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Application of Response Surface Methodology for Optimization of Chromium Biosorption from an Aqueous Solution onto *Syzygium cumini* (java) Seed Powder

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Abstract

The present study was designed to evaluate the interactive effects of pH, initial chromium ion concentration, biosorbent dosage and temperature of the aqueous solution on biosorption of chromium onto *Syzygium cumini* (java) seed powder. Based on four level Central Composite Design (CCD) involving pH from (2-6), initial chromium ion concentration from (10-30) mg/L, biosorbent dosage from (10-70) g/L and temperature of the aqueous solution from (293-313) K, a Response Surface Methodology (RSM) was standardized. The experiments designed by CCD were modeled using a polynomial equation as function of these parameters. Quadratic model proposed in the present study using CCD en suite is well suitable for analysis of experimental data. Further it was used to navigate the design space according to analysis of variance (ANOVA) results. The experimental values were on part with predicted values. Using the above methodology, we observed 90.50% removal of chromium from *Syzygium cumini* (java) seed powder at optimum conditions i.e. pH of 3.877, initial chromium ion concentration of 18.3201 mg/L biosorbent dosage of 36.3788 g/L and temperature of 302.3553 K STATISTICA 6.0 was used for implementing the CCD.

Keywords: *Syzygium cumini* seed powder; Biosorption; Response Surface Methodology; Optimization; Central Composite Design

Introduction

Heavy metal ions such as Cu, Cd, Hg, Zn, Pb, Cr etc., are natural components of the earth's crust and cannot be degraded and have been recognized as ecotoxicological hazards. Their chronic toxicities and ability to accumulate in living organism have been of great interest (Cay et al., 2004). The discharge of heavy metals into aquatic ecosystems is a concern over the last few decades. Effective removal of heavy metals from aqueous waste is one of the most important concerns among the world environment (Loukaidon et al., 2004). Heavy metal ions are commonly removed by chemical precipitation, ion exchange, solvent extraction, adsorption and chemical oxidation-reduction. These are relatively expensive and ineffective especially when the concentration of heavy metal exceeds 1 mg/L (Krauter et al., 1996; Lodeiro et al., 2006). These high-end processes have significant disadvantages including incomplete metal removal, requirement of expensive equipment and monitoring systems, high energy requirements and/or generation of toxic sludge or other waste products that require disposal (Volesky and Holan, 1995). Biosorption is emerging as an effective alternative for the removal of heavy metals from polluted water. Various plant and microorganism including sawdust (Shukla et al., 2002), solid residue of olive mill products (Gharaibeh et al., 1998), straw xanthate (Kumar et al., 2000), yeast (Huang and Morehart, 1990; Volesky et al., 1993), inactivated bacteria (Chang et al., 2003), fungus (Tunali et al., 2006), algae (Vilar et al., 2006) and aquatic plants, seaweeds (Mohanty et al., 2006; Suzuki et al., 2005) have been used as an efficient biosorbents since long time.

Chromium has been considered as the 16th toxic pollutant and it has become a serious health concern (Kowalski, 1994) because of its carcinogenic and teratogenic characteristics. Chromium is mainly released into environment from various industries such as metal

finishing industry, iron and steel industries, inorganic chemical production industry (Dakiky et al., 2002) etc. In addition, extensive use of chromium coated variables results in the accumulation of large quantities of chromium-containing effluents in water bodies which need an exigent treatment.

The conventional method used for optimization is the "one factor at a time" method in which one independent variable is varied while fixing all others at a specific level. This may lead to unreliable results and less accurate conclusions. This method requires a large number of experiments which might result in predicting the false optimum values. These drawbacks can be eliminated by optimizing all the affecting parameters collectively by Central Composite Design (CCD) (Box and Wilson, 1951) using Response Surface Methodology (RSM). A detailed account of this technique has been outlined (Cochran and Cox, 1957). This optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model. This technique has been applied in biosorption studies for optimization of different parameters (Farshid and Habibollah, 2008; Preetha and Viruthagiri, 2007; Kumar and Singh, 2009). The present study also applied CCD to optimize

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chromium biosorption in an aqueous solution onto *Syzygium cumini* (java) seed powder.

Experimentation

Preparation of *Syzygium cumini* seed powder

S cumini seeds were collected from the College of Engineering, Andhra University, Visakhapatnam, India. After thorough washing with deionised water, seeds were dried for 10 days, cut into small pieces, powdered and sieved. In the present study 53µm size seed powder was used as biosorbent without any pretreatment.

Fourier transform infrared spectroscopy

The samples of the *S cumini* before and after biosorption of chromium were dried overnight to remove humidity and encapsulated into dry potassium bromide (KBr) discs. These discs were then scanned into transmission mode using a Fourier transform infrared spectrometer, in a wavelength range of 400 to 4000cm⁻¹.

Metal solution

A standard stock of chromium solution (1000 mg/L) was prepared by dissolving 2.828 g of 99.9 % analytical grade K₂Cr₂O₇ in 1000 mL of distilled water. The working concentration of chromium in the aqueous solution was varied from 20 to 160 mg/L by diluting the chromium stock solution with deionized water. The pH of the working solution was adjusted using either 0.1 N HCL or 0.1N NaOH was added for varying.

Experimental procedure

10 g/L of biosorbent was added to 50 mL of the chromium ion solution in each of 250 mL Erlenmeyer flasks. The flasks were incubated in shaker at a speed of 180 rpm for different agitation times (1, 5, 10, 20, 30, 40, 50, 60, 90 and 120 min). The samples were filtered by Whatman filter paper and analyzed for chromium concentration in an Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer Analyst 200 model). Air-acetylene flame was used at a wave length of 357.87 nm with a slit width of 0.7 nm and sensitivity of 0.078mg/l.

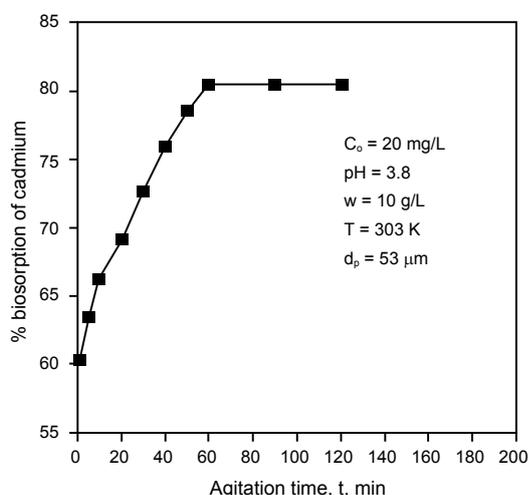


Figure 1: Effect of agitation time on chromium biosorption.

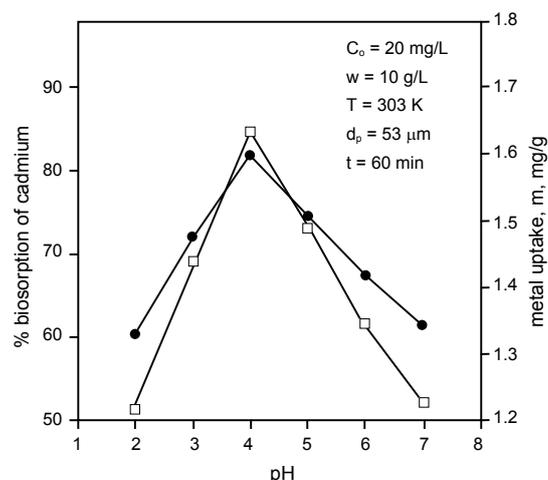


Figure 2: % Biosorption of chromium as a function of pH.

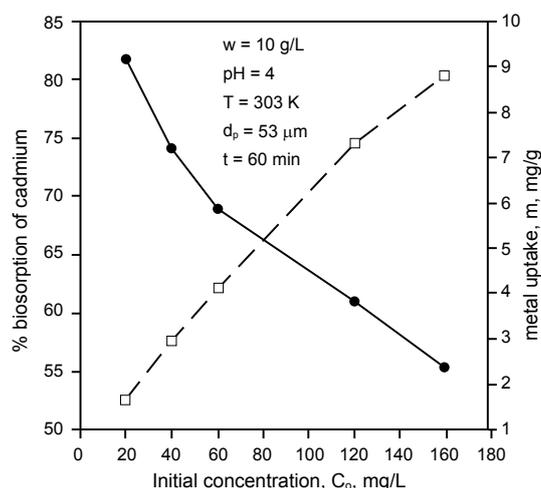


Figure 3: Effect of initial concentration on chromium biosorption.

Chromium biosorption is calculated from the relation $\frac{C_o - C_i}{C_o} \times 100$ where C_o = initial concentration of chromium in the aqueous solution (mg/L)

C_i = final concentration of chromium in the aqueous solution (mg/L)

It is found from Figure 1 that 60 min is the equilibrium agitation time for biosorption of chromium onto *S cumini* seed powder.

Preliminary experimental runs for the biosorption of chromium using *S cumini* seed powder has been developed by assigning four experimental modes, as listed below, varying one parameter and keeping the other three parameters constant for the equilibrium agitation time period of 60 min.

Mode 1: pH was varied as 2, 3, 4, 5, 6, and 7 keeping the initial chromium ion concentration, biosorbent dosage and temperature constant.

Mode 2: Keeping pH, biosorbent dosage and temperature as constant, initial chromium ion concentration was varied as 20 mg/L, 40 mg/L, 60 mg/L, 120 mg/L and 160 mg/L.

Mode 3: Biosorbent dosage was varied as 10 g/L, 20 g/L, 40 g/L and 80 g/L, keeping the other parameters constant.

Mode 4: The data was generated by keeping three variables constant and the temperature of the aqueous solution was varied as 293 K, 303 K, 313 K and 323 K.

Central composite design (CCD)

With the identification of the parameters having the statistically significant influence on the response a CCD (Box and Wilson, 1951) was used to optimize the levels of these parameters. The full CCD, based on three basic principles of an ideal experimental design, primarily consists of (i) a complete 2^n factorial design, where n is the number of test parameters, (ii) n_0 center points ($n_0 \geq 1$) and (iii) two axial points the axis of each design parameters at a distance of $2^{n/4}$ from the design center. The total number of design points is $N = 2^n + 2n + n_0$. For statistical calculations, the parameters X_i are coded as x_i according to Eq. (1):

$$X_i = \frac{X_i - \bar{x}_i}{\Delta x_j} \quad (i=1, 2, 3, \dots, k) \quad (1)$$

where x_i is dimensional value of an independent parameter, X_i is the real value of an independent parameter, \bar{x}_i is the real value of the independent parameter at the center point and Δx_j is the step change. The second degree polynomials (Eq. 2) are calculated with the statistical package STATISTICA 6.0 (Stat-Ease Inc., Tulsa, OK, USA) to estimate the response of the dependent variable:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 \quad (2)$$

where Y is predicted response, X_1, X_2, X_3, X_4 are independent parameter, b_0 is offset term, b_1, b_2, b_3, b_4 are linear effects, $b_{11}, b_{22}, b_{33}, b_{44}$ are squared effects and $b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34}$ are interaction terms.

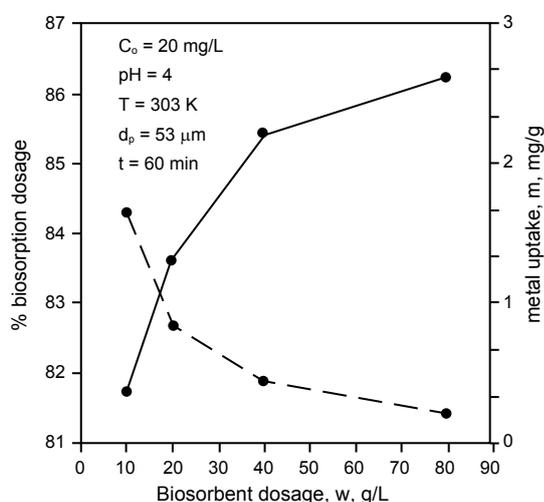


Figure 4: Variation of chromium biosorption with biosorbent dosage.

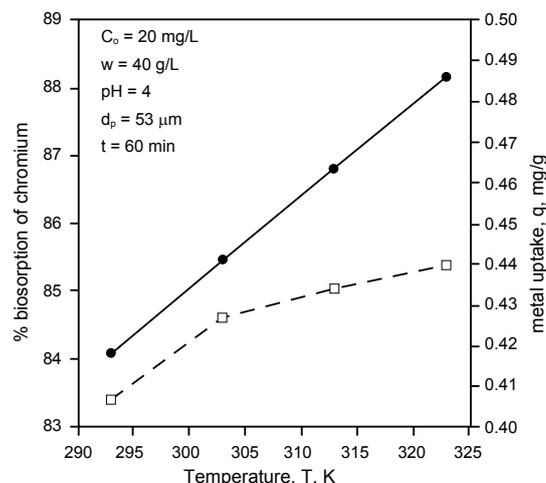


Figure 5: Dependence of temperature on biosorption of chromium.

Results and Discussion

Effect of pH

The effect of pH on % biosorption was investigated and results were shown in Figure 2. It reveals that % biosorption increased from 60.3 % to 81.7 % as pH is increased from 2 to 4 and then decreased beyond pH value of 4 reaching 61.3 % for pH value of 7. Low pH depresses biosorption of chromium, which may be due to competition with H^+ ions for appropriate sites on the biosorbent surface. However, with increasing pH, this competition weakens and chromium ions replaces H^+ ions bound to the biosorbent or forming part of the surface functional groups such as OH, COOH, etc. (Bhattacharya and Sharma, 2004).

Effect of initial chromium concentration

Figure 3 shows the effect of initial chromium concentration in the aqueous solution on the percentage biosorption of chromium. The percentage biosorption of chromium decreased from 81.7 % to 55.12 % as the initial chromium concentration increased from 20 mg/L to 160 mg/L. The decrease in percentage biosorption may be attributed due to lack of sufficient surface area to accumulate much more metal available in the solution.

Effect of biosorbent dosage

The influence of biosorbent dosage in biosorption of chromium is shown in Figure 4. The biosorption of chromium increases from 81.7 % to 86.2 %, with an increase in biosorbent dosage from 10 g/L to 80 g/L. Such behavior is obvious because the number of active sites available for chromium removal would be more.

Effect of temperature

The effect of temperature on biosorption of chromium was studied between 293 K to 323 K and results were depicted in Figure 5. The results show that biosorption increased from 84.1 % to 88.15 % with the rise in temperature.

Independent parameters	Range and level				
	-2	-1	0	+1	+2
pH (X_1)	2	3	4	5	6
Initial concentration (X_2), mg/L	10	15	20	25	30
Adsorbent dosage (X_3), g/L	10	25	40	55	70
Temperature (X_4), K	293	298	303	308	313

Table 1: Experimental range and levels of the independent parameters.

Run no.	Coded values				Real values				% biosorption of chromium	
	x_1	x_2	x_3	x_4	X_1	X_2	X_3	X_4	Experimental	Predicted
1	-1	-1	-1	-1	3	15	25	298	83.96	84.24292
2	-1	-1	-1	1	3	15	25	308	81.68	80.01500
3	-1	-1	1	-1	3	15	55	298	79.89	81.01333
4	-1	-1	1	1	3	15	55	308	77.96	77.77292
5	-1	1	-1	-1	3	25	25	298	73.14	73.16167
6	-1	1	-1	1	3	25	25	308	73.97	75.21625
7	-1	1	1	-1	3	25	55	298	74.42	74.51958
8	-1	1	1	1	3	25	55	308	78.02	77.56167
9	1	-1	-1	-1	5	15	25	298	75.98	77.74167
10	1	-1	-1	1	5	15	25	308	73.05	74.62125
11	1	-1	1	-1	5	15	55	298	73.46	73.88458
12	1	-1	1	1	5	15	55	308	70.47	71.75167
13	1	1	-1	-1	5	25	25	298	73.14	74.99792
14	1	1	-1	1	5	25	25	308	77.98	78.16000
15	1	1	1	-1	5	25	55	298	72.76	75.72833
16	1	1	1	1	5	25	55	308	78.49	79.87792
17	-2	0	0	0	2	20	40	303	62.74	63.99542
18	2	0	0	0	6	20	40	303	64.04	59.81042
19	0	-2	0	0	4	10	40	303	83.78	82.97042
20	0	2	0	0	4	30	40	303	82.18	80.01542
21	0	0	-2	0	4	20	10	303	85.74	84.59875
22	0	0	2	0	4	20	70	303	84.92	83.08708
23	0	0	0	-2	4	20	40	293	83.15	80.36708
24	0	0	0	2	4	20	40	313	80.48	80.28875
25	0	0	0	0	4	20	40	303	90.37	90.27000
26	0	0	0	0	4	20	40	303	90.19	90.27000
27	0	0	0	0	4	20	40	303	90.23	90.27000
28	0	0	0	0	4	20	40	303	89.98	90.27000
29	0	0	0	0	4	20	40	303	90.42	90.27000
30	0	0	0	0	4	20	40	303	90.43	90.27000

X_1 = pH; X_2 = initial concentration; X_3 = biosorbent dosage; X_4 = temperature

Table 2: CCD matrix showing coded and real values along with the experimental values for percentage biosorption of chromium.

Term	Coefficient	Value	Standard error of coefficient	t-value	p-Value
Constant	b_0	-8656.49	1474.300	-5.8716	0.000031 ^a
pH	b_1	30.99	31.784	0.9750	0.345008
pH × pH	b_{11}	-7.09	0.397	-17.8504	0.000000 ^a
Initial concentration	b_2	-17.95	6.357	-2.8241	0.012821 ^a
Initial concentration × initial concentration	b_{22}	-0.09	0.016	-5.5231	0.000058 ^a
Biosorbent dosage	b_3	-0.72	2.116	-0.3381	0.739971
Biosorbent dosage × biosorbent dosage	b_{33}	-0.01	0.002	-4.0443	0.001059 ^a
Temperature	b_4	58.64	9.653	6.0746	0.000021 ^a
Temperature × temperature	b_{44}	-0.10	0.016	-6.2562	0.000015 ^a
pH × initial concentration	b_{12}	0.42	0.104	4.0071	0.001143 ^a
pH × biosorbent dosage	b_{13}	-0.01	0.035	-0.3016	0.767111
pH × temperature	b_{14}	0.06	0.104	0.5323	0.602332
Initial concentration × biosorbent dosage	b_{23}	0.02	0.007	2.2048	0.043496 ^a
Initial concentration × temperature	b_{24}	0.06	0.021	3.0194	0.008625 ^a
Biosorbent dosage × temperature	b_{34}	0.00	0.007	0.4746	0.641907

^a Significant ($p < 0.05$)

Table 3: Coefficients, t-statistics and significance probability of the model.

Optimization of the selected parameters using CCD

The experiments with different pH values of 2–6, different chromium concentrations of 10–30 mg/L, different biosorbent dosages of 10–70 g/L and different temperatures of 293–313 K were coupled to each other and varied simultaneously to cover the combination of parameters in the CCD. The levels and ranges of the chosen independent

Source of variation	Sum of squares (SS)	Degree of freedom (D.F.)	Mean squares (MS)	F-value	Probe>F
Model	1646.457	14	117.6041	27.16478	0.000000
Error	64.939	15	4.3293		
Total	1711.397				

$R = 0.98084$; $R^2 = 0.96205$; Adjusted $R^2 = 0.92663$
 $F_{0.01(14,15)} = S^2_e / S^2 = 27.16478 > F_{0.01(14,15)Tabular} = 3.56$
 $P_{model} > F = 0.000000$

Table 4: ANOVA for the entire quadratic model.

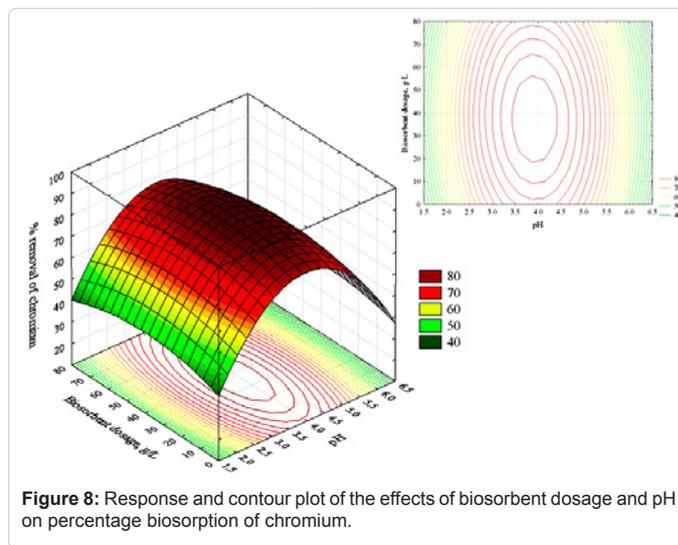


Figure 8: Response and contour plot of the effects of biosorbent dosage and pH on percentage biosorption of chromium.

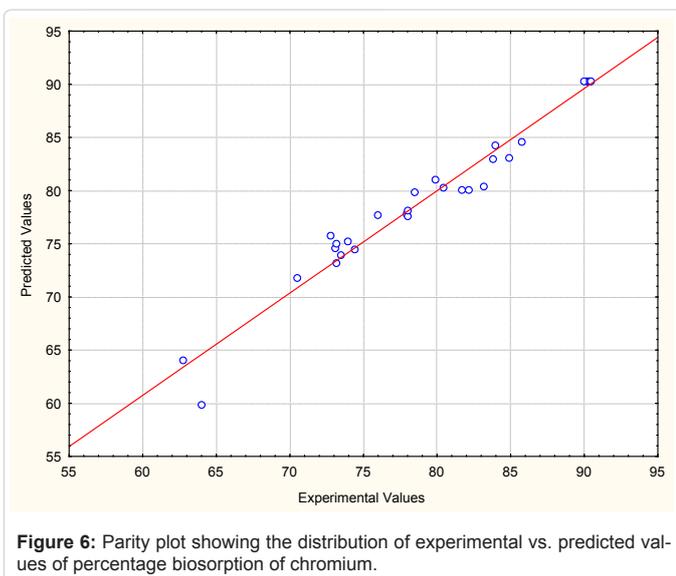


Figure 6: Parity plot showing the distribution of experimental vs. predicted values of percentage biosorption of chromium.

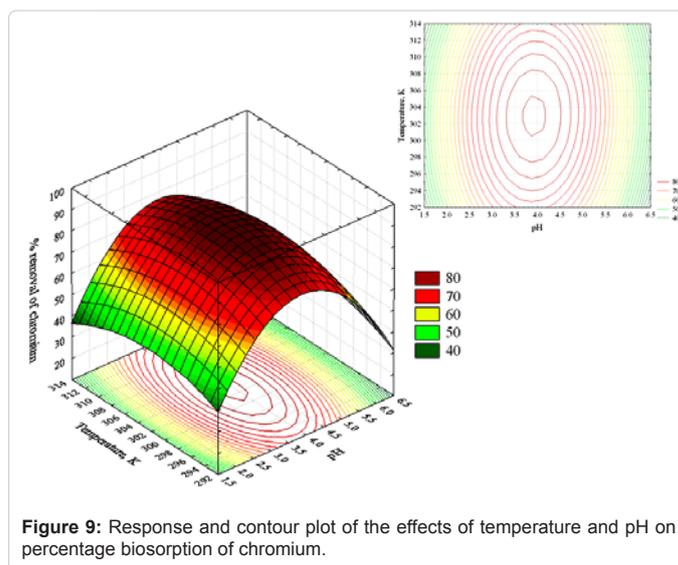


Figure 9: Response and contour plot of the effects of temperature and pH on percentage biosorption of chromium.

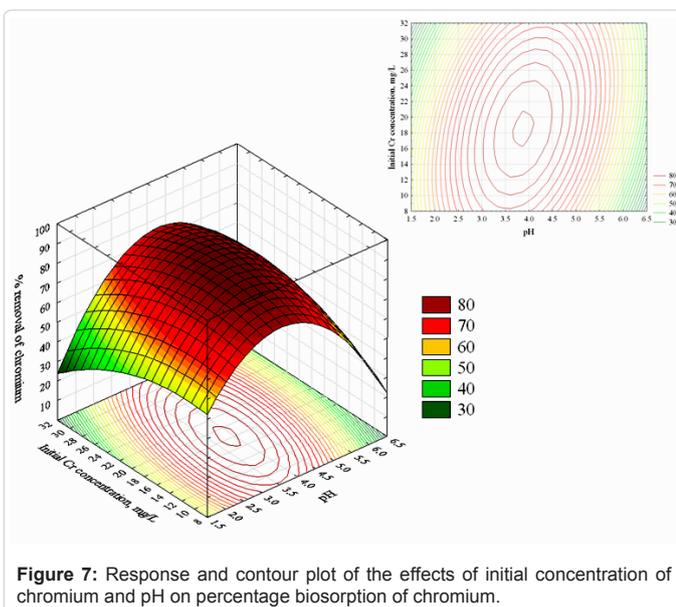


Figure 7: Response and contour plot of the effects of initial concentration of chromium and pH on percentage biosorption of chromium.

parameters used in the experiments for the removal of chromium were given in Table 1. A 2^4 – factorial CCD design, with eight axial points ($\alpha = \sqrt{4}$) and six replications at the center points ($n_0 = 6$) leading to a total number of 30 experiments (Table 2) was employed for the optimization of the parameters. The calculated regression equation for the optimization of medium constituents showed that percentage removal of chromium (Y) was function of the pH (X_1), biosorption dosage (X_2), initial chromium concentration (X_3) and temperature (X_4).

Multiple regression analysis of the experimental data resulted in the following equation for the biosorption of chromium:

$$Y = -8656.49 + 30.99 X_1 - 17.95 X_2 - 0.73 X_3 + 58.64 X_4 - 7.09 X_1^2 - 0.09 X_2^2 - 0.01 X_3^2 - 0.10 X_4^2 + 0.42 X_1 X_2 - 0.01 X_1 X_3 + 0.06 X_1 X_4 + 0.02 X_2 X_3 + 0.06 X_2 X_4 \quad (3)$$

The coefficients of the regression model were calculated and listed in Table 3. They contain one block term, four linear, four quadratic and six interaction terms. The significance of each coefficient was

determined by student's *t*-test and *p*-values and listed in Table 3. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant was the corresponding coefficient. This implies that the linear, quadratic and interaction effects of pH, biosorption dosage, initial concentration of chromium and temperature are highly significant as is evident from their respective *p*-values in (Table 4). The parity plot (Figure 6) showed a satisfactory correlation between the experimental and predicted values of percentage removal of chromium indicating good agreement of model data with the experimental data.

The results of the second order response surface model, fitting in the form of ANOVA were shown in Table 4. The Fisher variance ratio, the *F*-value ($= S_r^2 / S_e^2$), is a statistically valid measure to test the significance and adequacy of the model. The greater the *F*-value above unity, it is more certain that the factors adequately explain the variation in the data about its mean, and the estimated factor effects are real.

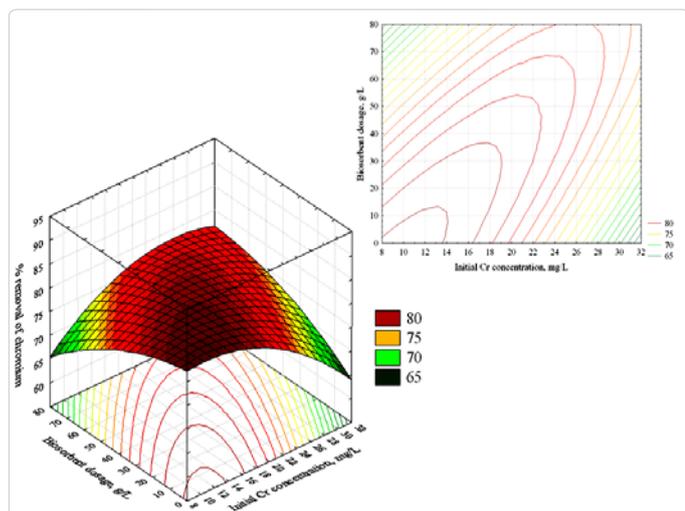


Figure 10: Response and contour plot of the effects of biosorbent dosage and initial concentration of chromium on percentage biosorption of chromium.

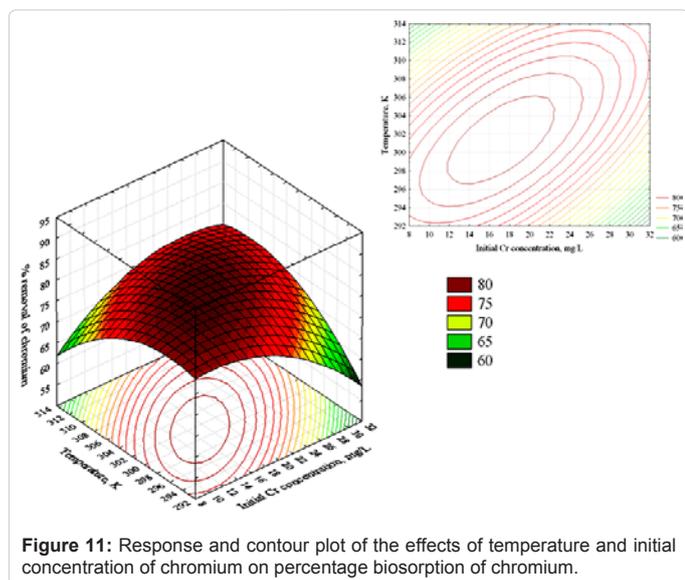


Figure 11: Response and contour plot of the effects of temperature and initial concentration of chromium on percentage biosorption of chromium.

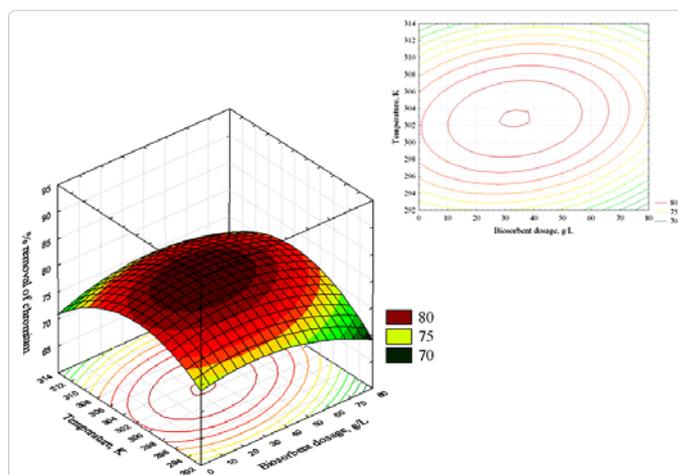


Figure 12: Response and contour plot of the effects of temperature and biosorbent dosage on percentage biosorption of chromium.

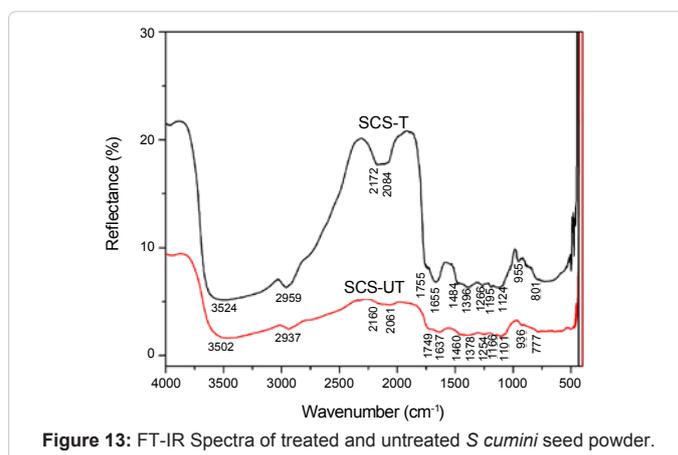


Figure 13: FT-IR Spectra of treated and untreated *S cumini* seed powder.

The ANOVA of the regression model demonstrated that the model was highly significant, as is evident from the Fisher's *F*-test ($F_{\text{model}} = 27.164$) and a very low probability value ($P_{\text{model}} > F = 0.000000$). Moreover, the computed *F*-value ($F_{0.01(14,15)} = S_r^2 / S_e^2 = 27.164$) was greater than the tabular *F*-value ($F_{0.01(14,15)\text{tabular}} = 3.56$) at the 1% level, indicating that the treatment differences were significant.

The correlation coefficient (R^2) provides a measure of the models variability in the observed response values. The closer the R^2 value to 1, the stronger the model is and it predicts the response better. In this present study, the value of the correlation coefficient ($R^2 = 0.9620$) indicated that 96.20 % of the variability in the response could be explained by the model. In addition, the value of the adjusted correlation coefficient ($\text{Adj } R^2 = 0.9266$) was also very high to advocate for a high significance of the model. A higher value of the correlation coefficient ($R = 0.9808$) justified an excellent correlation between the independent parameters.

The response surface contour plots of percentage biosorption of chromium versus the interactive effect of pH, initial chromium concentration, biosorbent dosage and temperature were shown in the Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12.

Each contour plot represents a number of combinations of two test parameters with the other parameter maintained at zero levels. The maximum percentage biosorption of chromium is indicated by the surface confined in the smallest curve (circular or elliptical) of the contour plot. The optimal set of conditions for maximum percentage biosorption of chromium is pH = 3.877, initial chromium concentration = 18.3201 mg/L, biosorbent dosage = 36.3788 g/L, and temperature = 302.3553 K. The extent of biosorption of chromium at these optimum conditions was 90.50 %.

Analysis of the biosorbent

The biosorption mechanism was investigated by using Fourier Transform Infrared Spectroscopy (FT-IR) analysis of *S cumini* and chromium loaded *S cumini*. The FTIR spectrum (Figure 13) of the free biomass exhibit the presence of amine and hydroxyl groups on the surface revealed by strong bands in the range of 3502 cm^{-1} -2937 cm^{-1} . The band at 1749 cm^{-1} suggests the presence of stretching C=O vibrations arising from groups such as lactone, quinine (Serrano et al., 1994) and carboxylic acids (George and McIntyre, 1987). The amide band (Jin and Bai, 2002) (Amide I) stretching mode at 1637 cm^{-1} and shifted to 1655 cm^{-1} . Sulphonamide bands and asymmetric bending of CH_3 group on the surface ligand at 1460 cm^{-1} shifted to 1484 cm^{-1} . The vibration at 1254 cm^{-1} can be attributed to deformation of C=O and stretching formation of -OH of carboxylic acids and phenols (Van der Mass, 1969). The bands at 1166 cm^{-1} and 1101 cm^{-1} bands may be due to presence of >C=S group. It is well indicated from FTIR spectrum of SCS that carboxyl and hydroxyl groups are present in abundance.

After treatment of *S cumini* with chromium the shifting of bands at 3502 cm^{-1} and 2937 cm^{-1} to 3524 cm^{-1} and 2959 cm^{-1} reveal that the involvement of amine and hydroxyl groups. The band at 1749 cm^{-1} shifted to 1755 cm^{-1} due to the involvement of stretching C=O vibrations from lactone, quinine (Serrano et al., 1994) and carboxylic acids (George and McIntyre, 1987). The bands 1460 cm^{-1} , 1378 cm^{-1} shifted to 1484 cm^{-1} , 1396 cm^{-1} noticed that direct involvement of -COO- group in the ion-exchange process. Further peak shifted from 1254 cm^{-1} , 1166 cm^{-1} to 1266 cm^{-1} , 1195 cm^{-1} due to the involvement of C=O and >C=S group respectively. These groups in biopolymers may function as proton donors, hence de-protonated hydroxyl and carboxyl groups may be involved in coordination with metal ions. Some bands in the fingerprint region could be attributed to the phosphate groups.

Conclusions

The present study involves the use of statistical experimental design to optimize process conditions for maximal biosorption of chromium from aqueous solutions. These parameters were optimized using CCD involving RSM. The significant interactions between the four parameters were observed from the response and contour plots. The maximum biosorption of chromium (90.50 %) onto *S cumini* seed powder was observed when the processing parameters were set as follows: pH = 3.877, initial concentration of chromium in aqueous solution = 18.3201 mg/L, biosorbent dosage = 36.3788 g/L and temperature = 302.3553 K. This methodology could therefore be effectively used to study the importance of individual, cumulative and interactive effects of the test parameters in biosorption and other processes.

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