International Journal of Basic and Applied Biology (IJBAB)
Print ISSN: 2349 – 5820; Online ISSN: 2349 – 5839 ; Volume 2, Number 1; October, 2014 pp. 57 – 64
© Krishi Sanskriti Publications
http://www.krishisanskriti.org/ijbab.html

Optimization of Reaction Conditions of L-Glutaminase from *Bacillus Cereus* MTCC1305 using RSM and ANN model

Priyanka Singh[1], Rathindra Mohan Banik[2]

[1]*Department of Bioscience and Biotechnology, Banasthali University, Rajashthan*
[2]*School of Biochemical Engineering, IIT (BHU), Varanasi, India*

Abstract **: Microbial glutaminase has been commonly used as flavor enhancing agent in food industry. In this study, the production of L-glutaminase from many bacteria and yeasts was tested and Bacillus cereus MTCC 1305 was selected as potent strain. The optimization of reaction conditions was considered as primarily important factor to get maximum activity of any enzyme. In this study, statistical methods like response surface methodology (RSM) and Artificial neural network (ANN) were employed to optimize reaction conditions viz., time, temperature, pH of reaction mixture, enzyme volume and substrate concentration. ANN model was superior to RSM model with higher value of coefficient of determination (0.9999_{ANN}>0.98172_{RSM}), lower value of root mean square error (0.6697_{ANN}>24.6061_{RSM}) and absolute average deviation ($0.086\%_{ANN}$<$3.239\%_{RSM}$). A multilayer perceptron neural network trained with an error back-propagation algorithm was incorporated to get a predictive model. Levenberg Marquardt (LM) training algorithm was used to train ANN and topology of ANN was obtained as 5-3-1. Optimum assay conditions predicted by ANN were pH of reaction mixture (7.5), reaction time (20 minutes), incubation temperature (35˚C), substrate concentration (40mM) and enzyme volume (0.5ml) with a maximum predicted activity of L-glutaminase 633.7349 U/l which was close to experimental activity of L-glutaminase 634.00 U/l at simulated optimum assay condition. The activity of L-glutaminase was enhanced by 1.499 fold after optimization of reaction conditions.**

1. INTRODUCTION

Glutaminase (Glutamine amidohydrolase, EC 3.5.1.2) catalyzes the hydrolysis of L glutamine to L-glutamic acid and ammonia, which plays an essential role in cellular metabolism [1]. This enzyme has been reported to be present in diverse group of microorganism, plant and animal sources. Microbial glutaminases are commonly used as flavor enhancing agent in food industry [2-5]. Many methods were reported to estimate its activity either by estimating concentration of released products ammonia [6-7] or glutamic acid [8-9]. Among all these methods, assay method proposed by Imada et al. (1973) was found to be simple, easy, and economical method to estimate glutaminase activity and was being reported in many recent papers. The reaction conditions like pH of reaction mixture, incubation temperature, reaction time, substrate concentration and enzyme volume were considered as significant factors for assay method of glutaminase. These reaction parameters were needed to be optimized to get optimum activity of glutaminase. The optimization of bioprocess parameters using statistical tools (RSM and ANN) are generally preferred over the conventional method of "one variable at one time" because of accounting interactive effects of the variables with screening and prediction of large experimental domain [10-13]. ANN is found as more accurate modeling technique as compared to RSM for prediction and modeling of nonlinear relationships of variables [14] and [12]. The objective of this study was to select a potent microbial strain producing high amount of L-glutaminase and develop an efficient estimation method for estimation of glutaminase. The assay parameters were optimized with respect to pH, temperature, reaction time, substrate concentration and enzyme volume. Further the statistical tools like ANN and RSM were employed to improve the production of L-glutaminase.

2. MATERIALS AND METHOD

2.1. Microorganism and culture conditions

Microbial strains like *Staphylococcus aureus* MTCC 3160, *Streptococcus lactis* MTCC 460, *Streptococcus sp.* MTCC 389, *Escherichia coli* MTCC 2893, *Bacillus megaterium* MTCC 2412, *Bacillus subtilis* MTCC 1789, *Bacillus cereus* MTCC 1305, *Bacillus licheniformis* MTCC 1483, were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. *Bacillus stearhothermophilus* NCIM 2235, *Pseudomonas aeruginosa* NCIM 2948, *Pseudomonas Putida* NCIM 2650, *Pseudomonas fluorescens* NCIM 5096, *Lactobacillus casei* NCIM 2364, *Lactobacillus plantarum* NCIM 2373, and *Aeromonas formicans* NCIM 2319 were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. The bacterial strains like *Bacillus sp.*, *Pseudomona sp.*, *Escherichia coli*, and *Aeromonas sp.* were grown in nutrient media (pH-7.0) containing beef extract (1g/l), yeast extract (2g/l), peptone (5g/l), and agar (15g/l) at 35˚C. *Lactobacillus* and *Streptococcus* were grown in MRS

broth media (pH-7.0) containing glucose (20g/l), peptone (10g/l), beef Extract (10g/l), yeast extract (5g/l), CH_3COONa (5g/l), Tween 80 (1g/l), ammonium citrate (2g/l), $MgSO_4$ (0.1g/l) and $MnSO_4$ (0.05g/l). Inoculated slants were incubated at 35˚C for 24h for microbial growth and stored at 4±1˚C in refrigerator for further use. The production of L-glutaminase from bacterial strains was studied in basal medium of pH 7.0 containing glucose (1g/l), L-glutamine (3g/l), $Na_2HPO_4 \cdot 2H_2O$ (3g/l), KH_2PO_4 (2g/l), NaCl (0.5g/l), $MgSO_4 \cdot 7H_2O$ (0.5g/l), $CaCl_2 \cdot 2H_2O$ (0.015g/l) and yeast extract (5g/l) [15-19]. The inoculum was prepared by adding a loop full of freshly prepared pure culture into 50ml growth media in 250ml Erlenmeyer flask. The enzyme production experiments were performed in 250ml conical flasks containing 100ml of medium (pH-7.0) by incubating at 35˚C, 120rpm for 36h in an orbital shaker (Scigenics, India) after adding inoculum of 2ml (2.13×10^5cell/ml, 10h age). Cell free broth was collected after centrifugation at 10,000×g at 4±1˚C for 10minutes and used as an enzyme source.

2.2. Estimation of glutaminase activity

The extracellular activity of L-glutaminase was determined using cell free broth by modified method of Imada et al. (1973), in which reaction mixture (pH of 7.5) containing 0.5ml of crude extract of enzyme, 0.5ml of 40mM L-glutamine solution, 1.0ml of 0.1M phosphate buffer was incubated at 37˚C for 30minutes. The reaction was terminated by the addition of 0.5ml of 1.5M Trichloro-acetic acid to reaction mixture. Reagent blank and substrate blank were also prepared simultaneously. 3.7 ml of distilled water was added to 0.1ml reaction mixture and then 0.2ml of Nessler's reagent was added. The absorbance of the blank and test sample was measured at 450nm. One unit of glutaminase activity was defined as enzyme required for deamination of 1.0µmole of glutamine per minute per ml of enzyme solution at pH of 7.5 and temperature 35˚C [20]. A standard graph using NH_4Cl (12×10^{-4}M) was plotted for computation of the concentration of ammonia

2.3. Optimization of reaction conditions using RSM and ANN methodologies

The statistical optimization strategy was applied for five assay parameters viz., pH, reaction time, temperature, substrate (L-glutamine) concentration, and volume of L-glutaminase enzyme. The assay parameters with upper and lower limits for experiments designed to estimate optimum activity of L-glutaminase using RSM and ANN methodologies are shown in Table1. All experiments designed by RSM (MINITAB version 15) for estimation of L-glutaminase activity were performed in triplicates. The experimental data was further analyzed on basis of multiple regressions and ANOVA. The following second order polynomial model was fitted for prediction of optimal levels (equation 1):

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \ldots\ldots (1)$$

Where, Y=Predicted response, β_0=Intercept coefficient, β_i=Linear coefficient, β_{ii}=Quadratic coefficient and β_{ij}=Interaction coefficient

The effect of interaction of significant variables on activity of L-glutaminase was further studied using contour plots.

Table1. Optimization of assay parameters of L-glutaminase using RSM and ANN model

Assay parameters	Design matrix by RSM	Design matrix for ANN
Time (10-30 min.),Temperat-ure (20-50ºC), pH of reaction mixture (5-10), Glutamine concentration (20-60mM), Volume of L-glutaminase (0.2-0.8ml)	(i)Central Composite Design with 2^3 level factorial, α=2.366, (ii)Thirty two experiments were designed with eight replication at centre points (iii) Optimum level of assay parameters were obtained after Regression Analysis, ANOVA, contour plots	(i)CCD data of 24 was divided into three sets: Training (16), Testing (4) and Validation (4). (ii)3 neurons were selected as Optimum number of neurons in hidden layer. (iii)Back propagation network with Multi-layer perceptron (MLP) based on Levenberg Marqua-rdt algorithm and sigmoid transfer function was used. (iv)MLP network architecture 5-3-1 with 5 input neurons, 3 hidden neurons and 1 output neuron.

ANN software (neurosolutions version 6) was further applied to provide a nonlinear mapping between the input variables and output variables. ANN was used for simulating same set of experimental data used for RSM except the replicated data obtained at centre point. These replicates do not improve the prediction ability of ANN network (Bas and Boyaci, 2007). The experimental data fed in neural network was categorized into three sets: Training, Testing and Validation. A multi-layer perceptron (MLP) together with back propagation was used to approximate nonlinear function to desired accuracy by changing the number of layers and number of neuron in each layer (Lou and Nakai, 2001). The performance of the network was measured in terms of mean squared error (MSE), which is the difference between output variable and pre specified external desired signal. The optimum number of neurons in hidden layer was determined on the basis of minimum value of MSE. The MLP network architecture designed for analysis of data was categorized in input layer, hidden layer and an output layer. The input layer comprised of neurons for input

variables, while the output layer of one neuron represents predicted response. Neurons of successive layers in MLP network are connected to each other by connection weight (W_{ij}) and threshold for activation of these neurons was introduced in term of bias (θ_j). Input data (X_i) was passed through input layer to hidden layer along with the weights. These weighted outputs ($X_i W_{ij}$) were then summed and added to bias term (θ_j) to produce neuron input (I_j) in the output layer according to equation 2:

$$I_j = \sum X_i W_{ij} + \theta_j \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

This neuron input was then passed through an activation function f(I_j) and transformed to output neuron by using sigmoid transform function as shown in equation 3:

$$f(I_j) = \frac{1}{1 + e^{-I_j}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The following equation was the outcome of the MLP neural network training, relating the input variables (X_1, X_2, X_3, X_4, X_5 and X_6) to the output variables (y) in terms of weight and biases (Prasanthi et al., 2008).

$$Y = w_2 \times \left(\frac{2}{1+e^{\left(-2 \times (w_1 \times xt^1 + b_1)\right)} - 1} \right) + b_2 \quad \ldots (4)$$

Where, w_1 and w_2=Weights, b_1 and b_2=Bias terms. Y=Predicted L-glutaminase activity, and xt=Row vector of six independent variables, xt^1=Transpose of the vector with a dimension of 3x1.

The performance of the ANNs was statistically measured in terms of coefficient of determination (R^2), root mean squared error (RMSE) and the absolute average deviation (AAD) which were determined by using following formulae:

$$RMSE = \left(\frac{1}{n} \sum_{i=1}^{n} (y_i - y_{di})^2 \right)^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i - y_{di})^2}{\sum_{i=1}^{n} (y_{di} - y_m)^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$AAD = \left\{ \left[\sum_{i=1}^{n} (y_i - y_{xi} / y_{di}) \right] / n \right\} \times 100 \quad \ldots\ldots\ldots (7)$$

Where, n =Number of points, y_i =Predicted value, y_{di} =Actual value, and y_m=Average of the actual values.

R^2 is a measure of the amount of the reduction in the variability of response obtained by using the repressor variables in the model. Since R^2 alone is not a measure of the model's accuracy, it is necessary to use RMSE and AAD analysis which are direct method for describing the deviations. Evaluation of R^2, RMSE and AAD values together would be better to check the accuracy of the model. The adequacy of model would be considered good with values of R^2 close to 1.0 and small values of RMSE and AAD [12] and [23].

3. RESULTS AND DISCUSSION

Glutaminases produced from different microbial strains are generally preferred for their application in food industry in comparison to those produced from plant and animal sources [6-7] and [24]. The extracellular nature of L-glutaminase is of much significance for considering its potential in vitro applications. Most of the bacterial strains [16-17] and [25-27] have been reported to secrete extracellular L-glutaminase and on this basis fifteen bacteria strains were used in this study to get high yielding potent bacteria. **Table2. Estimation of L-glutaminase activity among different bacterial strains**

Bacterial strains	Glutaminase activity (U/l)
Pseudomonas aeruginosa NCIM 2948	220
Pseudomonas putida NCIM 2650	198
Pseudomonas fluorescens NCIM 5096	134
Lactobacillus casei NCIM 2364	0.000
Lactobacillus plantarum NCIM 2373	170
Bacillus megaterium MTCC2412	248
Bacillus subtilis MTCC1789	023
Bacillus cereus MTCC 1305	425
Bacillus licheniformis MTCC1483	013
Bacillus stearothermophilus NCIM 2235	043
Aeromonas formicans NCIM 2319	116
Streptococcus lactis MTCC 460	013
Streptococcus sp. MTCC 389	0.00
Staphylococcus aureus MTCC 3160	043
Escherichia coli MTCC 2893	389

Among these bacterial strains, *Bacillus cereus* MTCC 1305 produced considerably high level of L-glutaminase (425U/l) after conducting the experiments under the reported assay conditions viz., pH of reaction mixture (7.5), reaction time (30minutes), reaction temperature (37˚C), glutamine's concentration (40mM), and enzyme volume (0.5ml) as shown in Table2 and selected for further study. The extractive fermentative production of L-glutaminase from *Bacillus cereus* MTCC 1305 using different PEG/dextran system [28] and optimization of cultural conditions [29] was already studied. Response surface methodology (RSM) was employed to optimize these five significant assay parameters and thirty two experiments were designed using central composite design (CCD) as shown in Table3.

Table 3.Central composite design for 5 variables with experimental and predicted activity of L-glutaminase using RSM and ANN model

Run order	X_1	X_2	X_3	X_4	X_5	Activity (U/l)		
						Exp	CCD	ANN
1	5.0	10	50	60	0.8	125	126.71	124.99 TRD
2	7.5	20	35	0	0.5	103	151.24	101.33

								TRD
3	10.0	10	50	20	0.8	34	12.712	33.82 TRD
4	7.5	20	35	40	0.5	634	627.36	628.03 TRD
5	5.0	10	20	60	0.2	69	48.879	68.99 TRD
6	10.0	30	20	20	0.8	103	98.879	104.02 TRD
7	7.5	20	35	40	1.1	369	353.90	368.83 TRD
8	7.5	20	35	40	0.5	634	627.36	-
9	5.0	10	20	20	0.8	104	87.212	105.18 TRD
10	5.0	30	50	60	0.2	119	124.71	118.72 TRD
11	5.0	30	20	60	0.8	231	249.88	231.09 TRD
12	7.5	20	35	40	0.5	634	627.36	-
13	7.5	20	65	40	0.5	109	120.24	109.21 TRD
14	5.0	10	50	20	0.2	23	-6.955	23.787 TRD
15	7.5	20	35	40	0.5	634	627.36	-
16	5.0	30	50	20	0.8	189	198.04	188.73 TRD
17	7.5	20	5	40	0.5	64	92.576	62.714 TRD
18	7.5	0	35	40	0.5	267	329.90	266.994 TRD
19	2.5	20	35	40	0.5	34	36.242	32.894 TRD
20	7.5	40	35	40	0.5	538	514.91	538.358 TRD
21	5.0	30	20	20	0.2	28	15.21	29.5497 TRD
22	10.0	30	50	20	0.2	142	124.71	141.995 TRD
23	10.0	30	20	60	0.2	329	321.54	328.572 TTD
24	7.5	20	35	40	-0.1	98	152.90	98.321 TTD
25	7.5	20	35	40	0.5	634	627.36	-
26	10.0	10	20	20	0.2	39	-4.121	38.647 TTD
27	10.0	30	50	60	0.8	487	501.37	486.893 TTD
28	7.5	20	35	40	0.5	634	627.36	-
29	10.0	10	20	60	0.8	403	391.54	403.134 VD
30	7.5	20	35	80	0.5	529	520.57	528.935 VD
31	12.5	20	35	40	0.5	209	246.57	209.002 VD
32	10.0	10	50	60	0.2	263	238.37	263.474 VD

Where, X_1= pH of reaction mixture, X_2=Reaction time (Minutes), X_3=Reaction temperature (˚C), X_4=Glutamine concentration (mM), X_5=Enzyme volume (ml), TRD=Training Data, TTD=Testing Data, VD=Validation Data

Table4. Regression analysis of CCD data in terms of test (T) and probability (p)

Term	Coef	SE Coef	T	p
Constant	-1965.07	189.261	-10.383	0.000
X_1	265.47	24.855	10.681	0.000
X_2	15.83	5.689	2.782	0.018
X_3	39.04	3.897	10.018	0.000
X_4	9.59	2.845	3.371	0.006
X_5	1184.87	185.107	6.401	0.000
$(X_1)^2$	-19.44	1.215	-15.999	0.000
$(X_2)^2$	-0.51	0.076	-6.748	0.000
$(X_3)^2$	-0.58	0.034	-17.151	0.000
$(X_4)^2$	- 0.18	0.019	-9.596	0.000
$(X_5)^2$	-1038.76	84.372	-12.312	0.000
$X_1 X_2$	0.19	0.411	0.462	0.653
$X_1 X_3$	0.05	0.274	0.170	0.868
$X_1 X_4$	1.20	0.206	5.860	0.000
$X_1 X_5$	-13.00	13.709	-0.948	0.363
$X_2 X_3$	0.17	0.069	2.529	0.028
$X_2 X_4$	0.01	0.051	0.267	0.794
$X_2 X_5$	2.50	3.427	0.729	0.481
$X_3 X_4$	-0.03	0.034	-0.924	0.375
$X_3 X_5$	-1.22	2.285	-0.535	0.603
$X_4 X_5$	2.79	1.714	1.629	0.132
R^2 = 98.83% R^2(Predicted) = 70.05% R^2 (adjacent) = 96.70%				

Regression analysis data in Table4 showed that all individual variables had linear positive significant effect ($p<0.05$) on L-glutaminase activity but pH of reaction mixture and enzyme volume as individual factors had high coefficient value which indicates their high degree of significant effect on the activity of L-glutaminase. The interaction effect of pH-glutamine concentration and time-temperature also showed linear positive significant effect with $p<0.05$, whereas interaction of other variables were found to be insignificant with $p>0.05$. Among these insignificant interactive variables, the interaction of pH-enzyme volume, temperature-glutamine concentration and temperature-enzyme volume had negative coefficient value which showed that interaction of these variables decreased the activity of L-glutaminase.

The statistical testing of the model was done by Fisher's F test and probability (p) value for analysis of variance (ANOVA) and data is shown in Table5. A highly significant quadratic regression model was obtained with high value of F and very low value of P. This indicates that the combined effects of all the independent variables significantly contributed to maximize the production of L -glutaminase. Coefficient of determination (R^2) after regression analysis was obtained as

98.83 indicating that the sample variation of only 1.17% of the total variation is not explained by the model.

Table5. Analysis of Variance (ANOVA) for analyzing model fitness in terms of Fischer (F) and probability (p)

Source	DF	Seq SS	Adj SS	Adj MS	F	p
Regression	20	1570484	1570484	78524	46.4	0
Linear	5	384058	316919	63384	37.5	0
Square	5	1108160	1108160	221632	131.0	0
Interaction	10	78266	78266	7827	4.6	0.01
Residual Error	11	18605	18605	1691		
Lack of Fit	6	18605	18605	3101	*	*
Pure Error	5	0	0	0		
Total	31	1589089				

The predicted activity of L-glutaminase (Y) was determined after substituting central values of variables in following second-order regression equation:

$$Y=(-1965.07)+265.47X_1+15.83X_2+39.04X_3+9.59X_4+1184.87X_5-19.44X_1^2-0.51X_2^2-0.58X_3^2-0.18X_4^2-1038.76X_5^2+0.19X_1X_2+0.05X_1X_3+1.20X_1X_4-13X_1X_5+0.17X_2X_3+0.01X_2X_4+2.50X_2X_5-0.03X_3X_4-1.22X_3X_5+2.79X_4X_5 \quad \text{(3.8)}$$

The interactive effect of these variables on L-glutaminase production with prediction of their optimum values was analyzed using contour plots. The contour plots for positive significant effect of interaction of variables like enzyme volume-glutamine concentration, enzyme volume-temperature, enzyme volume-time, enzyme volume-pH, glutamine concentration-temperature, glutamine concentration-time, glutamine concentration-pH, temperature-time, temperature-pH, time-pH on activity of L-glutaminase was shown in Figure 1a, 1b, 1c, 1d, 1e, 1f, 1g, 1h, 1i and 1j respectively. These plots showed increase of L-glutaminase activity with simultaneous increase of values of the variables up to an optimum point and decrease of its activity beyond this optimum level. The optimal levels for assay conditions were obtained as pH (7.5), time (20minutes), temperature (35˚C), enzyme volume (0.5ml), glutamine concentration (40mM) and predicted activity was determined as 626.67 U/l by using second order equation.

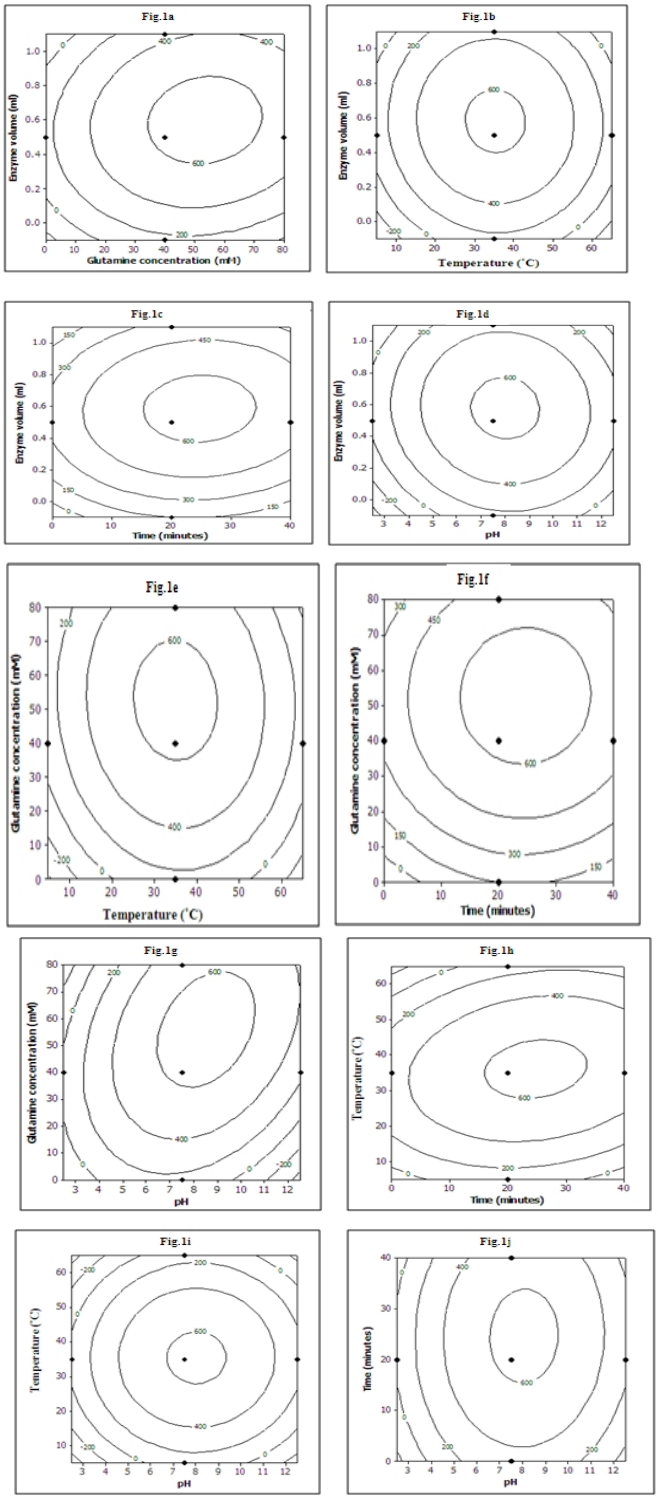

Figure1. Contour plots showing interactive effect of selected variables on activity of L-glutaminase (a) Enzyme volume versus Glutamine concentration; (b) Enzyme volume versus Temperature; (c) Enzyme volume versus Time (d) Enzyme volume versus pH; (e) Glutamine concentration versus Temperature; (f) Glutamine concentration versus Time; (g) Glutamine concentration versus pH; (h) Temperature versus time; (i) Temperature versus pH; (j) Time versus pH

Artificial Neural Network was further applied to analyze response in term of high degree of nonlinearity between input variables (pH, reaction time, incubation temperature, substrate concentration, and enzyme volume) and output variable (L-glutaminase activity). Multilayer perceptron with Levenberg-Marquardt algorithm and sigmoid transfer function was used to determine predicted activity of L-glutaminase. The minimum value of mean squared errors (MSE) was obtained for three neurons as shown in Figure2 and hence was selected as optimum number of neurons in hidden layer for this study.

The data of central composite design was passed into the input layer and then was propagated from input layer to hidden layer and finally to output layer of the network. The architecture of MLP network was obtained as "5-3-1" with five input neurons, three neurons in hidden layer and one output neuron as shown in Figure3.

The predicted activity was determined as 628.035U/l after substituting values of weight on synaptic nodes variance and bias term (Table6) in equation 4. The value of coefficient of determination (R^2), root mean squared error (RMSE) and average absolute deviation (AAD) was determined as 0.98172, 24.606, 3.239% respectively for RSM and 0.9999, 0.6697, 0.086 % respectively for ANN. The high value of R^2, low value of RMSE and low value of AAD for ANN showed better predictor of this model in comparison to RSM.

Table6. Weight and bias values of nonlinear function at optimum assay parameters in ANN model

Weight on synaptic connection between input and hidden nodes					
pH	Time	Temp.	glutamine conc.	Enzyme volume	Bias term (b_1)
-0.34	-0.63	-0.13	0.11	3.48	-0.31
-0.34	-0.16	-0.73	-0.18	-10.90	-0.33
-0.82	-1.69	2.28	0.96	3.50	0.61
Weight on synaptic connection between hidden and output nodes are -4.8728, -1.09035, Bias term (b_2=9.99078)					

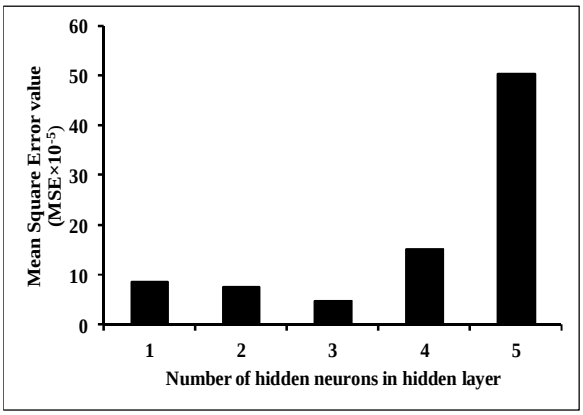

Figure 2. Determination of number of hidden layer's neurons for artificial neural network on basis of MSE values

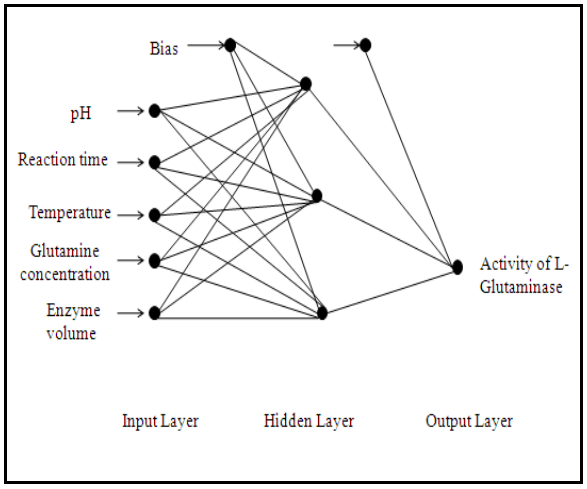

Figure 3. Multilayer perceptron neural network architecture for L-glutaminase

The predicted condition was experimentally verified in triplicate by conducting experiment at predicted optimum culture condition obtained by the model for validation. The experimental L-glutaminase activity was obtained as 628.035U/l at predicted optimum assay conditions (pH=7.5, reaction time=20min, incubation temperature=35˚C, concentration of L-glutamine=40mM, and volume of L-glutaminase =0.5ml), which was enhanced by 1.485 times than the activity of L-glutaminase obtained under unoptimized assay conditions (423U/l).

4. CONCLUSION

Bacillus cereus MTCC 1305 was selected as potent L-glutaminase producing bacteria. The activity of L-glutaminase was enhanced by 1.485 times after optimizing the reaction conditions, viz., pH of reaction mixture (7.5), reaction time (20min), incubation temperature (35˚C), substrate concentration (40mM), and enzyme volume (0.5ml). The statistical tools of RSM and ANN both were employed for optimization of reaction conditions. ANN model was obtained as better predictor than RSM with high value of coefficient of determination, low value of average absolute deviation.

5. ACKNOWLEDGEMENT

Authors would like to thank DST-INSPIRE to provide financial support to carry this work.

6. REFERENCES

[1] Sinsuwan, S, Yongsawatdigul, J, Chumseng, S and Yamabhai, M, Efficient expression and purification of recombinant glutaminase from *Bacillus licheniformis* (GlsA) in *Escherichia coli*, Protein Expression and Purification, 83, May 2012, pp. 52-58.

[2] Moriguchi, M, Sakai, K, Tateyama, R, Furuta, Y and Wakayama, M, Isolation and characterization of salt-tolerant glutaminase from *Micrococcus luteus* K-3, Journal of *Fermentation* and *Bioengineering*, 77, 6, 1994, pp. 621-625.

[3] Tachiki, T, Yamada, T, Ueda, M, Naemura, Y, Imamura, N, Hamada, Y and Shiode, J, Purification and some properties of glutaminase from *Pseudomonas nitroreducens* IFO12694, Bioscience, Biotechnology, and Biochemistry, 60, Jun 1996, pp. 1160-1164.

[4] Sabu, A, Chandrasekaran, M and Pandey, A, Biopotential of microbial glutaminases. Chemistry Today, 18, 2000, pp. 21-25.

[5] Kumar, L, Singh, B, Adhikari, DK, Mukherjee, J and Ghosh D, A Temperature and Salt-Tolerant L-glutaminase from Gangotri Region of Uttarakhand Himalaya: Enzyme Purification and Characterization, Applied Biochemistry and Biotechnology, 166, February 2012, pp. 1723-1735.

[6] Imada, A, Igarasi, S, Nakahama, K and Isono, M, Asparaginase and glutaminase activities of Microorganisms, Journal of General Microbiology, 76, May 1973, pp. 85-99.

[7] Brown, G, Singer, A, Dementieva, I, Proudfoot, M, Kuznetsova, E, Skarina, T, Gonzalez, CF, Kim, Y, Joachimiak, A, Chang, C, Savchenko, A and Yakunin, AF, Functional and Structural Characterization of Four Glutaminases from *Escherichia coli* and *Bacillus subtilis*, Biochemistry, 47, May 2008, pp. 5724-5735.

[8] Nierlich, DP and Magasanik, B, Regulation of Purine Ribonucleotide Synthesis by End Product Inhibition : The Effect of Adenine And Guanine Ribonucleotides on the 5'-Phosphoribosylpyrophosphat Amidotrans-ferase of *Aerobacter Aerogenes*, Journal of Biological Chemistry, 240, 1, January1965, pp. 358-365, 1965.

[9] Mongin, AA, Garcia, MCH, Vibcent, MY and Keller, Jr., RW, A simple method for measuring intracellular activities of glutamine synthetase and glutaminase in glial cells, American Journal of Physiology: Cell Physiology, 301, October 2011, pp. C814-C822.

[10] Rathi, P, Saxena, R and Gupta, R, A novel alkaline lipase from *Burkholderia cepacia* for detergent formulation, Process Biochemistry, 37, 2, October 2001, pp. 187-192.

[11] Sim, JH and Kamaruddin, AH, Optimization of acetic acid production from synthesis gas by chemolithotrophic bacterium-*Clostridium aceticum* using statistical approach, Bioresource Technology, 99, 8, May 2008, pp. 2724-2735.

[12] Bas, D and Boyaci, IH, Modeling and optimization I: Usability of response surface methodology, Journal of Food Engineering, 78, 3, February 2007, pp. 836-845.

[13] Dutta, JR, Dutta, PK and Banerjee, R, 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, 12, October 2004, pp. 2193-2198.

[14] Haykin, S, Neural Networks and Learning Machines. Pearson Education Inc, New Jersey, pp. 122-218, 2009.

[15] Nelson ,GEN, Peterson, RE and Ciegler, A, Glutaminase Production by Bacteria, Development in industrial Microbiology, Development In Industrial Microbiology, Publication of the Society for Industrial Microbiology, American Institute of Biological Sciences, Washington, 16, Chapter 42, 1975.

[16] Harayama, F and Yasuhira, H, Effects of glutaminase in rice Koji and from *Bacillus subtilis* GT strain on miso fermentation, Journal of Brewing Society of Japan, 86, September 1991, pp. 529-535.

[17] Klein, M, Kaltwasser, H and Jahns, T, Isolation of a novel, phosphate-activated glutaminase from *Bacillus pasteurii*, FEMS Microbiology Letter, 206, January 2002, pp. 63-67.

[18] Wakayama, M, Yamagata, T, Kamemura, A, Bootim, N, Yano, S, Tachiki, T, Yoshimune, K and Moriguchi, M, Characterization of salt-tolerant glutaminase from *Stenotrophomonas maltophilia* NYW-81 and its application in Japanese soy sauce fermentation, Journal of Industrial Microbiology and Biotechnology, 32, 9, September, pp. 383–390.

[19] Sathish, T and Prakasham, RS, Enrichment of glutaminase production by *Bacillus subtilis* RSP-GLU in submerged cultivation based on neural network–genetic algorithm approach, Journal of Chemical Technology and Biotechnology, 85, January 2010, pp. 50–58.

[20] Curthoys, NP and Watford, M, Regulation of glutaminase activity and glutamine metabolism, Annual Review of Nutrition, 15, July 1995, pp. 133-159.

[21] Lou, W and Nakai, S, Application of artificial neural networks for predicting the thermal inactivation of bacteria: a combined effect of temperature, pH and water activity, Food Research International, 34, 7, 2001, pp. 573-579.

[22] Prasanthi, V, Nikku, MY, Vuddaraju, SP, Nalla, KK, Raju, CAI, Donthireddy, RR, Optimization of the Fermentation Media using Statistical Approach and Artificial Neural Networks for the Production of an Alkaline Protease from *Bacillus subtilis*, International Journal of Natural and Engineering Sciences, 2, 3, September 2008, pp. 51-56.

[23] Ebrahimpour, A, Rahman, RNZRA, Kamarudin, NHA, Basri, M and Salleh, AB, Lipase production and growth modeling of a novel thermophilic bacterium: *Aneurinibacillus thermoaerophilus* strain AFNA, Electronic Journal of Biotechnology, 14,4, July 2011, pp. 1-16.

[24] Nandakumar, R, Yoshimune, K, Wakayama, M and Moriguchi, M, Microbial glutaminase: biochemistry, molecular approaches and applications in the food industry, Journal of Molecular Catalysis B*:* Enzymatic, 23, September 2003, pp. 87–100.

[25] Renu, S and Chandrasekaran, M, Extracellular L-glutaminase production by marine bacteria, Biotechnology Letter, 14, June 1992, pp. 471–474

[26] Keerthi, TR, Suresh, PV, Sabu, A, Rajeev, KS and Chandrasekaran, M, Extracellular production of L-glutaminase by alkalophilic *Beauveria bassiana* BTMF S10 isolated from marine sediment, World Journal of Microbiology and Biotechnology, 15, December 1999, pp. 751-752, 1999.

[27] Dura, MA, Flores, M and Toldra, F, Purification and characterisation of a glutaminase from *Debaryomyces* spp, International Journal of Food Microbiology, 76,June 2002, pp. 117– 126.

[28] Singh, P. and Banik, R.M., Partitioning studies of L-glutaminase production by *Bacillus cereus* MTCC 1305 in different PEG–salt/dextran. Bioresource Technology, 114, June 2012, pp. 730-734.

[29] Singh, P., Shera, S.S., Banik, J., Banik, R.M., Optimization of cultural conditions using response surface methodology versus artificial neural network and modeling of L-glutaminase production by *Bacillus cereus* MTCC 1305, Bioresource Technology 137, June 2013, pp. 261-269.