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Experimental Investigation and Artificial Neural Network-Based Modeling of Batch Reduction of Hexavalent Chromium by Immobilized Cells of Newly Isolated Strain of Chromium-Resistant Bacteria

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Abstract The batch bioreduction of Cr(VI) by the cells of newly isolated chromium-resistant *Acinetobacter* sp. bacteria, immobilized on glass beads and Ca-alginate beads, was investigated. The rate of reduction and percentage reduction of Cr(VI) decrease with the increase in initial Cr(VI) concentration, indicating the inhibitory effect of Cr(VI). Efficiency of bioreduction can be improved by increasing the bioparticle loading or the initial biomass loading. Glass bioparticles have shown better performance as compared to Ca-alginate bioparticles in terms of batch Cr(VI) reduction achieved and the rate of reduction. Glass beads may be considered as better cell carrier particles for immobilization as compared to Ca-alginate beads. Around 90% reduction of 80 ppm Cr(VI) could be achieved after 24 h with initial biomass loading of 14.6 mg on glass beads. Artificial neural network-based models are developed for prediction of batch Cr(VI) bioreduction using the cells immobilized on glass and Ca-alginate beads.

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1 Introduction

Chromium is a transition metal most commonly found in the environment in its trivalent [Cr(III)] and hexavalent [Cr(VI)] forms (James and Bartlett 1983). Naturally occurring Cr is almost exclusive in the trivalent state, as the energy required for its oxidation is high. Industrial processes release chromium in its most dangerous hexavalent form. Hexavalent chromium is highly water soluble and can be carcinogenic to both plants and animals (Venitt and Levy 1974). The Cr(VI) salts can easily penetrate into the circulation system through lung, infiltrate cells, complex with macromolecules and eventually form the carcinogen (O'Brien et al. 2003; Shakoori et al. 2004). If untreated water is discharged into the water bodies, it adversely affects the aquatic ecosystem and its inhabitants (Trumble and Jensen 2004).

The maximum levels permitted for trivalent chromium in wastewater is 5 ppm. According to Indian standards (Baral et al. 2006), the permissible limits of Cr(VI) are 0.05 and 0.1 ppm for potable and industrial discharge water, respectively. In order to comply with this limit, it is essential that industries treat their effluents to reduce the Cr(VI) to acceptable levels.

Though physical and chemical remediation processes are available to ameliorate the effect of this hexavalent chromium in water, they are either inefficient or suffer from high capital and operating costs (Poopal and Laxman 2008). Owing to the operational difficulties and treatment expenditure of removing Cr from water in developing countries, the above methods were found unaffordable for large scale treatment of wastewater rich in Cr(VI) (Devaprasath et al. 2007). The use of biological methods to remediate metal contaminated wastewater is an emerging field in environmental technology. Bioreduction and biosorption of Cr(VI) using bacterial, fungal, yeast or plant biomass are among the most lucrative strategies currently employed for removal of chromium by biological means (Kinnari et al. 2010).

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the cell. The complete mechanism of metal take up is not fully understood. They may be metabolism-dependent or metabolism-independent (Ahalya et al. 2003). In the case of Cr(VI), certain species of bacteria were found to reduce Cr(VI) to a less toxic Cr (III) form. Recently, many researchers discovered the potential of using Cr(VI)-reducing bacterial strains for detoxifying Cr(VI) contaminated environments (Lovley 1995; Cervantes et al. 2001; Basu et al. 1997; Shakoori et al. 2000; Francisco et al. 2002; Raicevic et al. 2010; Kinnari et al. 2010). This offers an attractive option for the removal and recovery of chromium ions from wastewater.

Bacteria may be used in suspended cell or immobilized cell systems for bioremediation of wastewater. Generally, batch or continuous stirred tank reactors with suspended cells are utilized in the Cr(VI) removal studies. The advantage of these reactors is that operation and control are simple. It is vulnerable to shock and washout and it takes a long time to recover from perturbation (Kim et al. 2002). On the other hand, attached growth or immobilized cell bioreactors may be used to overcome most of the problems encountered in suspended cells bioreactors. Use of immobilized cells is gaining a lot of importance, due to several advantages it has over the free cell (suspended cell) systems, such as no cell wash out problems, high biomass loading, resistance to toxic shock loading and no necessity of sludge separation unit (Shetty et al. 2007). Immobilization may also provide favorable microenvironmental conditions (i.e., cell–cell contact, nutrient–product gradients and pH gradients) for cells, resulting in better performance of the biocatalysts. In some cases, immobilization improves genetic stability. For some cells, protection against shear damage is important (Shuler and Kargi 1992). Meyer and Wallis (1997), in a study on treatment of heavy metal containing effluents, reported that metal uptake by biofilms was, on an average, 17 times better than that by the free living cultures. For the removal of Cr(VI), Morales et al. (2007) found that *Streptomyces*, in the form of biofilm, was a promising candidate for detoxification of metal contaminated sites. Li et al. (2005) found that immobilized cells of *Micrococcus roseus* in porous spherical beads exhibited an excellent tolerance to pH and temperature changes and were also more resistant to heavy metal stress compared with free cells.

Several natural and synthetic support materials such as agar, alginate, carrageenan, cellulose, and its derivatives like collagen, gelatin, polyacrylamide, polyester, polystyrene, and polyurethane have been used for cell immobilization (Munjal and Sawhney 2002). Dermou et al. (2007) studied Cr(VI) removal in trickling filters using plastic media and calcitic gravel as cell supports. There are reports on the efficient use of glass beads for cell immobilization (Shetty et al. 2007).

Cr(VI) removal using immobilized cells have been previously reported by many authors. Immobilized cells of *Microbacterium liquefaciens* MP30 (Pattanapipitpasal et al. 2001), *Bacillus* sp. ES 29 (Camargo et al. 2004), *Serratia marcescens* as a stable biofilm (Bruijn and Mondaca 2000), *Pseudomonas* (Konovalova et al. 2003), *Desulfovibrio vulgaris* (Humphries et al. 2006), *Intrasporangium* sp. strain Q5-1 (Yang et al. 2009), and *Bacillus* sp. (Kathiravan et al. 2010) are known for Cr(VI) removal.

With this background, the present study was undertaken to evaluate the hexavalent chromium reduction ability of a laboratory isolate of *Acinetobacter* sp. immobilized on two different types of carrier material like glass beads and calcium alginate (Ca-alginate) beads to remove Cr(VI) from water. This study is important with a view of selection of suitable carrier material for the selected strain of bacteria to be utilized in large scale industrial bioreactors like packed bed or fluidized bed bioreactors for the removal of Cr(VI) from industrial effluents.

Biological processes are both time variant and nonlinear in nature, and their complexity can be understood as the composition of many different and interacting elements governed by nondeterministic rules and influenced by external factors (Coruzzi et al. 2009; Gago et al. 2010). It is important to point out that many times the behavior of a biological system over a time period is difficult to understand and interpret, and additionally, genetic and environmental factors show a very high degree of intra- and interindividual variability, yielding a wide spectrum of biological responses (Karim et al. 1997; Guegan et al. 1998). Bioprocesses are typical multiple input multiple output (MIMO), unstructured and nonlinear systems, which are not easy to model (Shetty et al. 2008).

The artificial neural network (ANN) modeling technique is a true multiple input multiple output (MIMO) algorithm that has the ability to mimic the human learning process and can store large amounts of information through knowledge indexing. The artificial neural network's ability to recognize and reproduce cause and effect relationships through training, for multiple input/output systems makes it efficient to represent even the most complex systems (Aleboyeh et al. 2008). Prediction with ANN is made by learning experimentally generated data or using validated models (Fagundes-Klen et al. 2007). The main appeal of ANNs is that they offer the potential of a generic approach to the modeling of nonlinear systems.

Because of their reliable, robust and salient characteristics in capturing the nonlinear relationships existing between variables (multiinput/output) in complex systems, ANN models have been successfully used to predict the performance of bioprocesses (Baughman and Liu 1995; Syu and Hou 1996; Teissier et al. 1996; Massimo et al. 1991; Chen et al. 2004; Boareto et al. 2007; Shetty et al. 2008). Batch reduction of Cr(VI) by using immobilized bacteria is influenced by several factors, and as any other biological process, it is nonlinear in nature. So for accurate prediction of batch reduction efficiency as a function of different factors, ANN model can prove to be satisfactory. So in the present study, ANN models have been developed to predict the batch biological reduction of Cr(VI) by the cells immobilized on different support materials.

2 Material and Methods

2.1 Bacterial Source

The bacteria used in the present study for Cr(VI) reduction was isolated from the aerator liquid of an activated sludge process in the effluent treatment facility of a nearby dye/pigment-based specialty chemical industry and was identified as *Acinetobacter* sp. by partial 16sRNA sequencing (GenBank (NCBI) accession number: JF461086). The bacteria had a minimum inhibitory concentration (MIC) of 1,100 ppm. The bacteria were subcultured on Nutrient Agar every 15 days and stored at 4°C.

2.2 Biomass Production

Chromium reducing bacteria was grown in nutrient broth (pH: 7) supplemented with 200 ppm Cr(VI). Twenty-four-hour old cell culture was used for immobilization.

2.3 Immobilization of Bacterial Cells

2.3.1 Immobilization of Cells on Glass Beads

Cells were immobilized on the surface of glass beads of 3-mm diameter. To every 100 glass beads, 12.5 ml of actively growing 24-h-old *Acinetobacter* sp. cell suspension was added and it was refrigerated at 4°C for 2 days with occasional stirring. The glass beads with immobilized cells were then filtered, washed and used in the experiment. The initial weight of biomass on glass beads were then calculated using the following procedure: samples of 100 bioparticles (glass beads with the attached cells) were randomly collected from the flask. These bioparticles were washed with distilled water and dried at 105°C for 24 h and weighed. The biofilms were then completely removed from glass beads by heating the dried particles in 0.25 M NaOH solution. Then the beads were washed with distilled water, dried at 105°C for 24 h and weighed. The process of heating, washing and drying was repeated till constant weight was obtained, ensuring complete removal of biomass. The dry biomass weight in 100 bioparticles was then obtained by the difference between the weight of dried bioparticles and the weight of dried glass beads (Shetty et al. 2007). As the total number of

bioparticles taken for an experimental run was known, initial attached biomass dry weight in the flask was then calculated. The immobilization of biomass on glass beads were carried out at different batches and the attached biomass dry weight was measured. The average biomass dry weight for 100 glass beads was 2.92±0.08 mg. The glass beads with immobilized cells are referred to as glass bioparticles throughout the text.

2.3.2 Entrapment of Bacterial Cells in Calcium Alginate Beads

For immobilization, a 24-hour-old culture was selected. A known volume (5 ml) aliquot of the 24-h-old broth culture was centrifuged using a preweighed centrifuge tube in a refrigerated centrifuge (Remi, India). The supernatant was decanted and the tubes were kept in a hot air oven at 60°C overnight to dry the biomass. The mass of biomass was calculated by the difference between weight of the dried tube with the pelleted residue and that of the empty tube and then the biomass concentration in the 24-h-old grown cell culture was determined. This broth culture is used for the preparation of immobilized cells in alginate beads. A 6% w/v solution of sodium alginate was mixed with equal volume of actively growing *Acinetobacter* sp. cell suspension. The above mixture was added as drops by using a syringe to a beaker containing 3% calcium chloride solution. The ratio of cell alginate and calcium chloride solution used was 1:5. The beads formed were of diameter 3±0.2 mm. The beads thus formed in the calcium chloride solution were kept for 2 h in a refrigerator for solidification. Later, the calcium alginate beads were separated from solution and were kept in sterile water for an hour. As the calcium alginate beads were prepared by using a specific volume of 24-h grown cell broth and the number of particles formed per unit volume of the broth could be counted, the average amount of biomass per 100 beads was calculated. One hundred Ca-alginate beads contained 5.6±0.2 mg of biomass. The Ca-alginate beads with immobilized cells are referred to as Ca-alginate bioparticles throughout the text.

2.3.3 Experimental Methodology for Cr(VI) Reduction Experiments

Cr(VI) reduction experiments were conducted with 100 ml of nutrient broth containing different initial concentrations of Cr(VI) (80, 130, 180, 225 and 275 ppm). Potassium dichromate stock solution was used as a source of Cr(VI). Experiments were conducted with 100 or 500 bioparticles (glass or Ca-alginate beads previously immobilized with the bacteria). The flasks were not shaken continuously in order to avoid breakage of beads in the case of the Ca-alginate bioparticle experiments and the biofilm shear due to attrition between the bioparticles in the case of glass bioparticle experiments. Since industrial application of immobilized cell systems involves packed bed bioreactors, where the shear effects are minimal except the shear due to fluid flow, the laboratory nonshaking conditions are more realistic with reference to industrial scale applications. However, intermittent shaking was provided in order to ensure utility of all the Cr(VI) in the solution by the biomass in the beads through proper contact. Five milliliters of the liquid samples was withdrawn at different intervals of time. In order to prevent the effect of volume change, four flasks were used for each initial concentration experiment and sampling was done from each flask for only two-time intervals. The collected samples were analyzed for the residual Cr(VI) concentration. All the experiments were performed in triplicates and the deviations were found to be less than ±2%.

2.4 Analytical Method for Chromium Estimation

Samples were centrifuged at 10,000 rpm for 10 min at 4°C to remove suspended biomass, and the concentration of Cr(VI) in the supernatant was determined spectrophotometrically at 540 nm using diphenylcarbazide reagent in acid solution as the complexing agent for Cr(VI) (APHA 1998). Absorbance was measured using a Hitachi U2000 model spectrophotometer.

2.5 Modeling Strategy for Batch Cr(VI) Bioreduction Process by ANN

The prediction of output for a given set of inputs is a goal for any modeling strategy. In the present work, it is necessary to model the batch Cr(VI) reduction by the immobilized cells so as to predict the performance of immobilized cell batch reactor. Based on nonlinearity that may be involved in the bioreduction process by immobilized cells, ANN-based model is proposed for performance prediction.

For every ANN, the first layer constitutes the input layer (independent variables) and the last one forms the output layer (dependent variables). Between them, one or more neurons in layers, called hidden layers, can be located. The hidden layers act as predictors and, in theory, there can be more than one hidden layer. Information in an ANN is distributed among multiple neurons and connections between the neurons (weights). In neurons, the individual element inputs are multiplied by weights and the weighted values are fed to the summing junction. The neuron has a bias, which is summed with the weighted inputs to form the net input that is the argument of the transfer function or activation function; hence, generating the output. The behavior of ANN depends on both the weights and the input–output function (activation function) that is specified for the units (Shetty et al. 2008). Most of the commonly used ANNs for process modeling are layered feed-forward neural networks that are trained from input–output data using a back propagation algorithm.

Based on experimentation, initial immobilized biomass loading, initial Cr(VI) concentration and the incubation time were found to influence the percentage of Cr(VI) removal in the batch bioreduction process. So considering percentage Cr(VI) reduction as a measure of the batch process performance, it was chosen as the model output (dependent) variable. The initial immobilized biomass loading (X_m), initial Cr (VI) concentration(S_I) and the incubation time (t) were chosen as the model input (independent) variables. The input–output data were obtained by conducting batch experiments at different initial concentrations and initial biomass loading (corresponding to number of beads used) as presented in Section "2.3.3". The network architecture comprised of three neurons representing three input variables in the input layer and one neuron in the output layer representing the output neuron. Hornik et al. (1989) have shown that a multilayer ANN with as few as one hidden layer and with sigmoid transfer functions can map any function of practical importance. So a three-layer network with one hidden layer was chosen in the present study for both the cases of batch bioreduction using glass immobilized or Ca-alginate immobilized cells. A total of 70 sets of data were used for each of the cases. Forty-eight data points were used for training the network and 22 data points were used for validation of the network. The values of input variables and the corresponding values of percentage Cr(VI) reduction obtained by the experiments, which were used for training the networks, are presented in Tables 1 and 2, respectively for glass bioparticles and Ca-alginate bioparticles. Similarly, the data used for validation are shown in Tables 3 and 4, respectively. The experimental data with both glass bioparticles and Ca-alginate bioparticles were subjected to ANOVA to test the statistical significance of the input variables (factors) using MINTAB 15 software. The p-value obtained (<0.05) indicated that the experimental data are statistically significant. A computer code has been written using the Neural Networks Tool Box of MATLAB version 7.11 for training and testing the ANN. The Levenberg–Marquardt Back Propagation (LMBP) training algorithm was adopted to train the neural networks. Linear activation function ("purelin" function of MATLAB) was used for the output layer neuron. The number of neurons in the hidden layer and the activation functions for the same were chosen based on trial and error approach. The network was trained by varying the number of neurons in the hidden layer as well as the activation function. After the network was trained, it was tested using a part (50%) of the validation data set. The network, which gave the coefficient of correlation for training as well as testing greater than 0.99, was chosen. Hence, the numbers of neurons selected for the hidden layer were seven and the activation function for the hidden layer neurons was hyperbolic tan sigmoid transfer function ("tansig" function of MATLAB). The trained network was again tested with another 50% of the validation data set to confirm the validity of the neural network model developed.

3 Results and Discussions

3.1 Studies on Time Course Variation of Cr(VI) Reduction

The time course variations of percentage Cr(VI) reduction with different initial Cr(VI) concentrations are presented in Figs. 1 and 2 for different bioparticle loading of 100 and 500 glass bioparticles, respectively. Similarly, Figs. 3 and 4 show the time course variations of percentage Cr(VI) reduction during the bioreduction experiments with Ca-alginate bioparticles

Table 1 Experimental and ANN model predicted % Cr(VI) reduction for training data set for CASE I (with glass bioparticles)

Data set no.	X_m (mg)	S_I (ppm)	t (min)	% Cr(VI) reduction Experimental	% Cr(VI) reduction Predicted
1	2.92	80	30	5.97	5.99
2	2.92	80	240	28.75	29.49
3	2.92	80	360	32.93	32.54
4	2.92	80	1,440	39.53	39.49
5	2.92	130	30	6.3	4.92
6	2.92	130	60	9.77	10.87
7	2.92	130	90	12.77	15.61
8	2.92	130	240	28.68	27.53
9	2.92	130	360	30.7	30.51
10	2.92	130	1,440	36.09	35.39
11	2.92	180	60	11.6	15.88
12	2.92	180	90	22.65	20.47
13	2.92	180	120	26.79	24.05
14	2.92	180	240	30.38	31.83
15	2.92	180	360	33.7	34.39
16	2.92	180	1,440	35.36	35.63
17	2.92	225	60	17.53	16.6
18	2.92	225	90	21.64	20.9
19	2.92	225	120	25.76	24.24
20	2.92	225	240	29	31.37
21	2.92	225	1,440	34.63	35.32
22	2.92	275	30	4.64	4.71
23	2.92	275	60	10.54	10.05
24	2.92	275	90	12.86	14.26
25	2.92	275	120	16.24	17.55
26	2.92	275	240	24.46	24.66
27	2.92	275	360	28.93	27.11
28	2.92	275	1,440	30.89	30.54
29	14.6	80	30	19.39	20.3
30	14.6	80	90	40.6	38.46
31	14.6	80	240	62.42	63.99
32	14.6	80	1,440	90.91	90.63
33	14.6	130	90	29.8	32.36
34	14.6	130	120	42.26	39.36
35	14.6	130	1,440	83.14	83.61
36	14.6	180	30	13.78	13.65
37	14.6	180	60	21.34	23.363
38	14.6	180	240	57.56	56.03
39	14.6	225	30	8.7	9.13
40	14.6	225	90	24.67	26.49
41	14.6	225	120	37.66	33.04
42	14.6	225	240	45.74	49.15
43	14.6	225	360	57.02	56.24
44	14.6	275	90	23.13	23.47

Table 1 (continued)

Data set no.	X_m (mg)	S_I (ppm)	t (min)	% Cr(VI) reduction Experimental	% Cr(VI) reduction Predicted
45	14.6	275	120	31.65	29.39
46	14.6	275	240	39.65	43.33
47	14.6	275	360	48.86	48.81
48	14.6	275	1,440	55.13	53.44
Mean squared error (MSE)				3.176	
Root mean squared error (RMSE)				1.78	
Coefficient of correlation (R)				0.9914	

for different bioparticle loading of 100 and 500 beads, respectively. In all the cases, faster rates of reduction were observed at all the initial concentrations, up to 120 min and then the rate of reduction decreased. Initial fast rates may be owing to the sorption of Cr(VI) onto the biofilm on the glass beads or onto the gel matrix in the case of Ca-alginate beads. However, with the passage of time, the cells in the biofilm or within the gel matrix consume the nutrients and carbon in the solution. As the biofilm on glass beads thicken or as the biomass grow within the gel matrix, the bacteria attribute much to the removal of Cr(VI) and hence bioreduction may be the dominant mode of Cr(VI) removal. As bioreduction is always slower as compared to sorption, the rate decreases at later times when bioreduction is the dominant mode of removal. Very slower rates, observed after around 400 min, may be owing to reduction in the concentration of carbon source and the nutrients in the solution as a result of utilization by the microorganisms.

It can be found from Figs. 2 and 4 that, though the initial rates of reduction are almost similar at different initial concentrations, at later times the rates of reduction decreased as the initial concentrations have increased. The rates are highest at 80 ppm initial concentration and least at 275 ppm initial concentration. Reduction in rate with increase in initial concentration may be attributed to the inhibitory effect of Cr(VI) to the bacteria at high concentrations. High concentrations of Cr(VI) may inhibit the rate of growth of the bacteria in the biofilm (in the case of glass bioparticles) or inside the gel matrix (in the case of Ca-alginate bioparticles) and hence leading to a lowered rate of Cr(VI) reduction. At lower biparticle loading of 100 bioparticles and with initial concentrations of 80 to 225 ppm, as can be seen in Figs. 1 and 3, the rates seem not to be varying significantly with the initial concentrations. However, the rate is found to be the lowest at an initial concentration of 275 ppm, once again indicating the inhibitory effect at higher concentration.

3.2 Effect of Initial Cr(VI) Concentration

Figures 5 and 6 show the effect of initial Cr(VI) concentration on the Cr(VI) reduction at the end of 1,440 min (24 h) with glass bioparticles and Ca-alginate bioparticles, respectively. It can be found that the percentage reduction of Cr(VI) has decreased with the increase in initial Cr(VI) concentration. Percentage reduction has decreased from around 40 to 31% with 100 glass bioparticles and from 91 to 55% with 500 glass bioparticles, as the initial concentration increased from 80 to 275 ppm. Similarly, in the case of Ca-alginate bioparticles, percentage reduction has decreased from around 37 to 31% with 100 particles and from 89 to 55% with 500 particles, as the initial concentration increased from 80 to 275 ppm. When the initial Cr(VI) concentration is high, the bacteria in the biofilm or within the gel matrix get exposed to higher Cr(VI) levels and hence their growth will be inhibited. Lower growth leads to lower rates of reduction and hence leading to lower percentage reduction at the end of 24 h.

3.3 Effect of Bioparticle Loading on Cr(VI) Reduction

It can be seen from Figs. 5 and 6 that the percentage reductions achieved at the end of 24 h are considerably higher with bioparticle loading of 500 beads as

Table 2 Experimental and ANN model predicted % Cr(VI) reduction for training data set for CASE II (with Ca-alginate bioparticles)

Data set no.	X_m (mg)	S_I (ppm)	t (min)	% Cr(VI) reduction Experimental	% Cr(VI) reduction Predicted
1	5.6	80	120	23.22	22.33
2	5.6	80	240	28.57	29.34
3	5.6	80	360	32.73	32.61
4	5.6	80	1,440	36.91	38.52
5	5.6	130	30	5.26	4.51
6	5.6	130	120	23.79	21.98
7	5.6	130	1,440	35.33	35.34
8	5.6	180	30	4.98	5.64
9	5.6	180	60	9.97	13.73
10	5.6	180	90	22.71	19.46
11	5.6	180	120	25.75	23.49
12	5.6	180	240	29.91	30.77
13	5.6	180	360	32.68	33.16
14	5.6	180	1,440	34.62	34.42
15	5.6	225	30	10.89	10.32
16	5.6	225	90	20.04	21.97
17	5.6	225	120	24.61	24.81
18	5.6	225	240	28.32	27.83
19	5.6	225	360	32.46	27.5
20	5.6	225	1,440	33.76	32.35
21	5.6	275	30	4.3	2.25
22	5.6	275	60	9.49	10.12
23	5.6	275	120	16.12	19.47
24	5.6	275	240	23.83	26.01
25	5.6	275	360	28.49	27.9
26	5.6	275	1,440	30.64	32.39
27	28	80	90	43.47	41.43
28	28	80	120	50.93	49.06
29	28	80	240	62.74	63.53
30	28	130	120	43.51	42.82
31	28	130	240	52.29	56.18
32	28	130	360	61.07	62.37
33	28	130	1,440	77.48	77.98
34	28	180	60	19.12	26.24
35	28	180	120	48.91	42.92
36	28	180	240	55.46	56.04
37	28	180	360	65.03	61.32
38	28	180	1,440	74.04	73.29
39	28	225	30	7.92	4.85
40	28	225	60	13.7	16.88
41	28	225	240	41.54	43.07
42	28	225	360	48.82	49.32
43	28	225	1,440	58.45	58.8
44	28	275	60	14.93	13.44

Table 2 (continued)

Data set no.	X_m (mg)	S_I (ppm)	t (min)	% Cr(VI) reduction Experimental	% Cr(VI) reduction Predicted
45	28	275	90	23.43	23.22
46	28	275	120	30.9	30.28
47	28	275	360	48.43	48.93
48	5.6	80	120	23.22	22.33
Mean squared error (MSE)				5.16	
Root mean squared error (RMSE)				2.27	
Correlation coefficient (R)				0.992	

compared to 100 beads, both with glass as well as Ca-alginate bioparticles and at all initial concentrations of 80 to 275 ppm. Maximum percentage reduction of around 90% (with glass bioparticles) or 89% (with Ca-alginate bioparticles) could be achieved with 500 beads whereas only 40% (with glass bioparticles) or 37% (with Ca-alginate bioparticles) could be achieved with 100 beads for 80 ppm initial Cr(VI) concentration. When the numbers of bioparticles are larger, the initial inoculum of bacteria will be higher. With 100 glass or Ca-alginate bioparticles, the amount of initial biomass loading was 2.92 mg and 5.6 mg, respectively. With 500 glass or Ca-alginate bioparticles, the amount of initial biomass loading were 14.6 mg or 28 mg, respectively. A large amount of immobilized bacteria being present initially causes the reduction process to get accelerated, as they can utilize the Cr (VI) present in solution at a faster rate. Higher number

Table 3 Experimental and ANN model predicted % Cr(VI) reduction for validation data set for CASE I (with glass bioparticles)

Data set no.	X_m (mg)	S_I (ppm)	t (min)	% Cr(VI) reduction Experimental	% Cr(VI) reduction Predicted
49	2.92	80	90	17.97	17.08
50	2.92	80	120	22.75	20.96
51	2.92	130	120	18.79	19.33
52	14.6	80	60	35.14	30.09
53	14.6	80	120	50.9	45.52
54	14.6	130	360	66.03	66.67
55	14.6	180	90	36.21	31.61
56	14.6	180	360	65.94	64.42
57	14.6	180	1,440	75.13	76.28
58	14.6	225	60	15.53	18.56
59	14.6	275	30	8.34	7.41
60	2.92	80	60	14.37	12.16
61	2.92	180	30	5.79	10.1
62	2.92	225	30	10.61	11.14
63	2.92	225	360	33.55	33.67
64	14.6	80	360	76.97	73.86
65	14.6	130	30	11.31	14.28
66	14.6	130	60	18.11	24.04
67	14.6	130	240	54.71	57.49
68	14.6	180	120	48.91	38.5
69	14.6	225	1,440	60.21	66.21
70	14.6	275	60	14.95	16.19

Table 4 Experimental and ANN model predicted % Cr(VI) reduction for validation data set for CASE II (with cells immobilized on Ca-alginate beads)

Data set no.	X_m (mg)	S_I (ppm)	t (min)	% Cr(VI) reduction Experimental	% Cr(VI) reduction Predicted
49	5.6	80	30	8.93	4.7538
50	5.6	80	60	13.1	12.77
51	5.6	130	60	9.39	12.5
52	5.6	130	90	13.15	18.1
53	5.6	130	240	28.55	28.43
54	5.6	130	360	32.19	29.76
55	5.6	225	60	15.25	17.37
56	5.6	275	90	12.18	15.64
57	28	80	30	20.5	16.86
58	28	80	60	33.54	30.95
59	28	80	360	76.4	69.36
60	28	80	1,440	89.44	87.84
61	28	130	30	8.77	12.78
62	28	130	60	15.64	26.03
63	28	130	90	28.63	35.79
64	28	180	30	12.29	13.05
65	28	180	90	34.97	35.94
66	28	225	90	19.27	25.53
67	28	225	120	30.19	31.61
68	28	275	30	7.98	4.34
69	28	275	240	39.06	43.57
70	28	275	1,440	54.86	53.88

of bioparticles will also lead to increased total surface availability for the growth of bacteria and the sites for mass transfer of Cr to biofilm or the gel matrix increase per unit volume of the reaction mixture. All these factors enhance the Cr(VI) reduction process leading to increased percentage reduction with increase in bioparticle loading. So the efficiency of the bioreduction process may be increased by using high bioparticle loading into the reactor. Optimizing the number of bioparticles in order to achieve high percentage reduction or low Cr(VI) concentration in the effluent in order to maintain the effluent quality within the standards set by the statutory bodies is very

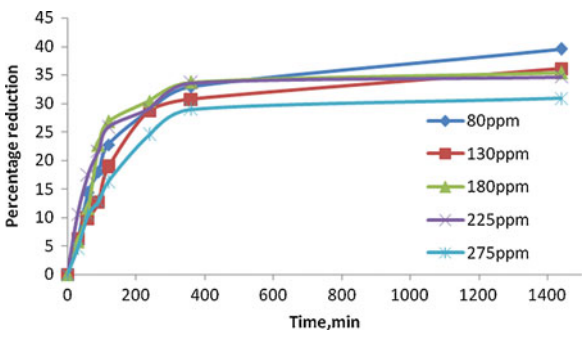

Fig. 1 Time course variation of percentage reduction of Chromium(VI) by glass bioparticles (100 beads) at different initial Cr(VI) concentrations of 80, 130, 180, 225 and 275 ppm

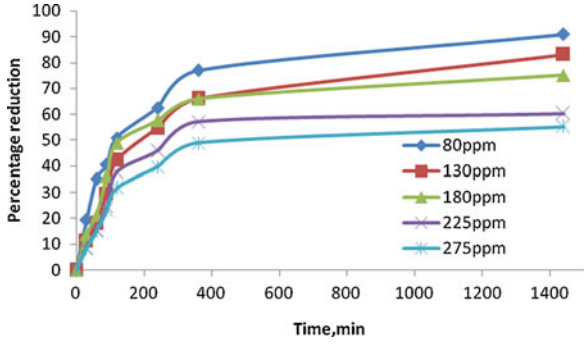

Fig. 2 Time course variation of percentage reduction of Chromium(VI) by glass bioparticles (500 beads) at different initial Cr(VI) concentrations of 80, 130, 180, 225 and 275 ppm

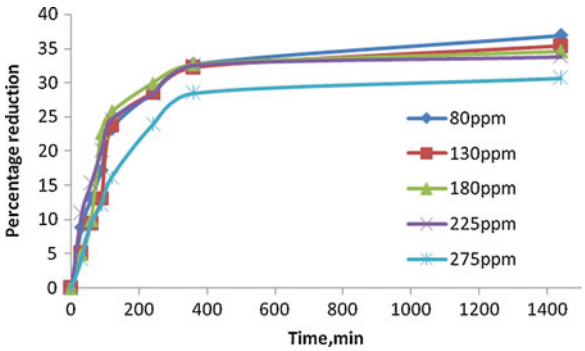

Fig. 3 Time course variation of percentage reduction of Chromium(VI) by Ca-alginate bioparticles (100 beads) at different initial Cr(VI) concentrations of 80, 130, 180, 225 and 275 ppm

important in developing an industrial scale application of the process.

3.4 Artificial Neural Network Model

Neural network models were developed for the prediction of percentage Cr(VI) reduction by batch bioreduction process using the cells of newly isolated strain of Cr(VI) resistant bacteria immobilized on CASE I: glass beads and CASE II: Ca-alginate beads. The input variables to the network are initial biomass loading (X_m), initial Cr(VI) concentration (S_I) and incubation time (t). Neural network development for each of the CASE I and II were based on training using 48 experimental data sets and validation using 22 data sets obtained by conducting batch experiments at different initial concentrations and initial biomass loading (corresponding to number of beads used). For both cases I and II, one hidden layer

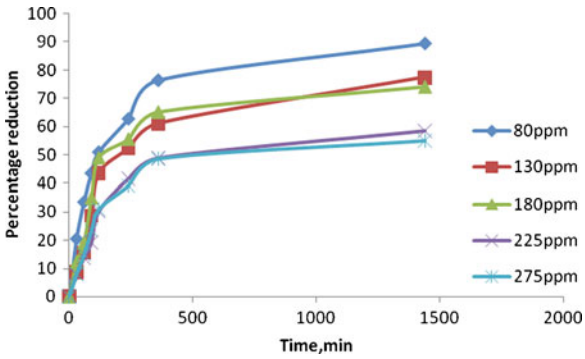

Fig. 4 Time course variation of percentage reduction of Chromium(VI) by Ca-alginate bioparticles (500 beads) at different initial Cr(VI) concentrations of 80, 130, 180, 225 and 275 ppm

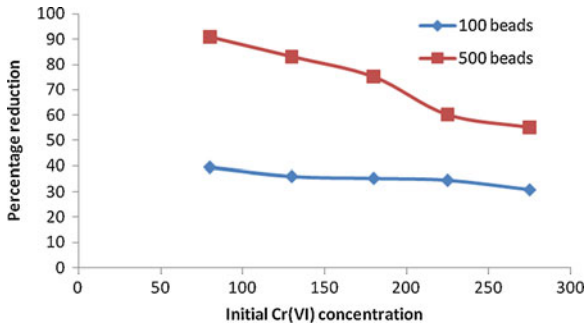

Fig. 5 Effect of initial Cr(VI) concentartion on percentage Cr (VI) reduction at the end of 24 h of batch run with 100 and 500 glass bioparticle loading

network, with three neurons in the input layer, seven sigmoidal neurons in the hidden layer, and one linear neuron in the output layer was found to be suitable for forecasting the batch biodegradation process. The network architecture being similar for both cases is presented in Fig. 7. The network parameters like the weights and bias values and the transfer functions used are presented in Tables 5 and 6, respectively for Case I and Case II. The experimental and ANN predicted values of percentage Cr(VI) removal for different sets of input variable values used for training the neural network for Case I and Case II are presented in Tables 1 and 2, respectively. The mean squared error (MSE), root mean squared error (RMSE) and the coefficient of correlation (R) values for training set are presented in the tables for both the cases. R values greater than 0.99 and small values of MSE and RMSE signify the good fit of the network to the experimental data. After the training was completed, the network was tested using the validation data set. The predicted values of percentage Cr(VI)

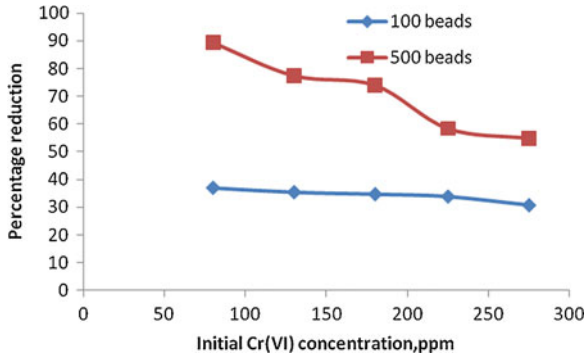

Fig. 6 Effect of initial Cr(VI) concentartion on percentage Cr (VI) reduction at the end of 24 h of batch run with 100 and 500 Ca-alginate bioparticle loading

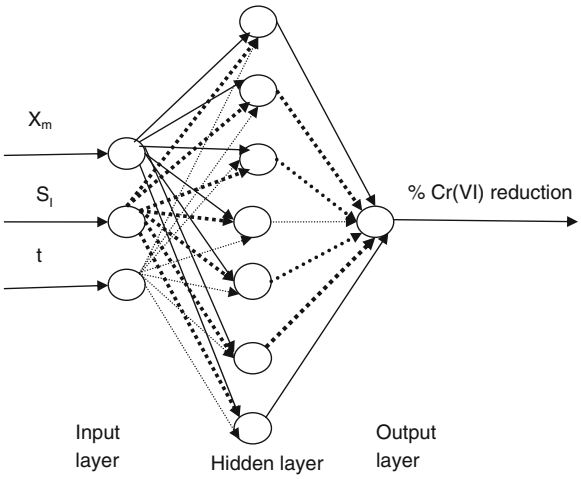

Fig. 7 Neural network architecture for bioreduction process with CASE I: glass bioparticles and CASE II: Ca-alginate bioparticles

removal for validation data set inputs for each of Cases I and II are presented in Tables 3 and 4, respectively. The plots of predicted % reduction vs. experimental percentage reduction for validation data set inputs, respectively for Case I and Case II are presented in Figs. 8 and 9, which show that the slopes of the fitted line for these points are 0.988 and 0.996, respectively with correlation coefficients of 98.5 and 97.7%, respectively. The slopes being very close to unity with high values of correlation coefficients indicate that the developed neural network models are valid within the range of input variables used for training the network. Hence, the neural network models were developed for prediction of performance of batch bioreduction process using glass bioparticles and Ca-alginate bioparticles, followed by validation. These models can be used for prediction of time course variation of percentage reduction at any initial Cr(VI) concentration and initial biomass loading based on number of beads used. Figure 10 is a representative comparison of time course variation of percentage Cr(VI) reduction during batch bioreduction process obtained experimentally and that which is predicted by ANN, at initial Cr(VI) concentration of 80 ppm and with biomass loading of 14.6 mg (500 glass bioparticles). The figure clearly supports the validity of ANN model developed for the prediction of time course variation during batch Cr(VI) bioreduction.

3.5 Effect of Type of Bioparticle

Two different types of bioparticles, one with cells immobilized on the surface, as a biofilm and another with cells entrapped in gel matrix were used in this study to compare the effect of mode of immobilization or immobilized cell location on the efficiency of bioreduction process. Glass beads with surface

Table 5 Network weights and biases for the ANN model (for glass bioparticles)

Input layer to hidden layer weights

	W_1	W_1	W_1	b
Neuron 1	1.4134	3.2121	1.6866	1.9161
Neuron 2	3.1163	0.5566	1.6764	−1.4532
Neuron 3	2.7793	−3.3871	0.2121	−1.1644
Neuron 4	−2.0719	−2.3822	0.1468	0.6847
Neuron 5	−0.5218	3.3249	1.2931	1.0672
Neuron 6	1.1463	1.3266	0.4371	2.1373
Neuron 7	−0.1369	0.0468	3.5079	4.2830

Transfer function for hidden layer neurons: hyperbolic tan sigmoid (tansig in MATLAB)

Hidden layer to output layer weights

W_2	W_2	W_2	W_2	W_2	W_2	W_2	b
−0.0217	0.4433	0.2684	0.2507	0.0546	0.0636	2.0491	−1.9609

Transfer function for output layer neurons: Pure linear (purelin in MATLAB)

Table 6 Network weights and biases for the ANN model (for Ca-alginate bioparticles)

Input layer to hidden layer weights

	W_1	W_1	W_1	b
Neuron 1	3.3137	0.1119	2.9165	−2.1951
Neuron 2	0.1560	−0.0068	−4.7752	−5.7710
Neuron 3	−1.2708	6.6989	−4.5185	3.3452
Neuron 4	5.0183	−3.3772	−1.8475	−1.9571
Neuron 5	1.3789	3.7641	3.3835	−1.3012
Neuron6	0.1910	6.5545	3.4819	0.1547
Neuron 7	−3.9454	−4.3176	1.7319	−4.3037

Transfer function for hidden layer neurons: hyperbolic tan sigmoid (tansig in MATLAB)

Hidden layer to output layer weights

W_2	W_2	W_2	W_2	W_2	W_2	W_2	b
0.2523	−3.2817	−0.0972	0.1477	0.0584	−0.1946	−0.1623	−3.1542

Transfer function for output layer neurons: pure linear (purelin in MATLAB)

immobilized cells and Ca-alginate beads with entrapped cells were used in the experiments. Figure 11 shows the comparison of the type of bioparticles on percentage reduction at different initial Cr(VI) concentrations with 500 bioparticle loading. The initial inoculum biomass loading were 14.6 mg and 28 mg respectively for glass and Ca-alginate beads in the case of 500 bioparticles. The initial inoculum biomass loadings were 2.92 mg and 5.6 mg, respectively for glass and Ca-alginate beads in the case of 100 bioparticles. It can be found that the initial inoculums with Ca-alginate beads are approximately twice that with glass beads. In spite of lower initial inoculums biomass amount, the percentage reduction achieved with glass beads were higher than those with Ca-alginate beads at all the initial concentrations. Similar observations were made with 100 bioparticles (figure not shown).

A true comparison is the one in which the performance with the same amount of initial biomass loading for the two cases are compared, rather than that with the same number of beads. So time course variation of percentage reduction with the Ca-alginate bioparticles was predicted using the ANN model developed, taking the initial biomass loading to be 14.6 mg, which is the same as the biomass present with 500 glass bioparticles. The ANN-predicted time course variation for Ca-alginate bioparticles with 14.6 mg initial biomass loading was compared with the experimental time course variation with 500 glass bioparticles (14.6 mg biomass loading) at 80 ppm initial Cr(VI) concentration. The resultant plots are

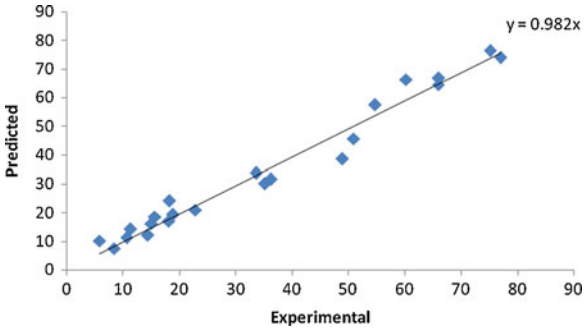

Fig. 8 Plot of ANN predicted vs. experimental percentage Cr (VI) reduction by glass bioparticles for validation data set

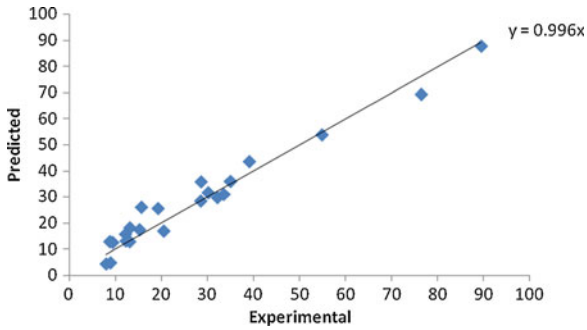

Fig. 9 Plot of ANN predicted vs. experimental percentage Cr (VI) reduction by Ca-alginate bioparticles for validation data set

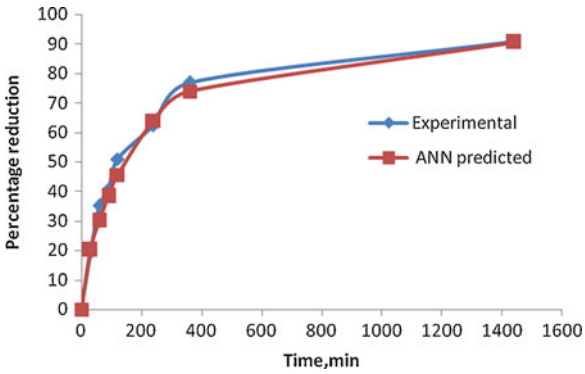

Fig. 10 Representative plot for comparison of experimental and ANN predicted time course variation of percentage reduction during batch bioreduction process using glass bioparticles with S_I=80 ppm and X_m=14.6 mg

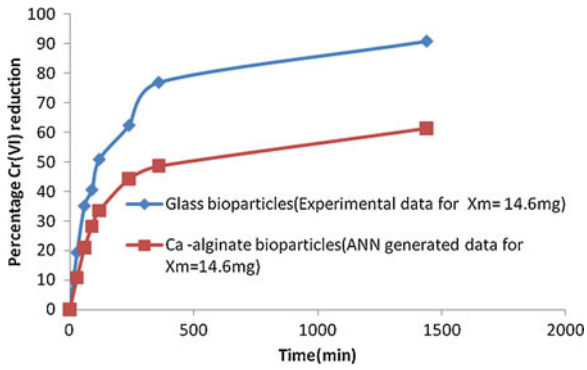

Fig. 12 Effect of type of bioparticles (glass and Ca-alginate bioparticles) used on time course variation of percentage Cr(VI) reduction with X_m=14.6 mg and S_I=80 ppm

shown in Fig. 12. It can be seen from Fig. 12 that the percentage reduction with glass bioparticles are always higher than those with Ca-alginate bioparticles indicating the higher rate of reduction with the glass bioparticles. The percentage reduction at the end of 24 h with glass bioparticles is around 91% whereas that with Ca-alginate bioparticles is only around 61%. Similar observations were made when experimental time course variations with 100 Ca-alginate bioparticles (5.6 mg initial biomass loading) were compared with ANN-predicted time course variations with 5.6 mg initial biomass loading (figure not shown).

Figure 13 shows the comparison of glass bioparticles and Ca-alginate bioparticles in terms of percentage reductions obtained at the end of 24 h with different initial Cr(VI) concentrations with initial biomass loading of 14.6 mg as a reference. For both the bioparticle types, ANN-predicted values of Cr(VI) reduction were used. It can be seen that the percentage reductions obtained with glass bioparticles were around 30 to 10% higher than those achieved with the Ca-alginate bioparticles, as the initial Cr(VI) concentration varied in the range of 80 to 275 ppm. Figures 11 and 13 show that glass beads are better supports to be used for cell immobilization as compared to Ca-alginate beads. It can be seen that higher rates of reduction as observed from time course variations of percentage Cr(VI) reduction and higher percentage reduction as observed at the end of the 24-h period could be achieved with the glass bioparticles as compared to Ca-alginate bioparticles.

The lower rates of reduction and percentage reductions in the case of Ca-alginate bioparticles may be attributed to the diffusional resistance offered by Ca-alginate gel matrix for the transfer of Cr(VI) from the bulk liquid to the cells that are entrapped in the matrix.

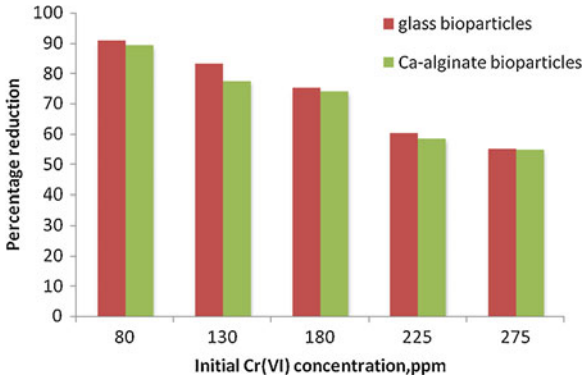

Fig. 11 Effect of type of bioparticles (glass and Ca-alginate bioparticles) used on percentage Cr(VI) reduction (experimental) at the end of 24 h of batch run with different initial Cr(VI) concentrations and bioparticle loading of 500 beads

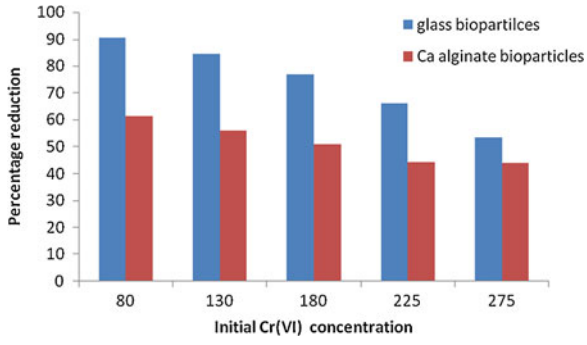

Fig. 13 Effect of type of bioparticles (glass and Ca-alginate bioparticles) used on percentage Cr(VI) reduction (ANN predicted)at the end of 24 h of batch run with different initial Cr(VI) concentrations and with X_m=14.6 mg

In the case of glass bioparticles, as the bacteria are in the biofilm on the surface of glass beads, the outer layers of cells in the biofilm get the nutrients easily and also get easily exposed to Cr(VI), leading to high rate of growth and bioreduction process. The resistance to mass transfer is the biofilm diffusional resistance that may play a role in the transfer of nutrients and Cr(VI) into the inner core of the biofilm only. The diffusional resistance offered by the gel matrix may be significantly higher than that offered by the biofilm. So faster rate of Cr(VI) reduction is observed with glass beads as compared to Ca-alginate beads. Significant improvement in percentage reduction may be obtained by cells immobilized on glass beads if suitable immobilization strategies are adopted to increase the amount of immobilized biomass.

4 Conclusions

Batch bioreduction of Cr(VI) were carried out with the cells of newly isolated chromium resistant *Acinetobacter* sp. bacteria, immobilized on glass beads and Ca-alginate beads. The rates of Cr(VI) bioreduction during the batch process and the percentage bioreduction at the end of 24 h, decreased with the increase in initial Cr(VI) concentration, indicating the inhibitory effect of Cr(VI) at higher concentrations. Efficiency of bioreduction can be improved by increasing the bioparticle loading or the initial biomass loading. Glass bioparticles have shown better performance as compared to Ca-alginate bioparticles in terms of batch Cr(VI) reduction achieved and the rate of reduction, both under the conditions of the same initial biomass loading and the same bioparticle loading. Significant improvement in percentage reduction can be obtained by using glass bioparticles as compared to Ca-alginate bioparticles. So glass beads may be considered as better cell carrier particles for immobilization as compared to Ca-alginate beads. Around 90% reduction of 80 ppm Cr (VI) could be achieved after 24 h with initial biomass loading of 14.6 mg on glass beads. Optimizing the number of bioparticles or initial biomass loading in order to get high percentage reduction or low Cr(VI) concentration in the effluent in order to maintain the effluent quality within the standards set by the statutory bodies is very important in developing an industrial scale application of the process. Artificial neural network-based models that are developed in the present study for the prediction of batch Cr(VI) bioreduction using immobilized cells of *Acinetobacter* sp. for each of the cases with glass bioparticles and calcium-alginate bioparticles have exhibited fairly good performance, as demonstrated by the predictions for validation data sets. So the model can be used for any further predictions of batch Cr(VI) bioreduction process with the bacteria within the range of conditions used for the model development.

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