

# Prediction of Biosorption of Total Chromium by *Bacillus* sp. Using Artificial Neural Network

Farhana Masood · Masood Ahmad ·  
Mujib Ahmad Ansari · Abdul Malik

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**Abstract** An artificial neural network (ANN) model was developed to predict the biosorption efficiency of *Bacillus* sp. for the removal of total chromium from aqueous solution based on 360 data sets obtained in a laboratory batch study. Experimental parameters affecting the biosorption process such as pH, contact time and initial concentration of chromium were studied. A contact time of 2 h was generally sufficient to achieve equilibrium. At optimal conditions, metal ion uptake increased with increasing initial metal ion concentration. The Freundlich model was applied to describe the biosorption isotherm. Chromium biosorption was most significantly influenced by pH, followed by the initial metal concentration of the solution. The findings indicated that the ANN model provided reasonable predictive performance ( $R^2 = 0.971$ ) of chromium biosorption.

**Keywords** Artificial neural network · Biosorption · Total chromium

Chromium (Cr) pollution has become of considerable concern due to the fact that chromium has been widely used in metal finishing, electroplating, leather tanning, stainless steel production, textile industries, and chromate preparation (Kumar et al. 2007). Chromium exists in the environment in either its hexavalent form Cr(VI) or trivalent form Cr(III). The metal species Cr(VI) is considered to be highly toxic in that it may act as a carcinogen, mutagen and teratogen in the biological system (Hu et al. 2005). Although Cr(III) is considered essential to mammals, the Cr(III) species is also known to be toxic to fish, specifically when the concentration exceeds 5.0 mg/L (Alloway and Ayres 1996). It has also been noted that prolonged exposure to Cr(III) may cause skin allergies and cancer in human beings (Yun et al. 2001).

Recently, the commonly used methods applied to remove excessive chromium from aqueous solutions have included ion exchange, chemical precipitation, activated carbon adsorption, evaporation and membrane processes. However, these methods have been found to be either inefficient or expensive when metal ions exist in low concentrations (<100 mg/L), and may also be associated with the generation of secondary environmental problems from waste disposal. The use of biological material in the removal of heavy metals from industrial effluents has gained importance during recent years because of its high efficiency, minimization of chemical/biological sludge, low operating cost, regeneration of biosorbents, and possibility of metal recovery (Nasernejad et al. 2005; Sethunathan et al. 2005).

Microorganisms including bacteria, algae, fungi and yeast uptake metal either actively (bioaccumulation) and/or passively (biosorption). More recently, attention has been paid on the use of microbial biomass particularly bacteria for removal of heavy metals from aqueous solutions

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F. Masood · A. Malik (✉)  
Department of Agricultural Microbiology,  
Faculty of Agricultural Sciences, Aligarh Muslim University,  
Aligarh 202002, India  
e-mail: ab\_malik30@yahoo.com

M. Ahmad  
Department of Biochemistry, Faculty of Life Sciences,  
Aligarh Muslim University, Aligarh 202002, India

M. A. Ansari  
Department of Civil Engineering, Zakir Husain College of  
Engineering and Technology, Faculty of Engineering and  
Technology, Aligarh Muslim University, Aligarh 202002, India

(Ahluwalai and Goyal 2007). Bacterial biomass isolated from industrial wastewaters, activated sludge or contaminated soils has been used for toxic metals removal (Nourbakhsh et al. 2002; Hussein et al. 2004; Ozdemir and Baysal 2004). Adsorption of heavy metals was shown to be dependent on various factors like metal species, initial concentration, pH and contact time.

An artificial neural network (ANN) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables. Application of ANN has been considered as a promising tool because of their simplicity towards simulation, prediction and modeling. The advantages of ANN models are that they require less time for development than the traditional mathematical models, the need for extensive experimentation is avoided as limited numbers of experiments are sufficient to predict the degree of non-linearity, and their ability to learn complex relationships without requiring the knowledge of the model structure (Pramanik 2004). ANN models may describe adsorption systems better than general rate models (Du et al. 2007). Even the adsorption isotherms can be represented by neural networks (Gao and Engell 2005). As a result, it may be preferable to use a non-parametric technique such as a feed forward back-propagation neural network to represent such an equilibrium relationship.

The present study describes the adsorption potential of *Bacillus* sp. for removal of total chromium from aqueous solution. Experimental parameters affecting the biosorption process such as pH, contact time and initial concentration of Cr ions were studied. On the basis of batch adsorption experiments, we implemented a three-layer ANN model for the prediction of biosorption efficiency, and the network results were compared with those obtained through experiments.

## Materials and Methods

Kanpur (UP), India, lies in the Indo-Gangetic plains between the parallels of 26°28'N and 80°24'E. About 310 tanneries are located at Jajmau (Kanpur), which is one of the major centers for the processing of raw hides. Treated effluent was collected from the release point of a Common Effluent Treatment Plant (CETP) in sterile plastic containers and stored at 4 ± 1°C. Twenty-five millilitres of tannery effluent were digested with nitric acid; perchloric acid as described in the Standard Methods (APHA 1995) for total metal analysis. Digested samples were analyzed by atomic absorption spectrophotometry (Model: GBC, 932 plus, Australia).

Tannery effluent (10 mL) was added in 90 mL sterile normal saline solution, serially diluted and 0.1 mL aliquots

of appropriate dilution were plated on Luria–Bertani (LB) agar plates. The plates were incubated overnight at 37°C and 20 morphologically distinct colonies sub-cultured onto fresh plates. The isolates were then tentatively identified on the basis of cell morphology, cultural and biochemical characteristics following Holt et al. (1994).

The minimum inhibitory concentration (MIC) was determined in a Tris-buffered mineral salts medium (Mergeay et al. 1985) amended with heavy metal salts such as CdCl<sub>2</sub>, CuSO<sub>4</sub>, CoCl<sub>2</sub>, CrCl<sub>3</sub>·6H<sub>2</sub>O, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, NiCl<sub>2</sub> and ZnCl<sub>2</sub> in varying concentrations ranging from 3.12 to 1,600 mg/L (Aleem et al. 2003; Ansari et al. 2008). The minimum concentration of the metal that completely inhibited growth was taken as the MIC.

Biosorption of total chromium by the selected bacterial isolate was determined according to the procedure of Ansari and Malik (2007). A stock solution of total chromium (1,000 mg/L) was prepared by dissolving K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in distilled water. All working solutions of varying concentrations were obtained by successive dilution. Fifty milligram (dry weight) of cells were added into 50 mL of metal solution of known initial concentration and the solution was continuously stirred on a shaker (150 rpm) at 37°C. Series of batch adsorption experiments were conducted to determine the effect of initial concentration of Cr, initial pH and contact time on adsorption performance of *Bacillus* sp. The influence of pH on the adsorption capacity was determined by agitating for a predetermined equilibrium time (2 h) 50 mL of 100 mg/L solution of chromium, with 50 mg of the adsorbent at pH values ranging from 4 to 9. All pH adjustments were made using reagent grade HCl and NaOH. To study the effect of contact time, the experiments were carried out for various sorption times (2, 3, 4, 5 and 6 h), at a constant initial concentration of 100 mg/L at pH 5. The effect of the initial metal ion concentration was determined by contacting 50 mg of the adsorbent in 50 mL of metal ion solution of different initial concentrations (100–400 mg/L) for 2 h at pH 5. All the experiments were conducted in duplicate, and mean values were used in the analysis. The samples were centrifuged at 5,000 rpm for 20 min at room temperature and the concentration of residual chromium in the supernatant was analyzed by AAS.

The equilibrium sorption capacity of the biomass at the corresponding equilibrium conditions was calculated using a mass balance equation as in Eq. (1).

$$Q_e = \frac{C_i - C_e}{M} \times V \quad (1)$$

where  $Q_e$  is the amount of the metal uptake by the biomass (mg/g) in the equilibrium;  $C_i$  is initial metal ion concentration in solution (mg/L);  $C_e$  is the equilibrium metal ion concentration in solution (mg/L);  $V$  is volume of the

medium (L); and M is the amount of the biomass used in the reaction mixture (g).

Recently, the use of neural networks has gained popularity for modeling biological wastewater treatment processes (Zhao et al. 1997; Lee et al. 2002). Neural networks can map a set of input patterns onto a corresponding set of output patterns after a series of past process data from a given system have been acquired. Moreover, neural network has a distinctive ability to learn nonlinear functional relationships without the requirement for structural knowledge of the process to be modeled. Among the various ANN models, the one of our interest was the feed forward back propagation network (Imandi et al. 2008; Pal et al. 2009). The feed forward back propagation neural network consisting of forward three neurons corresponding to the three process variables (pH, time and initial metal ion concentration) were used in the input layer, ten in the hidden layer and one in the output layer ( $Q_e$ ) of the network. The number of neurons per layer should be high enough to allow the network to reproduce the behavior of the system. However, too large of a neuron number can cause data overfitting, a situation that can be encountered when correlating experimental data. This is due to the fact that the large number of parameters to be adjusted when using too many neurons might induce the network to memorize the data used in the training while losing one of its more functional characteristics: generalization (Hornik 1991). Once the neural network was created, it was trained to accurately model the given phenomenon by using the experimental data in MATLAB version 7.1 (Mathworks Inc., Natick, US). The root mean square error (RMSE) was used as the error function and defined as:

$$\text{RMSE} = \sqrt{\frac{\sum (\hat{y} - y)^2}{n}} \quad (2)$$

where  $y$  is the measured values,  $\hat{y}$  the corresponding predicted values and  $n$  is the number of samples.

Sensitivity tests were conducted to ascertain the relative significance of each of the independent parameters (input neurons) on the removal efficiency (output) in the ANN model. In the sensitivity analysis, each input neuron was in turn eliminated from the model and its influence on prediction of removal efficiency ( $Q_e$ ) was evaluated in terms of correlation coefficient ( $R^2$ ), absolute percentage error (APE), mean absolute percentage error (MAPE), average absolute deviation (ADD) and RMSE criteria.

## Results and Discussion

The atomic absorption spectrophotometric analysis of heavy metals in tannery effluent revealed high levels of Cr, Ni, Zn,

Cu and Cd. Chromium was the highest (106.51 mg/L) in the effluent followed by Ni (21.7 mg/L), Zn (9.2 mg/L), Cu (2.78 mg/L), and Cd (0.35 mg/L). Other workers have also reported high concentrations of chromium in the same area (Khawaja et al. 2001; Singh et al. 2004). The concentrations of zinc, copper, cadmium and nickel were comparable to those reported by earlier workers in different regions of the country (Aleem et al. 2003; Ansari and Malik 2007).

A total of 20 morphologically different bacterial isolates were selected and were tested for their resistance against different metal ions that is  $\text{Cr}^{6+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$ . One bacterial strain displaying maximum MIC of 1,000 mg/L for  $\text{Cr}^{6+}$ , 1,000 mg/L for  $\text{Cr}^{3+}$ , 200 mg/L for  $\text{Ni}^{2+}$ , 400 mg/L for  $\text{Zn}^{2+}$ , 100 mg/L for  $\text{Cd}^{2+}$  and 400 mg/L for  $\text{Cu}^{2+}$  was tentatively identified as *Bacillus* sp. according to Bergey's Manual of Systematic Bacteriology (Holt et al. 1994). It was selected for further studies since it showed maximum tolerance. Morphological and biochemical characteristics of the isolate are presented in Table 1.

pH is an important factor controlling the process of biosorption. The pH affects not only the surface charge of the biosorbent, but also the degree of ionization and speciation of the heavy metal in solution (Green-Ruiz et al. 2008; Zamil et al. 2009). As pH in metal-containing water and wastewater can vary, solutions of different pH values were used to examine the effect on heavy metal sorption by *Bacillus*. In these tests, initial pH values of 100 mg/L aqueous metal solutions were adjusted in the range of 4.0–9.0, before addition of the biosorbent. As shown in Fig. 1a, the initial pH of the solution had a significant effect on chromium removal. The maximum chromium biosorption was obtained at pH 5.0 (26.7 mg/g dry wt) and thereafter decreased with further increases in pH. The drop in removal with increased pH is likely due to the lower availability of hydrogen ions for the protonation of the cell wall functional groups, which consequently reduces the interactions between the metal ions and the available binding sites.

The influence of contact time on the batch adsorption of chromium at optimum pH (5) and initial metal concentration (100 mg/L) dosage is shown in Fig. 1b. It can be observed that the adsorption of chromium ions quickly increased at the beginning of biosorption, but after 2 h, the adsorption slowed down. The result indicated that the maximum adsorbed amount of the chromium ions was achieved within 1–2 h and then followed by a longer equilibrium period. The kinetics of metal uptake is assumed to be a passive physical adsorption at the cell surface, is rapid and occurs in a very short time after the microorganisms have come into contact with metal ions (Singh et al. 2001). Similar results were observed in other studies on the uptake of various heavy metal ions using

**Table 1** Morphological, physiological and biochemical tests of *Bacillus* sp

Characteristic	Result
Cell shape	Rod
Diffusible pigments	None
Gram reaction	+ <sup>a</sup>
UV fluorescence	— <sup>b</sup>
Growth at temperature	25–50°C
Indole test	—
Methyl red test	+
Voges Proskauer test	—
Citrate utilization	—
Catalase test	+
Starch hydrolysis	+
Gelatin hydrolysis	+
Nitrate reduction	—
H <sub>2</sub> S production	—
Substrate utilization	
Glucose	+
Lactose	—
Maltose	+
Sucrose	+

<sup>a</sup> Positive result; <sup>b</sup> Negative result

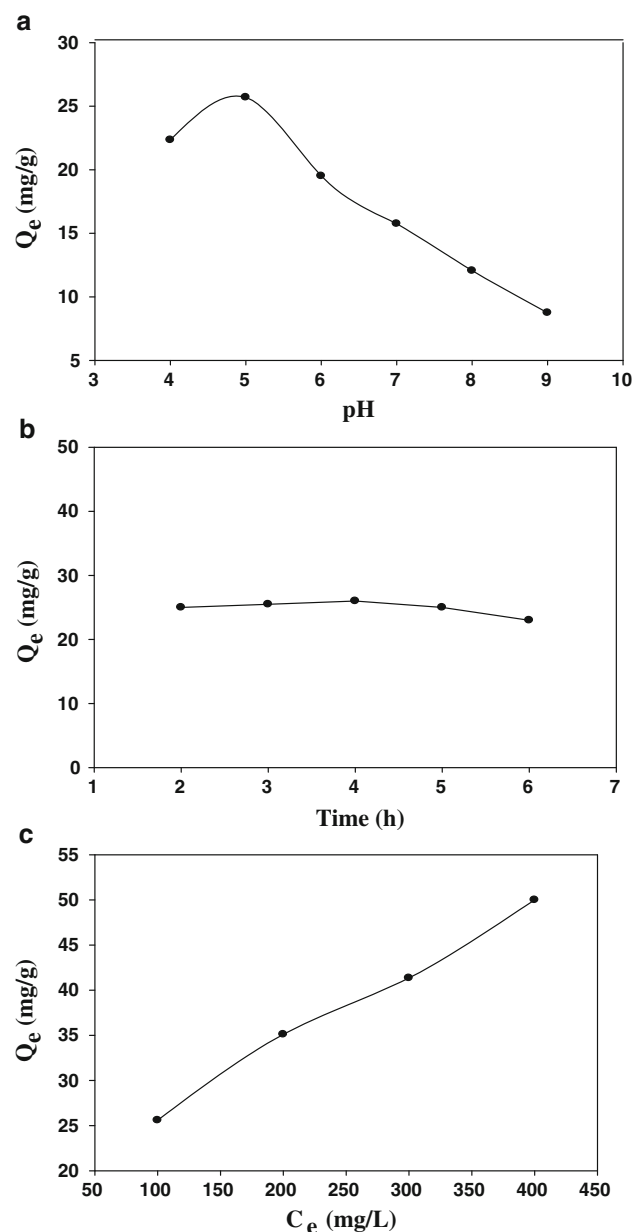
different biosorbents (Pan et al. 2006; Abu Al-Rub et al. 2006). Rapid sorption of metals is desirable for use of the biosorbents for practical applications.

The initial concentration of metal ion remarkably influenced the equilibrium metal uptake and adsorption yield. The higher the initial concentration of the metal ion, the larger amount of metal ion adsorbed (Fig. 1c). The chromium biosorption capacity of the *Bacillus* biomass increased from 16.5 to 50 mg/g as the initial chromium concentration was varied from 100 to 400 mg/L. Similar results were obtained in other studies with various metals and other biosorbent organisms (Öztürk 2007; Bueno et al. 2008; Uzel and Ozdemir 2009). The increased biosorption capacity with increased metal concentration may be attributed to a higher probability of interaction between metal ions and biosorbents (Öztürk et al. 2004).

The experimental data at optimum conditions were applied to the Freundlich isotherm model. The empirical linearized Freundlich equation is as follows:

$$\ln Q_e = \ln K_f + 1/n \ln C_e \quad (3)$$

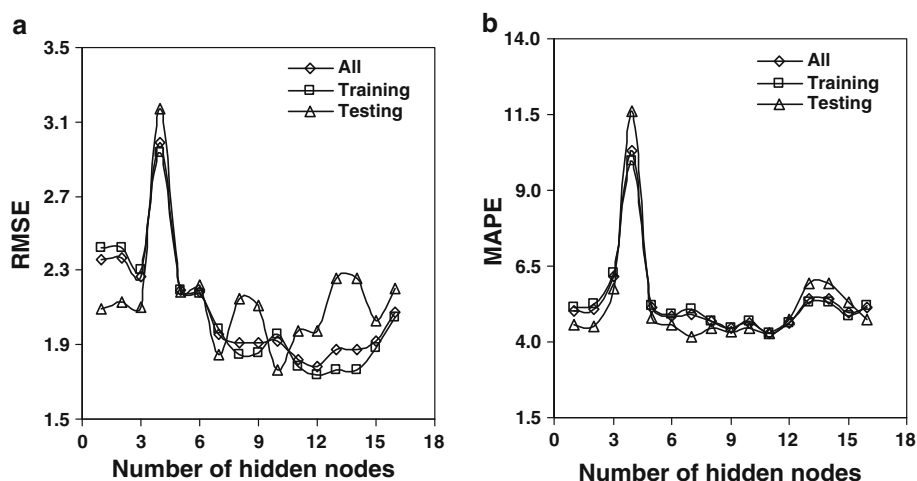
where  $Q_e$  is the biosorption capacity of metal per unit weight of biosorbent,  $C_e$  is the equilibrium concentration of metal ion in solution, and  $K_f$  and  $n$  are Freundlich biosorption isotherm constants, being indicative of the extent of the biosorption and the degree of nonlinearity between solution concentration and biosorption, respectively

**Fig. 1** Effect of pH (a), temperature (b) and initial chromium concentration (c) on the biosorption capacity

(Freundlich 1906). The plot of  $\ln Q_e$  versus  $\ln C_e$  for the biosorption of total chromium was employed to generate the intercept value of  $K_f$  and the slope of  $1/n$ . The values of  $K_f$  and  $1/n$  were found to be 21.345 and 0.144, respectively. According to Han et al. (2006),  $1/n$  values between 0.1 and 1 represent beneficial adsorption. The  $1/n$  value for the *Bacillus* sp. used indicates favorable adsorption of chromium. The coefficient of determination ( $R^2$ ) was found to be 0.9190 for total chromium biosorption, indicating that the biosorption of the metal ion fitted well the Freundlich model.

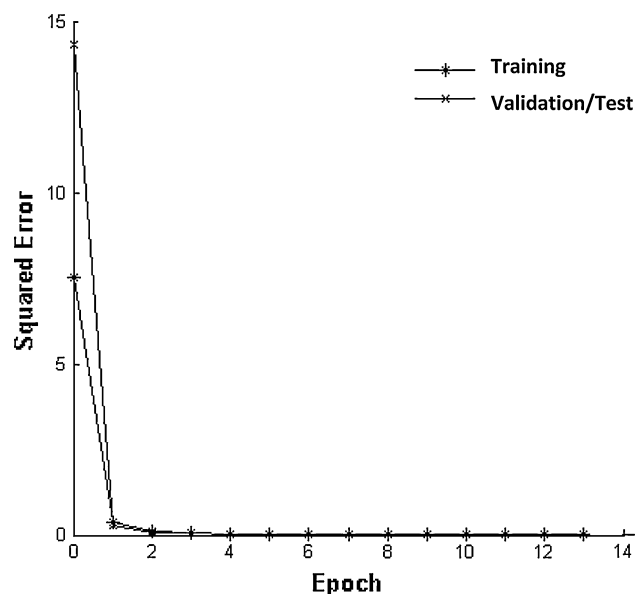
**Table 2** Biosorption capacity of chromium by various biosorbents

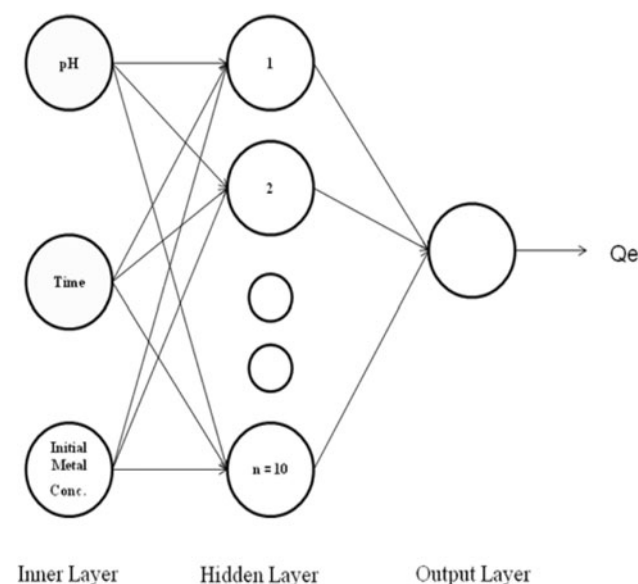
Biosorbent	Initial Cr conc. (mg/L)	Biosorption capacity (mg/g)	References
<i>Pseudomonas</i> sp.	5–450	95.0	Ziagova et al. (2007)
<i>Bacillus licheniformis</i>	20–300	69.35	Zhou et al. (2007)
<i>Escherichia coli</i>	10–100	4.60	Quintelas et al. (2009)
<i>Bacillus marisflavi</i>	200	5.783	Mishra and Doble (2008)
<i>Pseudomonas fluorescence</i> TEM08	24.9–494.5	40.80	Uzel and Ozdemir (2009)
Green taro ( <i>Colocasia esculenta</i> )	10–150	1.418	Elangovan et al. (2008)
Mangroves leaves	10–150	0.342	Elangovan et al. (2008)
Tea factory waste	50–400	54.65	Malkoc and Nuhoglu (2007)
<i>Bacillus</i> sp.	100–400	50.0	This study

**Fig. 2** Influence of the number of neurons in the hidden layer on the RMSE (a) and MAPE (b) during network training and testing

Biosorption capacities of various biosorbents for Cr as reported in the literature are summarized in Table 2. The sorption of Cr in the present study was comparable to several of the values for other species with high biosorption capacities. Present results indicate that *Bacillus* sp. is an efficient biosorbent for the removal of Cr from aqueous solutions.

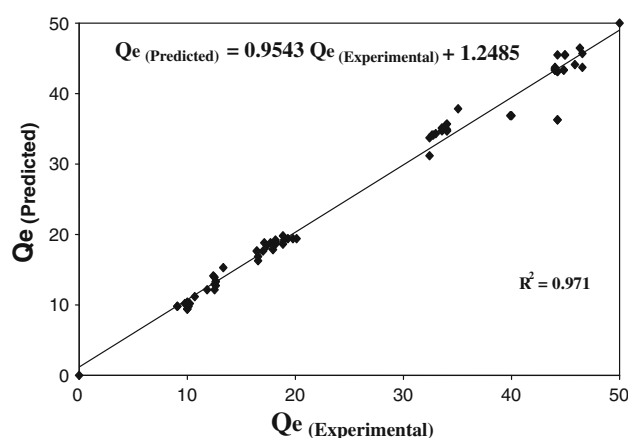
In this study a network topology using three input neurons (pH, time and initial metal concentration), one output neuron ( $Q_e$ ), and one hidden layer of neurons was used to describe the biosorption capacity of *Bacillus* sp. in removal of total chromium from aqueous solution. It must be stressed that a single layer of hidden sigmoid neurons constitutes the simplest structure capable to simulate any function with a finite number of discontinuities. In order to determine the optimum number of hidden nodes, a series of topologies was used, in which the number of nodes were varied from 1 to 17. Figures 2a, b report the relationship between network error and number of neurons in the hidden layer. As can be seen in Fig. 2a, the mean square error is minimal at about 10 neurons. Initially, the number of

**Fig. 3** Variation of squared error with epochs for training, testing and validation



**Fig. 4** Basic architecture of the artificial neural network proposed

neurons in the hidden layer varied from 1 to 17. During network training, the RMSE rapidly decreased when the number of neurons in the hidden layer was increased up to 10 neurons. Further increases did not cause a significant decrease in RMSE values. The number of neurons composing the hidden layer of the ANN was thus selected as



**Fig. 5** Comparison of the experimental results with those calculated via neural network modeling for the test sets

the maximum number of neurons providing a significant decrease in the RMSE between the experimental and model-predicted  $Q_e$  in the training data sets. The training of the network was stopped after reaching the minimum RMSE of 0.0001 between the experimental and model-predicted  $Q_e$  in the training data sets. Similarly during testing, the RMSE reached a minimum when the ANN was trained with 10 neurons in the hidden layer. The training was stopped after 10 iterations. Figure 3 illustrates training, validation and test mean squared errors. Out of 360

**Table 3** Performance evaluation of input variables with 10 neurons in the hidden layer for sensitivity analysis

Variable	$R^a$	$APE^b$	$MAPE^c$	$AAD^d$	$RMSE^e$
No time					
Performance all	0.9855	−1.980	6.2431	4.4047	2.3048
Performance training	0.9856	−1.6747	6.061	4.1014	2.3023
Performance testing	0.98566	−2.801	7.1514	5.6349	2.3148
Performance validation	0.98566	−2.801	7.1514	5.6349	2.3148
No pH					
Performance all	0.98734	−1.7948	4.8098	3.8679	2.1656
Performance training	0.98712	−1.8871	5.0102	3.8473	2.1823
Performance testing	0.98846	−1.4257	4.0085	3.9514	2.0978
Performance validation	0.98846	−1.4257	4.0085	3.9514	2.0978
No initial metal concentration					
Performance all	0.044095	−41.270	68.109	45.909	13.758
Performance training	0.12314	−43.015	68.519	45.240	13.614
Performance testing	−0.16476	−34.293	66.466	48.621	14.318
Performance validation	−0.16476	−34.293	66.466	48.621	14.318
All input variables					
Performance all	0.99013	−1.0273	4.6439	3.688	1.9169
Performance training	0.98971	−0.96463	4.6886	3.5631	1.9536
Performance testing	0.99211	−1.2778	4.4651	4.1948	1.7625
Performance validation	0.99211	−1.2778	4.4651	4.1948	1.7625

<sup>a</sup> Correlation coefficient; <sup>b</sup> Absolute percentage error; <sup>c</sup> Mean absolute percentage error; <sup>d</sup> Average absolute deviation; <sup>e</sup> Root mean square error



data sets generated during biosorption experiments, 80% (i.e. 288) were used to train the network and remaining 20% i.e. (72 data sets) were used for testing and validation of the ANN model. As the test data set and validation data set are same (20%) i.e. 72 data sets therefore there is no separate curve. Finally, the optimal ANN structure (having three neurons in the input layer, ten in the hidden layer and one in the output layer) is shown in Fig. 4.

In order to calculate training and test errors, all outputs were returned to their original scale, and then compared with experimental responses. Figure 5 shows a comparison between calculated and experimental values of the output variable for test sets by using neural network model. The plot for this figure has coefficient of determination of 0.971 for test set. This result confirmed that a neural network is a useful tool for accurate and cost-effective modeling of biological processes in the absence of other reasonably accurate process models. In this study, a sensitivity analysis was conducted to determine the degree of effectiveness of a variable (contact time, initial pH, and initial chromium concentration) using the proposed ANN model (Table 3). Findings of the sensitivity analysis showed that initial pH was the most significant parameter for the prediction of removal efficiency. The variables in order of decreasing level of sensitivity were: pH > initial metal concentration > contact time.

In conclusion, this study has shown the following: (1) tannery effluent contained high levels of various toxic metals, (2) *Bacillus* sp. isolated from tannery effluent was capable of biosorbing Cr from aqueous solution, (3) the biosorption process was affected by pH and initial metal concentration, (4) the experimental data fitted well to the Freundlich isotherm, and (5) predicted results from ANN model were in agreement with the experimental values. Based on the findings, it can be concluded that *Bacillus* sp. could be considered as a promising biosorbent for the removal of total Cr from aqueous solutions.

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