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Optimization of chromium biosorption in aqueous solution by marine yeast biomass of *Yarrowia lipolytica* using Doehlert experimental design

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Optimization of a chromium biosorption process was performed by varying three independent variables pH (0.5 to 3.5), initial chromium ion concentration (10 to 30 mg/L), and *Yarrowia lipolytica* dosage (2 to 4 g/L) using a Doehlert experimental design (DD) involving response surface methodology (RSM). For the maximum biosorption of chromium ion in an aqueous solution by *Y. lipolytica*, a total of fifteen experimental runs were set and the experimental data fitted to the empirical second-order polynomial model of a suitable degree. The analysis of variance of the quadratic model demonstrates that the model was highly significant. The model showed that chromium uptake in aqueous solution was affected by all the three variables studied. The optimum values of the variables were found to be 2.07, 18.76 mg/L and 3.39 g/L for pH, initial chromium ion concentration and biomass dosage, respectively at contact time of 30 min. At these optimal conditions, the maximum percentage biosorption of chromium was predicted to be 41.59. The experimental values were in good agreement with predicted values and the correlation coefficient was found to be 0.9891. Therefore, it is apparent that the DD involving RSM not only gives valuable information on interactions between the variables but also leads to identification of feasible optimum values of the studied variables.

Key words: Biosorption, Doehlert experimental design, response surface methodology, *Yarrowia lipolytica*.

INTRODUCTION

The presence of toxic heavy metals contaminated in aqueous streams, arising from the discharge of untreated metal containing effluent into water bodies, is one of the most important environmental issues (Hawari and Mulligan, 2006). Their presence in aquatic ecosystem causes harmful effect to living organisms (Xuejiang et al.,

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2006). In recent years, increased attention has focused on the use of microorganisms for the removal and possible recovery of metal ions from industrial wastes. In fact, it represents a potential alternative to existing technologies (chemical precipitation, reverse osmosis, solvent extraction), which have significant disadvantages such as high reagent or energy requirements and generation of toxic sludge or other products that need disposal (Bossrez et al., 1997). Biosorption, the passive binding of metals by living or dead biomass, can be used to remove toxic heavy metals from industrial wastewater.

Chromium and its compounds are ubiquitous and persistent environmental contaminants released into natural environment from a variety of anthropogenic sources, including the electroplating, leather tanning processes, chromite ore processing, wood preservation, alloys making, corrosion control, pigment and dyes, and metal finishing industries (Suksabye et al., 2008; Quintelas et al., 2009). Though the removal of Cr from wastewater is obligatory before discharging into aquatic environment, the rest in the effluent will still cause serious environmental impact. Therefore, the discharge of Cr into aquatic ecosystems has become a matter of concern over the last few decades (Massara et al., 2008; Suksabye et al., 2008; Ye et al., 2010). Strong exposure of Cr (VI) causes cancer in digestive tract and lungs and may cause epigastric pain, nausea, vomiting, severe diarrhea and hemorrhage (Mohanty et al., 2006). It is therefore very urgent to control chromium contamination. Considerable efforts have thus been devoted to developing available technologies which can remove chromium from the effluents of those industries.

In recent years, *Yarrowia lipolytica* has emerged as important non-conventional yeast with significant biological relevance and biotechnological applications (Barth and Gaillardin, 1997; Fickers et al., 2005). This yeast has been used in the remediation of various polluted environments (Margesin and Schinner, 1997; Zinjarde and Pant, 2002; Jain et al., 2004) and is also applied in the degradation of different wastes (Johnson et al., 1994; De Felice et al., 1997; Oswal et al., 2002; Lanciotti et al., 2005). *Y. lipolytica* is able to utilize a variety of renewable carbon sources and the biomass of the fungus has been used as single cell protein or as single cell oil (Achremowicz et al., 1977; Papanikolaou et al., 2002). However, there are few reports available regarding how this yeast can survive metal stress and accumulate different metals (García et al., 2002; Strouhal et al., 2003; Ito et al., 2007; Agnihotri et al., 2009; Bankar et al., 2009). This microorganism therefore, displays a potential for the bioremediation of metal polluted environments. Biosorption of chromium ions by different living and nonliving biomass have been studied by several authors (Aksu and Balibek, 2007; Yin et al., 2008; Aksu et al., 2009; Khambhaty et al., 2009; Ye et al., 2010).

Optimization of biosorption of heavy metals by the classical method involves changing one independent variable (that is *Y. lipolytica* dosages, pH, heavy metal concentration, temperature) while maintaining all others at a fixed level which is extremely time consuming and expensive for a large number of variables. These drawbacks of single parameter optimization process can be eliminated by optimizing all the affecting parameters collectively by Doehlert experimental design (DD) (Doehlert, 1970) of Response Surface Methodology (RSM). Basically, this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, predicting the response and checking the adequacy of the model. Several researchers have applied various designs for optimization of different process parameters (Preetha and Viruthagiri, 2007; Kumar et al., 2009; Kiran and Thanasekaran, 2011; Mona et al., 2011; Bermúdez et al., 2012). The objective of the present study was to optimize biosorption of chromium (VI) ions in aqueous solution onto *Y. lipolytica* in a batch experiment. For better understanding of different stages of biosorption at varying heavy metal concentration, pH and sorbent dosages, RSM was used to optimize heavy metal uptake.

MATERIALS AND METHODS

Microorganism and growth conditions

Yarrowia lipolytica NCIM 3589, obtained from National Chemical Laboratory, Pune, India, was used throughout the study. The culture was maintained on MGYP slants having the composition (%): malt extract 0.3, glucose 1.0, yeast extract 0.3, peptone 0.5 and agar agar 2.0. The pH of the medium was adjusted to 6.4 to 6.8 and culture was incubated at 30°C for 48 h. Subculturing was carried out once in two weeks and the culture was stored at 4°C.

Microbial cultivation

The composition of culture medium was (grams per liter): peptone: 5; yeast extract: 3 and NaCl: 3 (Imandi et al., 2008). The medium was sterilized by autoclaving at a pressure of 1.5 atm and temperature of 121°C for 20 min. The yeast cells were grown for 16 h (at end of exponential phase) and then filtered (Imandi et al., 2010).

Preparation of biosorbent

Yeast biomass was deactivated by heating in an oven at 80°C for 24 h (Schiewer and Volesky, 1995). The dried yeast was ground and screened through a sieve with 100 mesh. The pretreatment of the biosorbent was carried out with nonviable yeast cells in 700 g/L ethanol solution for 20 min at room temperature. Then, it was centrifuged at 3600 rpm for 10 min and the ethanol solution was discarded. The ethanol washed biomass was rinsed several times with deionized water to remove excess ethanol and adsorbed nutrient ions. The rinsed yeast was again centrifuged and the remaining biomass was dried at 70°C for 12 h (Gőksungur et al., 2005). The dried cells were ground and screened as mentioned above. The purpose of grinding dried yeast was to make a

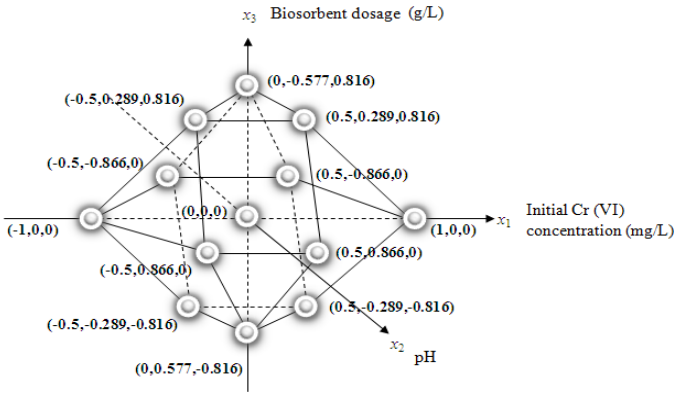

Figure 1. 3D view of the experimental domain. The dots represent the experimental runs of the Table 2.

homogenized yeast biomass in order to destroy biomass aggregates and increase uptake capacity (Bahadir et al., 2007).

Preparation of chromium solution

All the chemicals used were of analytical grade. Stock solution of 1000 mg/L was prepared by dissolving appropriate amount of $K_2Cr_2O_7$ in 1 L of deionized water. The pH of the metal solution was adjusted to a desired value with 0.1 N NaOH and 0.1 N HCL. Chromium solution of different concentrations was prepared by suitable dilution of the stock solution to known volumes.

Experimental procedure

2 g/L of biosorbent was added to 50 mL of the chromium ion solution in each of 250 mL Erlenmeyer flasks. The flasks were incubated in orbital shaker at a speed of 180 rpm for different agitation times (1, 2, 5, 10, 20, 30, 40, 50, 60, 90 and 120 min). The resulting samples were filtered by Whatman filter paper and analyzed for chromium concentration in flame atomic absorption spectrometer (novAA® 350, Analytikjena, Germany).

$$\text{The percent chromium biosorbed is calculated from the relation} = \frac{C_o - C_i}{C_o} \times 100 \quad (1)$$

Where, C_o is the initial concentration of chromium in the aqueous solution (mg/L); C_i is the final concentration of chromium in the aqueous solution (mg/ L).

Preliminary experimental runs for the biosorption of chromium using *Yarrowia lipolytica* has been carried out by assigning three experimental strategies, as listed below, varying one parameter and keeping the other two parameters constant for an equilibrium agitation time and temperature at 303 K.

Strategy 1: pH was varied as 1, 2, 3, 4, 6 and 7 keeping the biosorbent dosage and initial chromium ion concentration at constant values.

Strategy 2: Initial chromium ion concentration was varied as (mg/L) 20, 50, 100, 150 and 200, keeping the other parameters constant.

Strategy 3: Keeping pH and initial chromium ion concentration as constants, the biosorbent dosage was varied as (g/L) 1, 2, 3, 4 and 5.

Doehlert experimental design

Once the variables having the statistically significant influence on the responses were identified, a Doehlert experimental design (Doehlert, 1970) was used to optimize the values of these variables. The number of experiments required (N) is given by $N = n^2 + n + n_0$, where, n is the number of variables and n_0 is the number of center points. Replicates at the central level of the variables were performed in order to validate the model by means of an estimate of experimental variance. For statistical calculations, the natural variables X_i were coded as x_i according to Equation (2)

$$x_i = \left(\frac{X_i - X_{oi}}{\Delta X_i} \right) \alpha_i \quad (2)$$

Where, x_i is the coded value of the i^{th} variable, X_i the natural value, X_{oi} the value at the center point, ΔX_i the step change value, and α_i is the maximum value of the coded variable (that is 1.0, 0.866 and 0.816 for five levels, seven levels, and three levels, respectively).

The second degree polynomial (Equation 3) was fitted to the experimental data by using the statistical software STATISTICA 6.0 (Stat-Ease Inc., Tulsa, OK, USA) to estimate the response of the dependent variable and the regression coefficients.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (3)$$

Where, Y is the predicted response; X_1, X_2, X_3 are the independent variables; b_0 is the offset term; b_1, b_2, b_3 are the coefficients for linear effects, b_{11}, b_{22}, b_{33} are the coefficients for squared effects and b_{12}, b_{23}, b_{13} are the coefficients for interaction terms.

Doehlert design offers the following advantages over the other designs of RSM. Firstly, this design needs fewer experiments; secondly, the number of levels is not the same for all variables, which allows flexibility to assign a large or a small number of levels to the selected variables depending on their relative importance. Generally it is preferable to choose the variable with the stronger effect as the variable with seven levels in order to obtain maximum information of the system. Thirdly, considering that the efficiency of any experimental design is defined as the number of coefficients of the model divided by the number of experiments, Doehlert design is more efficient than Central Composite design or Box-Behnken design. Fourthly, Doehlert design is also more efficient in mapping the space: adjoining hexagons can fill a space completely and efficiently, since the hexagons fill space without overlap (Imandi et al., 2007).

Figure 1 shows the graphical representation of this network in the space defined by the factors. This uniform distribution of experimental points (shown as dots) allows interpolation by the mathematical model of responses anywhere within the experimental domain. Furthermore, the quality of the interpolation remains constant since the network is uniform. The Doehlert design shows great flexibility compared to other classical designs used in the process optimization.

RESULTS AND DISCUSSION

Effect of contact time

Time course profile for the biosorption of Cr (VI) for a solution of 20 mg/L is shown in Figure 2. The data showed that a contact time of 30 min was required to achieve an

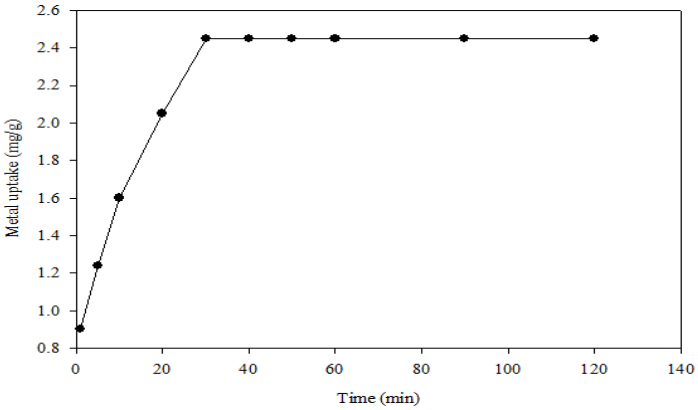

Figure 2. Effect of contact time on biosorption of chromium by *Y. lipolytica*.

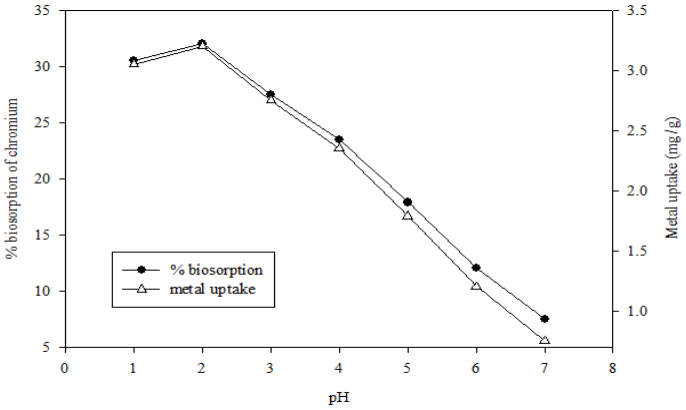

Figure 3. Effect of pH on chromium biosorption for 20 mg/L of metal and 2 g/L of biomass concentration.

optimum biosorption and there were no significant change in the removal of metal ions with further increase in contact time. Therefore, the uptake and unabsorbed chromium concentrations at the end of 30 min are given as the equilibrium values, q_{eq} (mg/g) and C_{eq} (mg/L). For further studies of biosorption with other variable parameters the optimum time of 30 min for Cr (VI) was chosen for contact period.

Effect of pH

Earlier studies on heavy metal biosorption has shown that pH of the solution is most important physical parameter influencing the adsorption process. Batch biosorption experiments are conducted at different pH (1.0 to 7.0) values and the results are depicted in Figure 3. The biosorption capacity for the *Y. lipolytica* was greater at pH 2.0 when compared to higher values of pH (Figure 3). All subsequent experiments were therefore carried out at pH 2.0. The results also suggest that active processes displayed by live cells may not be involved in biosorption of Cr (VI). Such observations on enhanced biosorption at acidic pH has been previously reported with other biosorbents such as bacteria (Zhou et al., 2007), fungi (Özer and Özer, 2003; Sen et al., 2005), milled peat (Dean and Tobin, 1999), cone biomass (Ucun et al., 2002), pods, leaves and bark of an ornamental plant (Abbas et al., 2008) and the husk of Bengal gram (Ahalya et al., 2005). The reason for the enhanced adsorption of Cr (VI) at low pH was that negatively charged $[HCrO_4]^-$, $[Cr_2O_7]^{2-}$, $[Cr_4O_{13}]^{2-}$ and $[Cr_3O_{10}]^{2-}$ ions are the dominant species under such conditions. The surfaces of yeast cell walls at low pH are surrounded by hydronium ions (H_3O^+). The negatively charged ion species are thus effectively adsorbed on the positively charged active sites on the sorbent (Özer and Özer, 2003). With an increase in pH, the binding of ions decreased on account of repulsive forces between the biosorbent (*Y. lipolytica*)and Cr (VI) ions. It is therefore proposed that in a manner similar to *Bacillus thuringiensis*, in *Y. lipolytica* also, the interactions may be primarily electrostatic or coordinative in nature (Şahin and Öztürk, 2005).

Effect of initial chromium ion concentration

The relationship between the percentage biosorption of Cr (VI) and its initial concentrations using different dosages of yeast biomass are presented in Figure 4. The initial metal ion concentration remarkably influenced the equilibrium metal uptake and biosorption yield. The amount of metal ion adsorbed increased with increase in initial concentrations (due to higher availability of metal ions for sorption) while the percentage biosorption of metal ion decreases with an increase in initial metal ion concentration. The increase of metal uptake is a result of the increase in the driving force, that is concentration gradient, with an increase in the initial metal ion concentrations (from 20 to 200 mg/L). However, the percenttage biosorption of Cr (VI) on yeast biomass decreased from 32 to 23%, respectively. Though an increase in metal uptake was observed, the decrease in percentage biosorption may be attributed to lack of sufficient surface area to accommodate much more metal available in the solution. At lower concentrations, all metal ions present in solution could interact with the binding sites and thus the percentage biosorption were higher than those at higher initial metal ion concentrations. At higher concentrations, lower biosor-ption yield is due to the saturation of biosorption sites.

Effect of biosorbent dosage

Studies on the effect of yeast biomass dosage for Cr (VI) removal is important to get the trade-off between the

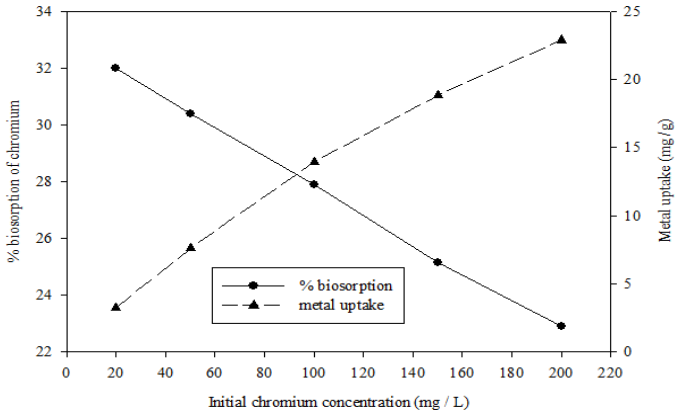

Figure 4. Effect of Cr (VI) ion concentrations on biosorption of chromium by *Y. lipolytica* at 2 g/L of biosorbent concentration (pH 2).

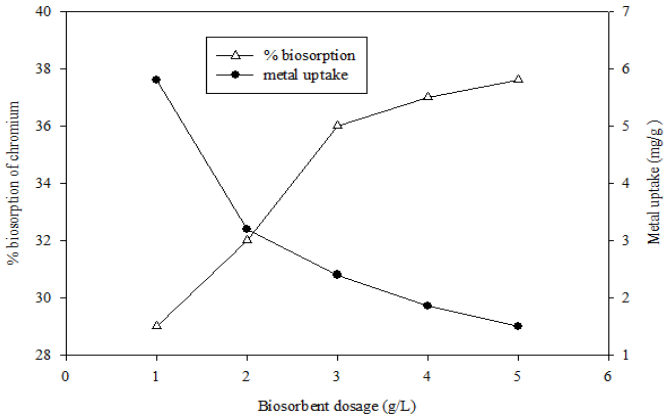

Figure 5. Effect of biosorbent dosage on biosorption of chromium for 20 mg/L of metal concentration (pH 2).

adsorbent capacity and percent removal of metal ions resulting in an optimum biosorbent concentration. The percent of biosorption and adsorption capacity for Cr (VI) as a function of biosorbent dosage was investigated (Figure 5). The removal of chromium was also dependent on the concentration of biomass used in the biosorption medium. The result shows that with the increase in the yeast biomass dosage, the metal removal efficiency also increased. The percentage biosorption of chromium increased 29 to 37%. Results show that the biosorption efficiency is highly dependent on the increase in biomass dosage of the solution. This is expected because higher dosage of adsorbent in the solution, increase the availability of exchangeable sites for the ions. It was earlier reported that the increase in the efficiency of metal ions removal with an increase in the biosorbent dosage was due to the increase in the number of adsorption sites (Vinod et al., 2010). The drop in adsorption capacity is basically due to the sites remaining unsaturated during the adsorption process.

Optimization of the process variables using DD

The selection of the range for process variables is extremely important when planning the experimental design; otherwise, after completion of the experimental runs, the optimal conditions, obtained either by response surface methodology, might not be found inside the experimental region. The following experiments were carried out to study the variables in such a range so that reasonable percentage removal of chromium would be achieved within that range. From the results of preliminary experimental runs, the three variables (initial chromium concentration, pH, and biosorbent dosage) have been identified as the potential variables for the percentage biosorption of chromium. Out of them, the pH had shown stronger effect on percentage biosorption of chromium and hence it was assigned seven level, followed by initial chromium concentration assigned five level and biosorbent dosage assigned three levels. A summary of the independent variables and their range and levels is presented in Table 1.

Fifteen (15) experimental runs (Table 2) including three replicates at the center point were carried out for 30 min of contact time. By using multiple regression analysis (STATISTICA 6.0) the coefficients of equation (4) was estimated, and gave the following equation.

$$Y = -61.7717 + 3.0454X_1 + 27.5904X_2 + 27.6128X_3 - 0.0846X_1^2 - 4.6417X_2^2 - 3.4006X_3^2 - 0.0137X_1X_2 - 2.7006X_2X_3 + 0.0468X_1X_3 \quad (4)$$

The predicted percentage biosorption of chromium resulted from equation (4) are in close agreement with the experimental values as evident from the last column of Table 2, and hence the above equation was deemed to be adequate in representing the percentage biosorption of chromium under the specified range of experiments. For quadratic models, the optimum point can be characterized as maximum, minimum, or saddle. It is possible to calculate the coordinates of the optimum point through the first derivate of the mathematical function, which describes the response surface and equates it to zero. The above quadratic equation obtained for three variables as described below:

$$\frac{\partial Y}{\partial X_1} = 3.0454 - 0.1692X_1 - 0.0137X_2 + 0.0468X_3 = 0 \quad (5)$$

$$\frac{\partial Y}{\partial X_2} = 27.5904 - 0.0137X_1 - 9.2834X_2 - 2.7006X_3 = 0 \quad (6)$$

$$\frac{\partial Y}{\partial X_3} = 27.6128 + 0.0468X_1 - 2.7006X_2 - 6.8012X_3 = 0 \quad (7)$$

Table 1. Experimental range and levels of the variables.

Variable	Range and level						
Coded variable, x_1	–1	–0.5	0	0.5	1		
Initial chromium concentration, X_1 (mg/L)	10	15	20	25	30		
Coded variable, x_2	–0.866	–0.577	–0.288	0	0.288	0.577	0.866
pH, X_2	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Coded variable, x_3	–0.816	0	0.816				
Biosorbent dosage, X_3 (g/L)	2.0	3.0	4.0				

Table 2. Doehlert three variable experimental design along with experimental and predicted values.

Experiment number	Coded value			Natural value			% biosorption of chromium	
	x_1	x_2	x_3	X_1	X_2	X_3	Experimental	Predicted
1	1	0	0	30	2.0	3.0	29.26	30.3487
2	–1	0	0	10	2.0	3.0	34.21	34.1212
3	0.5	0.866	0	25	3.5	3.0	28.42	27.8775
4	–0.5	–0.866	0	15	0.5	3.0	26.65	28.1925
5	0.5	–0.866	0	25	0.5	3.0	25.35	26.5112
6	–0.5	0.866	0	15	3.5	3.0	30.13	29.9687
7	0.5	0.288	0.816	25	2.5	4.0	33.48	32.9337
8	–0.5	–0.288	–0.816	15	1.5	2.0	31.26	29.8062
9	0.5	–0.288	–0.816	25	1.5	2.0	28.27	29.0200
10	0	0.577	–0.816	20	3.0	2.0	31.43	33.1337
11	–0.5	0.288	0.816	15	2.5	4.0	35.67	35.9200
12	0	–0.577	0.816	20	1.0	4.0	38.28	36.5762
13	0	0	0	20	2.0	3.0	40.15	40.1966
14	0	0	0	20	2.0	3.0	40.32	40.1966
15	0	0	0	20	2.0	3.0	40.12	40.1966

X_1, Initial chromium concentration (mg/L); X_2, pH, X_3, biosorbent dosage (g/L).

Thus, to calculate the coordinate of the optimum point, it is necessary to solve the first grade system formed by Equations. (5), (6) and (7) and to find the $X_{1,\,opt}$, $X_{2,\,opt}$ and dosage = 3.39 g/L. The extent of biosorption of chromium at these optimum conditions was 41.59%.

The significance of each coefficient in equation (4) was determined by student's *t*-test and *p*-values which were also listed in Table 3. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient (Lazić, 2004). This implies that the linear and quadratic effects of initial chromium concentration, pH and biosorbent dosage were highly $X_{3,\,opt}$ values. The optimal set of conditions for maximum percentage biosorption of chromium is pH = 2.07, initial chromium concentration = 18.76 mg/L, and biosorbent significant as is evident from their respective *p*-values. The interaction effect of pH and biosorbent dosage was found to be significant ($p \leq 0.05$). The remaining two interaction terms that is, initial chromium concentration × pH and biosorbent dosage × initial chromium concentration were found to be insignificant ($p > 0.05$) which were also presented in Table 3. The parity plot (Figure 6) showed a satisfactory correlation between the experimental and predicted values of percentage biosorption of chromium, wherein, the points cluster around the diagonal line which indicated the good fit of the model because the deviation between the experimental and predicted values is less.

The results of the second order response surface model fitting in the form of Analysis of Variance (ANOVA) are given in Table 4. It is required to test the significance and adequacy of the model. The Fisher variance ratio, the *F*-value ($= S^2_r / S^2_e$), is a statistically valid measure of how well the factors describe the variation in the data about its mean. The greater the *F*-value is from unity, the more certain it is that the factors explain adequately thevariation in the data about its mean, and the estimated factor effects are real. The ANOVA of the regression model demonstrates that the model is highly significant,

Table 3. Model coefficients estimated by multiple linear regression (significance of regression coefficients).

Term	Coefficient	Value	Standard error of coefficient	*t*-value	*p*-value
Constant	b_0	–61.7717	12.62456	–4.8930	0.004502*
Initial Cr (VI) concentration	b_1	3.0454	0.59717	5.0996	0.003771*
pH	b_2	27.5904	3.44779	8.0023	0.000492*
Biosorbent dosage	b_3	27.6128	5.33202	5.1787	0.003529*
Initial Cr (VI) concentration × initial Cr (VI) concentration	b_{11}	–0.0846	0.01144	–7.3944	0.000712*
pH × pH	b_{22}	–4.6417	0.38144	–12.1687	0.000066*
Biosorbent dosage × biosorbent dosage	b_{33}	–3.4006	0.72374	–4.6986	0.005344*
Initial Cr (VI) concentration × pH	b_{12}	–0.0137	0.08357	–0.1635	0.876502
pH × biosorbent dosage	b_{23}	–2.7006	0.76289	–3.5399	0.016564*
Biosorbent dosage × initial Cr (VI) concentration	b_{31}	0.0468	0.13214	0.3544	0.737472

*Significant at $p \leq 0.05$.

Table 4. ANOVA for the entire quadratic model.

Source of variation	Sum of square (SS)	Degree of freedom (d.f.)	Mean square (MS)	*F*-value	Probe>*F*
Model	353.8888	9	39.32098	25.02308	0.001226
Error	7.8569	5	1.57139		
Total	361.7457	14			

R = 0.98908063; R^2 = 0.97828049; Adjusted R^2 = 0.93918536. $P_{model} > F$ = 0.001226.

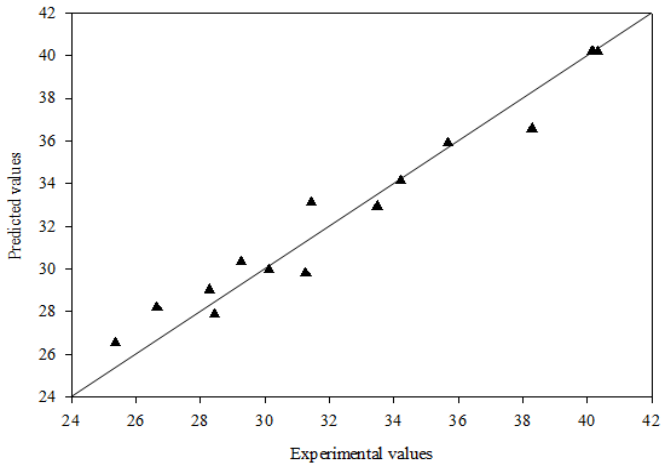

Figure 6. Parity plot showing the distribution of experimental vs. predicted values of percentage biosorption of chromium.

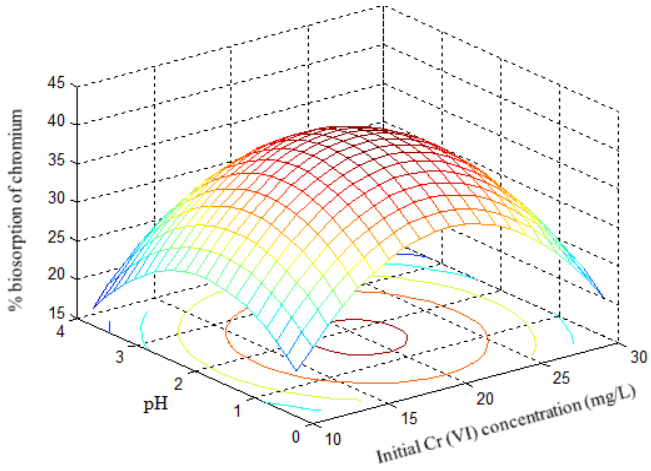

Figure 7. Response surface and contour plot of initial chromium concentration vs. pH on percentage biosorption of chromium (biosorbent dosage was kept constant at (3 g/ L)).

as is evident from the Fisher's *F*-test (F_{model} = 25.02308) and a very low probability value ($P_{model} > F$=0.001226). The goodness of fit of the model was checked by the determination coefficient (R^2). The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental variables and their interactions. The R^2 value is always between 0 and 1. The closer the R^2 value is to 1, the stronger the model is and the better it predicts the

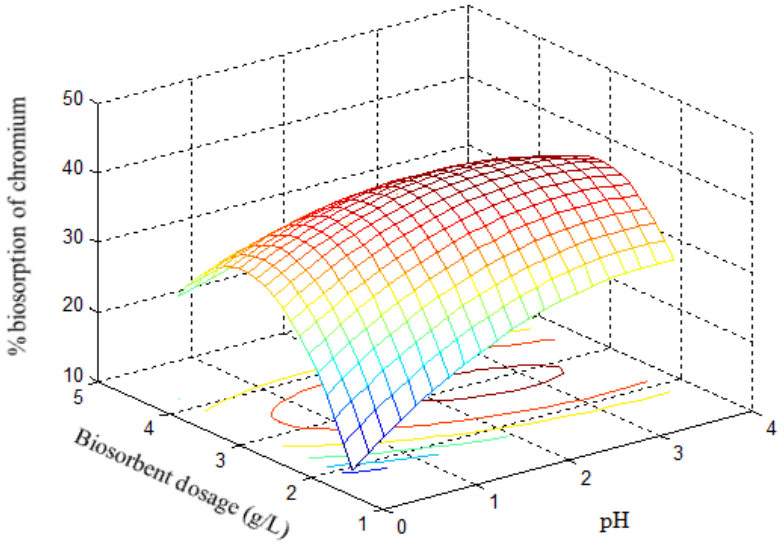

Figure 8. Response surface and contour plot of pH vs. biosorbent dosage on percentage biosorption of chromium (initial Cr (VI) concentration was kept constant at 20 mg/L).

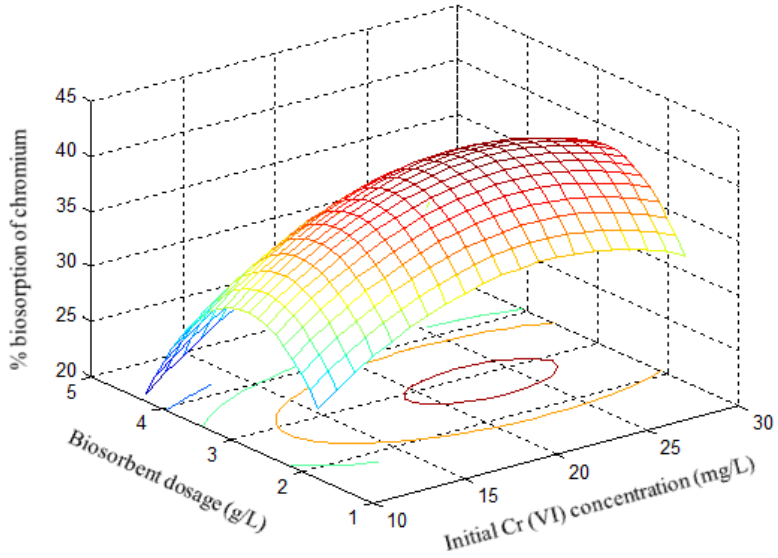

Figure 9. Response surface and contour plot of biosorbent dosage vs. initial chromium concentration on percentage biosorption of chromium (pH was kept constant at 2).

response. In this case, the value of the determination coefficient (R^2 = 0.9783) indicates that 97.83 % of the variability in the response could be explained by the model. In addition, the value of the adjusted determination coefficient (Adj R^2 = 0.9392) is also very high to advocate for a high significance of the model. Also a higher value of the correlation coefficient (R=0.9891) justifies an excellent correlation between the independent process variables. The response surface contour plots of percentage biosorption of chromium versus the interactive effect of pH, initial chromium concentration, and biosorbent dosage are shown in the Figures 7 to 9.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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