L, and 7.5 respectively with predicted 4105.76 mg/L of avermectin B1b production. The experiments performed at optimum levels of three variables predicted by ANN and RSM (4077.40 and 3080.556, respectively) revealed that the observed production of avermectin B1b was much closer to the value predicted by ANN (Table 4). According to the RSM analysis, NaCl has maximum effect, followed by KCl and pH, respectively. In ANN method maximum effect was from pH. The second most effect was from NaCl and the least effect from KCl.

For comparison of ANN and RSM, four evaluation parameters were named R^2, Adjusted R^2, AAD, and RMSE (Table 5). The values of R^2 and Adjusted R^2 calculated from selected ANN model are much higher as compared to those calculated from RSM. The values of AAD and RSME are sufficiently lower for ANN as compared to the RSM. These results revealed that ANN is much better technique for the optimization of avermectin B1b production from mutant strain *S. avermitilis* 41445 UV 45(m)3 of *S. avermitilis* 41445.

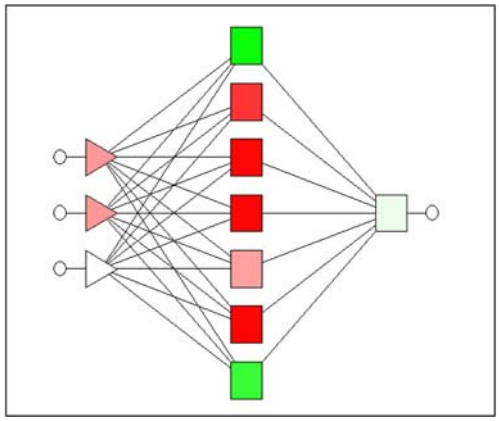

Fig. 1 Topology of neural networks for avermectin B1b production. Triangles represent the input (neurons added for ANN processing): KCl, NaCl and pH. The squares represent the hidden and the output layer (neurons generated during ANN processing). Small open circles represent the input and output layers (the neurons that can be observed in the form of numerical values).

Table 3 Sensitivity analysis by ANN for optimization of avermectin B1b production from S.avermitilis UV 45(m)3

Parameter	KCl (X1) g/L	NaCl (X2) g/L	pH (X3)
Ratio	1.445599	1.518385	2.470794
Rank	3.000000	2.000000	1.000000

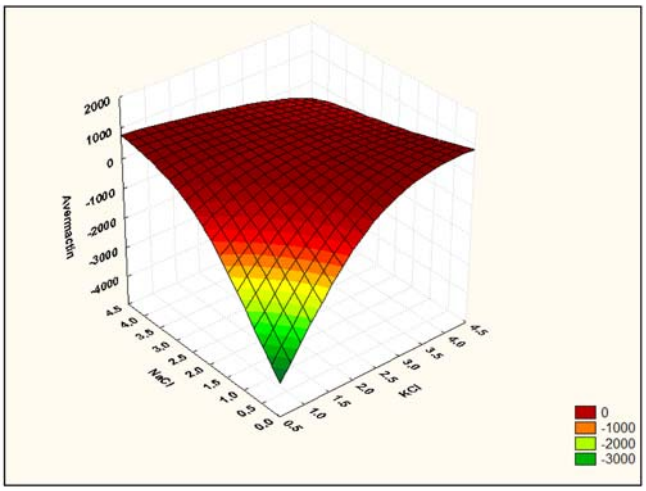

Fig. 2 Surface plot obtained optimization using ANN for the combined effect of KCl and NaCl on avermectin B1b production by keeping all other variables constant.

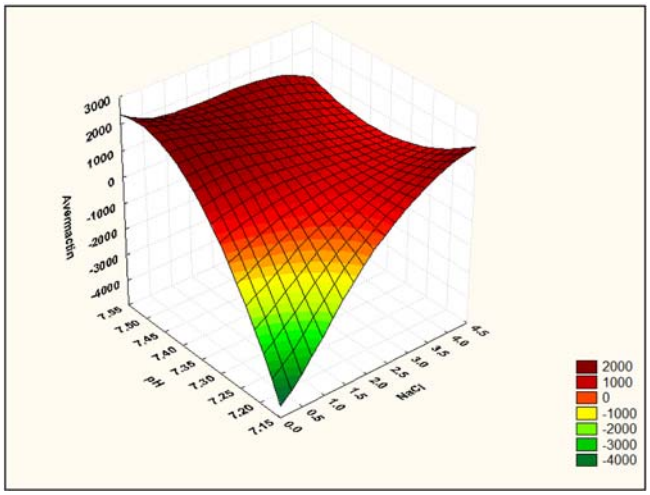

Fig. 4 Surface plot obtained optimization using ANN for the combined effect of NaCl and pH on avermectin B1b production by keeping all other variables constant.

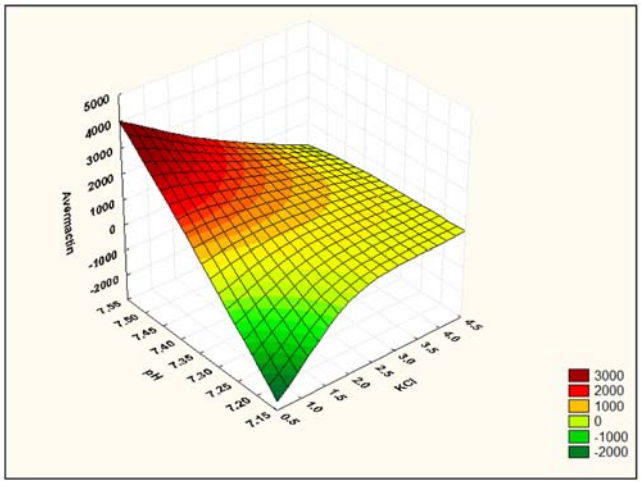

Fig. 3 Surface plot obtained optimization using ANN for the combined effect of KCl and pH on avermectin B1b production by keeping all other variables constant.

Discussion

The present study was conducted for comparison of two methods for the production of avermectin B1b from *S. avermitilis* 41445 UV 45(m)3 obtained from ultra violet irradiation of *S. avermitilis* 41445. Statistical optimization of medium and process parameters using the two methods ANN and RSM were performed using KCl (X1), NaCl (X2), and pH (X3) as significant variables. In routine optimization processes, one parameter at one time optimization has been employed most of the time. Single parameter at a time optimization technique is unable to predict the optimal conditions and correlation between different parameters for a bioprocess. RSM is advantageous over conventional single parameter optimization. ANN is better method for expressing the non-linearities in much simpler and better way as compared to the RSM, and has been successfully used as the most suitable technique for medium as well as process parameter optimization for many bioproducts (Dutta et al., 2004; Chen et al., 2009a; Nelofer et al., 2012).

Comparison analysis of RSM and ANN models on avermectin B1b production. The value of coefficient of determination (R^2) higher than 0.9 for a regression model characterizes the high correlation (Chen et al., 2009b). The integrity and accuracy of any statistical model can be well explained from the values of R^2 and adjusted R^2 (Elibol, 2004). In a study conducted by Song et al., 2012 and Zheng et al., 2008, it is reported that values of R^2 and adjusted R^2 play their role in the determination of statistical model sufficiency. About 99.25% of system variation and model efficiency can be explained well if the value of R^2=0.9925 (Baoxin et al., 2011). In the presently conducted research for medium and process parameter optimization for avermectin B1b production from *S. avermitilis* 41445 UV 45 (m)3 through RSM, multiple

Table 4 The predicted optimum levels and avermectin B1b production obtained from optimization by *S.avermitilis* 41445 UV 45(m)3 using ANN and RSM

Sr. no.	Method	KCl X1 g/L	NaCl X2 g/L	pH X3	Predicted by ANN	Predicted by RSM	Experimental data
1	ANN predicted optima	1.0	0.5	7.46	4105.76	--	4077.40
2	RSM predicted optima	2.1	2.56	7.5	--	4105.76	3080.556
3	Optima before optimization	1	2.6	7.3	117.531	374.126	107.5976

Table 5 Comparison of optimization and prediction capability by ANN and RSM for avermectin B1b production obtained from optimization of *S. avermitilis* 41445 UV 45(m)3

Sr. No.	Statistics	ANN	RSM
1	R^2	0.998	0.4214
2	Adjusted R^2	0.997	0.338
3	AAD	7.586	127.68
4	RMSE	0.105	1.73

correlation coefficients (R) and determination of coefficient (R^2) have been used for the verification of the required model. Value of multiple correlation coefficients (R) in the present study is very small, hence the selected RSM model is not significant for the production of avermectin B1b from *S. avermitilis* 41445 UV 45(m)3. Also the values of determination of coefficient (R^2) and adjusted R^2 were very small and not significant for the model. Good values of R^2 and adjusted R^2 were obtained (Gao et al., 2009), for the medium optimization of avermectin B1a production from *S. avermitilis* 14-12A using Response surface methodology. However, the medium and conditions used were different, which could be the possible reason for the different results from the present work. In a study conducted by Guo et al. (2012) value of R^2=0.9285 with 92.85% response variability determined the significance of statistical model.

In the ANOVA test, the F and P values determined the significance of the input variables. Higher values for the calculated F and p values smaller than 0.05 indicated the significance of a model (Rao et al., 2000; Li et al., 2008b; Gao et al., 2009). Guo et al. (2012) reported that p-value greater than 0.05 shows lack of fit for the applied statistical model. Also the adopted model is significant with F-value <0.5 (Li and Liu et al., 2008). In the present work the values of F and p also indicate the non-significance of RSM model. Therefore, it is concluded that RSM method could not be used for optimization and prediction of the present process. Significance of all coefficients and the interaction between all variables can be determined efficiently with the help of student's t-test and p values (Lee and Wang, 1997; Elibol, 2004; Zheng et al, 2008). Higher F- and lower p-values will determine how well the experimental results are in accordance with experimental results (Jo et al., 2008).

During the application of ANN modeling, the main step is the designing of network topology (Hornik et al., 1989). The statistical optimization procedures are highly affected by different designing parameters including the choice of activation function, training algorithms, training parameters, no. of hidden layers, no. of neurons in each hidden layer, initial weights, and training durations. Customarily one hidden layer with large number of hidden neurons depicts the precise estimations to any continuous nonlinear function (Nagata and Chu, 2003). During ANN, the neurons of hidden layer compute the strong association between input and output variables (Nagata and Chu, 2003). In the present study, during ANN modeling the developed topology network consisted of three layers: (1) an input layer consisting of three neurons, (2) a hidden layer with seven neurons, and (3) output layer of one neuron. ANN technique is more adaptable and it uses informal experimental designs generalizing better than regression models as compared to the other statistical approaches. The ANN approach does not include the lower significant variables during data analysis, thus is more accurate and reliable (Jayati et al., 2004).

During statistical process parameter and medium optimization for avermectin B1b production from *S. avermitilis* 41445 UV 45 (m)3 through ANN, value of R^2 obtained for ANN was 0.9353, which represented the goodness of fit of the model for the optimization. The value of adjusted R^2 (0.8706) also advocates the high significance of the model. For a model to be significant, the difference between coefficient of determination (R^2) and adjusted R^2 should be very small. In the present case, this difference is 0.0647; thus proving the model to be highly significant for the process parameter and medium optimization for avermectin B1b production from *S. avermitilis* 41445 UV 45 (m)3.

Values of ADD and RMSE are also important for a statistical method to be significant for the given optimization. For a method to be good the value of ADD should be as small as possible, and the value of RSME should be close to zero. In the present study the calculated values of ADD and RSME for RSM were higher than those of ANN values. The lower value of RSME for ANN as compared to the RSM revealed the model accuracy and proved the model to be more reliable. Based on the above parameters it can be concluded that ANN model showed much better performance for the optimization of avermectin B1b production from *S. avermitilis* 41445 UV 45 (m)3, in contrast to the RSM.

RSM determines the effects from factors alone and interaction effects of different factors in the form of regression analysis. On the other hand, ANN method offers sensitivity analysis, which tells about the ranking order of factors and the significance of factors. The ratio and ranking of each variable from ANN sensitivity analysis represents the network sensitivity for a fastidious variable (Lou and Nakai, 2001). The ratio equal to one or less than one indicates the variable to be less significant for output as compared to the variables with ratio higher than one. Chiu et al. (2006) predicted from their research work that predictive error ratio for each input variable can be calculated according to their degree of validity, which will demonstrate the contributions of distinctive variables in predicting the outcome. Ratios are ranked in order of descending importance. In the present study conducted for the production of avermectin B1b from *S. avermitilis* 41445 UV 45(m)3, the sensitivity analysis revealed maximum effect on production was from pH followed by NaCl and KCl.

Effect of pH on avermectin B1b production. In the case of *Streptomycetes*, the production of secondary metabolites is restricted to stationary phase cell biomass. Various factors and process parameters including carbon source, nitrogen source, pH, temperature, aeration, and incubation time are therefore responsible for the suppression of antibiotic production (James et al., 1991).

Selection of suitable medium, pH, incubation period, and temperature affect the growth of microorganism and the production of secondary metabolites. Maximum antibiotic production was obtained at optimum pH with higher growth rate of microorganism. With increasing the pH of the medium, the antibiotic production and growth began to decrease (Sood, 2011). The pH of medium strongly affects the yield of cell biomass. Cell biomass was decreased from 0.8 g/L to 0.6 g/L when pH of medium was raised upto 8.0 (da Silva et al., 2012). The pH found suitable for the enhanced production of granaticine from *Streptomyces thermoviolaceus* was between 7.3 and 7.5 (James et al., 1991).

The prominent effect on enhanced production of secondary metabolites is attributed to the pH of fermentation medium. About 1.6-fold increase in the production of avermectin was observed from *S. avermitilis* using high-throughput screening method (Gao et al., 2009). This enhanced production is attributed to the pH of medium being maintained at 7.5 in 360 m^3 fermentation batch study. In another study (Gao et al., 2009) it was observed that the production of avermectin B1a in optimized medium was 5128± 144 mg/L at pH 7.0. Enhanced production of oligomycin was obtained (Lin et al., 2009) in production medium adjusted at pH 7.0–7.2. Avermectin B1a production of 5228 U/mL was obtained using OUR monitoring of glucose feeding rate (Liang et al., 2010) using a medium with pH adjusted at 7.5 on industrial scale. Effect of pH on the production of antibiotics from *Streptomyces* spp. isolated from soil was reported by da Silva et al. (2012). They revealed that production is best observed at pH 7–8.5 through statistical optimization using RSM. In the present study the pH was optimized at 7.5 and 7.46 for the maximum production of avermectin B1b through RSM and ANN network respectively; these values are within the range of pH used by the other researchers.

Effect of NaCl and its concentration on avermectin B1b production. Production of secondary metabolites is directly influenced by medium composition (da Silva et al., 2012). Salinity is the promising environmental factor affecting the production of metabolites from the microorganism. High salt stress prompts the overall quantity of secondary metabolites produced and the metabolite profile, hence regulation is required in a strictly controlled manner (Wang et al., 2011). Good avermectin production was observed in earlier studies, with a transformed strain of *S. avermitilis* in a medium containing 0.1 g NaCl (Wang et al., 2011). Production of avermectin from *Streptomyces avermitilis* (Rezanka and Votruba, 1998) studied by a research group at varied concentrations of NaCl, and the production remained constant up to 0.5% salt concentration in the cultivation medium. A decrease in production reaching to zero occurred above 0.5–2.5% NaCl concentration. All of the above studies indicate that a higher concentration of salts inhibits the growth. NaCl was required in minute quantities for an optimal growth of *S. avermitilis.* In the present study the optimum level of NaCl depicted by ANN was very low as compared to RSM. Therefore, the lower concentration predicted by ANN is found to be more productive than the RSM-predicted NaCl concentration.

Effect of KCl and its concentration on avermectin B1b production. In the present study, the least significant effect on avermectin production was shown by KCl as predicted by the ANN method. The optimum level of this variable in the cultivation medium was almost half of the RSM predicted level. Medium salinity affects the growth of microorganism (Huang et al., 2011). The production and composition of secondary metabolites also decreased in medium containing higher KCl concentration. A research conducted for the enhanced production of oligomycin A from *S. avermitilis* (Lin et al., 2009), maximum production of oligomycin was obtained in a medium containing 4g/L KCl. In the present study, the product is different, which may be the possible reason for the lower optimal level of KCl.

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References

Azaman S, Ramakrishnan N, Tan J, Rahim R, Abdullah M, and Ariff A (2010) Optimization of an induction strategy for improving interferon-alpha2b production in the periplasm of *Escherichia coli* using response surface methodology. *Biotech Appl Biochem* **56,** 141–50.

Baoxin Z, Xiangjing W, and Wensheng W (2011) Optimization of fermentation medium for enhanced production of milbemycin by a mutant of *Streptomyces bingchenggensis* BC-X-1 using response surface methodology. *African J Biotechnology* **10(37),** 7225–35.

Bas D and Boyaci IH (2007) Modeling and optimization I: Usability of response surface methodology. *J Food Eng* **78,** 836–45.

Bezerra MA, Santelli RE, Oliveira EP, Villar LS, and Escaleira LAI (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* **76,** 965–77.

Burg RW, Miller BM, Baker EE, Birnbaum J, Currie SA, Hartman R et al. (1979) Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. *Antimicrobial agents Chemother* **15,** 361–7.

Chen HL, Yao J, Wang L, Wang F, Bramanti E, Maskow T et al. (2009a) Evaluation of solvent tolerance of microorganisms by microcalorimetry. *Chemosphere* **74,** 1407–11.

Chen XC, Bai JX, Cao JM, Li ZJ, Xiong J, Zhang L et al. (2009b) Medium optimization for the production of cyclic adenosine 3, 5-monophosphate by *Microbacterium* sp. no. 205 using response surface methodology. *Bioresource tech* **100,** 919–24.

Chen Z, Wen J, Song Y, Wen Y, and Li J (2007) Enhancement and selective production of avermectin B by recombinants of *Streptomyces avermitilis* via intraspecific protoplast fusion. *Chinese Sci Bulletin* **52,** 616–22.

Dasari VRRK, Donthireddy SRR, Nikku MY, and Garapati HR (2009) Optimization of medium constituents for cephalosporin C production using response surface methodology and artificial neural networks. *J Biochem Techn* **1,** 69–74.

da Silva I, Martins M, Carvalho C, de Azevedo J, and de Lima ProcÃ³pio R (2012) The Effect of Varying Culture Conditions on the Production of Antibiotics by *Streptomyces* spp. iIsolated from the Amazonian Soil. *Ferment Technol* **1(3)**.

Deepak V, Kalishwaralal K, Ramkumarpandian S, Babu SV, Senthilkumar S, and Sangiliyandi G (2008) Optimization of media composition for Nattokinase production by *Bacillus subtilis* using response surface methodology. *Bioresource tech* **99,** 8170–4.

Dey G, Mitra A, Banerjee R, and Maiti B (2001) Enhanced production of

amylase by optimization of nutritional constituents using response surface methodology. *J Biochem Eng* **7,** 227–31.
Dutta JR, Dutta PK, and Banerjee R (2004) Optimization of culture parameters for extracellular protease production from a newly isolated *Pseudomonas* sp. using response surface and artificial neural network models. *Process Biochemistry* **39,** 2193–8.
Elibol M (2004) Optimization of medium composition for actinorhodin production by *Streptomyces coelicolor* A3 (2) with response surface methodology. *Process Biochemistry* **39,** 1057–62.
Gao B, Su E, Lin J, Jiang Z, Ma Y, and Wei D (2009) Development of recombinant *Escherichia coli* whole-cell biocatalyst expressing a novel alkaline lipase-coding gene from *Proteus* sp. for biodiesel production. *J Biotech* **139,** 169–75.
Guo Z, Shen L, Ji Z, and Wu W (2012) Enhanced production of a novel cyclic hexapeptide antibiotic (NW-G01) by *Streptomyces alboflavus* 313 using response surface methodology. *Int J Mol Sci* **13,** 5230–41.
Hamsaveni D, Prapulla S, and Divakar S (2001) Response surface methodological approach for the synthesis of isobutyl isobutyrate. *Process Biochemistry* **36,** 1103–9.
Hill T and Lewicki P (2006) In *Statistics: Methods and applications: A comprehensive reference for science, industry, and data mining*: StatSoft, Inc., Southern Africa.
Hornik K, Stinchcombe M, and White H (1989) Multilayer feedforward networks are universal approximators. *Neural networks* **2,** 359–66.
Hounsa C, Aubry J, Dubourguier H, and Hornez J (1996) Application of factorial and Doehlert designs for optimization of pectate lyase production by a recombinant *Escherichia coli*. *J Appl Microbio Biotechn* **45,** 764–70.
Huang J, Lu C, Qian X, Huang Y, Zheng Z, and Shen Y (2011) Effect of salinity on the growth, biological activity and secondary metabolites of some marine fungi. *Acta Oceanologica Sinica* **30,** 118–23.
Ikeda H and Omura S (1997) Avermectin biosynthesis. *Chem Rev* **97,** 2591–609.
James P, Edwards C, and Dawson M (1991) The effects of temperature, pH and growth rate on secondary metabolism in *Streptomyces thermoviolaceus* grown in a chemostat. *J General Micro* **137,** 1715–20.
Jayati RD, Pranab KD, and Rintu B (2004) Optimization of culture parameters for extra cellular protease production from a newly isolated *Pseudomonos* sp. under response surface methodology and artificial neural network models. *Process Biochem* **39**, 2193–8.
Jo JH, Lee DS, Park D, Choe WS, and Park JM (2008) Optimization of key process variables for enhanced hydrogen production by *Enterobacter aerogenes* using statistical methods. *BioResource Technology* **99,** 2061–6.
Lee CL and Wang WL (1997) *Biological Statistics*, Science Press, China.
Liang JG, Chu XH, Chu J, Wang YH, Zhuang YP, and Zhang SL (2010) Oxygen uptake rate (OUR) control strategy for improving avermectin B1a production during fed-batch fermentation on industrial scale (150 m 3). *Afr J Biotechnol* **9,** 7186–91.
Li XY, Liu ZQ, and Chi ZM (2008a) Production of phytase by a marine yeast *Kodamaea ohmeri* BG3 in an oat medium: optimization by response surface methodology. *BioResource Technology* **99,** 6386–90.
Li Y, Jiang H, Xu Y, and Zhang X (2008b) Optimization of nutrient components for enhanced phenazine-1-carboxylic acid production by gacA-inactivated *Pseudomonas* sp. M18G using response surface method. *J Appl Microbio Biotech* **77,** 1207–17.
Lin X, Wen Y, Li M, Chen Z, Guo J, and Song Y (2009) A new strain of *Streptomyces avermitilis* produces high yield of oligomycin A with potent anti-tumor activity on human cancer cell lines in vitro. *J. Appli Microbio Biotech* **81,** 839–45.
Liu GQ and Wang XL (2007) Optimization of critical medium components using response surface methodology for biomass and extracellular polysaccharide production by *Agaricus blazei*. *J Appl Microbio Biotech* **74,** 78–83.
Lou W and Nakai S (2001) Application of artificial neural networks for predicting the thermal inactivation of bacteria: a combined effect of temperature, pH and water activity. *J. Food Res Int* **34,** 573–9.
Nagata Y and Chu KH (2003) Optimization of a fermentation medium using neural networks and genetic algorithms. *Biotechnology Letters* **25,** 1837–42.
Nelofer R, Ramanan R, Rahman R, Basri M, and Ariff A (2011) Sequential optimization of production of a thermostable and organic solvent tolerant lipase by recombinant *Escherichia coli*. *Annals of Microbiology* **61,** 535–44.
Nelofer R, Ramanan RN, Rahman RNZRA, Basri M, and Ariff AB (2012) Comparison of the estimation capabilities of response surface methodology and artificial neural network for the optimization of recombinant lipase production by *E. coli* BL21. *J Indust Microbio Biotech* **39,** 243–54.
Noorossana R, Davanloo Tajbakhsh S, and Saghaei A (2009) An artificial neural network approach to multiple-response optimization. *Inter J Adva Manufac Tech* **40,** 1227–38.
Rao KJ, Kim CH, and Rhee SK (2000) Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology. *Process Biochemistry* **35,** 639–47.
Rezanka T and Votruba J (1998) Effect of salinity on the formation of avermectins, odor compounds and fatty acids by Streptomyces avermitilis. *Folia Microbiologica* **43,** 47–50.
Rusli FM, Ramanan RNZRA, Mohamad R, Puspaningsih NNT, and Ariff AB (2010) Enhanced production of xylanase by recombinant *Escherichia coli* DH5 through optimization of medium composition using response surface methodology. *Annals of Microbiology* **60,** 279–85.
Sayyad SA, Panda BP, Javed S, and Ali M (2007) Optimization of nutrient parameters for lovastatin production by *Monascus purpureus* MTCC 369 under submerged fermentation using response surface methodology. *Applied Microbiology and Biotechnology* **73,** 1054–8.
Song Y, Li Q, Zhao X, Sun Y, and Liu D (2012) production of 2.3-Butanediol by *Klesbsiella pneumoniae* from enzymatic hydrolyzate of sugarcane bagasse. *BioResources* **7(4),** 4517–30.
Sood M (2011) Effect of Tempeature of Incubation on the Growth, Sporulation and Secondary Metabolites Production of *Aspergillus umbrosus. J Phyto* **3,** 35–7.
Wang Y, Lu Z, Sun K, and Zhu W (2011) Effects of high salt stress on secondary metabolite production in the marine-derived fungus *Spicaria elegans. Marine drugs* **9,** 535–42.
Xiong ZQ, Tu XR, and Tu GQ (2008) Optimization of medium composition for actinomycin X2 production by *Streptomyces* spp JAU4234 using response surface methodology. *J Indus Micro Biotech* **35,** 729–34.
Yong J and Byeon W (2005) Alternative production of avermectin components in *Streptomyces avermitilis* by gene replacement. *J Microbio* **43,** 277–84.
Zheng ZM, Hu QL, Hao J, Xu F, Guo NN, Sun Y et al. (2008) Statistical optimization of culture conditions for 1,3-propanediol by *Klebsiella pneumoniae* AC 15 via central composite design. *BioResource Technology.* **99(5),** 1052–6.