Parametric Optimization of Selenite and Selenate Biosorption Using Wheat Bran in Batch and Continuous Mode

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The present work has successfully optimized the biosorption of selenite and selenate species using wheat bran in both batch and continuous column mode with the help of central composite design (CCD). In batch mode the parameters which were taken into account were pH, biomass dose, initial selenium concentration, and temperature, whereas in continuous mode bed height, flow rate, and initial selenium concentration were studied. A second-order polynomial regression model was used, and results showed high regression coefficients (R^2, that is, 97.65 % for both Se(IV) and Se(VI) in batch mode and 97.95 % and 97.61 % for Se(IV) and Se(VI), respectively, in continuous mode), indicating good agreement with experimental data. Low values of standard deviations were also obtained, showing the adequacy of the proposed regression models in both batch and continuous mode. Contour plots predicted the maximum uptake of Se(IV) (49.56 $\mu g \cdot g^{-1}$) and Se(VI) (41.37 $\mu g \cdot g^{-1}$) at pH 2, initial selenium concentration of 1000 $\mu g \cdot L^{-1}$, temperature of 20 °C, and biomass dose of 20 $g \cdot L^{-1}$ in batch mode, whereas it was (73.05 and 58.29) $\mu g \cdot g^{-1}$ for Se(IV) and Se(VI) at a bed height of 25 cm, flow rate of 1.66 $mL \cdot min^{-1}$, and initial selenium concentration of 1000 $\mu g \cdot L^{-1}$ in continuous mode.

Introduction

The tremendous increase in the use of heavy metal ions over the last few decades has inevitably resulted in an increased flux of metallic substances into the aquatic environment. Acid mine drainage, industrial and domestic effluents, agricultural runoff, acid rain, and so forth have all contributed to the metal loads in water bodies.[1] The effects of metals in water and wastewater range from beneficial through troublesome to dangerously toxic. Selenium, which is an essential nutrient for the health of humans and animals, shows only a marginal line between the nutritious requirement and toxic effects upon exposure.[2] The primary source of selenium is the weathering of rocks that contain selenium. Excessive irrigation can lead to the dissolution of these rocks and speed up weathering. In addition, mining increases the potential for selenium toxicity through exposure of seleniferous rocks to air, which leads to the solubilization of the selenium. Selenium may also enter water through the disposal of fossil fuel wastes and agricultural irrigation of arid, seleniferous soils.[3] High concentrations of selenium in water systems lead to the poisoning of fish and wildlife and the threatening of public health.[2,4−7]

A variety of treatment technologies have been reported for selenium removal from contaminated waters.[8,9] However, most of the systems have technical and/or economical constraints. At this juncture advances in the knowledge of biosorption has gained important credibility over recent years because of its eco-friendly nature, excellent performance, and low cost technique for remediating even heavily metal-loaded water.[10]

The optimization of a biosorption process can be done by using the conventional "one-variable-at-a-time" technique in which the effect of one independent variable is seen on the response, while other factors are kept constant. This method assumes that the various treatment parameters do not interact and that the response variable is only a function of the single varied parameter. However, the response obtained from a waste treatment method, for example, results from the interactive influences of the different variables. Thus, the conventional technique does not depict the complete effects of the parameter on the response.[11] Another disadvantage of the one-factor optimization is the increase in the number of experiments necessary to conduct the research, which leads to an increase of time and expense as well as an increase in the consumption of reagents and materials.[12−14] These limitations of the traditional method can be eliminated by optimizing all parameters which affect the process collectively by using a multivariate statistic technique, which has many advantages such as a reduction in the number of experiments that need to be executed resulting in lower reagent consumption and considerably less laboratory work. Moreover, this method allows the development of mathematical models that permit the assessment of the relevance as well as statistical significance of the factor effects being studied and evaluation of the interaction effects between the factors. Among the available multivariate techniques, the most relevant is the response surface methodology (RSM). RSM is useful in process design and optimization as well as for the improvement of existing design.[15] The application of RSM to the sorption process involves a factorial search by examining simultaneous, systematic, and efficient variation of important components. It helps to predict a model for the process, identify possible interactions or higher-order effects, and determine the optimum operational conditions for the maximum removal of the pollutant species from water or wastewater.[14]

As far as the authors are aware of the current research on the biosorption of selenium, very few attempts[16−19] have been made in this direction, and thus, there still lies a strong challenge in

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developing low cost biosorbents for selenium removal with high uptake efficiency. The present study shows the successful utilization of low cost agro-industrial waste wheat bran for the sorption of selenium. Moreover, in all earlier attempts the conventional one-variable at-a-time technique has been used for the optimization of the biosorption process, whereas in the present study the process has been optimized with the help of a multivariate technique in both batch and continuous mode. In this connection full factorial central composite design (CCD) of RSM was used to optimize the variables for the maximum biosorption of selenite (Se(IV)) and selenate (Se(VI)). In addition to this the interactive effects of these variables were also investigated, in batch as well as continuous treatment mode. For the interpretation of the data regression analysis, graphical analysis and analysis of variance (ANOVA) were performed with the help of a statistical and graphical software package, Minitab Release 15.

Materials and Methods

Wheat bran was collected from a local wheat mill and was used in experiments with double washing with double-distilled water to remove soluble lighter materials. The untreated wheat bran was dried in an oven at 60 °C over a period of 24 h with crushing and sieving to < 178 μm. The physicochemical characterization of the biosorbent has been reported in ref 20, and the Fourier transform infrared (FTIR) analysis of the sorbent before and after sorption has also been conducted, which is reported in ref 21.

All of the chemicals used in the study were of analytical grade and used without further purification. Standards for calibration of AAS were prepared from selenium atomic absorption standard solution (1000 $\text{mg}\cdot\text{L}^{-1}$) purchased from Sigma Aldrich. Se(IV) and Se(VI) stock solutions (1000 $\text{mg}\cdot\text{L}^{-1}$) for experiments were prepared by dissolving 0.5531 g of Na_2SeO_3 and 0.6104 g of Na_2SeO_4 (Loba Chemie) in deionized double-distilled water to make a solution volume of 250 mL. For the experiments, the stock solutions were further diluted by adding distilled water as desired.

Batch Sorption Experiments. Batch experiments were carried out in triplicate in Erlenmeyer flasks by adding the desired amount of wheat bran in 50 mL of selenium solutions (Se(IV) or Se(VI)) of the desired initial metal concentration and pH at the desired temperature. The initial pH of the solution was adjusted by adding 0.1 M HCl and 0.1 M NaOH solution as required. The flasks were gently agitated in an electrically thermostatted reciprocating shaker at 200 rpm for a period of 120 min. The content of each flask was separated from biosorbent by centrifuging at 15000 rpm and was analyzed for remaining selenium concentration in the sample with the help of an atomic absorption spectrophotometer (Shimadzu AA-6300). The amount of selenium sorbed per unit mass of the biosorbent ($\mu\text{g}\cdot\text{g}^{-1}$) was evaluated by using the following equation:

$$q_t = \frac{(C_i - C_t)V}{W} \qquad (1)$$

where C_i and C_t are the selenium concentrations in $\mu\text{g}\cdot\text{L}^{-1}$ initially and at a given time t, respectively; V is the volume of the selenium solutions in mL; W is the weight of biosorbent in mg.

Continuous Up-Flow Fixed-Bed Column Sorption Experiments. Continuous flow sorption experiments were conducted in triplicate in a borosilicate glass column with an internal diameter of 2 cm and length of 30 cm. A known quantity of the biosorbent was packed in the column to yield the desired bed height of the sorbent. This bed of the sorbent was supported between two layers of 1 cm glass wool to prevent the sorbent from floating. The selenium solution having the desired initial concentration was then pumped through the column at desired flow rates with the help of a peristaltic pump (Miclins PP-10) in an up-flow mode at room temperature (± 30 °C). Samples were collected from the outlet of the column at a time interval of 10 min until the exhaustion of the column and were analyzed for the remaining selenium concentration. The operation of the column was stopped when the effluent selenium concentration exceeded a value of 99 % of the initial selenium concentration.

Central Composite Design (CCD). CCDs are optimized designs for fitting quadratic models.[22,23] It includes equal predictability in all directions from the center.[24] The number of experimental points in the CCD is sufficient to test the statistical validity of the fitted model and lack-of-fit of the model.[25]

The total number of experimental runs (N) in CCD, with n number of variables, can be represented by the following equation:[26]

$$N = 2^n + 2n + n_c \qquad (2)$$

The α-values for the axial points depend on the number of variables and can be calculated by $\alpha = [2^n]^{1/4}$. For three and four variables, they are, respectively, 1.68 and 2.00. In full factorial CCD all variables are studied at five levels (β, -1, 0, $+1$, $+\beta$).[27]

Data from CCD 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.[28]

$$y = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j + \varepsilon \qquad (3)$$

where y = 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.

Regression Analysis. Regression analysis is performed to fit the response function to the experimental data. The significance of the regression coefficients of each parameter is determined by the standard error of coefficient (SE coefficient), Student's t test and probability P-values. The standard error of coefficient is a measure of the variation in estimating the coefficient; T is the ratio of the coefficient to the standard error, and the P-value is defined as the smallest level of significance leading to the rejection of null hypothesis and is used to determine which of the variables in the model are statistically significant. Those effects are significant which are at the 5 % of probability level ($P < 0.05$). In general, the larger the magnitude of T and smaller the value of P, the more significant is the corresponding coefficient term.[29] Moreover, a positive sign of the regression coefficient represents a synergistic effect, while a negative sign indicates an antagonistic effect of the factor on the selected response.

Analysis of Variance (ANOVA). ANOVA subdivides the total variation in a set of data into component parts associated with specific sources of variation for determining the adequacy and significance of the quadratic model.[30] ANOVA is performed

Table 1. CCD Matrix of Four Variables along with Experimental and Predicted Response (Uptake, $\mu g \cdot g^{-1}$) for Se(IV) Sorption onto Wheat Bran in Batch Mode

run order	pH	conc. $\mu g \cdot L^{-1}$	temp. °C	b. dose mg	exp. response $\mu g \cdot g^{-1}$	pred. response $\mu g \cdot g^{-1}$	residual
1	4	800	35	1300	18.45	17.46	0.99
2	8	400	25	1100	9.60	8.99	0.60
3	4	800	25	1300	24.22	23.11	1.11
4	4	400	25	1100	14.31	15.77	−1.46
5	8	800	25	1300	12.50	11.40	1.10
6	8	800	35	1300	6.94	7.06	−0.12
7	8	800	35	1100	12.24	10.90	1.33
8	8	800	25	1100	16.85	16.82	0.03
9	8	400	35	1300	1.23	−0.81	2.04
10	4	400	35	1100	9.27	8.78	0.49
11	4	800	25	1100	29.87	30.31	−0.45
12	8	400	25	1300	1.15	3.29	−2.14
13	4	800	35	1100	23.64	23.08	0.56
14	8	400	35	1100	0.62	3.31	−2.69
15	4	400	25	1300	8.55	8.29	0.25
16	4	400	35	1300	1.27	2.88	−1.61
17	10	600	30	1200	3.12	3.18	−0.07
18	2	600	30	1200	20.43	20.36	0.06
19	6	1000	30	1200	18.54	20.81	−2.27
20	6	200	30	1200	0.67	−1.59	2.26
21	6	600	40	1200	5.15	5.63	−0.48
22	6	600	20	1200	17.45	16.96	0.48
23	6	600	30	1400	8.52	9.32	−0.80
24	6	600	30	1000	21.45	20.64	0.80
25	6	600	30	1200	13.50	13.52	−0.02
26	6	600	30	1200	13.23	13.52	−0.29
27	6	600	30	1200	13.96	13.52	0.43
28	6	600	30	1200	13.53	13.52	0.01
29	6	600	30	1200	12.98	13.52	−0.55
30	6	600	30	1200	14.22	13.52	0.70
31	6	600	30	1200	13.26	13.52	−0.27

Table 2. CCD Matrix of Four Variables along with Experimental and Predicted Response (Uptake, $\mu g \cdot g^{-1}$) for Se(VI) Sorption onto Wheat Bran in Batch Mode

run order	pH	conc. $\mu g \cdot L^{-1}$	temp. °C	b. dose mg	exp. response $\mu g \cdot g^{-1}$	pred. response $\mu g \cdot g^{-1}$	residual
1	4	800	35	1300	10.24	10.13	0.11
2	8	400	25	1100	1.94	2.74	−0.81
3	4	800	25	1300	16.48	15.06	1.42
4	4	400	25	1100	5.64	5.87	−0.23
5	8	800	25	1300	3.37	3.14	0.22
6	8	800	35	1300	0.94	0.16	0.78
7	8	800	35	1100	2.31	2.90	−0.60
8	8	800	25	1100	7.31	7.14	0.16
9	8	400	35	1300	0.83	0.52	0.31
10	4	400	35	1100	1.24	0.96	0.27
11	4	800	25	1100	19.32	20.35	−1.01
12	8	400	25	1300	0.05	1.03	−0.98
13	4	800	35	1100	15.67	14.12	1.53
14	8	400	35	1100	0.09	0.96	−0.87
15	4	400	25	1300	2.81	2.91	−0.09
16	4	400	35	1300	0.83	0.45	0.38
17	10	600	30	1200	0.98	0.17	0.81
18	2	600	30	1200	12.61	13.27	−0.66
19	6	1000	30	1200	11.27	12.65	−1.38
20	6	200	30	1200	0.08	−1.45	1.53
21	6	600	40	1200	0.94	1.38	−0.44
22	6	600	20	1200	8.67	8.08	0.59
23	6	600	30	1400	1.37	2.51	−1.14
24	6	600	30	1000	9.51	8.22	1.29
25	6	600	30	1200	6.22	6.71	−0.49
26	6	600	30	1200	6.51	6.71	−0.20
27	6	600	30	1200	6.83	6.71	0.12
28	6	600	30	1200	6.54	6.71	−0.17
29	6	600	30	1200	7.10	6.71	0.39
30	6	600	30	1200	6.88	6.71	0.17
31	6	600	30	1200	6.88	6.71	0.17

to test the statistical significance of the ratio of mean square due to regression and mean square due residual error, that is, the F value. The large Fisher's "F" value indicates that most of the variation in the response can be explained by the regression model equation. The associated P-value is used to judge whether F is large enough to indicate statistical significance. If the P-value for a larger F is lower than 0.05 (i.e., $\alpha = 0.05$, or 95 % confidence), then it indicates that the model is statistically significant.[31]

Response Surface Contour Plots. A contour plot is the projection of the response surface as a two-dimensional plane.[32] The values for two variables (x- and y- factors) are represented on the x- and y-axes (predictors), while the values for the z-factor (response) are represented by lines, called contours. Contour lines connect points with the same response value. For three or fewer responses, contour plots can be useful in identifying optimum settings. Individual contour plots can be used to identify best settings for each response.

Results and Discussion

Application of RSM in Batch Mode of the Sorption of Selenium. To study the effect of four variables (initial pH, initial selenium concentration, biosorbent dose, and temperature) which affect the biosorption of selenium, a two-level four-factor (2^4) full factorial CCD, leading to a total of 31 experimental runs, was applied. The different levels of the variables were pH at 2, 4, 6, 8, and 10; biosorbent dose (mg/50 mL) at 1000, 1100, 1200, 1300, and 1400; initial selenium concentration ($\mu g \cdot L^{-1}$) at 200, 400, 600, 800, and 1000; and temperature (°C) at 20, 25, 30, 35, and 40. Experiments were performed according to a CCD experimental plan, and the results thus obtained for each combination are given in Tables 1 and 2 for the sorption of Se(IV) and Se(VI), respectively, in batch mode. The results were also predicted with the help of CCD using the Minitab software and are given in the same tables.

Interpretation of the Regression Analysis. The response surface regression results obtained, namely, the SE coefficient, the T-value, and the P-value, along with the constant and coefficients (estimated using coded values) are given in Table 3 for the biosorption of both Se(IV) and Se(VI). The results indicated that the values of constants were 13.53 ± 0.02 with $T = 22.542$ and $P < 0.05$ for Se(IV) and 6.71 ± 0.02 with $T = 16.159$ and $P < 0.05$ for Se(VI), which implied that the average uptake of Se(IV) and Se(VI) on wheat bran was approximately (13.53 and 6.71) $\mu g \cdot g^{-1}$, respectively, and did not depend on the factors set in the experiment. It was also found that in both the cases of the selenium valences the uptake was significantly ($P < 0.05$) affected by the antagonistic effect of linear terms of pH, which meant the uptake of selenium decreased with an increase in the pH of the system. The linear term of concentration was also found to be significant in both cases and was found to positively affect the uptake of both selenium species, indicating that the uptake increased with the increase in initial selenium concentration. The linear terms of temperature and biomass dose were also found to effect the uptake of both Se(IV) and Se(VI) significantly ($P < 0.05$), and antagonistically, that meant a decrease in the uptake of both the selenium species with an increase in temperature and biomass dose. It was also observed from these results that the quadratic term of concentration was significant with $P = 0.005$ in the case of Se(IV); however, the higher values of T, that is, 17.288, and the parameter coefficients, that is, 11.2041, for the linear term of concentration showed that the linear term was more significant. The quadratic term of temperature was also found significant with $P = 0.029$ in the case of Se(VI); however, the greater T

Table 3. Estimated Regression Coefficients (Using Coded Units) for Experimental Response (Uptake, $\mu g \cdot g^{-1}$) for Selenium Sorption in Batch Mode

term	coef[a]	SE coef[b]	T[c]	P[d]
		Se(IV)		
constant	13.5257	0.6000	22.542	0.000
pH	−8.5891	0.6481	−13.253	0.000
conc. ($\mu g \cdot L^{-1}$)	11.2041	0.6481	17.288	0.000
temp. (°C)	−5.6658	0.6481	−8.742	0.000
b. dose (mg)	−5.6626	0.6481	−8.737	0.000
pH·pH	−1.7486	1.1875	−1.473	0.160
conc. ($\mu g \cdot L^{-1}$)·conc. ($\mu g \cdot L^{-1}$)	−3.9186	1.1875	−3.300	0.005
temp. (°C)·temp. (°C)	−2.2236	1.1875	−1.873	0.080
b. dose (mg)·b. dose (mg)	1.4614	1.1875	1.231	0.236
pH·conc. ($\mu g \cdot L^{-1}$)	−6.7128	1.5875	−4.228	0.001
pH·temp. (°C)	1.3127	1.5875	0.827	0.420
pH·b. dose (mg)	1.7773	1.5875	1.120	0.279
conc. ($\mu g \cdot L^{-1}$)·temp. (°C)	−0.2377	1.5875	−0.150	0.883
conc. ($\mu g \cdot L^{-1}$)·b. dose (mg)	0.2777	1.5875	0.175	0.863
temp. (°C)·b. dose (mg)	1.5823	1.5875	0.997	0.334
		Se(VI)		
constant	6.7086	0.4152	16.159	0.000
pH	−6.5521	0.4484	−14.611	0.000
conc. ($\mu g \cdot L^{-1}$)	7.0479	0.4484	15.717	0.000
temp. (°C)	−3.3514	0.4484	−7.474	0.000
b. dose (mg)	−2.8541	0.4484	−6.365	0.000
pH·pH	0.0137	0.8216	0.017	0.987
conc. ($\mu g \cdot L^{-1}$)·conc. ($\mu g \cdot L^{-1}$)	−1.1063	0.8216	−1.346	0.197
temp. (°C)·temp. (°C)	−1.9773	0.8216	−2.407	0.029
b. dose (mg)·b. dose (mg)	−1.3433	0.8216	−1.635	0.122
pH·conc. ($\mu g \cdot L^{-1}$)	−10.0443	1.0984	−9.144	0.000
pH·temp. (°C)	1.9447	1.0984	1.771	0.096
pH·b. dose (mg)	1.2628	1.0984	1.150	0.267
conc. ($\mu g \cdot L^{-1}$)·temp. (°C)	−2.4673	1.0984	−2.246	0.039
conc. ($\mu g \cdot L^{-1}$)·b. dose (mg)	−2.2953	1.0984	−2.090	0.053
temp. (°C)·b. dose (mg)	1.2558	1.0984	1.143	0.270

[a] Coefficient. [b] Standard error of the coefficient. [c] *T* test. [d] *P* value.

Table 4. ANOVA for the Response Surface Quadratic Model for Selenium Sorption in Batch Mode

source	DF[a]	seq SS[b]	adj SS[c]	adj MS[d]	F[e]	P[f]
			Se(IV)			
regression	14	1677.72	1677.72	119.837	47.55	0.000
linear	4	1580.82	1580.82	395.204	156.81	0.000
square	4	44.32	44.32	11.080	4.40	0.014
interaction	6	52.58	52.58	8.763	3.48	0.021
residual error	16	40.32	40.32	2.520		
total	30	1718.04				
			Se(VI)			
regression	14	801.815	801.815	57.273	47.47	0.000
linear	4	671.884	671.884	167.971	139.22	0.000
square	4	10.735	10.735	2.684	2.22	0.112
interaction	6	119.196	119.196	19.866	16.47	0.000
residual error	16	19.304	19.304	1.206		
total	30	821.119				

[a] Degree of freedom. [b] Sequential sum of squares. [c] Adjusted sum of squares. [d] Adjusted mean of squares. [e] *F* test. [f] *P*-value.

value, that is, −7.474, and the parameter coefficient, that is, −3.3515, for the linear term of temperature means that the linear terms were more significant. All of the interaction terms except that of pH and concentration ($P < 0.05$) for both Se(IV) and Se(VI) and concentration and temperature ($P = 0.039$) in the case of Se(VI) were found insignificant. The significance of these interaction effects between the variables were absent when the experiments were carried out by the conventional batch mode method where the effect of one variable was studied at a time.

On the basis of the results of regression analysis, the second order quadratic models were proposed to represent the empirical relationship between Se(IV) and Se(VI) uptake on wheat bran and the four test variables and are given by:

$$\text{Se(IV) uptake } (y) = 13.5257 - 8.5891x_1 + 11.2041x_2 - 5.6658x_3 - 5.6626x_4 - 1.7486x_1^2 - 3.9186x_2^2 - 2.2236x_3^2 + 1.4614x_4^2 - 6.7128x_1x_2 + 1.3127\text{x}_1x_3 + 1.7773x_1x_4 - 0.2377x_2x_3 + 0.2777x_2x_4 + 1.5823x_3x_4 \quad (4)$$

$$\text{Se(VI) uptake } (y) = 6.7086 - 6.5521x_1 + 7.0479x_2 - 3.3514x_3 - 2.8541x_4 + 0.0137x_1^2 - 1.1063x_2^2 - 1.9773x_3^2 - 1.3433x_4^2 - 10.0443x_1x_2 + 1.9447\text{x}_1x_3 + 1.2628x_1x_4 - 2.4673x_2x_3 - 2.2953x_2x_4 + 1.2558x_3x_4 \quad (5)$$

where y is selenium uptake (response) in $\mu g \cdot g^{-1}$, x_1, x_2, x_3, and x_4 are the coded values of the test variables, initial solution pH (x_1), initial selenium concentration (x_2) in $\mu g \cdot L^{-1}$, temperature (x_3) in °C, and biomass dose (x_4) in mg/50 mL. However, these regression equations (eqs 4 and 5) are applicable in the studied ranges of each parameter.

The goodness of the fit of the model was checked by the multiple coefficients of determination (R^2). The value of the multiple coefficients of determination was 97.65 % in case of both Se(IV) and Se(VI), which revealed that this regression was statistically significant, and only 2.35 % of the total variations was not explained by the model for both Se(IV) and Se(VI). At the same time, relatively lower values of the standard deviations, that is, 1.59 and 1.10, for Se(IV) and Se(VI), respectively, between the measured and the predicted results showed that the equation adequately represented the actual relationship between the response and significant variables. The predicted values of the response are shown in Tables 1 and 2 for Se(IV) and Se(VI), respectively, and it was found that predicted response, that is, uptake, was very close to the experimental results.

Interpretation of ANOVA. The results of ANOVA are presented in Table 4 for biosorption of both Se(IV) and Se(VI) onto wheat bran. It was found from the results that the *P*-values for all of the regressions in all cases of sorption, except that of the quadratic term of Se(VI), were lower than 0.05. The significance of these terms in the regression model indicated that at least one of the terms from the linear, quadratic or the interaction terms in the regression equation had a significant correlation with the response variable as has been shown in the interpretation of regression analysis. It was also observed from these tables that the *F*-values of 47.55 and 47.47 in the case of Se(IV) and Se(VI), respectively, were greater than the tabulated $F_{14,16}$ value, which is 2.38. This indicated that the second-order polynomial models presented in eqs 4 and 5 for the sorption of Se(IV) and Se(VI) were highly significant ($P < 0.05$) and were adequate to represent the actual relationship between the response, that is, the selenium uptake and the process variables. The associated Prob > *F*-value for the models was lower than 0.05 (i.e., $\alpha = 0.05$, or 95 % confidence) which indicated that the models were statistically significant. The ANOVA table also showed a term for residual error, which actually measured the amount of variation in the response data left unexplained by the model that has been discussed in the interpretation of regression analysis.

Interpretation of Response Surface Contour Plots. To investigate the interactive effect of two factors on the uptake Se(IV) and Se(VI) by wheat bran, and to gain insight about the effect of each variable, contour (2D) plots for the predicted

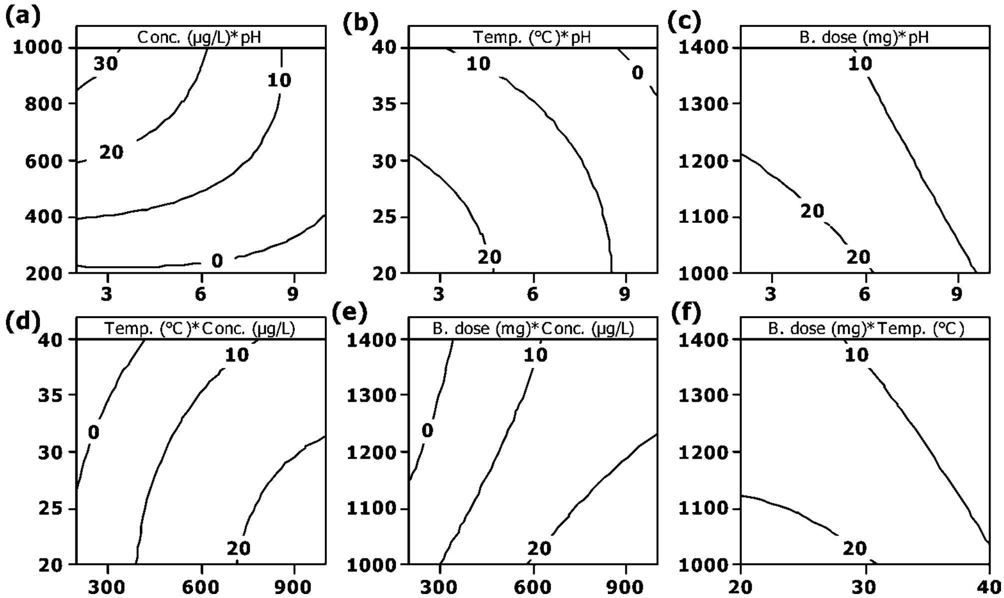

Figure 1. Response surface contour plot for the sorption of Se(IV) by wheat bran in batch mode (held values: pH = 6, conc. = 600 μg·L^{-1}, temp. = 30 °C, b. dose = 1200 mg).

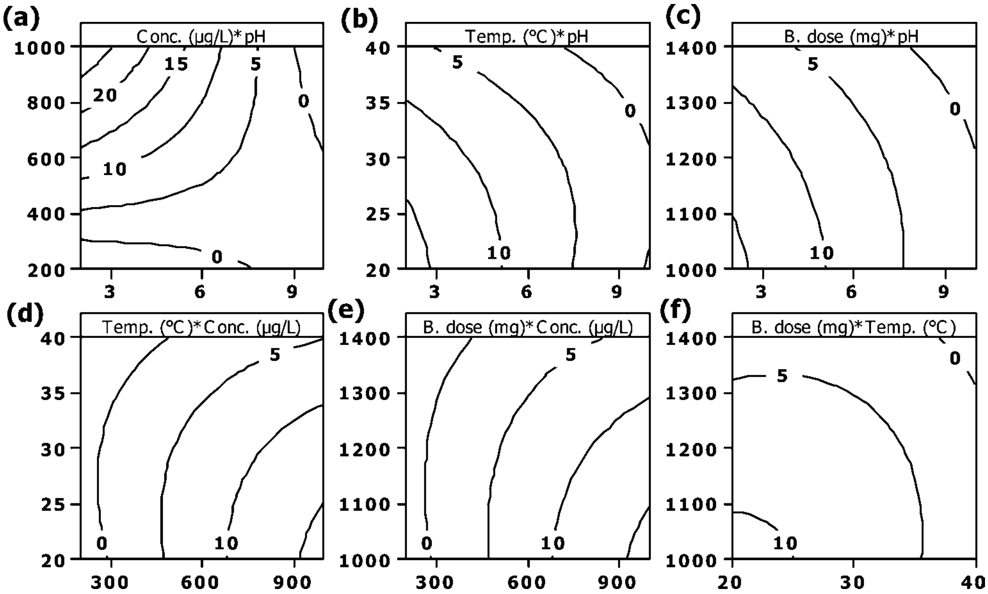

Figure 2. Response surface contour plot for the sorption of Se(VI) by wheat bran in batch mode (held values: pH = 6, conc. = 600 μg·L^{-1}, temp. = 30 °C, b. dose = 1200 mg).

responses were drawn, based on the model polynomial function to analyze the change in the response surface. Each plot is drawn between two independent variables and the response; the value of other variables were held and set at their middle values, and these were pH at 6, initial selenium concentration at 600 μg·L^{-1}, temperature at 30 °C, and biomass dose at 1200 mg in all sorption systems.

The contour plots of various combination of variables for Se(IV) and Se(VI) are presented in Figures 1 and 2, respectively. Figures 1a and 2a which represent the combined effect of pH of the system and initial selenium concentration on the uptake of Se(IV) and Se(VI), respectively, show that the uptake increased with the initial selenium concentration and decreased with the increase in the pH of the system. The maximum uptake was found at pH 2 and 1000 μg·L^{-1} for both selenite and selenate. The interactive effect of temperature and pH of the system is shown in Figures 1b and 2b for Se(IV) and Se(VI), respectively, and show that the uptake in both cases decreased with the increase in the temperature from (20 to 40) °C, whereas it decreased with the increase in pH in the case of both selenium species Se(IV) and Se(VI). Figures 1c and 2c represent the combined effect of biomass dose and pH for Se(IV) and Se(VI), respectively. In this case the uptake was found to decrease with an increase in the biomass dose from (1000 to 1400) mg/50 mL in all cases of the sorption system. The combined effect of temperature and concentration on the uptake of Se(IV) and Se(VI) was investigated and has been presented in Figures 1d and 2d, respectively, which showed that the uptake decreased with the increase of temperature and increased with an increase in initial selenium concentration in all of the cases. Figures 1e and 2e showed the uptake of Se(IV) and Se(VI), respectively, versus biomass dose and initial selenium concentration. The uptake in all cases showed a decreasing trend with biomass dose, while an increasing trend was observed with the initial selenium concentration. Figure 1f and 2f represent the uptake of Se(IV) and Se(VI), respectively, versus the biomass dose and temper-

Table 5. CCD Matrix of Four Variables along with Experimental and Predicted Response (Uptake, μg·g^{-1}) for Se(IV) Sorption onto Wheat Bran in Continuous Mode

run order	bed height cm	flow rate mL·min^{-1}	conc. μg·L^{-1}	exp. response μg·g^{-1}	pred. response μg·g^{-1}	residual
1	9.05	3.01	282.5	4.42	4.65	−0.23
2	20.95	3.01	282.5	12.75	15.62	−2.87
3	9.05	6.98	282.5	2.08	1.94	0.14
4	20.95	6.98	282.5	2.98	4.65	−1.67
5	9.05	3.01	817.5	22.52	23.56	−1.04
6	20.95	3.01	817.5	37.75	40.46	−2.71
7	9.05	6.98	817.5	5.65	5.35	0.29
8	20.95	6.98	817.5	11.51	13.84	−2.33
9	5.00	4.99	550.0	4.58	5.32	−0.74
10	25.00	4.99	550.0	26.06	21.69	4.37
11	15.00	1.66	550.0	28.86	26.03	2.83
12	15.00	8.33	550.0	2.20	1.39	0.81
13	15.00	4.99	100.0	3.02	1.59	1.43
14	15.00	4.99	1000.0	27.59	25.39	2.20
15	15.00	4.99	550.0	10.01	10.56	−0.55
16	15.00	4.99	550.0	10.56	10.56	0.00
17	15.00	4.99	550.0	11.01	10.56	0.45
18	15.00	4.99	550.0	9.74	10.56	−0.82
19	15.00	4.99	550.0	10.23	10.56	−0.33
20	15.00	4.99	550.0	11.15	10.56	0.59

Table 6. CCD Matrix of Four Variables along with Experimental and Predicted Response (Uptake, μg·g^{-1}) for Se(VI) Sorption onto Wheat Bran in Continuous Mode

run order	bed height cm	flow rate mL·min^{-1}	conc. μg·L^{-1}	exp. response μg·g^{-1}	pred. response μg·g^{-1}	residual
1	9.05	3.01	282.5	1.21	−1.02	2.23
2	20.95	3.01	282.5	7.85	10.01	−2.16
3	9.05	6.98	282.5	2.09	2.05	0.04
4	20.95	6.98	282.5	4.87	3.82	1.05
5	9.05	3.01	817.5	16.36	17.20	−0.84
6	20.95	3.01	817.5	32.73	32.57	0.16
7	9.05	6.98	817.5	6.65	4.29	2.36
8	20.95	6.98	817.5	8.37	10.39	−2.02
9	5.00	4.99	550.0	0.33	2.48	−2.15
10	25.00	4.99	550.0	18.75	16.89	1.86
11	15.00	1.66	550.0	17.33	16.87	0.46
12	15.00	8.33	550.0	0.07	0.82	−0.75
13	15.00	4.99	100.0	0.02	0.61	−0.59
14	15.00	4.99	1000.0	21.81	21.51	0.30
15	15.00	4.99	550.0	8.76	8.92	−0.16
16	15.00	4.99	550.0	9.51	8.92	0.58
17	15.00	4.99	550.0	9.01	8.92	0.08
18	15.00	4.99	550.0	8.27	8.92	−0.65
19	15.00	4.99	550.0	9.19	8.92	0.26
20	15.00	4.99	550.0	8.86	8.92	−0.06

ature and show a decreasing trend with both biomass dose and temperature in all cases of sorption. Thus, with the help of contour plots, the maximum uptake of Se(IV) and Se(VI) was predicted and was approximately (49.56 and 41.37) μg·g^{-1}, respectively, at pH 2, initial selenium concentration of 1000 μg·L^{-1}, temperature 20 °C, and biomass dose 1000 mg/50 mL.

Application of RSM in Continuous Mode of Sorption of Selenium. In the continuous mode of sorption of selenium, three parameters which were taken into account were bed height, flow rate, and initial selenium concentration, and a two-level three-factor (2^3) full factorial CCD, leading to a total of 20 experimental runs, was applied. The levels of the variables were bed height (cm) at 5, 9.05, 15, 20.95, and 25; flow rate (mL·min^{-1}) at 1.66, 3.01, 4.99, 6.98, and 8.33; and initial selenium concentration (μg·L^{-1}) at 100, 282.5, 550, 817.5, and 1000. Experiments were performed according to a CCD experimental plan, and the results thus obtained for each combination are given in Tables 5 and 6 for sorption of Se(IV) and Se(VI), respectively, by wheat bran in continuous mode. The results were also predicted with the help of the Minitab software and are given in the same tables.

Interpretation of the Regression Analysis. The regression coefficients in case of sorption of both species of selenium, that is, Se(IV) and Se(VI), are shown in Table 7. The values of the regression constants were found to be 10.54 ± 0.02 with $T = 10.424$ and $P < 0.05$ for Se(IV) and 8.92 ± 0.02 with $T = 12.352$ and $P < 0.05$ for Se(VI). Thus, the average uptake of Se(VI) on wheat bran in continuous mode was approximately 10.54 μg·g^{-1}, and that of Se(VI) was 8.91 μg·g^{-1}. The uptake in the case of both Se(IV) and Se(VI) was found to be significantly ($P < 0.05$) and synergistically affected by the linear term of bed height and initial selenium concentration, whereas the uptake was significantly ($P < 0.05$) and antagonistically affected by the linear term of flow rate, in both cases (Se(IV) and Se(VI)). This implied that the uptake in the case of both selenium species increased with the increase in bed height and initial selenium concentration and decreased with the increase in flow rate. The quadratic terms in the case of both Se(IV) and Se(VI) were found to be insignificant ($P > 0.05$), which implied that there was no curve relation between any of the variables and the response, that is, uptake. Among the interaction terms, the terms of bed height and flow rate with $P = 0.037$ for Se(IV) and $P = 0.004$ for Se(VI) and flow rate and concentration with $P = 0.001$ for Se(IV) and $P < 0.00001$ Se(VI) were found to be significant.

The regression models, based on the obtained values of the regression coefficients, for Se(IV) and Se(VI) uptake on wheat bran and the three variables are given as follows:

$$\text{Se(IV) uptake } (y) = 10.537 + 8.175x_1 - 12.316x_2 + 11.881x_3 + 2.946x_1^2 + 3.174x_2^2 + 2.935x_3^2 - 5.937x_1x_2 + 4.191x_1x_3 - 10.953x_2x_3 \quad (6)$$

$$\text{Se(VI) uptake } (y) = 8.9128 + 7.1952x_1 - 8.0237x_2 + 10.4297x_3 + 0.7610x_1^2 - 0.0669x_2^2 + 2.1357x_3^2 - 6.5393x_1x_2 + 3.0641x_1x_3 - 11.2961_2x_3 \quad (7)$$

where y is selenium uptake (response) in μg·g^{-1}, x_1, x_2, and x_3 are the coded values of the tests variables, bed height (x_1) in cm, flow rate (x_2) in mL·min^{-1}, and initial selenium concentration (x_3) in μg·L^{-1}. These regression equations (eqs 6 and 7) are applicable in the studied ranges of each parameter.

The values of R^2 were found to be 97.95 % and 97.61 % for Se(IV) and Se(VI), respectively, which revealed that the total variations which were explained by the residues are only 2.05 % and 2.39 % in the case of Se(IV) and Se(VI), respectively. The adequacy of the equation was also represented by the lower values of the standard deviations between the measured and the predicted results, and these were found to be 2.48 and 1.77 for Se(IV) and Se(VI), respectively. The predicted values of the responses, that is, selenium uptake, using the regression model are shown in Tables 5 and 6 for Se(IV) and Se(VI), respectively. It was found from these tables that the predicted values of the uptake were very close to the experimental results with lower values of the residuals.

Interpretation of ANOVA. The ANOVA was conducted for a second-order response surface model, and the results are given in Table 8 for both Se(IV) and Se(VI). It was found from these results that, in both cases, the linear terms were significant (*P*

Table 7. Estimated Regression Coefficients (Using Coded Units) for Experimental Response (Uptake, μg·g^{-1}) for Selenium Sorption in Continuous Mode

term	coef[a]	SE coef[b]	*T*[c]	*P*[d]
	Se(IV)			
constant	10.537	1.011	10.424	0.000
bed height (cm)	8.175	1.128	7.250	0.000
flow rate (mL·min^{-1})	−12.316	1.127	−10.925	0.000
conc. (μg·L^{-1})	11.881	1.128	10.531	0.000
bed height (cm)·bed height (cm)	2.946	1.846	1.596	0.142
flow rate (mL·min^{-1})· flow rate (mL·min^{-1})	3.174	1.846	1.719	0.116
conc. (μg·L^{-1})·conc. (μg·L^{-1})	2.935	1.847	1.589	0.143
bed height (cm)·flow rate (mL·min^{-1})	−5.937	2.474	−2.399	0.037
bed height (cm)·conc. (μg·L^{-1})	4.191	2.478	1.692	0.122
flow rate (mL·min^{-1})·conc. (μg·L^{-1})	−10.953	2.477	−4.422	0.001
	Se(VI)			
constant	8.9128	0.7216	12.352	0.000
bed height (cm)	7.1952	0.8049	8.940	0.000
flow rate (mL·min^{-1})	−8.0237	0.8047	−9.971	0.000
conc. (μg·L^{-1})	10.4296	0.8053	12.951	0.000
bed height (cm)·bed height (cm)	0.7610	1.3178	0.577	0.576
flow rate (mL·min^{-1})· flow rate (mL·min^{-1})	−0.0669	1.3176	−0.051	0.961
conc. (μg·L^{-1})·conc. (μg·L^{-1})	2.1357	1.3183	1.620	0.136
bed height (cm)·flow rate (mL·min^{-1})	−6.5393	1.7663	−3.702	0.004
bed height (cm)·conc. (μg·L^{-1})	3.0641	1.7685	1.733	0.114
flow rate (mL·min^{-1})·conc. (μg·L^{-1})	−11.2961	1.7679	−6.389	0.000

[a] Coefficient. [b] Standard error of the coefficient. [c] *T* test. [d] *P* value.

Table 8. ANOVA for the Response Surface Quadratic Model for Selenium Sorption in Continuous Mode

source	DF[a]	seq SS[b]	adj SS[c]	adj MS[d]	*F*[e]	*P*[f]
			Se(IV)			
regression	9	1952.59	1952.59	216.954	35.31	0.000
linear	3	1738.29	1737.59	579.196	94.28	0.000
square	3	41.20	41.20	13.732	2.24	0.147
interaction	3	173.10	173.10	57.701	9.39	0.003
residual error	10	61.44	61.44	6.144		
total	19	2014.03				
			Se(VI)			
regression	9	1276.47	1276.47	141.830	45.31	0.000
linear	3	1087.42	1086.45	362.150	115.69	0.000
square	3	8.96	8.96	2.988	0.95	0.451
interaction	3	180.09	180.09	60.031	19.18	0.000
residual error	10	31.30	31.30	3.130		
total	19	1307.77				

[a] Degree of freedom. [b] Sequential sum of squares. [c] Adjusted sum of squares. [d] Adjusted mean of squares. [e] *F* test. [f] *P*-value.

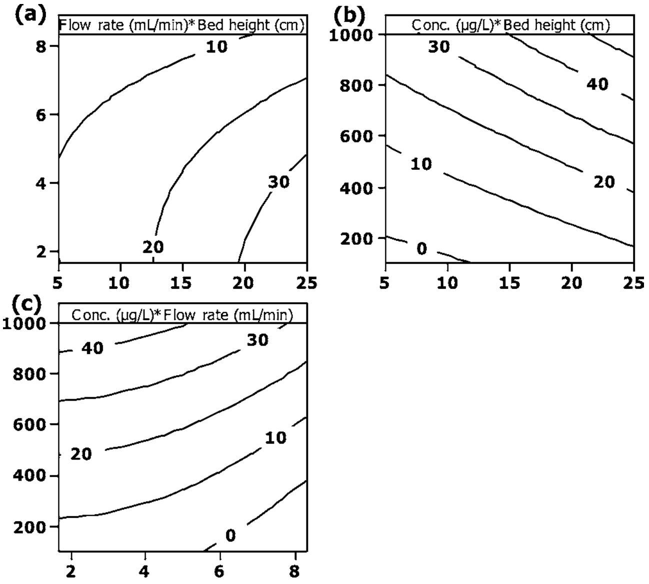

Figure 3. Response surface contour plot for sorption of As(III) by rice polish in continuous mode (held values: bed height = 15 cm, flow rate = 4.99 mL·min^{-1}, conc. = 550 μg·L^{-1}).

< 0.05); however, the quadratic terms were insignificant ($P > 0.05$), which indicate a linear relationship between the uptake and the variables that is, the uptake either increased or decreased with the increase in the variables, and there was no curvature in the relationship between the response and the variables. The interaction terms in the case of both Se(IV) and Se(VI) were found insignificant. It was also observed that the model *F*-values were 35.31 and 45.31 for Se(IV) and Se(VI) biosorption, respectively, and these were greater than the tabulated $F_{9,10}$, the value which is 3.02. The high *F*-values for the models presented in eqs 6 and 7 for the sorption of Se(IV) and Se(VI) respectively implied that the models adequately represented the actual relationship between the response and the process variables. The associated Prob > *F*-value for the models was lower than 0.05, which indicated that the models were statistically significant. Here also the residual error term measured the amount of variation in the response data left unexplained by the model.

Interpretation of Response Surface Contour Plots. Response surface contour plots, for the biosorption of Se(IV) and Se(VI) onto wheat bran in continuous up-flow fixed-bed column mode, were drawn to explore the relationship between the three variables on a single plot and to view combinations of different variables shown on *x* and *y* axes that produce desirable response values. The contour plots were drawn to study the relative effects of any two variables; the values of the remaining variable were kept constant. The held values of the remaining factors were set at their middle values, and these were bed height at 15 cm, flow rate at 4.99 mL·min^{-1}, and initial selenium concentration at 550 μg·L^{-1}.

The response surface contour plots to estimate the response surface over independent variables bed height, flow rate, and initial selenium concentration for Se(IV) and Se(VI) biosorption are shown in Figures 3 and 4, respectively. The combined effect of flow rate and bed height for Se(IV) and Se(VI) are shown in Figures 3a and 4a, respectively. These plots show that the uptake

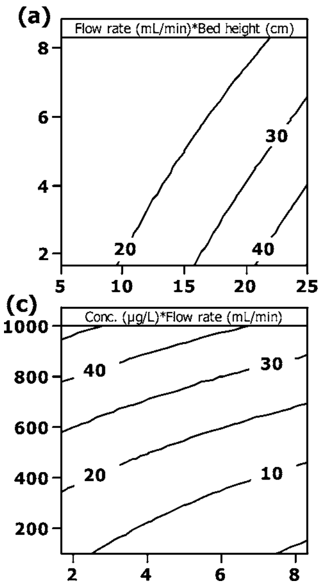

Figure 4. Response surface contour plot for sorption of As(V) by rice polish in continuous mode (held values: bed height = 15 cm, flow rate = 4.99 $mL \cdot min^{-1}$, conc. = 550 $\mu g \cdot L^{-1}$).

of selenium in both cases increased with the increase in the bed height from (5 to 25) cm, while it decreased with an increase in the flow rate from (1.66 to 8.33) $mL \cdot min^{-1}$. The maximum predicted uptake was indicated by the surface confined in the smallest curve of the contour diagram. The response surfaces of mutual interactions between the variables initial selenium concentration and bed height are presented in Figures 3b and 4b for Se(IV) and Se(VI), respectively. The uptake was found to increase linearly with the increase in initial selenium concentration from (100 to 1000) $\mu g \cdot L^{-1}$ and bed height from (5 to 25) cm. Figures 3c and 4c show uptake for Se(IV) and Se(VI), respectively, versus initial selenium concentration and bed height, and it was found that the uptake increased with the initial selenium concentration, while it showed a decreasing trend with the flow rate. Thus, contour plots predicted the maximum uptake of Se(IV) and Se(VI) onto wheat bran in continuous up-flow fixed-bed column mode to be approximately (73.05 and 58.29) $\mu g \cdot g^{-1}$ at a bed height of 25 cm, flow rate of 1.66 $mL \cdot min^{-1}$, and initial selenium concentration of 1000 $\mu g \cdot L^{-1}$.

Conclusions

1. In the present study CCD was employed successfully for the optimization of variables for the biosorption of Se(IV) and Se(VI) onto wheat bran in batch as well as continuous column mode. The predicted values of uptake, with the help of CCD in both batch and continuous modes of sorption, were found to be in good accordance with those obtained using experiments according to the CCD plan.

2. In batch mode it was found that the uptake of Se(IV) and Se(VI) decreased with the increase in pH, biomass dose, and temperature, whereas it increased with the increase in the initial selenium concentration. In continuous mode the uptake in both cases was found to increase with the increase in bed height and initial selenium concentration, whereas it decreased with the flow rate of solution through the column.

3. The maximum uptake predicted in case of batch mode of sorption was approximately 49.56 $\mu g \cdot g^{-1}$ for Se(IV) and 41.37 $\mu g \cdot g^{-1}$ for Se(VI) at pH 2, initial selenium concentration of 1000 $\mu g \cdot L^{-1}$, temperature of 20 °C, and biomass dose of 20 $g \cdot L^{-1}$, whereas in case of continuous mode of sorption it was (approximately 73.05 and 58.29) $\mu g \cdot g^{-1}$ for Se(IV) and Se(VI), respectively, at a bed height of 25 cm, flow rate of 1.66 $mL \cdot min^{-1}$, and initial selenium concentration of 1000 $\mu g \cdot L^{-1}$.

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