Optimization of Gentamicin Production: Comparison of ANN and RSM Techniques

M.Rajasimman and S.Subathra

Abstract**—**In this work, statistical experimental design was applied for the optimization of medium constituents for Gentamicin production by *Micromsonospora echinospora subs pallida* (MTCC 708) in a batch reactor and the results are compared with the ANN predicted values. By central composite design, 50 experiments are carried out for five test variables: Starch, Soya bean meal, K_2HPO_4, $CaCO_3$ and $FeSO_4$. The optimum condition was found to be: Starch (8.9,g/L), Soya bean meal (3.3 g/L), K_2HPO_4 (0.8 g/L), $CaCO_3$ (4 g/L) and $FeSO_4$ (0.03 g/L). At these optimized conditions, the yield of gentamicin was found to be 1020 mg/L. The R^2 values were found to be 1 for ANN training set, 0.9953 for ANN test set, and 0.9286 for RSM.

Keywords**—**Gentamicin, optimization, *Micromonospora echinospora,* ANN, RSM

I. INTRODUCTION

GENTAMICIN is an aminoglycoside antibiotic, and can treat many types of bacterial infections, particularly Gram negative infection. Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of certain microbiological growth media. Gentamicin is a basic and water-soluble antibiotic, first invented by Weinstein et al (1963) [1] from soil fungus M*icromonospora purpurea*. There are some studies on the gentamicin production [2-6].

Artificial Neural Networks (ANN) has been established as a tool for effortless computation. ANN have been successfully employed in solving problems in areas such as fault diagnosis, process identification, property estimation, data smoothing and error filtering, product design and development, optimization, dynamic modeling and control of chemical processes, for the prediction of vapor-liquid equilibrium (VLE) data and estimation of activity coefficients. ANN has remarkable ability to derive meaningful information from complicated or imprecise data. It can be used to extract patterns and detect trends, which are too complex to be noticed by other computational technique [7]. Neural networks, inspired by the information processing strategies of the human brain, are proving to be useful in a variety of engineering applications. ANN may be viewed as paralleled computing tools comprising of highly organized processing elements called neurons which control the entire processing system by developing association between objects in response to their environment. The researches have proposed many architectures of the network .Two widely used network for modeling the non-linear problems in engineering systems are the Backpropagation and Radial Basis Function (RBF) networks. Radial basis networks require lesser neurons than the standard feed forward back propagation networks and they can be trained in a fraction of time [8].

Response surface methodology (RSM) is an advanced tool, now a days commonly applied involves three factorial designs giving number of input (independent) factors and their corresponding relationship between one or more measured dependent responses. RSM is widely used for multivariable optimization studies in several biotechnological processes such as optimization of media, process conditions, catalyzed reaction conditions, oxidation, production, fermentation, etc., [9-14]. The objectives of this work are to find out the optimum production medium by response surface methodology for the production of gentamicin by *Micromonospora echinospora subs pallida* and to compare the RSM predicted values with ANN predicted values.

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II. MATERIALS AND METHODS

A. Microorganism

Micromonospora echinospora subs pallida (MTCC 708) obtained from MTCC, Chandigarh, is used for the batch studies. The Growth medium consist of : Beef extract - 3g/L, Glucose - 1g/L, Yeast extract - 5g/L, $CaCO_3$ - 4g/L, Soluble starch -24g/L, Agar -15g/L. The production medium consists of Starch – 5 to 10 g/L, Soya bean meal – 1 to 5 g/L, K_2HPO_4 – 0.6 to 1.0 g/L, $CaCO_3$ – 2 to 5 g/L, $FeSO_4$ – 0.01 to 0.05 g/L.

B. Experimental Design and Procedure

Response surface methodology is used in this study. The experimental variables at different levels used for the production of Gentamicin by *Micromonouspora echinospora subs pallida* using CCD is given in Table 1. A total of 50 runs are used to optimize the medium. The average from two replicated values of each run is taken as dependent variables or response or yield (production of gentamicin).

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The experimental design is carried out using Design Expert 7.1.5 (Stat Ease, USA). Central composite design (CCD) is used to identify the optimum operating condition in order to obtain maximum gentamicin production (Y_1) as response. The collection of experiments provides an effective means for optimization through these process variables. Besides, the design permits the estimation of all main and interaction effects. On the other hand, the purpose of the center points is to estimate the pure error and curvature. A second-degree quadratic polynomial can be used to represent the function in the range of interest.

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

where X_1, X_2, X_3, X_4, . . ., X_k are the input variables which affect the response Y and β_0, β_i, β_{ii} and β_{ij} are the constants. A second-order model is designed such that variance of Y is constant for all points equidistant from the center of the design. Production of Gentamicin was found by following the procedure given by Wang et al., 1993 [15].

TABLE I
EXPERIMENTAL VARIABLES AT DIFFERENT LEVELS USED FOR THE PRODUCTION OF GENTAMICIN BY *MICROMONOUSPORA ECHINOSPORA SUBS PALLIDA* USING CCD

Variable	Levels					
	Code	-2.38	-1	0	+1	+2.38
Starch (g)	X_1	6	7	8	9	10
Soyabean meal (g)	X_2	1	2	3	4	5
K_2HPO_4 (g)	X_3	0.6	0.7	0.8	0.9	1
$CaCO_3$ (g)	X_4	2	3	4	5	6
$FeSO_4$ (g)	X_5	0.01	0.02	0.03	0.04	0.05

C. Batch Experiments

Cell suspension was prepared from slant culture obtained from MTCC Chandigarh. The cell suspension was then added to the 50 ml of growth medium in a 250 ml Erlenmeyer flask. The medium was sterilized at 121°C for 20 minutes in an autoclave. The inoculated flask was kept in a rotary shaker at 150 rpm at 28°C. Growth period of *micromonospora echinospora* was two days. The grown medium was used for the production of gentamicin. Experiments were carried out according to the CCD given in Table 2.

D. Radial Basis Function Network

Radial basis networks require lesser neurons than the standard feed forward back propagation networks and they can be trained in a fraction of time. In this work, radial basis network function has been successfully incorporated for the prediction of Gentamicin production. The proposed technique of using radial basis function requires only limited experimental values to predict the behavior of the system. A simple well-trained neural network can be employed to overcome the modeling problems of reactor without prior knowledge of the relationships of process variables under

TABLE II
EXPERIMENTAL CONDITIONS AND OBSERVED RESPONSE VALUES OF 2^5 CENTRAL COMPOSITE DESIGN

Run .No	X_1	X_2	X_3	X_4	X_5	Gentamicin, mg/L		
						Experimental	RSM Predicted	ANN Predicted
1	2.38	0	0	0	0	695	695.61	695
2	-1	-1	1	-1	1	490	495.56	490
3	-1	-1	1	-1	-1	460	507.38	460
4	-1	1	-1	1	-1	475	520.77	475
5	-1	1	-1	1	1	490	520.82	490
6	0	0	2.38	0	0	580	532.68	580
7	-1	-1	-1	-1	-1	495	525.06	495
8	-1	-1	-1	1	-1	515	517.70	515
9	1	1	-1	-1	1	890	825.00	890
10	-1	-1	-1	-1	1	500	526.37	500
11	-1	-1	1	1	1	485	514.46	485
12	0	2.38	0	0	0	810	758.09	810
13	1	1	-1	-1	-1	640	711.19	640
14	0	0	0	0	0	875	866.24	875
15	0	0	0	0	0	870	866.24	870
16	0	0	0	0	0	870	866.24	870
17	-1	1	1	1	-1	510	571.84	510
18	1	1	1	1	1	770	810.59	770
19	0	0	0	0	0	875	866.24	875
20	-1	1	-1	-1	-1	520	523.75	520
21	-1	1	1	-1	-1	520	510.44	520
22	1	-1	-1	1	-1	700	687.65	700
23	-1	-1	1	1	-1	520	564.40	520
24	1	-1	-1	-1	-1	680	699.38	680
25	0	-2.38	0	0	0	750	691.35	750
26	0	0	0	0	-2.38	700	641.75	700
27	-2.38	0	0	0	0	300	188.84	300
28	-1	1	-1	-1	1	510	561.93	510
29	1	-1	1	-1	1	750	738.63	750
30	1	-1	-1	-1	1	740	776.31	740
31	0	0	0	0	0	870	866.24	870
32	1	1	-1	1	1	765	779.52	765
33	0	0	0	0	0	880	866.24	880
34	1	-1	1	1	1	750	753.14	750
35	1	-1	-1	1	1	700	726.46	700
36	0	0	0	0	0	875	866.24	875
37	0	0	0	0	0	875	866.24	875
38	1	-1	1	1	-1	710	727.48	710
39	-1	-1	-1	1	1	440	480.89	440
40	-1	1	1	1	1	600	558.77	600
41	***0***	***0***	***0***	***2.38***	***0***	***700***	*637.86*	*697*
42	***0***	***0***	***0***	***2.38***	***0***	***700***	*651.59*	*697*
43	***1***	***1***	***1***	***1***	***-1***	***765***	*748.04*	*769*
44	***0***	***0***	***0***	***0***	***0***	***880***	*866.24*	*880*
45	***1***	***1***	***1***	***-1***	***1***	***750***	***791.69***	*742*
46	***1***	***1***	***1***	***-1***	***-1***	***700***	***691.01***	*692*
47	***0***	***0***	***0***	***0***	***0***	***865***	***866.24***	*865*
48	***1***	***1***	***-1***	***1***	***-1***	***695***	***703.84***	*698*
49	***1***	***-1***	***1***	***-1***	***-1***	***660***	***674.83***	*662*
50	***-1***	***1***	***1***	***-1***	***1***	***460***	***535.50***	*461*

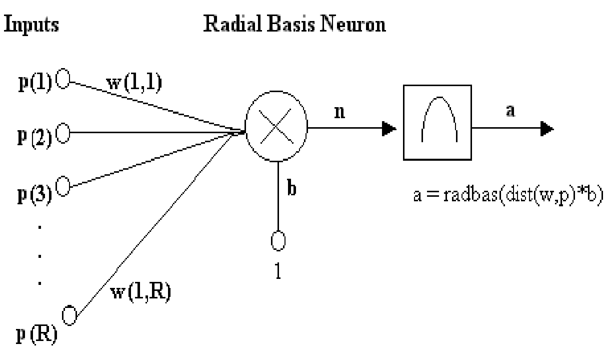

Fig. 1 Radial Basis Function Neuron Model

investigation. Radial basis function networks form one of the essential categories of neural networks. A RBF network is a two-layer network whose output units form a linear combination of the basis functions computed by the hidden units. A function is radially symmetric if its output depends on the distance of the input sample from another stored vector. Neural networks whose node functions are radially symmetric functions are referred to as Radial Basis Function Nets. The radial basis neural networks have been designed by the using the function newrb available in the neural network toolbox supported by MATLAB 7.0. The function newrb iteratively creates a radial basis network by including one neuron at a time. Neurons are added to the network until the sum squared error is found to be very small or the maximum numbers of neurons are reached. At each iteration the input vector, which will result in lowering the network error most, is used to create a radial basis neuron [16]. The following steps are repeated until the network's mean squared error falls below goal as given in Fig. 1. (i)The network is simulated, (ii) The input vector with the greatest error is found, (iii) A radbas neuron is added with weights equal to that vector and (iv)The purelin layer weights are redesigned to minimize error

III. Results and Discussion

A. Gentamicin Production

Experiments were performed according to the CCD experimental design given in Table 2 in order to search for the optimum combination of components of the medium. The Model F-value of 18.86 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The Lack of Fit F-value of 147.35 implies the Lack of Fit is significant. There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise.

The Fisher F-test with a very low probability value ($P_{model} > F = 0.0001$) demonstrates a very high significance for the regression model. The goodness of fit of the model was checked by the determination coefficient (R^2). The coefficient of determination (R^2) was calculated to be 0.9286 for gentamicin production. This implies that 92.86% of experimental data of the gentamicin production was compatible with the data predicted by the model (Table 1) and only 7.14% of the total variations are not explained by the model. The R^2 value is always between 0 and 1, and a value >0.75 indicates aptness of the model. For a good statistical model, R^2 value should be close to 1.0. The adjusted R^2 value corrects the R^2 value for the sample size and for the number of terms in the model. The value of the adjusted determination coefficient (Adj $R^2 = 0.8794$) is also high to advocate for a high significance of the model. Here in this case the adjusted R^2 value is 0.8794, which is lesser than the R^2 value of 0.9286. The Pred R^2 of 0.7035 is in reasonable agreement with the Adj R^2 of 0.8794. The value of CV is also low as 7.89 indicate that the deviations between experimental and predicted values are low. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this work the ratio is 19.900, which indicates an adequate signal. This model can be used to navigate the design space. The mathematical expression of relationship to the gentamicin production with variables are shown below

$$Y_1 = 865.31 + 106.54X_1 + 14.03X_2 + 3.35X_3 + 2.89X_4 + 15.96X_5 - 74.78X_1^2 - 24.84X_2^2 - 60.19X_3^2 - 38.98X_4^2 - 32.79X_5^2 + 3.28X_1X_2 - 1.72X_1X_3 - 1.09X_1X_4 + 18.91X_1X_5 + 1.09X_2X_3 + 1.09X_2X_4 + 9.22X_2X_5 + 16.09X_3X_4 - 3.28X_3X_5 - 9.53X_4X_5$$

The results of multiple linear regressions conducted for the second order response surface model are given in Table 2. The significance of each coefficient was determined by Student's *t*-test and *p*-values, which are listed in Table 3. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_1, X_5, X_1^2, X_2^2, X_3^2, X_4^2, X_5^2 are significant model terms. Values greater than 0.10 indicate the model terms are not significant. This implies that the linear effects of starch ($p < 0.0001$) and $FeSO_4$ ($p < 0.049$) are more significant than the other factors, i.e., ($p < 0.05$). Table 2 also indicate that the interaction between, starch and starch, soya bean meal and soya bean meal, K_2HPO_4 and K_2HPO_4, $FeSO_4$ and $FeSO_4$, $CaCl_3$ and $CaCl_3$ and interactive effects of starch and $FeSO_4$ ($p < 0.05$) had very significant influence on gentamicin yield by the *micromonouspora echinospora subs pallida* used in this study. These suggest that the concentrations of starch and $FeSO_4$ have a direct relationship with the production of gentamicin and interactive effects of starch and $FeSO_4$ in this particular complex production medium.

TABLE III
ANALYSIS OF VARIANCE (ANOVA) FOR RESPONSE SURFACE QUADRATIC MODEL

Source	Coefficient factor	Sum of squares	DF	F	P > F
Model	866.24	1.037E+006	20	18.86	< 0.0001[a]
X_1	106.54	4.916E+005	1	178.72	< 0.0001[a]
X_2	14.03	8526.29	1	3.10	0.0888
X_3	3.35	485.41	1	0.18	0.6775
X_4	2.89	360.74	1	0.13	0.7199
X_5	15.96	11039.39	1	4.01	0.0496
X_1* X_1	-74.96	3.107E+005	1	112.96	< 0.0001[a]
X_2* X_2	-25.02	34280.91	1	12.46	0.0014[a]
X_3* X_3	-60.37	2.013E+005	1	73.20	< 0.0001[a]
X_4* X_4	-39.16	84432.69	1	30.70	< 0.0001[a]
X_5* X_5	-32.97	59756.25	1	21.72	< 0.0001[a]
X_1* X_2	3.28	344.53	1	0.13	0.7260
X_1* X_3	-1.72	94.53	1	0.034	0.8542
X_1* X_4	-1.09	38.28	1	0.014	0.9069
X_1* X_5	18.91	11438.28	1	4.16	0.0500 [a]
X_2* X_3	1.09	38.28	1	0.014	0.9069
X_2* X_4	1.09	38.28	1	0.014	0.9069
X_2* X_5	9.22	2719.53	1	0.99	0.3283
X_3* X_4	16.09	8288.28	1	3.01	0.0932
X_3* X_5	-3.28	344.53	1	0.13	0.7260
X_4* X_5	-9.53	2907.03	1	1.06	0.3124
Residual		79769.25	29		
Lack of fit		79597.37	22	147.35	< 0.0001[a]
Pure Error		171.88	7		
Cor Total		1.117E+006	49		

Std. Dev.-52.45 ; R^2 0.9286; Mean - 664.70; Adj R^2-0.8794; C.V. %-7.89; Pred R^2 - 0.7035; Adeq Precision - 19.900

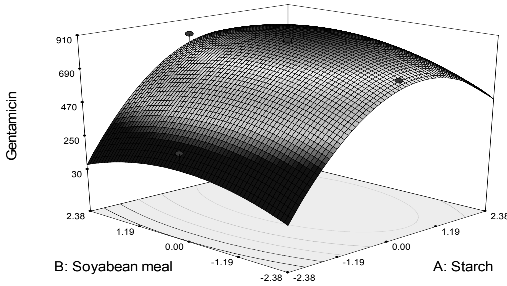

Fig. 2 3D plot of the combined effect of the starch and soya bean meal

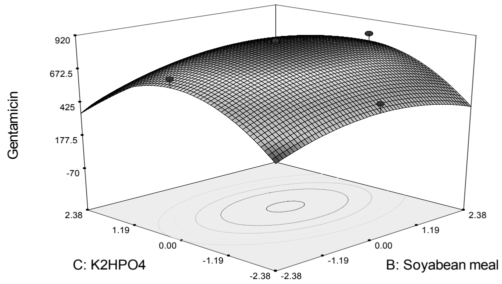

Fig. 6 3D plot of the combined effect of the soya bean meal and K_2HPO_4

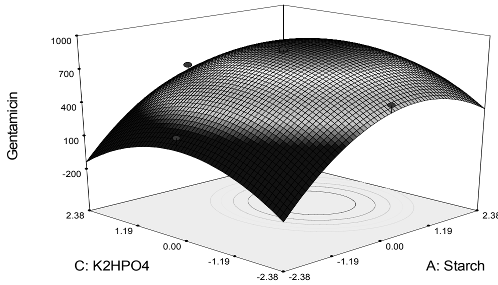

Fig. 3 3D plot of the combined effect of the starch and K_2HPO_4

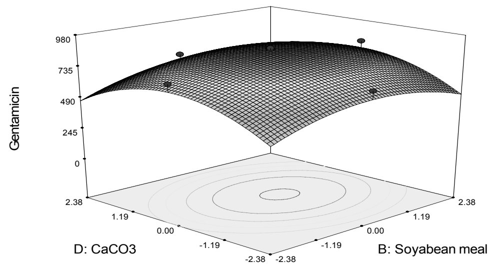

Fig. 7 3D plot of the combined effect of the soya bean meal and $CaCO_3$

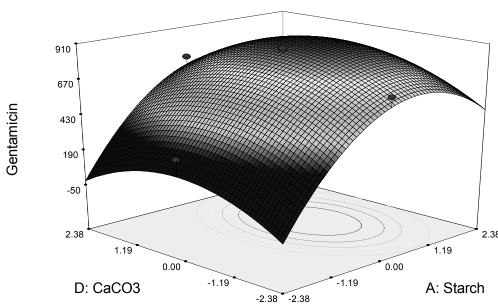

Fig. 4 3D plot of the combined effect of the starch and $CaCO_3$

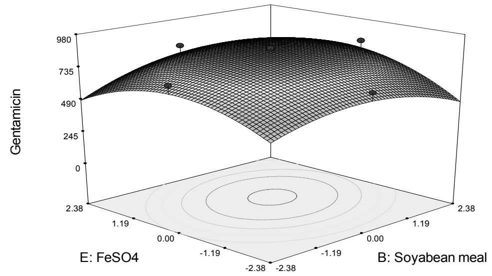

Fig. 8 3D plot of the combined effect of the soya bean meal and $FeSO_4$

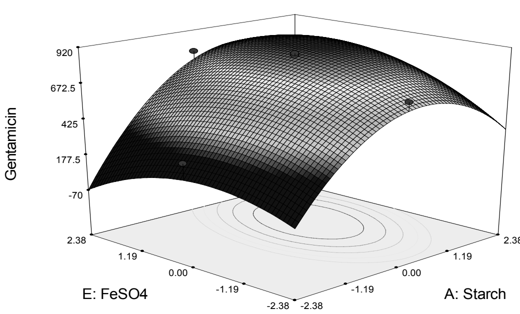

Fig. 5 3D plot of the combined effect of the starch and $FeSO_4$

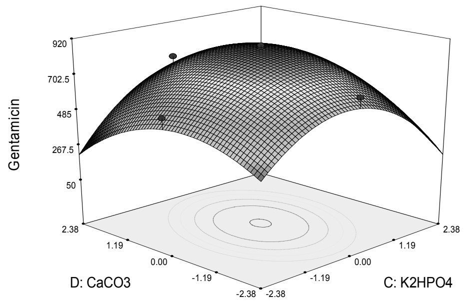

Fig. 9 3D plot of the combined effect of the K_2HPO_4 and $CaCO_3$

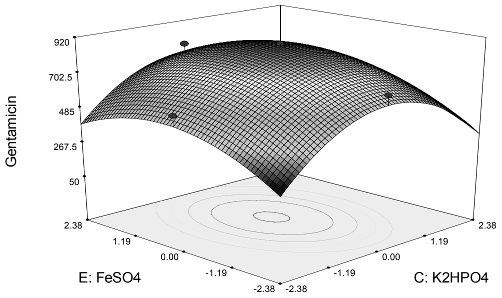

Fig. 10 3D plot of the combined effect of the K_2HPO_4 and $FeSO_4$

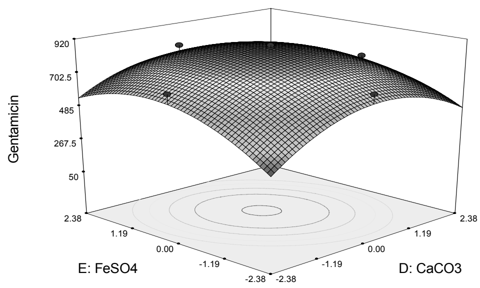

Fig. 11 3D plot of the combined effect of the $CaCO_3$ and $FeSO_4$

Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interaction effects of these two factors. These plots can be easily obtained by calculating from the model, the values taken by one factor where the second varies with constraint of a given Y value. The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response. The response surface curves for gentamicin production are shown in Figs 2 – 11. From all the figures, it was observed that the lower and higher levels of all the variables did not result in higher gentamicin yields. The shape of the response surface curves showed a moderate interaction between the variables. The studies of the contour plot also reveal the best optimal values of the process conditions lies within the range; starch: 8.5 –9.3 g/L, soya bean meal: 3-3.5 g/L, K_2HPO_4: 0.5 – 1.0 g/L, $CaCO_3$: 3.7-4.4 g/L and $FeSO_4$: 0.01-0.05 g/L.

The optimum values obtained by substituting the respective coded values of variables are: 8.9 g/L starch, 3.3 g/L soya bean meal, 0.8 g/L K_2HPO_4, 4.0g/L $CaCO_3$ and 0.031 g/L $FeSO_4$. The regression model fitted for the present CCD predicts that the maximum concentration of gentamicin can be obtained using the optimal concentrations of four test variables calculated previously is 950 mg/L, with a variation of 908 and 993 mg/L in the confidence limits of 95%. The optimized results for the five test variables are verified by carrying out shake flask experiments. The maximum concentration of gentamicin obtained experimentally was found to be 1020 mg/L. This is obviously in close relation with the model prediction. After optimization the gentamicin production was enhanced by 200 mg/L experimentally. The comparison of gentamicin production by *M. echinospora subs pallida* MTCC 708 before and after optimization is shown in Fig.12.

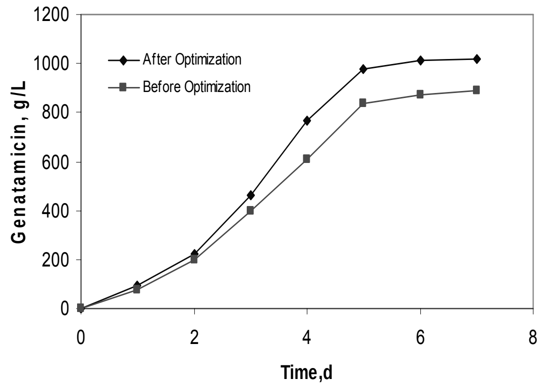

Fig.12 Production of gentamicin – before and after optimization

B. *Comparison of RSM and ANN Predicted Values*

An attempt was made to compare the ANN with RSM for the optimization of gentamicin production. Input variables and data for the ANN were given in table 2 in bold italics. Initially the data was trained by varying the number of neurons in the hidden layer from three to eight. Fig.1 shows the architecture of the given neural network. The experimental and predicted values of gentamicin production are given in Table 2. The correlation coefficient of 1 for training the model and 0.9953 for testing the model was obtained. The predicted output values of RSM and ANN are shown in Table 2. Though both the models based on RSM and ANN preformed well and offered stable responses in predicting the combined interactions of the independent variables with respect to the response, yet the ANN based approach was better in fitting to the measured response in comparison to the RSM model.

The absolute standard deviation and percentage root mean square error, used in this study are defined as

Absolute Standard Deviation (ABSD)

$$ABSD = \frac{\sum \left| (NN\ value - Experimental\ value) \right|}{\text{number of data points}}$$

Root Mean Square Error (RMSE)

$$\%RMSE = \sqrt{\frac{\sum \left(\frac{Experimental\ value - NN\ value}{Experimental\ value} \right)^2}{\text{number of data points}}} \times 100$$

The Absolute Standard Deviation and percentage RMSE were found for RSM and ANN and were tabulated in Table 4. From the Table it has been found that the deviations were well within the permissible limit and ANN predicts better than the RSM. Thus artificial neural network modeling of gentamicin production is highly justified for the batch production.

TABLE IV
COMPARISON BETWEEN ANN AND RSM MODEL

	Correlation coefficient		Average Absolute error		RMS error	
	ANN	RSM	ANN	RSM	ANN	RSM
Training data	1	-	0	8.6	0	3.12%
Testing data	0.9953	0.9286	3.67	25.18	4.3%	7.17%

IV. Conclusion

Response surface methodology was applied for the optimization of production medium components for the production of gentamicin. The model developed for CCD had R^2 values of 0.9286 for gentamicin production. The optimum values obtained by substituting the respective coded values of variables are: 8.9-g/L starch, 3.3-g/L soya bean meal, 0.88 g/L K_2HPO_4, 4.2 g/L $CaCO_3$ and 0.033 g/L $FeSO_4$. The regression model fitted for the present CCD predicts that the maximum concentration of gentamicin can be obtained using the optimal concentrations of four test variables calculated previously is 950 mg/L. The analysis of the data shows that optimized values of medium components give more production of gentamicin (1020 mg/L) in comparison with the conventional optimization methods. From the results it was also found that the ANN predicted values (R^2-0.9953) are closer than the RSM predicted values.

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