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Response surface methodological approach for optimizing heavy metal biosorption by the blue-green alga *Chroococcus disperses*

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ABSTRACT

In this study, the performance of live *Chroococcus dispersus* algae for the removal of As, Cd, Co, Cr, and Fe from aqueous solutions as batch was investigated. For this purpose, Box–Behnken design and response surface method were applied to evaluate and optimize the interactive effects of three operating variables including metal concentration, time of reaction, and algae dose on the bioaccumulation process. The regression equation coefficients were calculated, and the data confirmed the validity of second-order polynomial equation for the removal of As, Cd, Co, Cr, and Fe with *Chroococcus dispersus* algae. Analysis of variance showed a high coefficient of determination value (R^2) for As, Cd, Co, Cr, and Fe being, respectively, 0.996, 0.994, 0.996, 0.995, and 0.998. The optimum metal concentration, time of reaction, and algae dose were found to be, respectively, 5.03 mg L^{-1}, 114 h, and 2.96 g L^{-1} for As; 11.9 mg L^{-1}, 109 h, and 2.66 g L^{-1} for Cd; 11.5 mg L^{-1}, 102 h, and 2.87 g L^{-1} for Co; 7.75 mg L^{-1}, 105 h, and 2.51 g L^{-1} for Cr; and 10.4 mg L^{-1}, 118 h, and 2.73 g L^{-1} for Fe. Under optimal value of process parameters, maximum efficiencies for the removal of As, Cd, Co, Cr, and Fe were 96.7%, 97.12%, 98.55%, 95.82%, and 99.4%, respectively.

Keywords: Heavy metal removal; Optimization; Box–Behnken design; *Chroococcus dispersus* algae; Biosorption

1. Introduction

Heavy metals are serious environmental pollutants in many countries due to their toxicity and carcinogenicity and nonbiodegradable nature and may cause significant damage to the environment and public health [1–4]. Although some metals are nutritionally essential for the growth of living cells, they have toxic effects at high concentrations. This is due to their oxidative capacity to produce and release free radicals, and they may become substitutes for necessary metal ions in enzymes, interrupting their function [5–7]. Other nonessential metals are toxic even at low concentrations that readily accumulate in different tissues. According to the United States Environmental Protection Agency [8], mercury, chromium, lead, arsenic, copper, cadmium, cobalt, zinc, nickel, beryllium, manganese, and tin are the most toxic heavy metals. Heavy metals cannot be easily removed by conventional biological wastewater treatment [9–11]. Heavy metals and toxic pollutants can be removed from aqueous solution by chemical precipitation, membrane technologies, ion exchange, electrochemical treatment, and adsorption on activated carbon [12–15], but these are expensive techniques in terms of energy and chemical product consumption, often resulting in the generation of toxic sludge [16–21]. The need for nonexpensive, simple, effective, and efficient systems for the removal of heavy metals from wastewater has generated interest in evaluating biosorption and bioaccumulation processes [22,23]. Metal accumulative bioprocesses can be divided into two main processes: biosorption by nonliving biomass and bioaccumulation by living cells [24,25]. A large

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number of organisms such as bacteria, fungi, yeast, and algae have been studied for their ability for bioremediation and accumulating heavy metals and pollutants [12,26]. Algae play a key role in aquatic ecosystems, which *produce oxygen* and *organic compounds* from inorganic compounds as a by-product of photosynthesis. The effects of toxic pollutants on algae can be directly affected in both community *structure* and *ecosystem* function due to oxygen depletion and decrease in primary productivity [27,28]. Living cells have the advantage that the simultaneous removal of heavy metals is achieved with the growth of the organism. Additionally, they can remove metal through a combination of several reactions, such as surface reactions, intracellular and extracellular precipitation, and extracellular complexation reactions [29,30]. Also, their need for maintenance energy is very low to remain metabolically active [31]. Algae are suitable alternatives for heavy metal removal due to their large surface area and photosynthetic properties and simple handling and culture. They have very simple nutritional needs to stay alive [32,33].

The aim of the present study is the bioaccumulation of As, Cd, Co, Cr (VI), and Fe from aqueous solution using *C. disperses* algae in a continuous reactor. The Box–Behnken experimental design model was used for designing this experiment, optimization of variables, and determining the effects of independent parameters such as metal concentration, time of reaction, and algae dose.

2. Materials and methods

2.1. Isolation and growth condition of algae

The microalgae were collected from the paddy fields of Marvdasht in the south of Iran (Fars province). Primary culturing was done in BG-11 medium. Preserved samples were prepared and incubated in 50-mL conical flasks. Constant illumination was used at 25°C under continuous light of 60 µmol m^{-2} s^{-1} over the 400–700 nm wave band. Temperature was 24°C ± 2°C. Metal removal experiments were performed in batch tests. For each experiment, 250 mL acid-washed glass flasks were filled with 200 mL water containing *Chroococcus disperses*.

Each solution was supplemented with nutrients such as KNO_3 (0.5 mg L^{-1}), KH_2PO_4 (0.2 mg L^{-1}), $MgSO_4{\bullet}7H_2O$ (0.2 mg L^{-1}), and $CaCl_2{\bullet}2H_2O$ (0.1 mg L^{-1}). The $CuSO_4{\bullet}5H_2O$, Cd $(NO_3)_2$, Cr_2 $(NO_3)_2{\bullet}12H_2O$, As_2O_3, and $FeCl_3{\bullet}2H_2O$ were used to prepare the heavy metals stock solution of selected metals in deionized water. Stock solutions of heavy metals were prepared followed by appropriate dilutions to get desired concentrations. Heavy metal analysis in each experiment was carried out using Atomic Absorption Spectrophotometer (PERKIN-ELMER 2380). The biosorption efficiency was calculated using the following formula: Biosorption (%) = $(C_0 - C_e)/C_0 \times 100$, where C_0 and C_e are the initial and final concentrations of heavy metals.

2.2. Establishment of response surface methodology

The Box–Behnken design (BBD), which is the standard response surface methodology (RSM), was used to examine the relationship between response variables and optimize the individual and interaction effects of various variables for the optimization of the biosorption process. For statistical calculations, each of the independent variables was consecutively low, medium, and high coded as −1, 0, and +1. The complete design consisted of 17 runs, and these were performed in triplicate to optimize the levels of selected variables. The experimental range and levels of independent variables considered in this study are presented in Table 1.

The variables used in this study were metal concentration (mg L^{-1}) (X_1), time of reaction (X_2), and algae dose (g/L) (X_3). Heavy metal removal efficiency (Y) was considered as the dependent factor (response). The independent parameters and the dependent output response were modeled and optimized using analysis of variance (ANOVA) to justify the adequacy of the models.

The quadratic equation model for predicting the optimal conditions can be expressed according to Eq. (1)[34]:

$$Y_i = \beta_0 + \sum\beta_i x_i + \sum\beta_{ii} x_i^2 + \sum\beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the predicted response associated with each factor-level combination, β_0 is the intercept term, β_i is the slope or linear effect of the input factor X_i, β_{ii} is the quadratic effect, β_{ij} is the linear interaction effect between input factors, and ε is the residual term.

3. Results and discussion

3.1. Box–Behnken statistical analysis

RSM is an approach that joins mathematical and statistical tools and techniques and is valuable for developing, improving, and optimizing the processes [10, 35–37] and could represent the relative significance of several affecting factors even in the presence of complex interactions. Other advantage of the RSM is that it is employed to minimize a number of experiments when there are several factors incorporated in the study. The experimental results and predicted values for heavy metal removal efficiencies are presented in supplementary material. There were discrepancies in the variance trend for the five responses. Therefore, the operational conditions were optimized, respectively, for the different responses.

Using multiple regression analysis, the responses (As, Cd, Cr, Co, and Fe removal) were correlated with the three design factors using the second-order polynomial Eq. (1). The quadratic regression models for As removal (Y_1, %), Cd removal (Y_2, %), Cr removal (Y_3, %), Co removal (Y_4, %), and

Table 1
Experimental independent variables

Variables	Factor	Range and level		
		–1	0	+1
Metal concentration (mg L^{-1})	X_1	5	10	15
Time of reaction	X_2	30	75	120
Algae dose (g L^{-1})	X_3	1	2	3

Fe removal (Y_5, %) in terms of coded factors are given by Eqs. (2)–(6), respectively:

$$Y_1\ (\mathrm{As}) = 77.88 - 2.84X_1 + 22.51X_2 + 10.7X_3 - 0.42X_1^2 - 18.12X_2^2 - 2.8X_3^2 - 1.965X_1X_2 + 1.8X_1X_3 + 2.99X_2X_3 \quad (2)$$

$$Y_2\ (\mathrm{Cd}) = 83.54 + 3.11X_1 + 23.35X_2 + 6.91X_3 - 3.62X_1^2 - 15.33X_2^2 - 7.46X_3^2 - 0.37X_1X_2 + 4.5X_1X_3 + 3.88X_2X_3 \quad (3)$$

$$Y_3[38] = 82.48 - 1.93X_1 + 22.46X_2 + 9.97X_3 - 2.97X_1^2 - 15.1X_2^2 - 6.34X_3^2 - 0.96X_1X_2 - 0.32X_1X_3 + 2.38X_2X_3 \quad (4)$$

$$Y_4[32] = 87.68 - 3.75X_1 + 23.73X_2 + 8.28X_3 + 0.29X_1^2 - 20.53X_2^2 - 5.22X_3^2 - 0.75X_1X_2 + 4.95X_1X_3 + 3.82X_2X_3 \quad (5)$$

$$Y_5[14] = 90.38 - 1.95X_1 + 24.08X_2 + 9.99X_3 + 0.47X_1^2 - 19.35X_2^2 - 6.9X_3^2 - 0.1X_1X_2 + 1.38X_1X_3 + 1.77X_2X_3 \quad (6)$$

The statistical significance of the model equation was performed which is ascertained by applying ANOVA. Table 2 shows the ANOVA table for the responses Y_1, Y_2, Y_3, Y_4, and Y_5. The regression variables of the predicted response surface quadratic model are presented in Table 2.

The significance of each coefficient was evaluated by the *F* value and *P* value [39,40]. The *F* value is used as the test for comparing the model variance with residual (error) variance. *F* value from the model implied the significance of the model similarity, and the value of probability >*F* less than 0.05 indicated that the model terms are significant [41–43].

Table 2
ANOVA for the validation of the model

Metal ions	Source of variations	Degrees of freedom	Sum of squares	Mean square	*F* value	*P* (>*F*)
As	Regression	9	6,552.8	2,184.28	632.3	0.02
	Residual	7	24.2	3.45		
	Total	16	6,577			
	Lack of fit	3	18.7	6.23	4.54	0.088
	Pure error	4	5.5	1.37		
	R^2	0.996				
	Adjusted R^2	0.991				
Cd	Regression	9	6,345.6	2,115.23	444.58	0.006
	Residual	7	33.3	4.76		
	Total	16	6,378.9			
	Lack of fit	3	27.4	9.14	6.22	0.054
	Pure error	4	5.9			
	R^2	0.994				
	Adjusted R^2	0.988				
Cr	Regression	9	6,139.7	2,046.5	478	0.18
	Residual	7	30	4.28		
	Total	16	6,169.7			
	Lack of fit	3	23.5	7.82	4.8	0.081
	Pure error	4	6.5	1.63		
	R^2					
	Adjusted R^2					
Co	Regression	9	7,277.1	2,425.7	605.4	0.002
	Residual	7	28	4		
	Total	16	7,305.1			
	Lack of fit	3	22.9	7.63	5.9	0.06
	Pure error	4	5.2	1.29		
	R^2	0.996				
	Adjusted R^2	0.991				
Fe	Regression	9	7,338	2,446	1,365	0.06
	Residual	7	12.5	1.79		
	Total	16	7,350.5			
	Lack of fit	3	8.6	2.87	2.9	0.16
	Pure error	4	3.9	0.98		
	R^2	0.998				
	Adjusted R^2	0.996				

From the ANOVA for the quadratic model for heavy metal removal, for As removal, the model *F* value of 632.3 and $P > F$ of 0.02 showed that the model was highly significant. In this case, X_1, X_2, X_3, X_2X_3, X_2^3, and X_3^2 were significant model terms, whereas X_1X_2, X_1X_3, and X_1^2 were all insignificant in As removal. From the ANOVA for the response surface quadratic model for Cd removal, the model *F* value of 444.58 with a very low probability value $P > F$ of 0.006 indicated that the quadratic models were significant. In this case, X_1, X_2, X_3, X_2X_3, X_1X_3, X_1^3, X_2^2, and X_3^2 were significant model terms, whereas X_1X_2 was insignificant to Cd removal. The ANOVA of the regression model demonstrated that the second-order polynomial model fitted the experimental results well for Cr, Co, and Fe removal, as was evident from the very low probability value ($P > F$). Also for Cd removal, X_1, X_2, X_3, X_1^3, X_2^2, and X_3^2, for Co removal X_1, X_2, X_3, X_1X_3, X_2X_3, X_2^2, and X_3^2, and for Fe removal X_1, X_2, X_3, X_2X_3, X_2^2, and X_3^2 were significant model terms. The lack-of-fit was also calculated from the experimental error (pure error) and residuals. The *P* values of lack-of-fit for all the models were greater than 0.05, indicating that the *F* statistic was insignificant, implying the significance of the mathematical model correlation between the variable and process response.

The mathematical models were also inspected for validity by providing an adequate approximation to the real system. In this case, by applying the diagnostic plots such as the predicted versus experimental value plots, the goodness-of-fit of the model can be confirmed. The values of R^2 for actual and the predicted adsorption efficiencies were found to be 0.996, 0.994, 0.996, 0.995, and 0.998, respectively (Fig. 1). An adequate agreement is indicated between the predicted values obtained from the model and the experimental values.

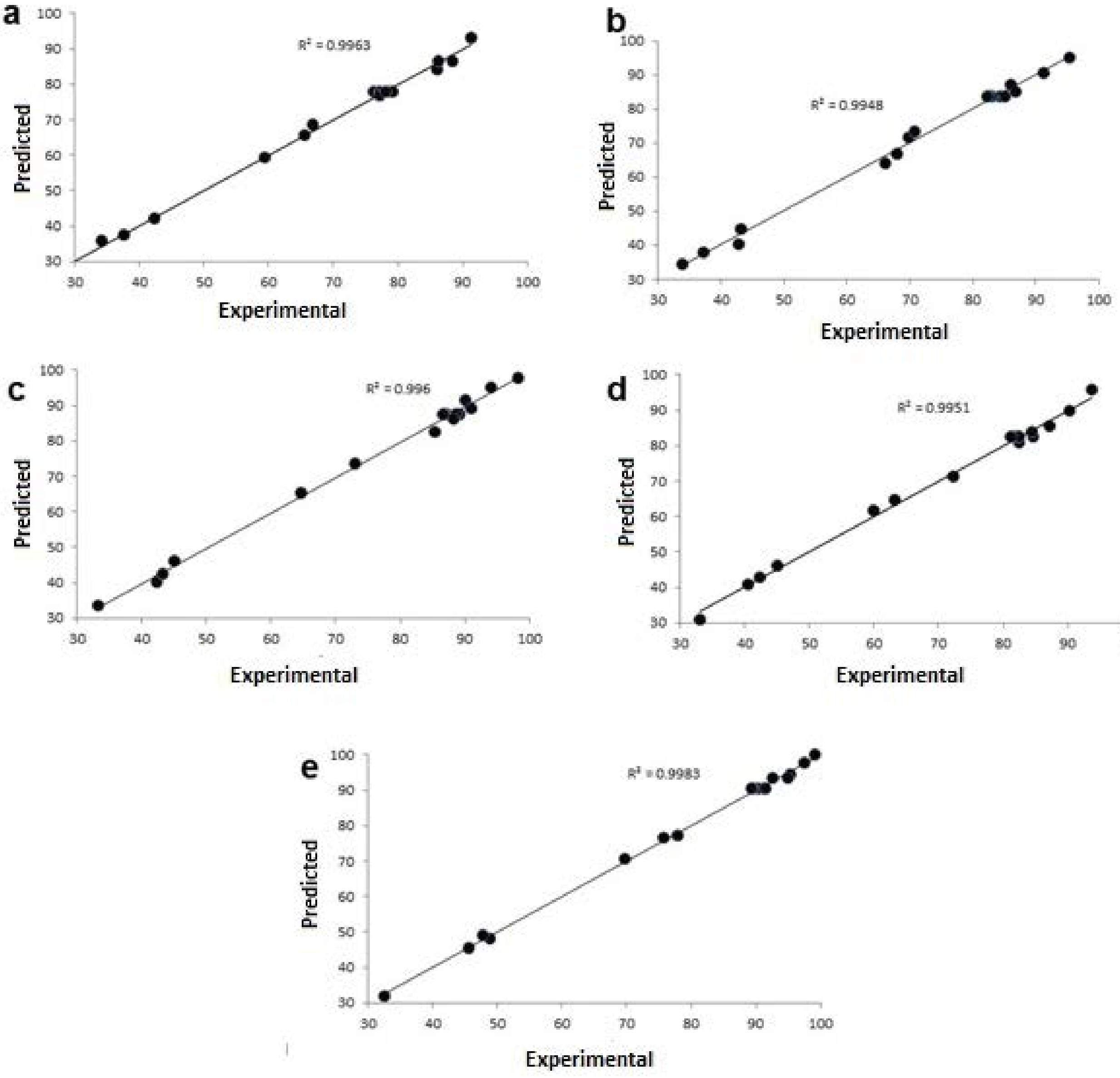

Fig. 1. Comparison of experimental (%) vs. predicted (%) data by RSM for As (a), Cd (b), Co (c), Cr (d), and Fe (e).

3.2. Effect of parameters on heavy metal removal

The effect of time of reaction and initial heavy metal concentration on heavy metal biosorption is shown in Fig. 2. As is obvious from Fig. 2(a), the percentage of As removal increased with the increase in time of reaction from 30 to 100 h, then smoothly steadily decreased.

Similar results were obtained in experiments on the removal of Cd, Co, Cr, and Fe (Figs. 2(b)–(e)). Fig. 2(b) shows a 3D plot of the simultaneous effects of time of reaction and initial Cd concentration on Cd removal while the *C. disperses* algae dose was kept constant at 2 g L^{-1}, being the central level. The removal capacity of *C. disperses* increased when initial Cd concentration was increased from 5 to 12 mg L^{-1}, and then no change was seen with further increase in initial Cd concentration, resulting in the saturation of the biosorbent surface for Cd.

These observed trends are due to the fact that an increase of initial metal ion concentration generates an important driving force to overcome all mass transfer resistances between the aqueous and solid phases [44]. But, at further concentrations of metal ions, the available superficial functional groups already are occupied and consequently the diffusion of metal ions on the unreacted functional groups is inhibited.

However, according to the contour plot in Fig. 2(d) and the surface plot in Fig. 2(a), (c), and (e), the heavy metal

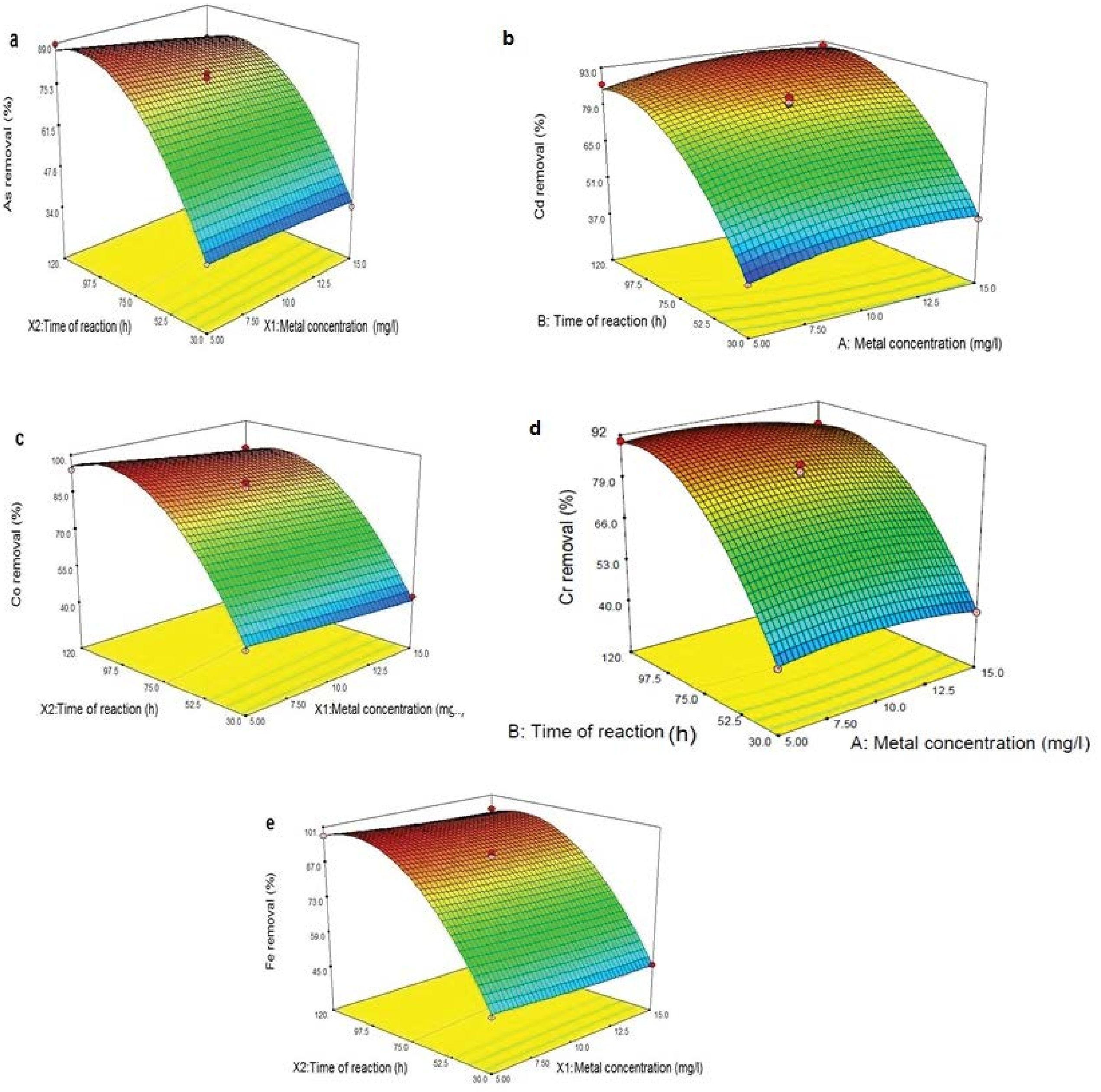

Fig. 2. (a–e) Effect of time of reaction and metal concentration on heavy metal removal.

removal capacity of *C. disperses* decreased as the initial heavy metal concentration increased. As can be seen from Fig. 2(d), the maximum Cr removal was observed at initial Cr concentration of 7.5 mg L^{-1} and contact time of 100 h.

In Fig. 3, the response surface and contour plots were developed as a function of the initial heavy metal concentration and *C. disperses* algae dose at a fixed time of reaction at 75 h.

According to Fig. 3(a), increasing the *C. disperses* dose increased As removal efficiencies somewhat. When the initial concentration of As was low, As removal increased as the Algae dose increased, reaching their maximum values (87%). The interaction showed that the initial concentration of As and the algae dose played an important role in As removal. Thus, a relatively lower initial concentration of As and a high algae biomass loading are conducive to efficiency in As removal. This is a reasonable result because a lower weight of the sorbent implies a smaller number of biosorption active sites and in this case proton–metal ion competition for occupation of the active sites becomes more critical. According to Fig. 3(b), the 3D plot of the second-order predicted model indicated that for initial Cd concentration, the percentage of Cd removal increases as the *C. disperses* dose increases. It can also be concluded that increasing the *C. disperses* dose has a greater effect when the initial Cd concentration is high. In the 3D plot of Fig. 3(c), the Co removal efficiency decreased as the initial concentration of Co increased at low *C. disperses* doses. This result showed that bioaccumulation of As, Co,

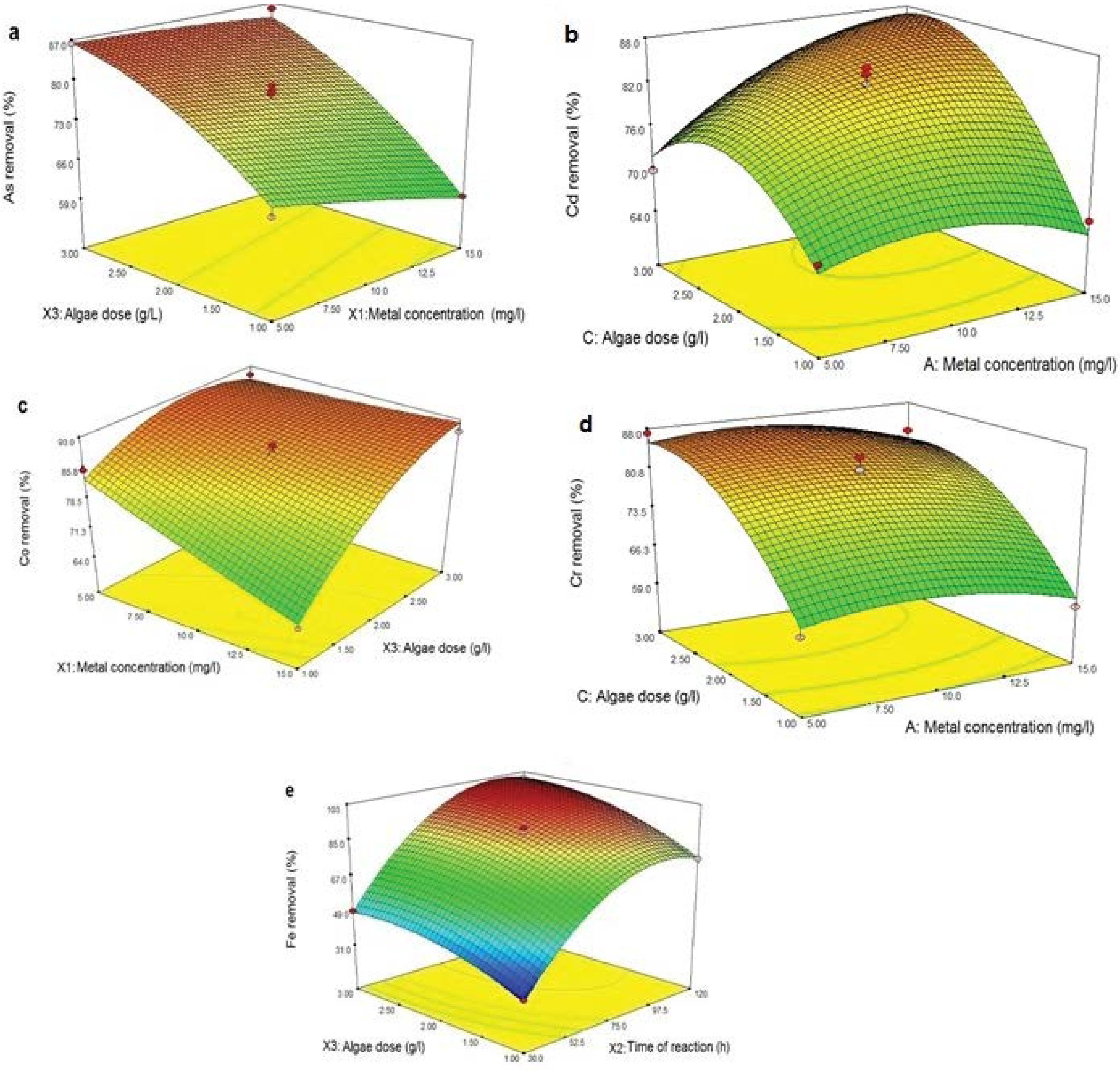

Fig. 3. (a–e) Effect of *C. disperses* algae dose and metal concentration on heavy metal removal.

Cr, and Fe in the *C disperses* was heavily dependent on the initial metal concentrations. At low concentrations of heavy metal, *C disperses* showed its effectiveness for As, Co, Cr, and Fe bioaccumulation, but at high concentrations of heavy metal, bioaccumulation efficiency decreased. However, Co removal increases as the *C. dispersus* dose and initial Co concentration increase. It can also be concluded that increasing the sorbent mass has a greater effect when the initial metal ion concentration is high [45–47]. Approximately 93% removal of Co could be achieved in 75 min reaction time with the initial Co concentration of 15 mg L^{-1} and 3 g L^{-1} of *C. dispersus* dose. This result is also acceptable because in the

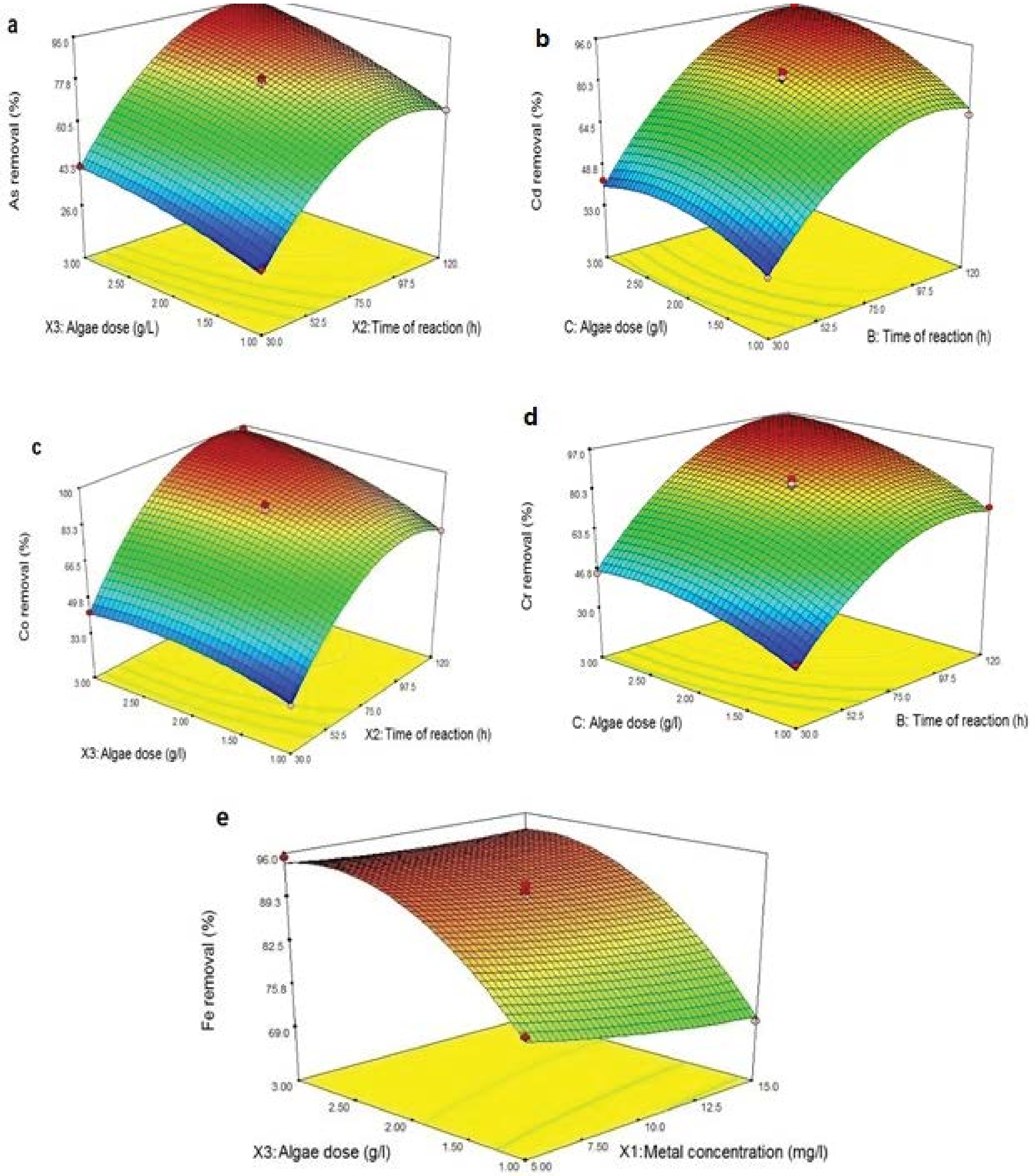

Fig. 4. (a–e) Effect of time of reaction and *C. dispersus* algae dose on heavy metal removal.

Table 3
Optimal operating conditions of the process variables for 100% Cr(VI) removal efficiency

Heavy metal	Initial metal concentration (mg L^{-1})	Time of reaction (h)	Algae dose (g L^{-1})	Removal (%)		% Error (ε)
				Observed	Predicted	
As	5.03	114	2.96	96.70	96.4	0.3
	5.03	114	2.96	96.51	96.4	0.11
Cd	11.9	109	2.66	96.82	97.4	–0.58
	11.9	109	2.66	97.12	97.4	–0.28
Co	11.5	102	2.87	98.25	99.9	–1.65
	11.5	102	2.87	99.55	99.9	–0.35
Cr	7.75	105	2.51	96.23	95.7	0.53
	7.75	105	2.51	95.82	95.7	0.12
Fe	10.4	118	2.73	99.4	100	–0.6
	10.4	118	2.73	98.21	100	–1.79

case of low initial metal ion concentration, even low mass of sorbent can adsorb it sufficiently, and therefore, increasing the sorbent mass has a marginally positive effect [6,40].

As can be seen in Fig. 3(d), the *C. dispersus* dose had a greater effect than the initial Cr concentration in removal efficiency. Cr removal efficiency increased as the *C. dispersus* dose increased at all applied initial Cr concentrations, reaching their maximum values (86.7% in 2.8 g L^{-1} of *Chroococcus disperses*). Fig. 3(e) shows that an increase in the algae dose causes a significant increase in the percentage of Fe removal.

The interactive effect of time of reaction and *C. dispersus* algae dose on the percentage of heavy metal removal are shown in Fig. 4. As is clear from the 3D plots, heavy metal removals increased sharply when the time of reaction increased in the range of 30–100 h and then remained constant as time increased. Also, heavy metal removal increased as the Algae dose increased, reaching their respective maximum values for As, Cd, Co, Cr, and Fe of 93.9%, 95.8%, 99.6%, 91.4%, and 99%.

Further increase in the algae dose generally does not influence significantly in biosorption efficiency. These observed trends are due to the fact that in higher algae dose, a partial agglomeration of biomass occurs, which decreases surface area-to-volume ratio for biosorption [48]. The kinetics of heavy metal biosorption by live algae commonly occur in two stages: first physical adsorption takes place at the cell surface in a short time, followed by low bioaccumulation through the active transport of intracellular metal complexes and chelation [27,49]. In this study, heavy metal biosorption via *C. dispersus* algae followed first a rapid phase of uptake during the 100 h of exposure and reached a maximum rate of heavy metal removal, after which came a light increase of the amount of total heavy metal removed. A similar pattern in the removal of metals by other species of algae, such as *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Scenedesmus subspicatus*, *Scenedesmus quadricauda*, and *Chlorella vulgaris*, has also been observed [50–52].

The main objective of the purpose of optimization was to determine the optimal values of variables for heavy metal removal using *C. dispersus* algae. The goal in terms of heavy metal removal efficiency was defined as that of maximizing removal efficiency to achieve the highest optimal removal rate. Therefore, two sets of experiments were repeated under optimal conditions to achieve maximum heavy metal removal experimentally (Table 3). As shown in Table 3, the percentage error obtained for this model for the difference between the predicted and the observed values as the optimum values was in the range of 0.11%–1.79%, which was in good agreement with the value predicted from the model.

4. Conclusion

The present study reports the performance of live *C. dispersus* algae in heavy metal removal from aqueous solution using RSM with Box–Behnken statistical analysis. The influence of operational parameters such as the initial metal concentration, *C. dispersus* algae dose, and reaction time was considered. The results revealed that the BBD could be efficiently and successfully applied for the modeling and optimization of the process variables and interactions in the response. The removal efficiency of As, Cd, Cr, Co, and Fe increases in proportion to increases in the dose of algae and time of reaction. Based on the numerical optimization performed by the Design Expert software, under optimum conditions, maximum efficiencies in the removal of As, Cd, Co, Cr, and Fe were 96.7%, 97.12%, 98.55%, 95.82%, and 99.4%, respectively. The main objective of purpose of optimization was to determine the optimal values of variables for heavy metal removal using *C. dispersus* algae. The goal in terms of heavy metal removal efficiency was defined as that of maximizing removal efficiency to achieve the highest optimal removal rate. Therefore, two sets of experiments were repeated under optimal conditions to achieve maximum heavy metal removal experimentally (Table 3). The second-order polynomial equation model, whose validity is agreed upon, was estimated using ANOVA statistical testing as the percentage error obtained for this model for the difference between the predicted and the observed values, which were in the range of 0.11%–1.79%.

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