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Optimization of Cr(VI) removal by sulfate-reducing bacteria using response surface methodology

R. Ahmadi[a], A. Rezaee[b,*], M. Anvari[c], H. Hossini[b], S.O. Rastegar[d]

[a]*Department of Biology, Science and Research Branch, Islamic Azad University, Rasht, Iran, Tel. +982182883590; Fax: +982182884555; email: rominahmd@yahoo.com*
[b]*Faculty of Medical Sciences, Department of Environmental Health, Tarbiat Modares University, Tehran, Iran, Tel. +982182883590; Fax: +982182884555; emails: rezaee@modares.ac.ir (A. Razaee), hoo.hosseini@gmail.com (H. Hossini)*
[c]*Department of Biology, Rasht Branch, Islamic Azad University, Rasht, Iran, Tel. +982182883590; Fax: +982182884555; email: m.anvari@yahoo.com*
[d]*Biotechnology Group, Chemical Engineering Department, Tarbiat Modares University, Tehran, Iran, Tel. +982182883980; Fax: +982182883517; email: so.rastegar@gmail.com*

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ABSTRACT

The aim of this work was to optimize Cr(VI) removal using sulfate-reducing bacteria from wastewater. Three effective factors including initial pH, initial Cr(VI) concentration, and inoculation percentage were optimized using a central composite design of response surface methodology. The optimum conditions were initial pH 7.5, initial Cr(VI) concentration 130 mg/l, and inoculation percentage 7.75%, and the maximum Cr(VI) removal was 82%. The kinetics study of Cr(VI) removal showed the pseudo-first-order model described experimental data better and was selected as an overall kinetic Cr(VI) removal.

Keywords: Cr(VI); Sulfate-reducing bacteria; Response surface methodology; Optimization; Kinetic

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1. Introduction

Hexavalent chromium, Cr(VI), is well known for its toxicity, and it is released into biotic environment specially aquatic ecosystems by electroplating, metal finishing, chromate preparation, chemistry, leather, wood, tannery, and fertilizer industries [1,2]. Wastewater containing Cr(VI) is treated by reducing to Cr(III) that is lower toxicity solid hydroxide [1]. Up to now, numerous techniques have been used to remove chromium from industrial effluents, including chemical precipitation, evaporation, reverse osmosis, adsorption, ion-exchange, and membrane separation [3]. Often, the treatment methods of Cr(VI), suffer from drawbacks such as high capital and operational costs or the disposal of the residual metal sludge [4]. Generally, heavy metals are toxic for microorganisms which are due to substitution of essential ions on cellular sites and block age of functional groups of important molecules such as enzymes [5]. Among microbial communities, sulfate-reducing bacteria (SRB) are known as high-tolerant bacteria (such as *Desulfovibrio desulfuricans*) that can be used for treating Cr(VI) [6,7]. The SRB are considered as an important member of bacterial which are in the group of chemoorganotrophic and strictly anaerobic bacteria. Appling the SRB has advantages in

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*Corresponding author.

economy, environment, and biotechnology [5,8]. Under anaerobic (without oxygen) conditions, SRB oxidize simple organic compounds by utilizing sulfate (SO_4^{2-}) as an electron acceptor and biogenically generate sulfide (S^{2-}) and alkalinity [9]. This produced sulfide can react with dissolved metals to form metal sulfide precipitates that have very low solubility [10]. Cheung et al. described the metabolic pathway for chromium reduction by a type of sulfate-reducing bacterium (*Desulfovibrio vulgaris*) [11]. During the ATP synthesis, sulfate reduced to sulfite, and then, hexavalent chromium reduced to trivalent form (insoluble precipitate) by transferring electron from cytochrome that is originally prepared by hydrogen oxidation. SRB first reduce the sulfate/thiosulfate/sulfite and convert them to sulfide (HS^- or H_2S) ions, and as a consequence, the sulfide ions reduce the Cr(VI) to Cr(III) and theirs oxide to S^0. The reaction is shown below:

$$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O \quad (1)$$

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (2)$$

$$4SO_3^{2-} + H^+ \rightarrow 3SO_3^{2-} + HS^- \quad (3)$$

The overall reaction is below:

$$3HS^- + Cr_2O_7^{2-} + 11H^+ \rightarrow 3S^0 + 2Cr^{3+} + 7H_2O \quad (4)$$

In the Cr(VI) removal process, some parameters such as initial pH, initial Cr(VI) concentration, time, bacterial inoculation percentage, and some other parameters were considered as effective factors, but a methodology is required to optimize these parameters and to identify their interactions. Response surface methodology (RSM) is an efficient experimental tool based on statistical analysis to determine optimal conditions for a multivariable system. Statistical optimization can determine the role of each component, and the interactions among the parameters, which can save time, decrease the need for instrumentation, chemicals, and manpower [12,13]. The aim of the present work was to evaluate the potential of SRB on the removal of Cr(VI). The effects of three parameters such as initial pH, initial Cr(VI) concentration, and bacterial inoculation percentage on the removal process were studied using RSM.

2. Materials and methods

2.1. Materials

All the chemicals used in the study were of analytical grade. Distilled water was used in preparation of all the chemical solutions. Stock solution of chromium (1,000 mg/L) was prepared by dissolving potassium dichromate ($K_2Cr_2O_7$) in distilled water. The working concentrations were provided by diluting the stock solution with distilled water. The pH was adjusted to the desired value with 1 M HCl and 1 M NaOH.

2.2. Bacterial culture

The SRB culture was prepared by the Iranian Research Institute of Petroleum Industry, Tehran, Iran. The samples were inoculated in the Postgate's Medium B in order to grow and store SRB for long time. Bioreduction experiments were performed in the 15-ml tube including 1 ml of inoculums with the bacteria. The flasks were shaken incubated in 37°C. Then, the Cr(VI) was added in certain concentration. Measurements were taken to determine the pH, bacteria count, and Cr(VI) content of the solutions. The pH was measured using a portable pH meter (Eutech, Singapore). The cell numbers in the liquid phase were enumerated using improved Neubauer counting chamber under a phase-contrast microscope (Carl zeiss, Germany). The chromium content was determined by colorimetric methods using a 1, 5 diphenylcarbazide [$CO(NH{\cdot}NHC_6H_5)_2$] reagent at $\lambda = 540$ nm (spectrophotometer, Rayleigh UV 9200, China).

2.3. Experimental design method

Experimental design was performed to investigate the effect of three main parameters including initial pH, initial chromium concentration, and inoculation percentage on the process efficiency and also to obtain optimal condition. Each factor in experimental designs based on the general factor was varied at five different levels (−α, −1, 0, +1, +α), while the other parameters were kept constant [14]. The range and the levels of the variables investigated in this study are given in Table 1. CCD is essentially a particular set of mathematical and statistical methods for designing experiments, building models, evaluating the effects of variables, and searching optimum conditions of variables to predict targeted responses [15]. In this study, using Design-Expert 7.1.4, a total of 20 experiments were designed for this procedure and are showed in Table 2. The behavior of the system is explained by the quadratic polynomial empirical model.

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<1}^{3} \sum_{j=1}^{3} \beta i_j X_i X_j + \varepsilon \quad (5)$$

where y is the expected value of the response variable; β_0, β_{ii}, and β_{ij} are the model parameters; and X_i and X_j

Table 1
Experimental variables at different levels

Factor	Units	Low axial (−α)	Low factorial (−1)	Center point (0)	High factorial (+1)	High axial (+α)
A: initial pH	–	6	6.5	7	7.5	8
B: initial Cr(VI) concentration	mg/l	10	130	255	375	500
C: inoculation	%	1	4	7	9	10

Table 2
Experimental plan based on CCD and the results

	Factor			
Run	*A*: initial pH	*B*: initial Cr(VI) concentration (mg/l)	C: inoculation percentage (%)	Cr(VI) removal (%)
1	6.5	133	3.25	63.23
2	7.5	378	3.25	40.28
3	7	255	10	85.33
4	7	500	5.5	60.08
5	7	255	5.5	63.24
6	7	10	5.5	72.88
7	7	255	5.5	62.55
8	7	255	5.5	63.35
9	7	255	5.5	61.78
10	7.5	378	7.75	76.22
11	6	255	5.5	74.98
12	6.5	133	7.75	68.11
13	7.5	133	7.75	79.5
14	6.5	378	7.75	72.34
15	7.5	133	3.25	52.82
16	8	255	5.5	57.77
17	7	255	5.5	64.09
18	7	255	5.5	63.65
19	6.5	378	3.25	58.45
20	7	255	1	48.77

are the coded factors evaluated [16]. In this study, y represents the amount of Cr(VI) removal using of SRB.

2.4. Confirmation experiment

In order to check the validation of obtained model, an experiment at optimal factor levels was performed and the experimental Cr(VI) removal was compared to predict the model.

3. Results and discussion

3.1. Statistical analysis

ANOVA results are showed in Table 3. It investigated the effect of all factors and also their interaction in responding the system. This statistical tool is required to test the significance and adequacy of the model. The mean squares (MS) are obtained as MS = SS/DF, where SS = sum of squares (SS) of each variation source and DF = the respective degrees of freedom (DF). The Fischer variation ratio (*F*-value) is a statistically valid measure of how well the factors describe the variation in the data about its mean. It can be calculated from ANOVA as *F*-value = MS (due to the model variation)/MS (due to error variance). Normally, the data have some variations around its mean value; the greater the *F*-value from unity, the more acceptable is this variation [17]. In general, the calculated *F*-value should be several times greater than the tabulated value. In fact, as shown in Table 3, the calculated p-value of the model is <0.0001. The results showed that this regression was statistically

Table 3
ANOVA for response surface models applied

Source	Sum of squares	df	Mean square	*F*-value	*p*-Value
Model	2,064.97	7	294.996	47.63	<0.0001 (significant)
A: pH	142.38	1	142.384	22.99	0.0004
B: initial Cr(VI) concentration	110.09	1	110.092	17.77	0.0012
C: inoculation percentage	1492.08	1	1492.083	240.93	<0.0001
AB	29.14	1	29.146	4.70	0.0508
AC	240.35	1	240.352	38.81	<0.0001
BC	41.72	1	41.724	6.73	0.0234
C^2	9.194	1	9.194	1.48	0.2465
Residual ($R^2 = 0.96$, $R^2_{adj} = 0.94$)	74.31	12	6.192		

significant at less than a 0.05% level (i.e. at 95% confidence interval (CI)). Table also shows that *p*-value's factors and their interactions are significant with less than 0.05.

3.2. Fitting model

By applying multiple regression analysis on the experimental data, the experimental results of the CCD were fitted with a modified quadratic model polynomial equation. Eq. (6) was obtained from the 20 batch runs by the application of RSM:

$$\text{Cr(VI) removal } (\%) = 64.01 - 2.98A - 2.62B + 9.66C - 1.91AB + 5.48AC + 2.28BC + 0.58C^2 \tag{6}$$

where *A*, *B*, and *C* are initial pH, initial Cr(VI) concentration, and inoculation percentage, respectively. It should be noted that polynomial models are reasonable approximations of the true functional relationship over relatively small regions of the entire space of independent variables [18]. Fig. 1 shows the predicted data (data were gathered from model) versus actual data (data that were gathered from experimental condition). The clustering of the points around the diagonal line indicates a satisfactory correlation between the experimental and predicted data, confirming the robustness of the model. The relatively high R^2 (0.96) and R^2_{adj} (0.94) values indicate that the second equation for the Cr(VI) removal is capable of representing the system under the given experimental conditions. Also Fig. 2 revealed that residuals vs predicted plot that has no obvious pattern and unusual structure. This is a plot of the residuals vs. the ascending predicted response values. It tests the assumption of constant variance. The plot should be a random scatter (constant range of residuals across the graph).

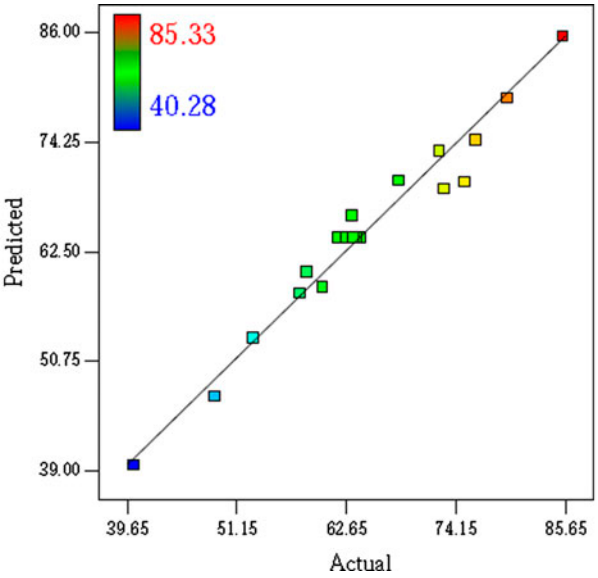

Fig. 1. Predicted vs. actual values for Cr(VI) removal.

3.3. Two-dimensional response plots

Fig. 3(a) represented the two-dimensional response surfaces of Cr(VI) removal (%) of the relationship between different parameters at the optimized values. Fig. 3(a) shows a combined effect of initial pH and inoculation percentage at the specific initial Cr(VI) concentration (500 mg/l). Figure illustrates that decreasing initial pH and increasing inoculation percentage have positive effect on Cr(VI) removal. The maximum Cr(VI) removal (73%) was observed for initial pH of 3.5 and inoculation of 7.75%. The relationship between initial Cr(VI) concentration and inoculation percentage at the specific initial pH of 5 are showed in Fig. 3(b). According to this figure, a maximum removal of Cr(VI) of >77% was observed in the initial Cr(VI) concentration 130 mg/l and inoculation percentage 7.75% at the constant initial pH 7.5.

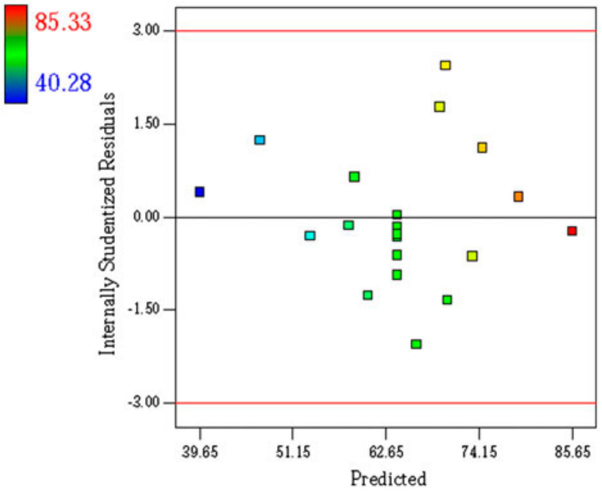

Fig. 2. Plot of residuals vs. predicted response for Cr(VI) removal.

According to this figure, amount of Cr(VI) removal will be increased by increasing inoculation from 3.25 to 7.75% at a constant initial pH.

3.4. Experimental optimization

It should be noted that the goal of optimization is to find a good set of experimental conditions. The optimum conditions proposed by the model were initial pH 7.5, initial Cr(VI) concentration 130 mg/l, and inoculation percentage 7.75%, at which maximum Cr(VI) removal of 79% was achieved. These values are all in agreement with the results obtained from the contour plots. It is necessary to note that qualitative and statistical analysis of time indicates that the removal Cr(VI) is favored with the increase in it, but it is restricted from an economical point of view.

3.5. Confirmatory experiments

Table 4 presents the results of the experiment conducted at the optimal conditions. Under these conditions, the experimental value for the Cr(VI) removal was found to be 82%. To test the validity of the optimized conditions given by the model, an experiment was carried out with the parameters suggested by the model. Results showed that verification experiment and predicted values from fitted correlations were in close agreement at a 95% CI. The 95% CI is the range in which the process average was expected to fall 95% of the time. The results of analysis indicated that the experimental values were in good agreement with the predicted values, and hence, the model is successful in predicting the responses. These results confirmed the validity of the model, and the experimental values were determined to be quite close to the predicted values.

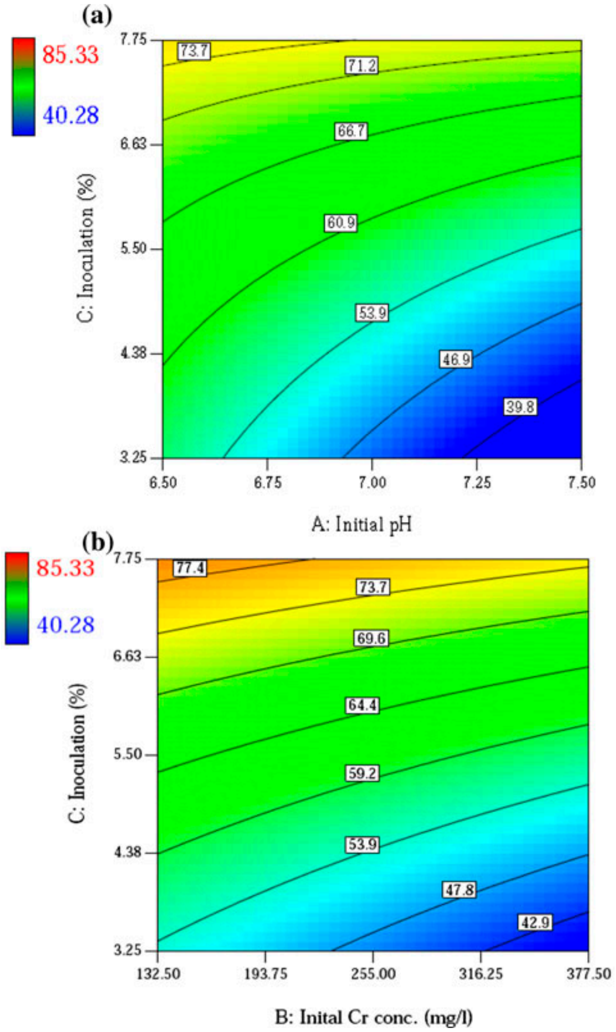

Fig. 3. Contour plots of the interactive effect for Cr(VI) removal: (a) effect of initial pH and inoculation at the constant initial Cr(VI) concentration 130 mg/l and (b) effect of initial Cr(VI) concentration and inoculation at the constant initial pH 7.5.

3.6. Kinetic study

Fig. 4 shows the trend of Cr(VI) removal efficiency under optimal conditions including initial pH 7.5, initial Cr(VI) concentration 130 mg/l, and inoculation 7.75%. Maximum removal efficiency was determined about 80% at endpoint of 11 d. To evaluate the kinetics of Cr(VI) removal, two of the most used kinetic models pseudo-first-order (Eq. 7) and

Table 4
Verification of the model at optimum condition

Initial pH	Initial Cr(VI) concentration (mg/l)	Inoculation (%)	Cr removal (%) (prediction)	Cr removal (%) (experiment)	95% CI low	95% CI high
7.5	130	7.75	79	82	74	83

pseudo-second-order (Eq. 8) were fitted to experimental results. The two used equations of kinetic are below [19,20]:

$$\ln C_t = \ln C_0 - k_1 t \tag{7}$$

$$\frac{1}{C_t} = k_2 t + \frac{1}{C_o} \tag{8}$$

where C_0 is the initial Cr(VI) concentration and C_t is the Cr(VI) concentration after time t; k_1 and k_2 are the first- and second-order kinetic constants, respectively. The linear equations of the kinetic plots and their correlation factor are shown in the Fig. 5. The kinetic constant values of the k_1 and k_2 were obtained about 0.151 d^{-1} and 0.003 $mg^{-1}\ d^{-1}$, respectively. The correlation factor (R^2) of the straight lines was 0.98 for the pseudo-first-order and 0.97 for the pseudo-second-order. It was evident that the correlation coefficient for the pseudo-first-order kinetic model was higher than pseudo-second-order kinetic model; therefore, the removal of Cr(VI) using SRB follows the pseudo-first-order kinetic model for the entire process.

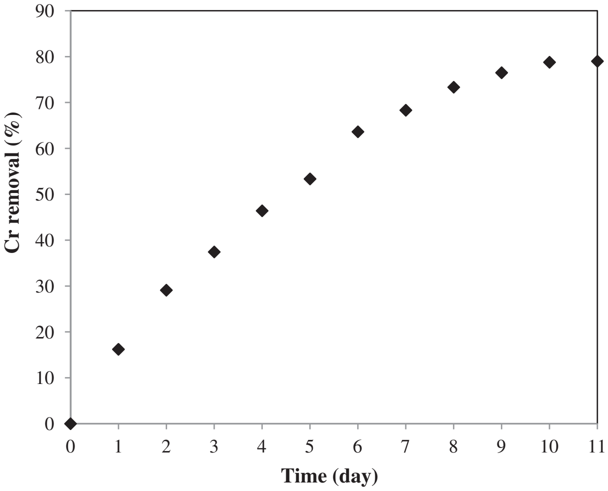

Fig. 4. Cr(VI) removal efficiency vs. time under optimal condition (initial pH 7.5, initial Cr(VI) 130 mg/l, and inoculation 7.7%).

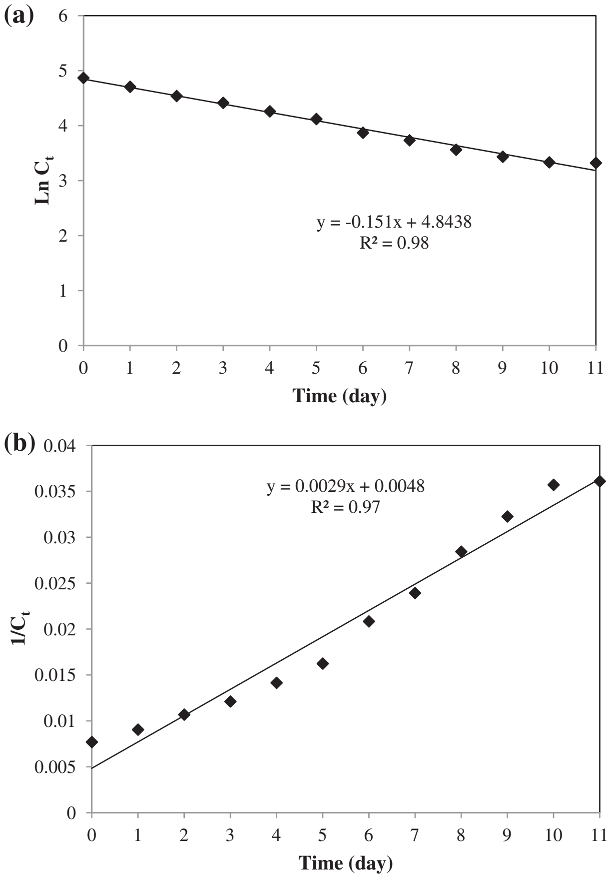

Fig. 5. The plots of the kinetic model (a) Pseudo-first-order and (b) Pseudo-second-order.

4. Conclusion

Removal of Cr(VI) by SRB has been studied. Effective parameters such as initial pH, initial Cr(VI) concentration, and inoculation percentage were examined to obtain the highest efficiency on Cr(VI) removal using RSM. This study has shown that the development of mathematical models for process simulation based on statistics can be useful for predicting and understanding the effects of experimental factors. Results showed that the best model for removal of Cr(VI) was the reduced quadratic model. It was found that at the optimum condition, initial pH 7.5, initial Cr(VI) concentration 130 mg/l, and inoculation

7.75%, the maximum Cr(VI) removal of 82% has been obtained. The kinetics of Cr(VI) removal was investigated using the pseudo-first-order and pseudo-second-order models. Results showed the experimental data were better described by pseudo-first-order model and was selected as overall kinetic removal of Cr(VI).

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