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Biosorption of Cr(VI) from Water Using Biomass of *Aeromonas hydrophila*: Central Composite Design for Optimization of Process Variables

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Abstract The potential use of biomass of *Aeromonas hydrophila* for biosorption of chromium from aqueous solution was investigated. The variables (pH, initial Cr(VI) concentration, biomass dose, and temperature) affecting process were optimized by performing minimum number of experimental runs with the help of central composite design. The results predicted by design were found to be in good agreement (R^2=99.1%) with those obtained by performing experiments. Multiple regression analysis shows that uptake decreases with increase in pH and biomass dose, whereas it increases with increase in temperature and concentration. The maximum removal of Cr(VI) predicted by contour and optimization plots was 184.943 mg/g at pH 1.5, initial Cr(VI) concentration 311.97 mg/L, temperature 60 °C, and biomass dose 1.0 g. The removal of Cr(VI) was governed by adsorption of Cr(VI) as well as its reduction into Cr(III), which further gets adsorbed. The sorption capacity of biomass was calculated from experimental data using Langmuir sorption model and was found to be 151.50 mg/g at 40 °C and pH 1.5, which is comparable to other biosorbents. In addition to this, Dubinin–Radushkevich model was applied, and it was found that nature of sorption was chemisorption.

Keywords *Aeromonas hydrophila* · Hexavalent chromium · Biosorption · Optimization · Response surface methodology · Isotherm

Introduction

Discharge of heavy metals is a serious threat to the environment and public health because of their toxicity, accumulation in food chain, and persistency in nature [1, 2]. Chromium is one of them because of its extensive use as chromate and dichromate in electroplating,

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leather tanning, metal finishing, nuclear power plant, textile industries, and chromate preparation, etc. [3, 4]. The toxic, carcinogenic, and mutagenic nature of hexavalent chromium is well known and documented [5–7].

There are various tertiary treatment techniques for the removal of heavy metals from aqueous streams [8]; however, these processes are having technical and/or economical constraints [3]. In this context, biosorption has emerged as an alternative to these methods with the advantage of being technically easy, potential for regeneration, and sludge-free operation. In addition to this, it is eco-friendly in nature and is a low-cost domestic technique for remediating even heavily metal-loaded water [4, 9]. Various biosorbents such as agricultural byproducts and microorganisms have been reported for efficiently accumulating heavy metals [3, 9–17]. Among microorganisms, bacterial biomass (both live and dead) have gained attention of researchers for removal of metal from aqueous solution during recent years because of their ubiquity and smaller size, which leads to high surface area and fast rates [11, 12]. Several functional groups such as carboxyl, phosphonate, amine, and hydroxyl groups are reported to be present as bacterial cell wall component due to the fact that bacteria are capable of sorbing metal ions from aqueous solution [11]. Aeromonads (a bacterial strain) have been reported for remediation of metal because they are ubiquitous in fresh and marine water and tolerant toward heavy metals [18]. In this series, *Aeromonas caviae* has already been used for the removal of chromium [19] by conventional batch sorption method. The present investigation is primarily aimed to test the potentiality of biomass of *Aeromonas hydrophila* for the sorption of hexavalent chromium from water and to optimize the parameters affecting the sorption for its maximum removal via a 2^4 full factorial central composite response surface methodology (RSM) experimental design with the help of software MINITAB® Release 15. In the conventional method, there is variation of only one parameter at a time, keeping the other parameters constant, and thus, the cumulative effect of all the affecting parameters at a time cannot be studied. This method is also time consuming and requires a number of experiments to be performed to determine the optimum levels, which is unreliable [20, 21]. However, in RSM, the interactions of two or more variables can be studied simultaneously. In addition to this, it results in higher percentage yields, reduced process variability, closer confirmation of the output response to nominal and target achievement, and less treatment time with minimum costs [22].

Materials and Methods

All chemicals and reagents used were of analytical grade and purchased from E. Merck, India Ltd., Mumbai, India. The stock solution containing 1,000 mg/L of Cr(VI) was prepared by dissolving 1.4143 g of $K_2Cr_2O_7$ in 500 mL of deionized, double distilled water. Required initial concentration of Cr(VI) samples was prepared by appropriate dilution of the above stock Cr(VI) standard solution. The surface area of biomass was determined by a three-point N_2 gas adsorption method using quanta sorb surface area analyzer (model Q5-7, Quanta Chromo Corporation, USA). Density of biosorbent was determined using Densitometer. Particle size distribution analysis was carried out using a particle size analyzer (Model no. 11708, Malvern Instrument, USA). Atomic absorption spectrophotometer (Model no. AA 6300, Shimadzu, Japan) was utilized to determine total Cr (Cr(VI) and Cr (III)) in the standard and unknown solution. The pH of the solution was measured with Orion pH/ISE meter (model no. 960) previously calibrated with standard buffer solution.

Preparation of Biosorbent

The strain used in this study was *A. hydrophila* (MTCC 646), a pure culture obtained from the Microbial Type Culture Collection & Gene Bank, Institute of Microbial Technology, Chandigarh, India. The culture was routinely maintained at 4 °C on nutrient agar medium slant and aerobically cultivated in nutrient broth containing beef extract 10 g/L, NaCl 5 g/L, peptone 20 g/L. The initial pH of the culture was adjusted to 7.0 to 7.5. The flasks were incubated at 30 °C in a rotatory shaker agitated at 200 rpm for 24 h. The growing cells from the culture broth were separated from the liquid by centrifugation at 5,000 rpm for 5 min and washed with deionized water. The wet cell biomass was dried for 24 h at 60 °C in an oven. Dried cells were powdered by a blender in uniform size (100 μm) and used for experiment. The characterization of dried biomass of *A. hydrophila* was performed and given in Table 1.

Adsorption Studies

Batch experiments were carried out in Erlenmeyer flasks by adding dried cells of *A. hydrophila* in 50 mL of aqueous chromium solution of desired initial concentration. The pH of the solution was monitored by adding 0.1 M HCl and 0.1 M NaOH solution as required. The flasks were gently agitated in an electrically thermostated reciprocating shaker at 200 rpm for a period of 3 h. The content of flask was separated from biosorbent by centrifuging at 15,000 rpm and was analyzed for total chromium concentration in the sample. The amount of Cr(VI) sorbed per unit mass of the biosorbent (q_t in milligrams per gram) was evaluated by using the following equation:

$$q_t = (C_i - C_t) \times V/W \tag{1}$$

where C_i and C_t are the Cr(VI) concentrations in milligrams per liter initially and at a given time t, respectively; V is the volume of the Cr(VI) solutions in milliliters; W is the weight of biosorbent in milligrams.

Chromium Analysis in the Aqueous Solution

The concentration of Cr(VI) in the solution was measured using AAS. Total Cr concentration (Cr(VI) and Cr(III)) in the solution was measured by first converting the Cr(III) species to Cr(VI) at high temperature (130–140 °C) by the addition of excess potassium permanganate. The Cr(III) concentration was then calculated from the difference between the total Cr and Cr(VI) concentrations [23].

Table 1 Physical properties of dried *Aeromonas hydrophila* biomass.

Parameters	Values
Surface area (m^2/g)	0.676
Density (g/cm^3)	0.32
Particle size (μm)	100
Pore volume (cm^3/g)	0.849

Theory

Response Surface Methodological Approach

RSM is an empirical statistical technique employed for multiple regression analysis by using quantitative data. It solves multivariable data, which is obtained from properly designed experiments to solve multivariable equation simultaneously [24]. The graphical representation of their functions is called response surface, which was used to describe the individual and cumulative effect of the test variables and their subsequent effect on the response. An easy way to estimate response surface is thru factorial designs, which are the most useful schemes for the optimization of variables with a limited number of experiments. A variety of factorial designs are available to accomplish this task [25]. The most successful and best among them is the central composite design (CCD), which is accomplished by adding two experimental points along each coordinate axis at opposite sides of the origin and at a distance equal to the semi-diagonal of the hyper cube of the factorial design and new extreme values (low and high) for each factor added in this design [26]. If the factorial is a full factorial, then

$$\alpha = \left[2^k\right]^{1/4} \tag{2}$$

Since in this study four factors such as initial concentration of chromium, pH, temperature, and biomass dose were considered, thus, k=4, so α=2. Furthermore, the total number of experiments in CCD can be calculated from the following equation:

$$N = 2^n + 2n + n_c \tag{3}$$

where N is the total number of experiments, n is the number of variables, and 2^n is the number of factorial runs with $2n$ axial runs and n_c center runs. Thus, for this design, total number of experimental runs will be 31 (n=4; 2^n=16; $2n$=8; n_c=7).

Data from the central composite design were subjected to a second-order multiple regression analysis to explain the behavior of the system using the least squares regression methodology to obtain the parameter estimators of the mathematical model [26].

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j + \varepsilon \tag{4}$$

where Y is the response, β_0 is the constant, β_i the slope or linear effect of the input factor X_i, β_{ii} is the quadratic effect of input factor X_i, β_{ij} the linear by linear interaction effect between the input factor X_i, and ε is the residual term.

MINITAB® Release 15, developed by Minitab Inc., USA, a statistical software package [27], was used for regression analysis of the data obtained and used to estimate the coefficient of regression equation. Analysis of variance (ANOVA), which is statistical testing of the model in the form of linear terms, squared terms, and the interaction was also utilized to test the significance of each term in the equation and the goodness of fit of the regression model obtained [28]. This response surface model was also used to predict the result by isoresponse contour plots in order to study the individual and cumulative effects of the variables and the mutual interactions between the variables on the dependent variable [29]. Optimization curves were plotted in order to confirm the experimental results and to achieve the required removal of chromium by choosing the predicted conditions.

Table 2 Central composite design analysis for biosorption of hexavalent chromium.

Independent variable	Range				
	$-\alpha$	−1	0	+1	$+\alpha$
pH	1.5	2.5	3.5	4.5	5.5
Initial metal ion concentration (mg/L)	103.99	155.98	207.98	259.98	311.97
Temperature (°C)	20	30	40	50	60
Biomass dose (g)	1.0	1.5	2.0	2.5	3.0

Results and Discussions

Optimization of Biosorption Process Using RSM Approach

The range of variables affecting the sorption of Cr(VI) (i.e., pH, initial metal ion concentration, biomass dose, and temperature) as per CCD design is represented in Table 2. Since in the present investigation there are four variables, 31 experimental runs were required as per 2^4 full factorial design (Table 3). Experiments were performed according to the experimental plan (CCD), and the results thus obtained for each combination are given in Table 4. The results were also predicted with the help of Minitab software's CCD and are given in Table 4. Significant changes in uptake of chromium were observed for all the combinations, implying that all the variables were significantly affecting the sorption of chromium.

Interpretation of the Regression Analysis

The response surface regression results thus obtained from CCD, namely, the *T* and the *P* values along with the constant and coefficients (estimated using coded values), are given in Table 5. The *T* value is used to determine the significance of the regression coefficients of the parameters, and the *P* value is defined as the smallest level of significance leading to rejection of null hypothesis. In general, the larger the magnitude of *T* and the smaller the value of *P*, the more significant is the corresponding coefficient term [30].

The value of constant was found to be 82.61 and was significant (P=0.000, T=79.323). The constant, which does not depend on any variable and interaction of variables, shows that the average uptake of hexavalent chromium on *A. hydrophila* was 82.61 mg/g and that this average uptake is independent of the factors set in the experiment. The effect of all the linear factors, i.e., pH, concentration, temperature, and biomass dose were found to be highly significant (P=0.000) on the removal of hexavalent chromium, i.e., there is a linear relation of all these parameters with the uptake of Cr(VI). All the quadratic terms were not

Table 3 Number of experimental runs required and their details for 2^4 full factorial CCD.

Factors	Replicates	Central composite design		Base blocks	Total blocks
		Base runs	Total runs		
4	1	31	31	1	1
	2-Level factorial: full factorial				
Cube points	Center points in cube	Axial points	Center points in axial	Alpha	
16	7	8	0	2	

Table 4 Full factorial central composite design matrix of four variables (factors) along with experimental and predicted response (uptake; milligrams per gram).

Standard order	Run order	Pt type	Blocks	pH	Concentration (mg/L)	Temperature (°C)	Biomass dose (g)	Exp. uptake (mg/g)	Predicted (mg/g)	Residual
17	1	−1	1	2.5	259.98	50	2.5	106.43	105.56	0.87
3	2	1	1	4.5	155.98	30	1.5	42.53	44.34	−1.81
30	3	0	1	2.5	259.98	30	2.5	91.53	92.74	−1.21
8	4	1	1	2.5	155.98	30	1.5	70.24	69.44	0.79
28	5	0	1	4.5	259.98	30	2.5	73.42	74.59	−1.16
5	6	1	1	4.5	259.98	50	2.5	87.43	87.61	−0.18
6	7	1	1	4.5	259.98	50	1.5	102.43	103.73	−1.29
2	8	−1	1	4.5	259.98	30	1.5	85.67	85.28	0.38
26	9	0	1	4.5	155.98	50	2.5	60.46	58.84	1.61
25	10	0	1	2.5	155.98	50	1.5	89.27	89.03	0.23
15	11	1	1	2.5	259.98	30	1.5	108.26	110.81	−2.55
21	12	−1	1	4.5	155.98	30	2.5	48.76	44.46	4.29
29	13	0	1	2.5	259.98	50	1.5	125.34	129.05	−3.71
24	14	−1	1	4.5	155.98	50	1.5	65.96	64.14	1.81
18	15	−1	1	2.5	155.98	30	2.5	62.53	62.18	0.34
27	16	0	1	2.5	155.98	50	2.5	76.55	76.35	0.19
13	17	1	1	5.5	207.98	40	2.0	58.10	59.13	−1.03
4	18	1	1	1.5	207.98	40	2.0	105.29	102.18	3.10
2	19	1	1	3.5	311.97	40	2.0	122.84	117.38	5.45
12	20	1	1	3.5	103.99	40	2.0	43.86	47.24	−3.38
14	21	1	1	3.5	207.98	60	2.0	99.68	98.72	0.95
7	22	1	1	3.5	207.98	20	2.0	67.22	66.10	1.11
9	23	1	1	3.5	207.98	40	3.0	65.96	67.47	−1.51
20	24	−1	1	3.5	207.98	40	1.0	94.43	90.84	3.58
16	25	1	1	3.5	207.98	40	2.0	82.61	82.32	0.28
23	26	−1	1	3.5	207.98	40	2.0	82.61	82.32	0.28
11	27	1	1	3.5	207.98	40	2.0	82.61	82.32	0.28
31	28	0	1	3.5	207.98	40	2.0	82.61	82.32	0.28
19	29	−1	1	3.5	207.98	40	2.0	82.61	82.32	0.28
10	30	1	1	3.5	207.98	40	2.0	82.61	82.32	0.28
1	31	1	1	3.5	207.98	40	2.0	82.61	82.32	0.28

found to be significant ($P>0.05$). Quadratic terms are used to evaluate whether or not there is curvature (quadratic) in the response surface [26]. Because the quadratic terms were identified as non-significant ($P>0.05$), it means that the relationship between biosorption process and all the factors studied is a straight line rather than a curved line.

The interaction terms of pH and biomass dose ($P=0.016$) and concentration and biomass dose ($P=0.001$) were found to be significant. Very high value of parameter estimate (coefficients) for linear effects of pH, concentration, temperature, and biomass dose and relatively high values of coefficients of interaction terms of pH and biomass dose and concentration and biomass dose shows a high level of significance indicating the importance of these variables in the biosorption process. The first order effect of all linear terms and second-order main effect of interaction terms of pH and biomass dose and concentration and biomass dose were highly significant. A positive sign of the coefficient represents a synergistic effect, while a negative sign indicates an antagonistic effect. The linear variables pH and biomass dose and the interaction term concentration and biomass

Table 5 Estimated regression coefficients (using coded units) for experimental uptake (milligrams per gram).

Term	Coefficients	Standard error coefficients	*T* value	*P* value
Constant	82.610	1.041	79.323	0.000
pH	−21.489	1.125	−19.103	0.000
Concentration (mg/L)	35.178	1.125	31.275	0.000
Temperature (°C)	16.320	1.125	14.509	0.000
Biomass dose (mg)	−11.627	1.125	−10.337	0.000
pH×pH	−1.662	2.061	−0.807	0.432
Concentration (mg/L)×concentration (mg/L)	−0.008	2.061	−0.004	0.997
Temperature (°C)×temperature (°C)	0.092	2.061	0.045	0.965
Biomass dose (mg)×biomass dose (mg)	−3.162	2.061	−1.535	0.144
pH×Concentration (mg/L)	−0.432	2.755	−0.157	0.877
pH×temperature (°C)	0.217	2.755	0.079	0.938
pH×biomass dose (mg)	7.387	2.755	2.681	0.016
Concentration (mg/L)×temperature (°C)	−1.357	2.755	−0.493	0.629
Concentration (mg/L)×biomass dose (mg)	−10.796	2.755	−3.919	0.001
Temperature (°C)×biomass dose (mg)	−5.417	2.755	−1.966	0.067

dose had a negative significant relationship with the biosorption process. The effect of linear terms temperature and concentration and interaction term of pH and biomass dose had a positive effect on the uptake of Cr(VI), i.e., with an increase in temperature and initial metal ion concentration, there will be an increase in the uptake of Cr(VI), and the effect of pH on the biosorption depends upon biomass dose.

A regression equation was prepared using the coefficients, which is as follows:

$$\begin{aligned} Y = {} & 82.610 - 21.489 \times \mathrm{pH} + 35.178 \times \mathrm{concentration(mg/L)} + 16.321307 \\ & \times \mathrm{temperature}\ (^{\circ}\mathrm{C}) - 11.628 \times \mathrm{biomass\ dose\ (mg)} - 1.663 \times (\mathrm{pH})^2 - 0.008 \\ & \times (\mathrm{concentration(mg/L)})^2 + 0.092 \times (\mathrm{temperature}\ (^{\circ}\mathrm{C}))^2 - 3.163 \times (\mathrm{biomass\ dose\ (mg)})^2 \\ & - 0.433 \times \mathrm{pH} \times \ \mathrm{concentration\ (mg/L)} + 0.2178 \times \mathrm{pH} \times \mathrm{temperature}\ (^{\circ}\mathrm{C}) + 7.388 \times \mathrm{pH} \\ & \times \mathrm{biomass\ dose\ (mg)} - 1.357 \times \mathrm{concentration(mg/L)} \times \mathrm{temperature}\ (^{\circ}\mathrm{C}) - 10.797 \\ & \times \mathrm{concentration\ (mg/L)} \times \mathrm{biomass\ dose\ (mg)} - 5.418 \times \mathrm{temperature}\ (^{\circ}\mathrm{C}) \\ & \times \mathrm{biomass\ dose\ (mg)} \end{aligned} \tag{5}$$

where Y is the response variable, predicted amount of hexavalent chromium adsorbed (milligrams per gram). The low value of standard deviation (2.755) between the measured and predicted results shows that the equation adequately represents actual relationship between the response and significant variables. High values of R^2 (99.1%) and R^2 (adjusted) (98.2%) indicates a high dependence and correlation between the observed and the predicted values of response. The predicted values were found to be very close to the experimental results (Table 4).

The ANOVA was also performed, which demonstrates that the quadratic model was highly significant, as was evident from the calculated Fisher's "*F*" value (120.71) and a probability (*P*) value of 0.000. The large value of *F* indicates that most of the variation in the response can be explained by the regression equation. The associated *P* value is used to estimate whether *F* is large enough to indicate statistical significance. If $P>F$ value is lower than 0.05, then it indicates that the model is statistically significant [31]. It was also observed from ANOVA study that the coefficients for the linear (P=0.000) and interaction (P=0.008) effects were highly significant and thus confirm the applicability of the predicted model.

Interpretation of Residual Graphs

The normality of the data can be checked by plotting the normal probability plot (NPP) of the residuals. The NPP is a graphical technique for assessing whether or not a data set is approximately normally distributed [32]. The residual is the difference between the observed and the predicted value (or the fitted value) from the regression. If the points on the plot fall fairly close to the straight line, then the data are normally distributed. Figure 1a shows normal probability plot of residual values. It could be seen that the experimental points were reasonably aligned, suggesting normal distribution. The results can be shown (Fig. 1b) with the help of a histogram. A histogram of the residuals shows the distribution of the residuals for all observations. The figure shows an almost symmetrical histogram (bell-shaped, i.e., the errors are normally distributed with mean zero) [27]. Figure 1c plots the residuals versus the fitted values (predicted response). The residuals are scattered randomly about zero, i.e., the errors have a constant variance. Figure 1d plots the residuals in the order of the corresponding observations. The residuals appear to be randomly scattered about zero. Except for one point (run number 19 having a residual value of 5.06), all other points were found to fall in the range of +5 to −5.

Interpretation of Contour Plots

Contour plot is the projection of the response surface as a two-dimensional plane. This analysis gives a better understanding of the influence of variables and their interaction on the response. To investigate the interactive effect of two factors on the removal of

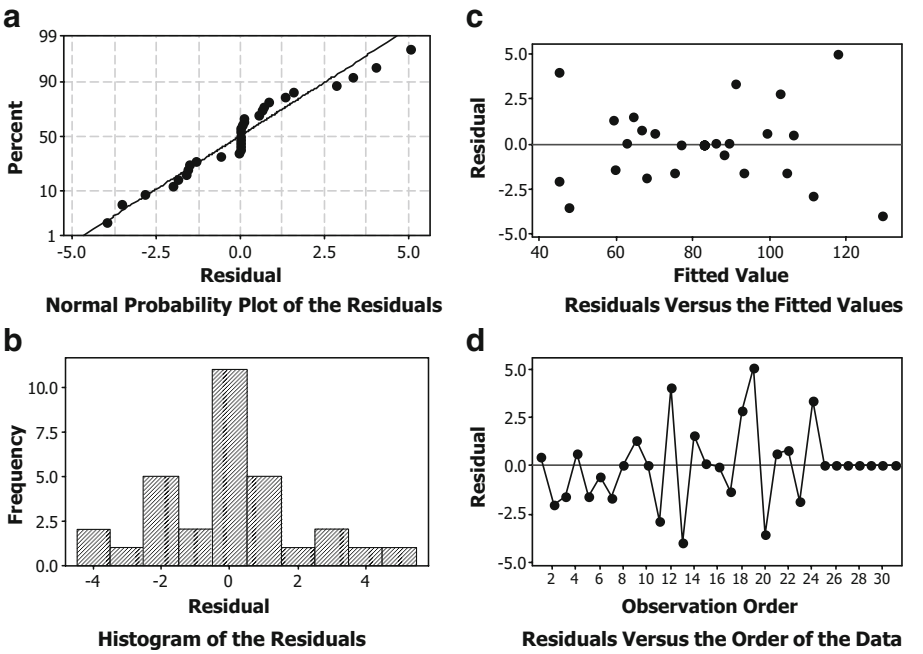

Fig. 1 Residual graphs. **a** Normal probability plot for residuals. **b** Histograms of residuals. **c** Residuals versus fitted values. **d** Residuals versus the order of the data

chromium, the RSM-CCD was used, and contour plots were drawn. The hold values of the remaining factors were set at their middle values (i.e., pH at 3.5, initial metal ion concentration at 208.0 mg/L, temperature at 40 °C, and biomass dose at 2.0 g). Figure 2 shows the combined effect of pH and initial metal ion concentration. It is clear from the figure that the uptake was decreased with the increase in pH and increased with an increase in initial metal ion concentration. The maximum uptake was achieved at pH 1.5 and concentration of 311.97 mg/L. At high pH, the surface of the biosorbent gets highly protonated, which favors the sorption as well as reduction of Cr(VI) in the form of anions. The uptake of chromium was found to increase as the initial metal ion concentration increased because the number of ions adsorbed from solutions of higher concentrations is more than that removed from less concentrated solutions. There is no curvature in the plot that means that there is linear relationship between uptake and pH and concentration, i.e., the square terms of pH and concentration are not significant. Figure 3 shows the combined effect of pH and temperature on the removal of chromium. It reveals that the uptake of chromium increased with increase in temperature and decreased with increase in pH, which was found to be in accordance with the experimental and predicted model values. The maximum uptake was found at temperature of 60 °C and pH 1.5. The increase in uptake with increasing temperature could be explained because the rise of temperatures favors the sorbate transport with in the pores of sorbent. Combined effect of pH and biomass dose was analyzed and presented in Fig. 4, showing that the uptake was decreased with the increase in both pH and biomass dose. The maximum uptake of Cr(VI) was found at a biomass of 1.0 g and pH 1.5. The decrease in the uptake of Cr(VI) with increasing sorbent dose was due to split in the flux or the concentration gradient between Cr(VI) concentration in the solution and the Cr(VI) concentration on the surface of the biosorbent, which finally reduces the uptake of Cr(VI) onto the unit weight of sorbent.

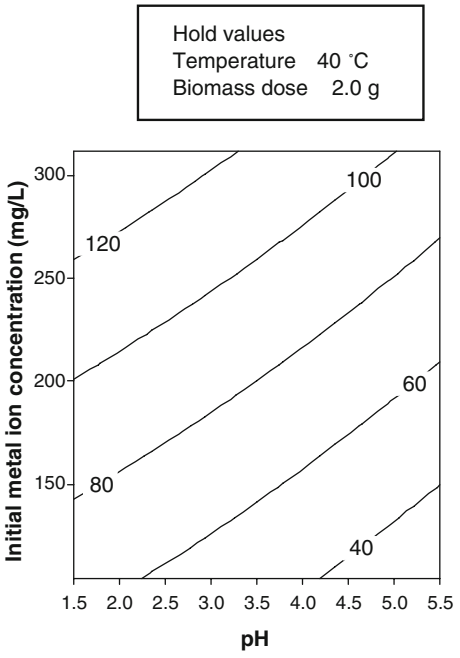

Fig. 2 Contour plot for uptake (milligrams per gram) vs. pH and concentration

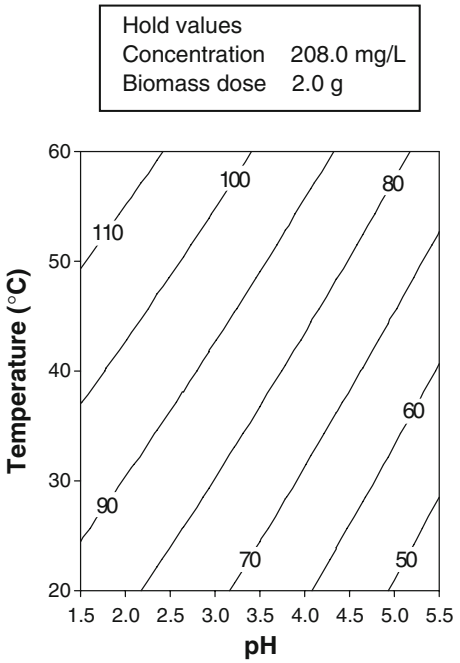

Fig. 3 Contour plot for uptake (milligrams per gram) vs. pH and temperature

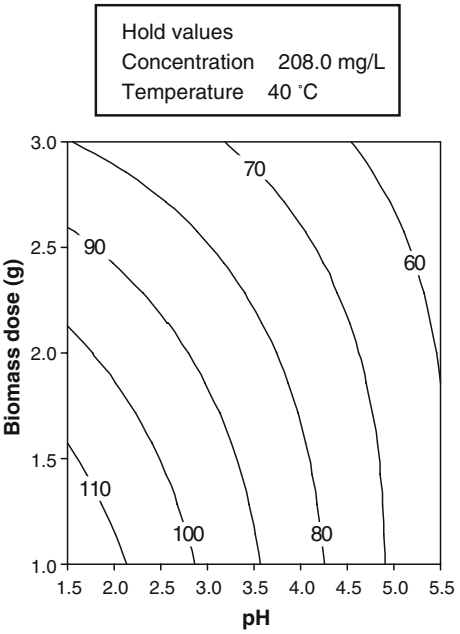

Fig. 4 Contour plot for uptake (milligrams per gram) vs. pH and biomass dose

Interpretation of Process Optimization Curve

Response optimization helps to identify the factor settings that optimize a single response or a set of responses. It is useful in determining the operating conditions that will result in a desirable response.

In the present study, the goal for Cr(VI) uptake using biomass of *A. hydrophila* was to obtain a value at or near the target value of 185 mg/g. Uptake values less than 10 and greater than 200 mg/g were unacceptable. Both weight and importance were set at 1. The global solution, which is defined as the best combination of factor settings for achieving the desired response, was found to be pH 1.5, initial metal ion concentration 311.97 mg/L, temperature 60 °C, and biomass dose 1.0 g for a predicted response of 184.943 mg/g, with a desirability score of 0.999 (Fig. 5). Thus, a maximum uptake of Cr(VI) of 184.94 mg/g can be achieved with *A. hydrophila* under the studied conditions. Other desired values can also be predicted by changing the current factor level setting in the optimization plot, and then these values can be achieved by performing experiments. In general, there are many advantages of optimization plot so as to achieve predicted response with higher desirability score, lower-cost factor settings with near optimal properties, to study the sensitivity of response variables to changes in the factor settings and to get required responses for factor settings of interest.

Biosorption Mechanism

During the optimization of pH during sorption of chromium, it has been found that the uptake of chromium decreases with the increase in the pH of the solution and the maximum sorption was achieved at a pH value of 1.5. This indicates that sorption of chromium is dependent on the pH of the solution, and it is governed by chromium ion speciation at various pH and the functional group present on the sorbent surface. At acidic pH, the predominant species of chromium will be $Cr_2O_7^{-2}$, $HCrO_4^{-}$, $Cr_3O_{10}^{-2}$,and $Cr_4O_{13}^{-2}$, and at higher pH, the presence of CrO_4^{-2} is reported [33]. The surface of the biomass becomes highly protonated under acidic conditions and hence favors the uptake of Cr(VI) in the form of anions. Whereas, with the increase of pH, the degree of protonation of the surface got reduced gradually, and sorption started decreasing. In addition to this, as the pH increases, there will be competition between OH^{-} and chromate ions (CrO_4^{-2}) since OH^{-}, being the more dominant species at higher pH, will cause hindrance in the sorption of CrO_4^{-2} ion on the surface of biomass. Furthermore, the net positive surface potential of sorbent decreases with the increase of OH^{-}, resulting in the weakening of electrostatic forces between sorbent and sorbate, which will ultimately reduce the sorption of chromium [34].

It has also been reported that in highly acidic conditions, the oxy species of chromium present in the solution in +6 oxidation state will act as very powerful oxidant. This will

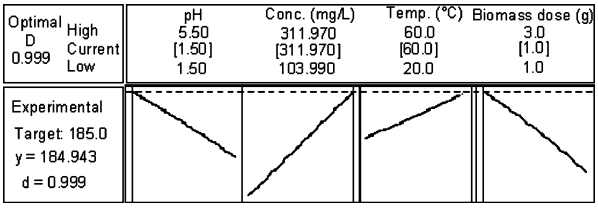

Fig. 5 Process optimization curve for a target value of 185 mg/g

oxidize the surface of the biosorbent, and all these oxy chromium species themselves will get reduced into Cr(III) form [35–37]:

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O \quad (6)$$

$$HCrO_4^- + 7H^+ + 3e^- \rightarrow Cr^{3+} + 4H_2O \quad (7)$$

Hence, it can be concluded that the removal of Cr(VI) from the aqueous solution occurs via two mechanisms, one is the direct sorption of Cr(VI) on the surface of the sorbent and other is the reduction of Cr(VI) into Cr(III) at low pH, and then the sorption of Cr(III) on the surface of the sorbent.

Adsorption Equilibrium Study

Various isotherm models have been utilized for describing sorption equilibrium for wastewater treatment. For the present work, Langmuir and Dubinin–Radushkevich equations are being used in order to evaluate the sorption capacity of the biomass and the nature of sorption of hexavalent chromium on the surface of the biomass respectively. The study of isotherm was carried out by varying initial metal ion concentration from 51.99 to 311.97 mg/L at various temperatures (20–40 °C).

Langmuir Sorption Isotherm

The Langmuir sorption isotherm describes that the uptake occurs on a homogeneous surface by monolayer sorption without interaction between sorbed molecules [38]. The linear form of the Langmuir isotherms may be represented as:

$$C_e/q_e = 1/Q^o b + C_e/Q^o \quad (8)$$

where Q^o and b are the Langmuir constants related to the monolayer sorption capacity (milligrams per gram) and free sorption energy (liters per milligram), respectively. The isotherm constants $Q°$ and b are calculated from the slope and intercept of plot between C_e/q_e and Ce (Fig. 6), respectively. The isotherm showed good fit to the experimental data with good correlation coefficients (Table 6). The sorption capacity of the biomass of *A. hydrophila* was found to be 151.50 mg/g at 40 °C and pH 1.5, which is found to be much better than other biosorbents already used for the removal of hexavalent chromium from water (Table 7).

Dubinin–Radushkevich (D-R) Sorption Isotherm

The data were also applied to Dubinin–Radushkevich (D-R) isotherm. This model envisages about the heterogeneity of the surface energies [39] and can be written in the following linear form:

$$\ln q_e = \ln X_m - \beta F^2 \quad (9)$$

$$F = RT \ln\left(1 + \frac{1}{Ce}\right) \quad (10)$$

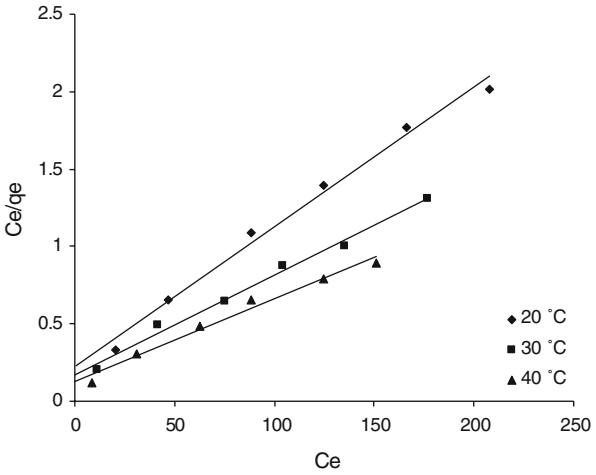

Fig. 6 Langmuir sorption isotherm

where q_e is the amount of sorbate sorbed by sorbent (moles per gram), X_m is the maximum sorption capacity of the sorbent (moles per gram), β is the constant related to energy (square moles per squares kilojoules), F is the polar potential, R is the universal gas constant [8.314 J/mol/K], T is the absolute temperature (Kelvin), and Ce the concentrations at equilibrium (moles per liter).

The constant β and X_m was obtained from slope and intercept of the plot of ln q_e against F^2 (Fig. 7), respectively, and the values are given in Table 6. Thus, mean sorption energy, E, which is defined as the free energy transfer of 1 mol of solute from infinity of the surface of the sorbent, can be calculated using the calculated value of β from:

$$E = \frac{1}{\sqrt{-2\beta}} \tag{11}$$

If the magnitude of E is between 8 and 16 kJ mol^{-1}, the sorption process is supposed to proceed via chemisorption, while for values of E<8 kJ mol^{-1}, the sorption process is of physical nature [40].

The isotherm showed good fit to the experimental data (Fig. 7) with good correlation coefficients (Table 6). The applicability of this isotherm model to the chromium sorption shows that there is possibility of heterogeneous energetic distribution of active sites on the surface of the sorbent. The estimated values of E (Table 6) for the present study were found

Table 6 Parameters of Langmuir and D-R isotherm for biosorption of Cr(VI) on *Aeromonas hydrophilla* at various temperatures.

Temperature (°C)	Langmuir constants			D-R constants			
	Q^0 (mg/g)	b (L/mg)	R^2	β (kJ^2/mol^2)	X_m (mol/g)	R^2	E (kJ/mol)
20	98.03	0.044	0.996	0.0029	0.00164	0.974	13.131
30	133.45	0.048	0.989	0.0027	0.00253	0.971	13.608
40	151.50	0.053	0.988	0.0024	0.00344	0.978	14.142

Table 7 Comparison of sorption capacities of the *Aeromonas hydrophila* for the removal of Cr(VI) with those of other biosorbents.

Sl no.	Biosorbent	Sorption capacities (mg/g)
1.	*Aeromonas hydrophila*	151.50 (at 40 °C, pH 1.5) (present study)
2.	*Staphylococcus xylosus*	143.00 (at pH 1.0) [11]
3.	*Aeromonas caviae*	124.46 (at 20 °C, pH 2.5) [9]
4.	Untreated *Rhizopus nigricans*	123.5 (at 30 °C, pH 2.0) [7]
5.	*Pseudomonas sp.*	95.0 (at pH 4.0) [11]
6.	*Bacillus thuringiensis*	83.30 (at 25 °C, pH 2.0) [6]
7.	*Lyngbya putealis*	69.0 (at 25 °C, pH 2) [41]
8.	*Rhizopus arrhizus*	58.1 (at 25 °C, pH 2.0) [7]
9.	*Mucor hiemalis*	51.0 (at 40 °C, pH 2.0) [7]
10.	Raw *Oedogonium hatei*	31.0 (at 45 °C, pH 2.0) [11]
11.	Acid treated *Oedogonium hatei*	35.2 (at 45 °C, pH 2.0) [11]
12.	Algae, *Chlorella vulgaris*	27.3 (at 25 °C, pH 2.0) [7]
13.	*Zoogloea ramigera*	27.5 (at 25 °C, pH 2.0) [11]
14.	*Aspergillus* biomass	23.6 (at 28 °C, pH 5.0) [7]
15.	*Lentinus sajor-caju* (untreated)	19.6 (at 25 °C, pH 2.0) [7]
16.	Immobilized, dried, activated sludge	18.9 (at 25 °C, pH 1.0) [7]
17.	Algae *Spirogyra*	14.7 (at 18 °C, pH 2.0) [7]
18.	*Chryseomonas luteola*	3.0 (at pH 4.0) [11]

in the range expected for chemical sorption. Thus, the sorption of Cr(VI) on the surface of the *A. hydrophila* was chemisorption.

Conclusion

The following conclusion can be drawn from this study:

1. The biomass of *A. hydrophila* was found to be a potential biosorbent for the removal of Cr(VI) from water. A 2^4 full factorial central composite design was utilized with the

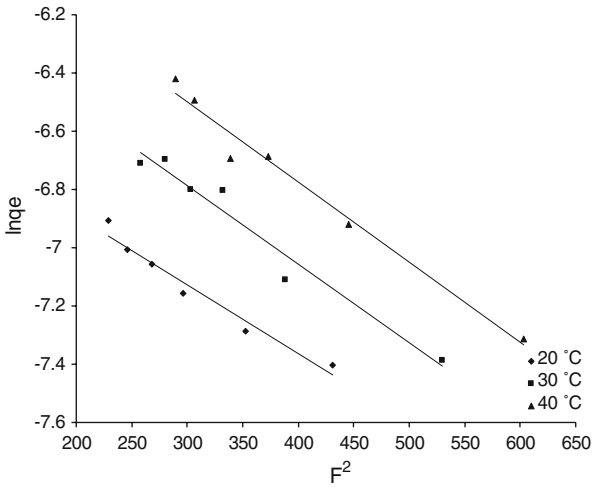

Fig. 7 D-R sorption isotherm

help of MINITAB® Release 15 software for predicting the results with 31 sets of experiments, and a high correlation has been found between the experimental and predicted values (R^2=99.1%).

2. The uptake of chromium was found to be very sensitive to the linear effect of all the factors, viz., pH, metal ion concentration, temperature, and biomass dose. The uptake was found to decrease with an increase in the pH and biomass dose, while there was an increase with concentration and temperature in the present study. The predicted model can be used for the better removal of hexavalent chromium from aqueous streams in wastewater treatment plants. In this study, the predicted maximum removal of Cr(VI), i.e., 184.943 mg/g was obtained at pH 1.5, initial metal ion concentration 311.97 mg/L, temperature 60 °C, and biomass dose 1.0 g.
3. During the sorption process, it was found that at low pH, the removal of Cr(VI) was not only due to adsorption but also due to reduction of Cr(VI) into less toxic form Cr(III), which is finally adsorbed.
4. The sorption of Cr(VI) on *A. hydrophila* was found to follow both Langmuir and D-R isotherm, suggesting the monolayer sorption as well as heterogeneous energetic distribution of active sites on the surface of the sorbent. The sorption capacity of biomass *A. hydrophila* was found to be 151.50 mg/g at 40 °C and pH 1.5, and the nature of sorption was found to be chemisorption.

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