Iranian Journal of Biotechnology. 2013 December; 11(4): 223-32. DOI: 10.5812 / ijb.11272
Published online 2013 November 20. **Research Article**

Modeling and Optimization of β-Cyclodextrin Production by *Bacillus licheniformis* using Artificial Neural Network and Genetic Algorithm

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Received: March 21, 2013; **Revised:** August 8, 2013; **Accepted:** September 9, 2013

Background: The complexity of the fermentation processes is mainly due to the complex nature of the biological systems which follow the life in a non-linear manner. Joined performance of artificial neural network (ANN) and genetic algorithm (GA) in finding optimal solutions in experimentation has found to be superior compared to the statistical methods. Range of applications of β-cyclodextrin (β-CD) as an enzymatic derivative of starch is diverse, where the complex performance of cyclodextrin glucanotransferase (CGTase) as the involved enzyme is not well recognized.
Objectives: The aim of the present work was to use ANN systems with different training algorithms and defined architectures joined with GA, in order to optimize β-CD production considering temperature of the reaction mixture, substrate concentration, and the inoculum's pH as the input variables.
Materials and Methods: Commercially Neural Power, version 2.5 (CPC-X Software, 2004) was used for the numerical analysis according to the specifications provided in the software. β-CD concentration was determined spectrophotometrically according to phenolphthalein discoloration technique, described in the literature.
Results: Randomly obtaining the experimental data for β-CD production in a fermentation process, could get explainable order using the ANN system coupled with GA. Changes of the β-CD as the function of each of the three selected input variables, were best quantified with use of the ANN system joined with the GA. The performance of the IBP learning algorithm was highly favorable (10300 epoch's number within 5 second, with the lowest RMSE value) while the sensitivity analysis of the results which was carried out according to the weight method, were indicative of the importance of input variables as follows: substrate concentration < temperature < inoculum's pH. For instance, small changes in the system's pH are associated with the large variation in the β-CD production as has been described by the suggested model.
Conclusions: Production of β-CD (enzymatic derivative of starch) by *B. licheniformis* was satisfactorily described based on multivariate data analysis application of the ANN system and the experimental data were optimized by considering ANN plus the GA where the IBP was used as the training method and with use of three neurons as the constructed variables in the hidden layer of the test network.

Keywords: Artificial Neural Network; *Bacillus licheniformis*; β-Cyclodextrin Production; Genetic Algorithm; Modeling; Optimization

1. Background

Cyclodextrins (CDs) are cyclic oligosaccharides which contain six, seven, eight or more glucopyranose units and are known as α-CD, β-CD, and γ-CD, respectively. They are produced during degradation of starch by cyclodextrin glucanotransferases (CGTase) (EC 2.4.1.19) which is commercially available or can be produced by some microorganisms especially diverse strains of *Bacillus* such as *Bacillus circulans* (1). *Bacillus macerans*, *Bacillus firmus* (2) and *Bacillus licheniformis* (3-5). The capability of cyclodextrins (CDs) to form inclusion complexes and modify the physical and chemical properties of the guest molecules, makes them extremely attractive for vast industrial applications such as food, chemical, pharmaceutical, and textile, as well as in biotechnology and agriculture. The complexity of the fermentation processes has been well documented in the literature and this is mainly due to the complex nature of biological systems which the manner they follow in the life processes is highly non-linear. With considering the other natural attitudes of living objects such as lack of preciseness of information (or fuzzy data), the need for deep recognition of the challenging matters and use of optimization methods in this regard,

Implication for health policy / practice / research / medical education:
In this work, I²-Cyclodextrin production by Bacillus licheniformis was modeled using the artifi•cial neural network (ANN) system. Effects of the reaction conditions such as temperature, substrate concentration of the reaction medium and inoculum characteristics in terms of pH were studied by means of various networks

became clearly evident (6). These handling methods based on mathematical modeling, which have been used traditionally in different types of biological processes, are single variable optimization, factorial and fractional factorial design of experiment, and response surface methodology (7). Experimental behavior of a process of the interest could thus be adequately predicted using an appropriate mathematical model. It is possible to obtain optimal cell growth and metabolite formation by varying the model parameters, according to the details given by a particular statistical design, instead of being extensively involved in experimentation. There are numerous advantages of developing experimental optimization methods for existing process with use of knowledge based procedure, and the management of information can be efficiently proceeded in this manner. Availability of experimental data and knowing the underlying theory in a particular subject are the two key elements in deciding whether or not to use the ANN system. These types of artificially managed information systems (the NN system) work best in areas with unclear theory but when one has accessibility to the relevant data (8).

The evolutionary method based on the natural selection of the best individual in a population is the concept followed in genetic algorithm (GA) computational program. In developing optimization method based on ANN system, it is highly desirable to couple neural network (NN) to the genetic algorithm (GA). The necessitousness of this coupling is to follow the four main steps in GA program: 1) randomly generating a population of the individual experiments, 2) evaluating each of them by assigning a specific fitness function, 3) selecting the individuals with the higher assigned fitness value, and 4) by hybridizing the selected individuals, the completion of the sorting process is achieved. Treating the new population as before and the sorting in GA program is continued until appearance of the system's satisfaction criteria (9, 10).

Growth of microorganism and its ability in producing of a particular metabolite proceed through the unstructured kinetics, in which the systems follow the saturation type of enzymatic mechanism. The usual practice in situations with unclear theory, is to record the cell responses to changing environment in terms of the substrate concentration, temperature and pH of the test system (*in vitro* studies). Mechanistic approach in solving these types of problems and handling the highly complex enzymes such as cyclodextrin glucanotransferase (CGTase), with unknown underlying mechanism, can be a real barrier in experimentation to find optimal positions of the chosen inputs relative to the output(s). Use of ANN joined with GA has been highly recommended in these situations for decreasing the extent of these types of problems. Thus predictive ability of the test system (ANN plus GA) is readily achievable.

2. Objectives

The aim of the present work was to describe an optimized model for production of β-CD by B. *licheniformis* using ANN system joined with the GA. Temperature of the reaction mixture, changes in maltodextrin concentrations as the reaction substrate, and the inoculum's pH have been considered as the input variables and the dependency of β-CD production on these variables was quantified in terms of the amount of β-CD produced in these fermentation processes.

The focusing point in ANN system was to examine different training algorithms namely, incremental back propagation (IBP), batch back propagation (BBP), quick propagation (QP), genetic algorithms (GA), and Levenberg-Marquardt (LM). The results of this section, in which IBP and BBP were the chosen algorithms, was taken as the basis of the training with considering different numbers of the constructed neurons in the hidden layer (2 to 10 neurons). The changes in the IBP with varying the number of neurons were less than those of the BBP training algorithm (comparisons of R^2 values). Thereafter, the ANN systems with IBP and BBP training algorithm both were examined to evaluate the system's responses in optimization section in which GA, particle swarm optimization (PSO), and rotation inherit optimization (RIO) were the methods of the choice. Artificial neural network with IBP as the training algorithm coupled with GA was found to be more capable in fitting of the experimental data (better predictive ability for ANN system 'having IBP training algorithm' joined with GA).

3. Materials and Methods

3.1. Materials, Microorganism, Cultivation Conditions and Quantification of the Metabolite Concentration (β-CD)

All chemicals used in the present study were analytical grade and purchased from the local suppliers (maltodextrin and β-cyclodextrin 'Sigma' and the other chemicals 'Merck'). A freeze dried culture of *Bacillus licheniformis* (PTCC 1320) was purchased from the Persian Type Culture Collection, Iranian Research Organization for Science and Technology, Tehran, Iran. The culture transfer was followed according to the directions provided by the supplier. For inoculum preparation, one loopfull of the biomass from the agar slant was transferred into 250 mL Erlenmeyer flask contained 50 mL of the main medium which consisted of the following ingredients (L^{-1}): 10 g soluble starch, 5 g peptone, 5 g yeast extract, 1 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$. The sodium carbonate compound concentration of 5 w/v % solution was used in different volume ratios for the adjustment of the pH of the medium. The addition of this solution to the main medium was done separately and after autoclaving of the test solution. The main medium's pH was adjusted to 8. The culture was incubated using a rotary shaker at 37 °C and at 150 rpm for 24 hours. Initially for the enzyme production, a 5 v/v % of the test inoculum (prepared as described above) was transferred into a 250 mL conical flask containing the main medium at the different pH (8, 9 and 10), the test cultures were incubated at 37 °C for 48 hours using a shaker incubator (150 rpm). The grown culture was used as the inoculum for examining effect of inoculums' pH on the production of β-CD. Thereafter the culture solution containing the enzyme of the interest solution was added to solutions of the maltodextrin as the test substrate. The three different initial concentrations of the substrate (1, 5 and 10 w/v %) were used in the present study. The production of β-CD was followed at different temperatures (37, 50, and 60 °C). Each production assay was carried out for 10 days. The reaction medium consisted of required amount of maltodextrin dissolved in 50 mM Tris–HCl buffer (pH = 8), and 5 mM $CaCl_2$, the volume of the solution was 50 mL in a conical flask of 250 mL. This solution was inoculated with the test bacterium solution (containing CGTase) at the ratio of 1:1 (v/v). The medium prepared as described was incubated in a shaker incubator (120 rpm). Amount of β-CD was determined at the end of the experiment using spectrophotometer for recording the color changes at 550 nm based on complexation made between β-CD and phenolphthalein, the details of procedure are given elsewhere (11).

Table 1 shows the data of β-CD production obtained experimentally, and partitioned through training and testing data subsets which were used for the training of the ANN system.

Table 1. Experimental Data of Training and Testing of Artificial Neural Network

Temperature (°C)	Substrate Concentration (w/v %)	Inoculum's pH	β-CD Production (mM)
Training Data			
60	1	10	0.168
60	10	10	0.266
37	10	10	0.822
60	1	8	0.503
50	1	8	0.750
50	10	8	1.007
37	1	8	0.579
60	1	9	1.455
50	5	10	1.106
60	5	9	1.603
37	1	9	1.021
37	5	9	1.119
50	10	9	1.020
60	10	9	1.833
37	5	8	0.799
50	1	9	0.710
50	10	10	1.420
37	5	10	0.562
37	10	8	0.455
60	5	8	0.712
Testing Data			
60	5	10	0.176
37	1	10	0.493
50	5	8	0.912
37	10	9	1.208
50	5	9	0.947
60	10	8	0.854
50	1	10	0.690

3.2. ANN linked to GA

The software package of Neural Power version 2.5 (CPC-X software, 2004) was used in the present study. The multilayer normal feed forward neural network was used to characterize production of β-CD from maltodextrin by B. *licheniformis*. The suggested ANN consisted of three layers, where the input layer comprised of three neurons (substrate concentration, temperature, and inoculum's pH), three in the hidden layer, and one in the output layer of the model (amount of β-CD produced in the fermentation process). Once the ANN was generated, it was trained to accurately model the test system of the interest. The ANN was trained by different learning algorithms, IBP, BBP, QP, GA, and LM. The structure of the suggested ANN used for the present study is shown in Figure 1. The transfer functions of hidden and output layers were hyperbolic tangent (Tanh). The training was continued until the network root mean squared error (RMSE) reached to the lowest value while the coefficient of determination (R^2) became close to one. Other parameters for the ANN were chosen according to Table 2.

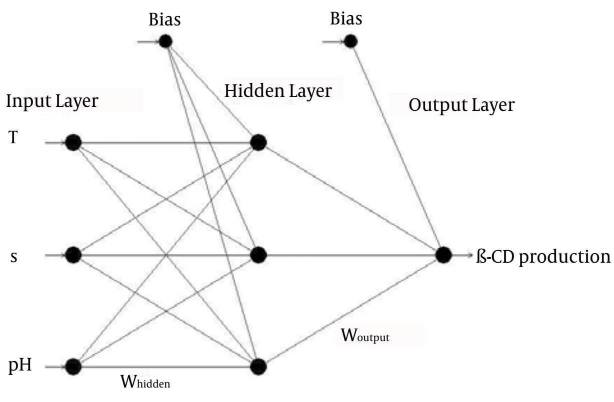

Figure 1. Structural Organization of the Neural Network Used for Estimation of β-CD Production by *B. licheniformis*.

Table 2. The Neural Network Characteristics Used to Evaluate the Training Algorithm

Training algorithm	Learning rate	Momentum
BBP	0.8	0.8 [a]
IBP	0.8	0.8 [a]
QP	0.8	0.8 [a]
LM	0.1	0.4
GA	0.15 [a]	0.8 [a]

[a] the default values of the software

The normalization of the data was performed at the first stage of the data fitting: Equation 1 (Figure 2)

$$data\ normalization: \theta = \frac{X - X_{min}}{X_{max} - X_{min}}$$

Figure 2. Equation 1

where the Xs show the values of input or output parameters, as needed and θ is the normalized form of the relevant data.

The expressions bellow were used for RMSE and R^2 values determination: Equation 2 (Figure 3), Equation 3 (Figure 4)

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(Y_{pre}^{i} - Y_{exp}^{i}\right)^2}{n}}$$

Figure 3. Equation 2

$$R^2 = 1 - \left(\frac{\sum_{i=1}^{n}(Y_{exp}^{i} - Y_{pre}^{i})^2}{{}_{i=1}^{n}(Y_{exp}^{i} - \bar{Y})^2}\right)$$

Figure 4. Equation 3

Where Y is the average Y over the n samples, and Y^{i}_{exp} and Y^{i}_{pre} are the ith experimental and predicted value. The process details in linking the ANN with GA are shown in Figure 5 where β-CD production was optimized by comparing ANN linked with either of the three algorithms, listed in the Neural Power software, namely GA, particle swarm optimization (PSO), and rotation inherit optimization (RIO).

4. Results

4.1. Artificial Neural network Analysis of β-CD Production

For minimization of the learning error during the neural network training it is necessary to iteratively update the network weights. Different learning algorithms

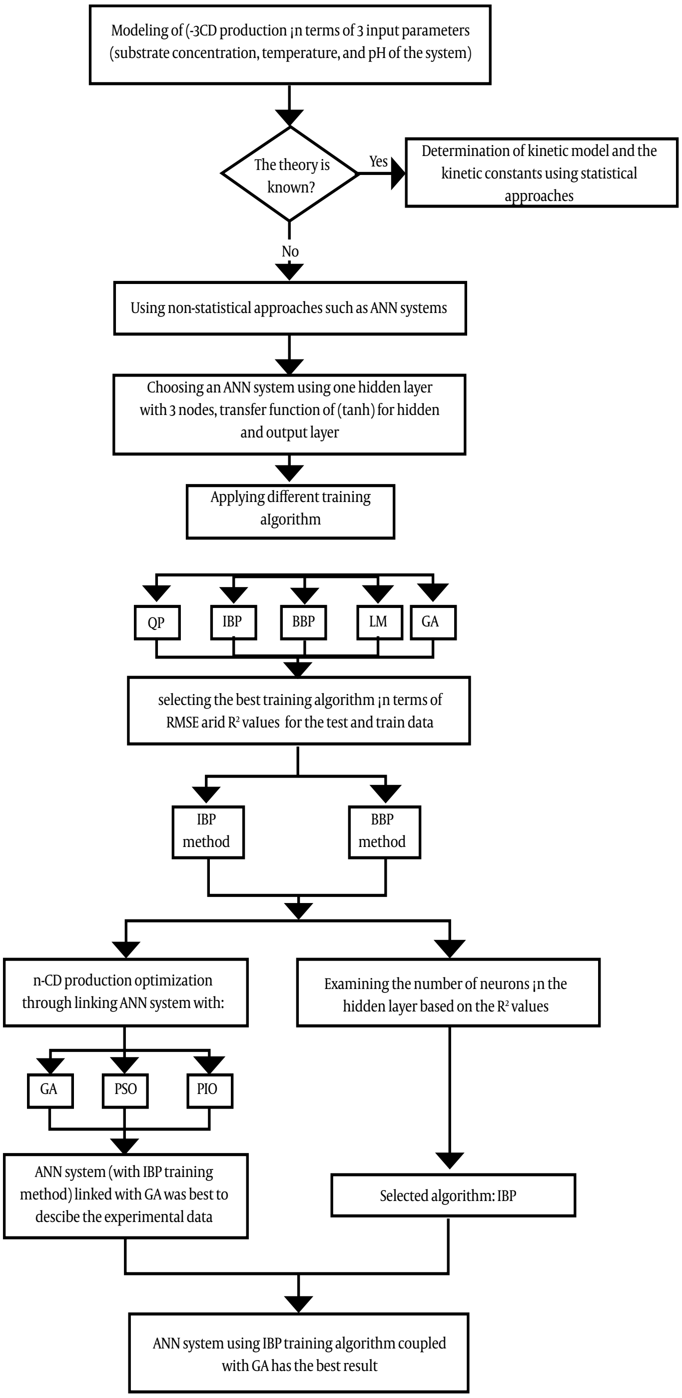

Figure 5. Algorithm of the Program Followed in the Present Study

namely IBP, BBP, QP, GA, and LM were used in this work for training multilayer normal feed forward for obtaining best result of the β-CD production from maltodextrin substrate. Figure 6 shows the results of

ANN performance on the basis of R^2 values obtained for the different training algorithms used in the present study. It is also notable to see the results presented in Table 3 where the decreasing order of the RMSE character is as follows: LM > BBP > IBP. Successive training cycles (epochs) are adjusted according to the learning rule and the error correction learning rule is based on the recording the difference between the ANN solution at any cycle of the training and the corresponding correct answer (8).

Table 3. ANN Performance Parameters During Training

Training Function	Iterations	Time Elapsed (s)	RMSE
BBP	45750	10	0.086
IBP	10300	5	0.058
QP	76750	42	0.095
LM	11250	9	0.093
GA	26150	28	0.1039

Modification of connections' weights on this basis thus can be used to gradually reduce the overall network error. According to Table 3, the IBP and BBP were the selected training algorithms and this was based on their low values of RMSE as compared with that of the LM. Selecting the training method on the basis of the RMSE value is the approach which has been used by the other researchers (12), although the performance of LM in many studies reported in the literature, was better mainly in terms of the completion time of the training (13).

Performance of feed forward error back propagation is based on finding error as a function of ANN weights with use of gradient descent. The iteration in back propagation (BP) thus proceeds to two steps: forward activation in producing a solution, and the completed error at the output is propagated backward through the hidden layer to reduce the error by modifying the weights at the input layer.

The convergence rate and complexity of a model is highly depended on the type of transfer function used, Table 4 presents some transfer functions commonly used in the ANN systems. By performing the trial an error process, it is possible to find the transfer function with the best performance. In the present study transfer functions for the hidden and output layer both were hyperbolic tangent (Tanh).

Table 4. Some Transfer Functions Commonly Used in the ANN Systems

Function Name	Mathematical Formula
Hyperbolic Tangent	$f(x)=\frac{1-exp(-ax)}{1+exp(-ax)}$
Sigmoid	$f(x)=\frac{1}{1+exp(-ax)}$
Gaussian	$f(x)=exp(-ax^2)$
Linear	$f(x)=ax$
Threshold linear	$f(x)=\begin{cases}0 & x<0\\ 1 & x>1\\ ax & 0<x<1\end{cases}$
Bipolar linear	$f(x)=\begin{cases}-1 & x<-1\\ 1 & x>1\\ ax & -1<x<1\end{cases}$

The key element for obtaining an appropriate accuracy of the suggested model in ANN system is to find the optimal number of neurons in the hidden layer. Too few neurons in the hidden layer limit the modeling ability of the ANN system while excessive number of neurons result over-fitting and decreases the predictability of the system. The optimal number of neurons in hidden layer is usually determined through trial and error process and in the present study the optimum number of neuron was chosen to three on the basis of the R^2 values. Table 5 shows the dependency of performance quality of the ANN system on the neuron numbers in the hidden layer. Performance of the BBP and IBP methods were comparable while, variation in the IBP performance in response to changes of the neuron number in the hidden layer was considerably lower than BBP (Table 5).

Figure 6 and Figure 7 are the result of assessing the performance of the trained ANN systems through training and testing and analysis of the error prediction. On the basis of selecting the best training method (Figure 6), the selection was finalized according to the testing stage of the ANN system. Figure 7 shows the correlation between the experimental values for the β-CD production and the values predicted by the ANN system.

Table 5. R^2 Values of the ANN Models With Respect to Training and Testing Data Obtained According to IBP and BBP Training Algorithms

BBP			
	Model	**R^2 Value (Train Data)**	**R^2 Value (Test Data)**
	3-2-1	0.9472	0.9736
	3-3-1	0.9896	0.9916
	3-4-1	0.9982	0.9877
	3-5-1	0.6272	0.4722
	3-6-1	0.8556	0.2803
	3-7-1	0.8264	0.6090
	3-8-1	0.8264	0.6147
	3-9-1	0.9981	0.7899
	3-10-1	1.0000	0.9506
IBP			
	Model	**R^2 Value (Train Data)**	**R^2 Value (Test Data)**
	3-2-1	0.9312	0.9194
	3-3-1	0.9956	0.9894
	3-4-1	0.9953	0.9541
	3-5-1	0.9961	0.9795
	3-6-1	0.9999	0.8699
	3-7-1	0.9988	0.9796
	3-8-1	0.9999	0.9063
	3-9-1	0.9994	0.9326
	3-10-1	0.9999	0.8133

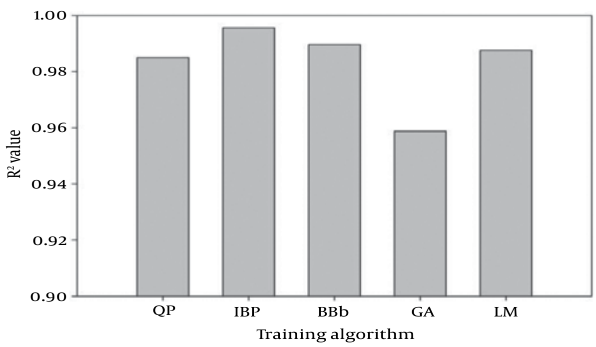

Figure 6. Comparison of the R^2 Values for Training Section in Various Training Algorithms.

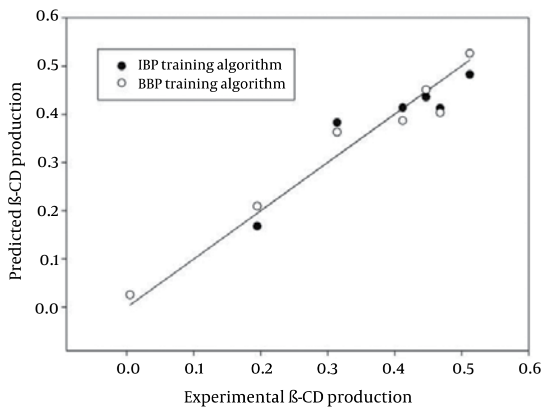

Figure 7. Correlation Between Experimental and Predicted β-CD Production by B. licheniformis Data of the Testing Section in the ANN System.

The R^2 values with the normalized data were 0.989 and 0.992 when IBP and BBP respectively, were used as the training method. The success of the neural network modeling, even though the number of data points is relatively small, may be partially attributed to the statistical experimental design procedure employed during the experimental work. The well-stretched distribution of the data in the entire experimental region may help to obtain a good model even with restricted number of the data points.

4.2. Optimization of β-CD Production

The maximization of the β-CD metabolite, with considering the experimental data, was best described using IBP in combination with GA, where the other two optimization methods offered by the NeuralPower software were PSO and RIO. Results of the optimization study are shown in Table 6. ANN linked with GA was selected and gave the best quality of the ANN performance. In each of these three cases the default values of the Neural Power software were used to handle each of the three cases. The model validation was carried out by running the GA several times with use of different randomly generated populations, where, as a result of successive runs, only slight variation in the value of β-CD production was observed.

4.3. Sensitivity Analysis

In a study, checking the responsiveness of the suggested model to change in any model parameter(s) has been termed sensitivity analysis. The result of this test indicates the extent of the change i. e. for instance larger the result of sensitivity analysis, means there is greater variation in the suggested model because of changes in the parameter of the interest. The following methods have been usually used to analyze the sensitivity of the suggested model in a particular ANN system (14): 'Partial derivatives (PaD)', 'weights', 'profile', and 'backward stepwise' methods. The details of these methods are given elsewhere (14). Evaluation of the suggested model quality (robustness of the model parameter) is carried out by finding out the extent of contribution of the selected functions in describing relationship between the input, the constructed (hidden), and the output variables (14). In the present study and according to the results of sensitivity analysis (weight method / NeuralPower software), the quantitative extent of contribution of each input variables on the amount of β-CD produced, was presented in Figure 8.

Table 6. Optimum Conditions for β-CD Production by Different Optimization Methods

Input Parameter	IBP			BBp			Experimental Optimum of β-CD Production
	GA	PSO	RIO	GA	PSO	RIO	
Temperature (°C)	60	59.97	60	60	59.94	60	60
Substrate Concentration (w/v %)	10	10	10	10	10	10	10
Inoculum's pH	8.9	8.78	8.78	8.66	8.67	8.66	9
β-CD Production (mM)	1.84	1.84	1.84	1.87	1.84	1.87	1.833

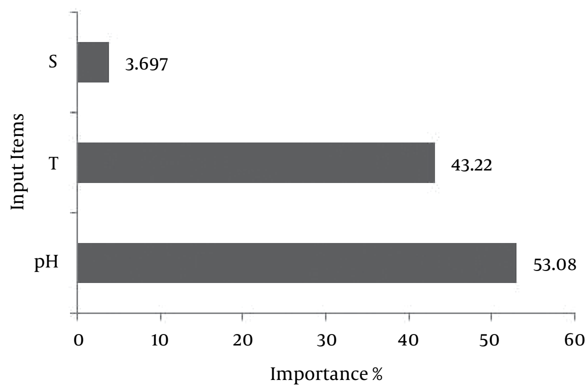

Figure 8. Result of Sensitivity Analysis in Quantitatively Defining the Effects of the Input Variables (Substrate Concentration, System's Temperature and pH) on the Output Parameter (Amount of Produced β-CD) (Importance of Input Parameters on the β-CD Production)

5. Discussion

Appropriateness of using ANN for living organisms is for those behaviors with unclear theory of the action (8). Growth dependency of *B. licheniformis*, in terms of the bacterial activity in production of β-CD, on the temperature changes of the test system is shown in Figure 9 A. Apparently this pattern followed a mechanism which cannot be explained with the data of Figure 9 A. Sigmoid shape of the growth curve presents the logistic model which is one of the well-known equations in areas of the microbial growth (15). The results presented in Figure 9 are linked to those seen in Figure 8 and in describing the robustness of the temperature variable in this study, more data are needed and small size of information reduce the chance of reaching the best decision in expressing the findings quality. In other words, higher value obtained for the sensitivity analysis (Figure 8) indicates that the small change in temperature variable is associated with the large variations in the suggested model behavior.

Trend of β-CD production as a function of maltodextrin concentration is seen in Figure 9 B, production of metabolite increases proportionally with the increase of substrate concentration, as expected in changes seen in microbial growth. In the present study no growth inhibition behavior due to dextrin as a growth promoting substrate concentration was observed. Growth inhibition character due to the substrate has been extensively studied for many compounds for different microbes. Figure 8 shows that the sensitivity analysis was the lowest for the substrate concentration parameter, i. e. large changes in the substrate level is associated with the small variation in the suggested model behavior.

Figure 9 C shows the effect of the inoculum's pH on the production of β-CD. According to the studies reported in the literature, almost all of the bacterial strains capable of producing β-CD (most of the *Bacillus* species) are alkalophilic (1 , 2) and the test bacterium used in the present study was not alkalophilic strain and an attempt was made to modify the pH of the growth medium. During adaptation of a bacterium to a new culture medium with different pH, it is likely to see fluctuations of the metabolite production (3). More works are needed to better understand the trend of effects of temperature and inoculum's pH on the β-CD production by the test bacterium used in the present study.

Production of β-CD by *B. licheniformis* was satisfactorily described in this work based on multivariate data analysis with considering application of the ANN system and among five training algorithms examined, performance of the IBP and BBP methods were comparable (with considering R^2 and RMSE values). While variation in the IBP performance in response to changes of the neuron number in the hidden layer was considerably lower than BBP. The experimental data were best optimized by using ANN system coupled to the GA (IBP training method). The results of the sensitivity analysis showed the following trend of importance of input variables: substrate concentration < temperature < inoculum's pH.

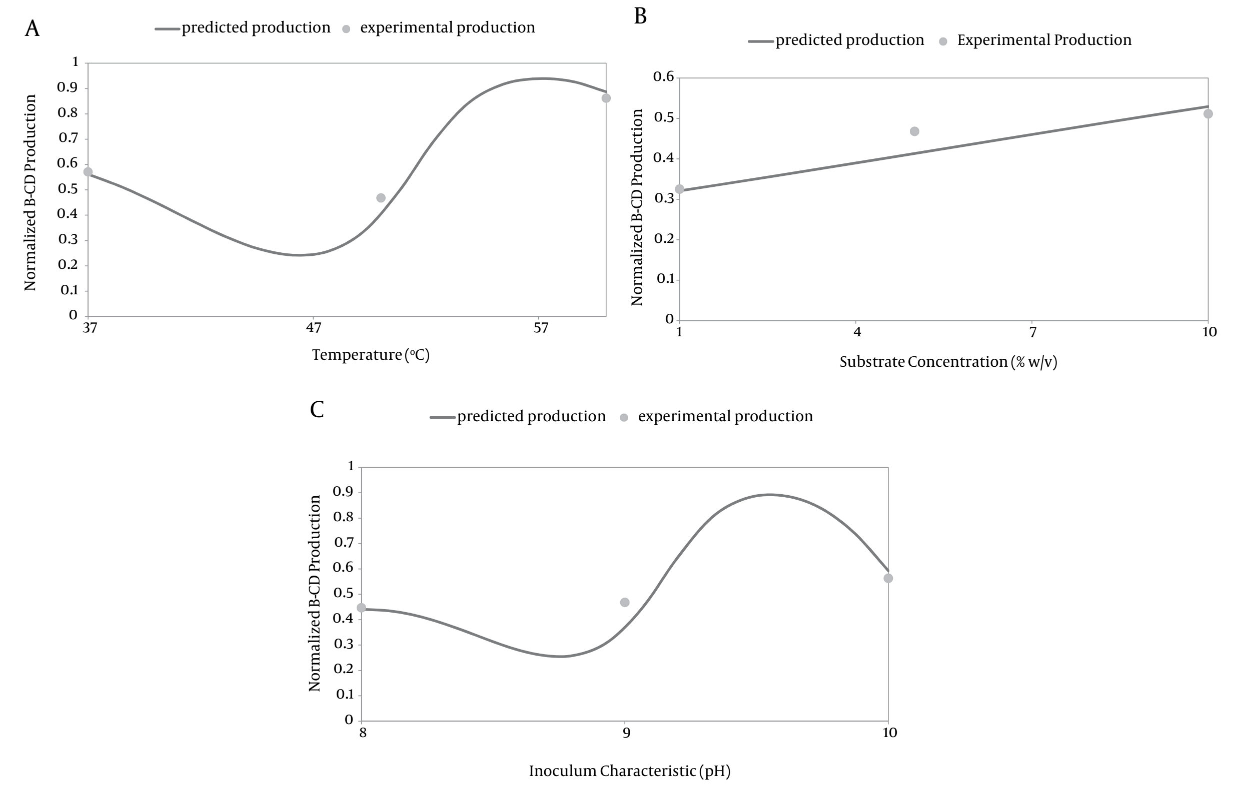

Figure 9. The effect of Input parameters on β-CD production predicted by the NeuralPower software: (a) Temperature effect at S = 5 w/v %; inoculums pH = 9; (b) Substrate concentration effect at T = 50 °C; inoculums pH = 9; (C) Inoculum's pH effect at T = 50 0C; S = 5 w/v %..

Acknowledgements

The authors would like to express their gratitude to Dr. F. Vahabzadeh, Professor, Chemical Engineering Department, Food Engineering and Biotechnology Group, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran, for the support of this work.

Authors' Contribution

Study concept: Naderifar, Sanjari. Analysis and interpretation of data: Pazuki, Sanjari. Drafting of the manuscript: Sanjari, Pazuki. Critical revision of the manuscript: Pazuki, Naderifar, Sanjari.

Financial Disclosure

There is no financial interest.

Funding / Support

Amirkabir University of Technology (Tehran polytechnic).

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