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Optimization Study on Cr (VI) Removal by Response Surface Methodology using *Artemia franciscana*

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Abstract
Response Surface Methodology was employed in the present investigation to obtain the optimized physical condition for the removal of Cr (VI) from the aqueous solution. The pH was varied from 8–12 and the salinity from 30–50‰. The pH of 10 and salinity 40‰ was found to be optimized physical condition for the 95% of Cr (VI) removal from the aqueous solution using Artemia franciscana by the process of bioaccumulation.

Keywords: *Artemia franciscana, RSM, Cr (VI), bioaccumulation, optimization*

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INTRODUCTION

Heavy metals are highly toxic at low concentrations and can accumulate in living organisms, causing both short-term and long-term adverse effects [1]. Among the various heavy metal ions, chromium is one of the most important heavy metal contaminants in the wastewater of industrial dyes and pigments, film and photography, galvanometry and electric, metal cleaning, plating and electroplating, leather and mining [2]. There exist three oxidation states for chromium in nature, namely Cr(II), Cr(III) and Cr(VI); soluble hexavalent chromium (Cr(VI)) is extremely toxic than the other two species and exhibits carcinogenic effects on biological systems due to strong oxidizing nature [3]. The maximum permissible limit of Cr(VI) in wastewater has been set as 0.05 mg/l by the US Environmental Protection Agency (EPA) and Bureau of Indian Standards (BIS) [4].

The present study illustrates the feasibility of using *Artemia* as a bioaccumulator in reducing the Cr (VI) from the aqueous solution. This crustacean has been widely used since the early 1970s as a test organism in short-term toxicity testing [5, 6]; and able to bioaccumulate quite large amounts of elements from the aquatic environment even when their concentration in this compartment is extremely low [7]. It has the broad tolerance to the environmental factors such as salinity, temperature, and dissolved oxygen in the water [8]. This organism possesses an uncommon adaptability to extreme conditions, thus being found in environments where other life forms are not sustainable [9]. The habitats in which the genus *Artemia* is found are characterized by the absence of predatory animal species. Therefore, in such environment, the evolution of *Artemia* populations is favored by the abundance of bacteria, protozoa and algae that are the basis of the *Artemia* diet [10]. There are several advantages of using *Artemia,* including their ready availability, ease of culturing, low cost and a large literature describing their morphological, biochemical and molecular characteristics [11, 12].

Response Surface methodology (RSM) is essentially a particular set of mathematical and statistical methods for experimental design and evaluating the effects of variables and searching optimum conditions of variables to predict targeted responses [13–15]. Its greatest applications have been in industrial research, particularly in situations where a large number of variables influencing the system feature. The RSM has already been successfully applied in other fields, i.e. food processing, biochemical engineering and adsorption processes for optimization [16–20]. Few researchers have done work on biosorption process optimization using RSM [21, 22]. There is no published report on optimization of bioaccumulation process for removal of heavy metal ion Cr (VI), with *Artemia franciscana* using response surface methodology. It is well suited approach to the study the main and interactive effects of distinct variable and optimization of the process. Response surface methodology was applied to the central-composite design (CCD) experimental design.

So, in the present investigation, the effect of physical parameters on the removal efficiency was observed and the RSM was performed to optimize the physical parameters for the removal of Cr (VI) by the process of bioaccumulation.

MATERIALS AND METHODS

Experimental Procedure

Artemia cysts were collected from the salt pans located at Kelambakkam, 12°47’N 80°South India. The cysts were processed and hatching of the cyst was carried out in the sea water with the 35 ppm concentration with a pH of 8.0–8.5. Exactly 1.00 gm of *Artemia* Cysts is measured out for testing. The viable cysts were hatched out within the 24 h time period. 10 days old *Artemia* has been used for the experimental study.

The Cr (VI) stock solution was prepared by dissolving accurately weighed potassium dichromate in synthetic sea-water. The experimental solutions were obtained by diluting the stock solution in accurate proportions to initial concentrations. The Cr (VI) concentrations were chosen for the experimental condition was 40 ppm respectively. The *Artemia* was introduced into the aqueous solution to the different salinity of 30–50‰ and different pH range of 8–14. Experimental studies were carried out at the room temperature. The aqueous solution was analyzed in atomic absorption spectrophotometer for the reduction of Cr (VI) before and after treatment.

Response Surface Methodology

The RSM package provides functions useful for designing and analyzing experiments that are done sequentially in hopes of optimizing a response surface. The function CCD can generate (and randomize) a central-composite design; it allows the user to specify an aliasing or fractional blocking structure, and does a sanity check to make sure it is suitable for estimating a second-order model.

The central composite design was widely used for fitting a second-order model. By using this method, modeling is possible and it requires only a minimum number of experiments. It is not necessary during the modeling procedure to know the detailed reaction mechanism since the mathematical model is empirical. Generally, the CCD consists of a 2^n factorial run with $2n$ axial runs and *NC* center runs (six replicates). These designs consist of a 2^n factorial or fractional (coded to the usual ±1 notation) augmented by $2n$ axial points (±¿, 0, 0, . . ., 0), (0, ±¿, 0, . . ., 0), . . ., (0, 0, . . ., ±¿), and *nc* center points (0, 0, 0, . . ., 0) [23]. Each variable is investigated at two levels. Meanwhile, as the number of factors, *n*, increases, the number of runs for a complete replicate of the design increases rapidly. In this case, main effects and interactions may be estimated by fractional factorial designs running only a minimum number of experiments. Individual second-order effects cannot be estimated separately by $2n$ factorial designs. Therefore, the central composite design was employed in this study. The responses and the corresponding parameters are modeled and optimized using ANOVA to estimate the statistical parameters by means of response surface methods.

Basically, this optimization process involves three major steps, which are, performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model.

$$Y=f (X_1, X_2, X_3, X_4, \ldots, Xn) \quad (1)$$

Where, *Y* is the response of the system and *Xi* is the variables of action called factors. The goal is to optimize the response variable (*Y*). It is assumed that the independent variables are continuous and controllable by experiments with negligible errors. It is required to find a suitable approximation for the true functional relationship between independent variables and the response surface [24].

The experimental sequence was randomized in order to minimize the effects of the uncontrolled factors. The response was used to develop an empirical model that correlated the response to the adsorption of chromium from aqueous solution using prepared activated carbon in a batch process variable using a second-degree polynomial equation as given by Eq. (2):

$$Y = b'_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n}\sum_{j>1}^{n} b_{ij} X_i X_j \tag{2}$$

Where, Y is the predicted response, b'_0 the constant coefficient, b_i the linear coefficients, b_{ij} the interaction coefficients, b_{ii} the quadratic coefficients and x_i, x_j are the coded values of the adsorption of chromium on prepared activated carbon variables. The number of tests required for the CCD includes the standard 2^n factorial with its origin at the center, $2n$ points fixed axially at a distance, say from the center to generate the quadratic terms, and replicate tests at the center; where n is the number of variables. The axial points are chosen such that they allow readability, which ensures that the variance of the model prediction is constant at all points equidistant from the design center [25]. Replicates of the test at the center are very important as they provide an independent estimate of the experimental error. For four variables, the recommended number of tests at the center is six [26]. Hence, the total number of tests (N) required for the four independent variables is:

$$N=2^n+2n+n_c=2^2+(2\times2)+1=9 \tag{3}$$

Once the desired ranges of values of the variables are defined, they are coded to lie at ±1 for the factorial points, 0 for the center points and $\pm_c$ for the axial points.

RESULTS AND DISCUSSION

Effect of Salinity and pH on the Removal Efficiency

The maximum removal of Cr (VI) was observed under the salinity condition in 40 ppt. The increasing salinity concentration decreases the reduction of Cr (VI). At the 40 ppt, the removal percentage of Cr (VI) was found to be 95% respectively. Whereas, at the salinity condition of 30 and 35‰, the removal percentage of Cr (VI) was found to be 85 and 90% and for higher range, it was found to be 72 and 68% respectively (Figure 1). The optimized pH condition for the present study was found to be 10, it brings out the removal efficiency of 95%, whereas in other pH conditions, the removal efficiency was found to be less (Figure 2).

Response Surface Methodology (RSM)

Analysis of Process Parameters by CCD

Response surface methodology was used to optimize the physical parameters such as salinity and pH for the efficient removal of Cr(VI) from the aqueous solution. The results of 20 runs using CCD are listed in the Table 1. The experimental data were analyzed by regression method and it was performed using the following quadratic polynomial model:

Y=+64.25; Number of days=–0.746; pH=+1.6; salinity=–7.4; Number of days^2=+5.57; pH^2=+1.65; salinity^2=+5.63; No. of days. pH=+1.2; No. of days. Salinity=+0.400; pH Salinity=–1.35.

The model efficiency was checked using analysis of variance (ANOVA) which includes F-test statistical analysis and the results are shown in Table 2. In the model, ANOVA showed the F-value of 1593.89 and a P-value (0.00) which indicates that the model is highly significant. The P-values are used to analyze the significance of each of the coefficients and inevitable to understand the pattern of the mutual interactions between the variables. The smaller the P value, the bigger is the significance of the corresponding coefficient [27]. The coefficient of determination, R^2 is 0.99 signifies that the sample variation of 99.98% for the removal of Cr (VI) from the aqueous solution and it's depends on the independent variables such as salinity and pH. The R^2 (0.99) value stipulates good correlation between the experimental and predicted values. The 2D (contour) plots for the removal efficiency were formulated for different combinations of two factors at one time, while other factors were kept constant.

Model Validation

The competency of the model equation for estimating the optimum response values was proved by using the selected optimal conditions. The contour plots for the removal of Cr (VI) are shown in the Figure 3. From these plots, it was inferred that the maximum removal efficiency of 95% of Cr (VI) was obtained at salinity of 40‰ and pH of 10. The stagnant point conveying a maximum removal efficiency had the following vital values: salinity: 40‰; pH: 10 and number of days: 5.

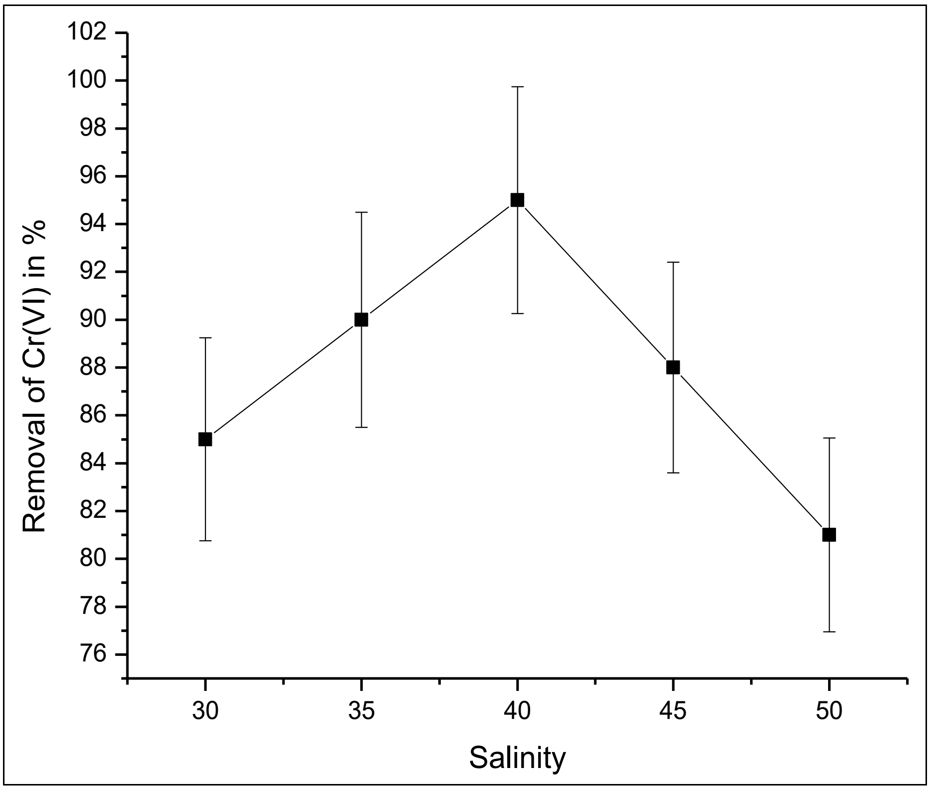

Fig. 1: *Effect of Salinity on the Removal of Cr (VI).*

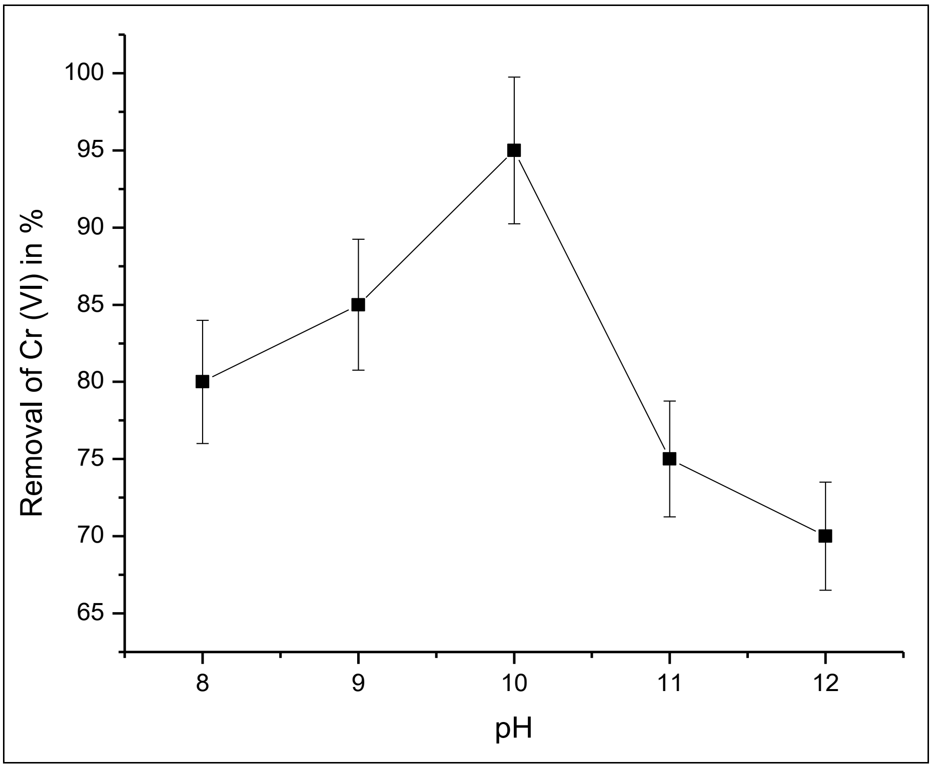

Fig. 2: *Effect of pH On the Removal of Cr (VI).*

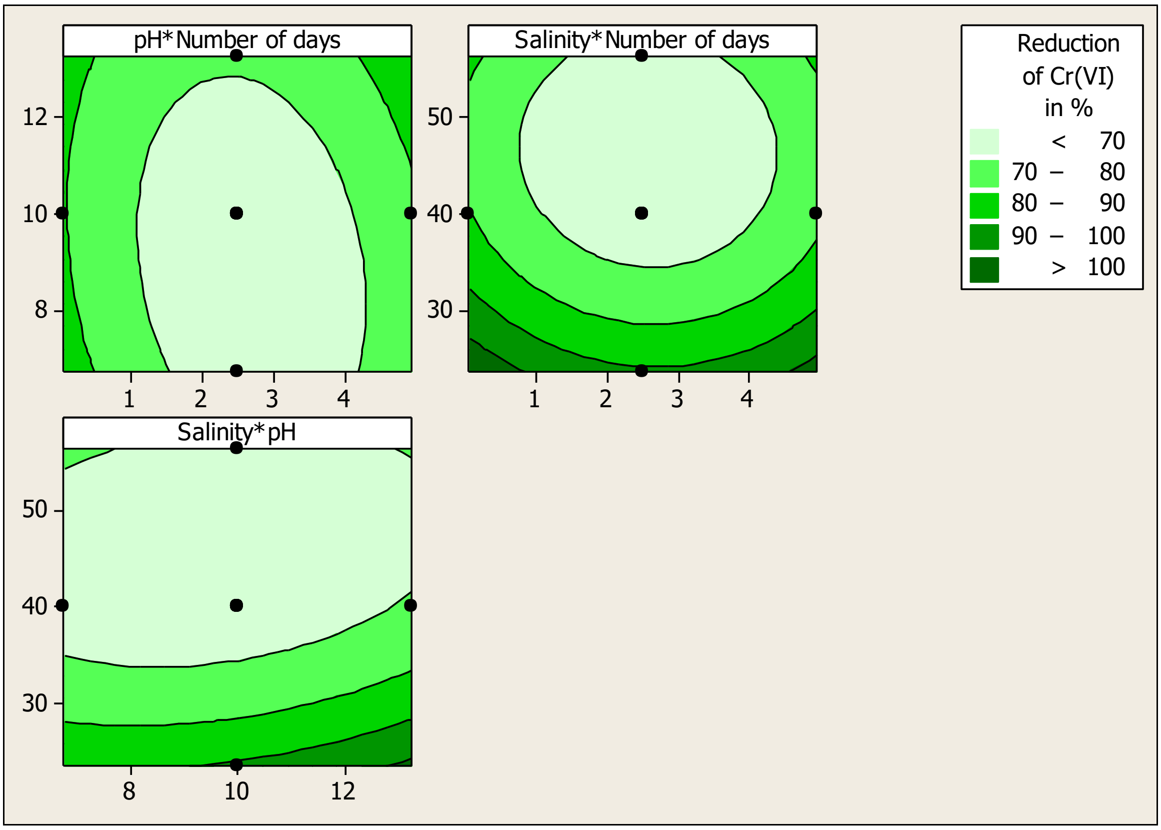

Fig. 3: *Contour Plot for the Reduction of Cr (VI) in % with Optimized pH and Salinity.*

Table 1: *RSM Design for Optimizing Significant Variables for the Removal of Cr (VI).*

Run order	Number of Days	Salinity	pH	Removal Efficiency in % (Experimental)	Removal Efficiency in % (Predicted)
1.	4.0	30.00	12.0	86.2	86.24
2.	1.0	30.00	8.0	82.4	82.49
3.	2.5	40.00	10.0	62.8	62.87
4.	2.5	40.00	10.0	62.8	62.87
5.	1.0	50.00	12.0	68.0	67.77
6.	4.0	50.00	8.0	66.5	66.43
7.	2.5	40.00	6.7	68.2	68.06
8.	4.9	40.00	10.0	80.1	80.02
9.	2.5	40.00	13.26	73.4	73.5
10.	2.5	56.33	10.0	69.0	69.25
11.	2.5	23.67	10.0	93.8	93.5
12.	2.5	40.00	10.0	66.5	66.37
13.	2.5	40.00	10.0	66.2	66.37
14.	0.05	40.00	10.0	82.4	82.46
15.	1.0	30.00	12.00	86.7	86.77
16.	4.0	30.00	8.00	78.2	78.43
17.	2.5	40.00	10.00	63.6	63.50
18.	2.5	40.00	10.00	63.6	63.50
19.	1.0	50.00	8.00	70.2	70.16
20.	4.0	50.00	12.00	70.2	70.11

***Table 2:** Analysis of Variance for the Removal Efficiency of Cr (VI) in %.*

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	1593.89	1593.89	177.09	3616.97	0.00
Linear	3	781.24	781.24	260.41	5318.51	0.00
Square	3	785.27	785.27	261.758	5346.00	0.00
Interaction	3	27.38	27.38	9.12	186.40	0.00
Residual Error	8	0.39	0.39	0.049	-	-
Lack-of-Fit	5	0.35	0.35	0.069	4.62	0.11
Pure Error	3	0.04	0.04	0.015	-	-
Total	19	1644.13	-	-	-	-

Seq SS, Sequential sum of squares; Adj SS, Adjacent sum of squares; Adj MS, Adjacent mean square.

CONCLUSION

It was concluded from the results, the software Response Surface Methodology (RSM) was employed to optimize the physical parameters to enhance the removal efficiency of Cr (VI). The contour plots inferred that salinity of 40‰ and pH of 10 were found optimized condition for the removal 95% of Cr (VI) from the synthetic waste water.

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