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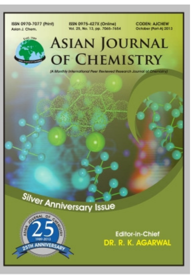

Optimization of Cr(III) Removal from Wastewater Using *Thespesia populnea* Particles by Response Surface Methodology

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In this study response surface methodology involving central composite design was employed to investigate the effects of different operating conditions (pH, biomass dose, biomass particle size and agitation time) on the removal of Cr(III) from wastewater using *Thespesia populnea*. The significance of the independent variables and their interactions were tested by the analysis of variance (ANOVA) and F-test statistics. Optimization of the variables for maximum Cr(III) reduction was performed using the quadratic model. The model predicted maximum reduction capacity of 86 % at the best identified conditions of pH = 2.81, biomass dose concentration of 0.91 g L^{-1}, particle size of 0.250 mm, agitation time of 52 min. The analysis of variance revealed that the quadratic polynomial proposed model demonstrated good agreement with experimental values. The results of this study indicate that *Thespesia populnea* seeds are a viable biosorbent for wastewater treatment.

Key Words: *Thespesia populnea*, Wastewater, Biosorption, Optimization, Response surface methodology.

INTRODUCTION

The heavy metals are pollutants which are non-biodegradable and have bio-accumulation characteristics[1]. Heavy metals vary in their biological function for humans and other organisms, because some of these metals are required in minute concentrations as micro-nutrients for the body to function properly. However, if the threshold limit is exceeded, heavy metals become toxic to the body as they are at the top of the trophic level[2].

The use of Cr(III) has increased in the industries like electroplating, metal finishing, tanning and textiles. The trivalent Cr(III) and hexavalent Cr(VI) species are mostly found in the effluents from these industries and these are very persistent and dangerous in the aquatic environment[3,4]. Chromium is extremely toxic and has carcinogenic and mutagenic activity. Its effect on health includes severe diarrhea, ulcers, eye irritations, skin irritations and kidney dysfunction and lung carcinoma. It, like other heavy metals, also has the potential to cause harmful effects on plants *e.g.*, decrease in plant growth and changes in plant morphology[5].

Many different techniques for the treatment of wastewater containing heavy metals have been developed and used globally. These techniques are divided into two broad categories: abiotic (precipitation, adsorption, ion exchange, membrane) and biotic (bioremediation)[6,7]. The main drawbacks of using abiotic techniques are expense and disposal while biotic techniques may provide sustainable and cost effective solutions and may also be used *in situ*[8-10]. One such biotic technique is the biosorption, which is not to be confused with the bioaccumulation. In the latter living cells are used while in the former the dead or inactive biomass is employed[11-14].

Milo (*Thespesia populnea*), a member of the family Malvaceae and the genus *Thespesia*, is a fast growing small tree. Commonly known as the Portia Tree, *T. populnea* (*T. populnea*), ranging in height from 6-10 m, is one of the best known species for producing useful wood and fibers. It is now widely distributed in coastal areas of the tropics and subtropics[15]. The potential uses of *T. populnea* may vary from food to medicinal and craft timber applications. The plant has been in use for producing rope, dye, mats and is valued as a shade tree and a windbreaker to control soil erosion[16]. To our best of knowledge, no previous literature reports on using *T. populnea* for waste water treatment application.

Response surface methodology (RSM) is essentially a combination of mathematical and statistical methods which can be used for: experimental design; assessment of the effects of the variables; search the optimum condition of variables to predict the targeted response and building models. It is one of the key branches of the experimental design and an important technology in the development of the new processes, optimizing the parameters for better performance, enhancement in designing methodology and synthesis of new products. It is particularly useful in the situation where multiple variables are affecting the process. This feature is basically described as response and usually measured on a continuous scale, which actually is the most important function of the process[17,18]. In literature, there is no published work on the optimization of Cr adsorption using *Thespesia populnea.* Therefore, the aim of this work is to investigate the use of the dried seed capsule of milo (*Thespesia populnea*) as a bio-sorbent for Cr(III) removal. The effect of operating parameters such as (pH; biomass dose; biomass particle size and agitation time) was optimized to maximize the removal of Cr(III) from aqueous using response surface methodology (RSM) approach along with the center composite design (CCD). Responses were best fitted using the ANOVA strategy.

EXPERIMENTAL

Collection of industrial effluents and bio-sorbent: This study was focused on the removal of Cr(III) ions from tannery wastewater. For this purpose tannery industry wastewater was collected from DADA Enterprises, Qasoor, Pakistan. These wastewater samples were stored at dark place in polythene bottles with air-tight lids. *Thespesia populnea* dried seed capsules (fruits) were collected from the University of Karachi, Karachi, Pakistan.

Bio-sorbent preparation: The bio-sorbent material was washed with distilled water three times and it dried at 60 ºC. Then the bio-sorbent was triturated and the particles were sieved to obtain a fraction with particles size 0.25-0.710 mm which were used in the present study. Ten grams of triturated bio-sorbent was dispersed in 50 mL of HNO_3 (1.0 M mol/L) solution and left agitating (120 rpm) for 1 h at room temperature. The material was filtered and washed with distilled water to removal excess acid from the material. Then the material was placed in 100 mL of a NaOH (0.75 M mol/L) solution under agitation (120 rpm) for 1 h at room temperature. The bio-sorbent was filtered and washed again repeatedly with distilled water. The material was dried at 60 ºC and stored in plastic bottles with airtight lids until further analysis[19].

Batch biosorption studies: Batch biosorption experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of industrial wastewater. Then pre-determined amount of bio-sorbent according to experimental design was added into the flasks containing the wastewater sample. Then flasks were agitated on an orbital shaker at room temperature for pre-determined time according to the experimental design at speed of 200 rpm. Then samples were filtered by using Whatman # 1 filter paper and the heavy metal ion concentrations remaining in supernatant were analyzed. Experiments were repeated for different initial pH, agitation time and bio-sorbent concentration values. The same procedure is used for the industrial effluents as proposed by Ozer *et al.*[20].

The residual Cr(III) concentrations in the properly digested samples of supernatant and untreated solutions were analyzed by using atomic absorption spectrophotometer with specific hollow cathode lamp for the respective metals, as suggested by Akan *et al.*[21]. First of all standard solutions of Cr(III), then a blank was analyzed. After the blank, unknown samples were analyzed. The absorbance values of treated samples were compared to the untreated original industrial wastewater samples.

The removal of metal was determined according to:

$$R\,(\%) = \frac{C_o - C_t}{C_o} \times 100 \quad (1)$$

where: R is the percentage of heavy metal adsorbed by biomass, C_o is the initial concentration of metal ions in mg L^{-1} and C_t is the concentration of metal ions at time t (equilibrium) in mg L^{-1}. At equilibrium, metal uptake was determined according to:

$$q = \frac{V(C_i - C_e)}{S} \times 100 \quad (2)$$

where: q is the metal uptake in mg/g biomass, V is the volume of metal-containing solution in contact with the bio-sorbent in mL, C_i and C_e are the initial and equilibrium (residual) concentration of metal in the solution in mg L^{-1}, respectively and S is the amount of added bio-sorbent on dry basis in "g"[22].

Experimental design and optimization: In the present study, central composite design (CCD) was used for the response surface methodology (RSM) in the experimental design, which is well suited for fitting a quadratic surface and normally works well for the process optimization. The CCD is an effective design, *i.e.*, it is ideal for sequential experimentation and allows a reasonable amount of information for testing the lack of fit, while not involving an unusually large number of design points[17,18].

Response surface methodology was used to evaluate the influence of three process factors: dose of bio-sorbent, agitation time and pH of the solution at fixed particle size. The factorial level was chosen by considering the properties of the reactants. In present study the particle size was fixed and two particle sizes (0.250 and 0.710 mm) were used. Three factors varied by selecting each particle size. Therefore, CCD with three factors was applied using Design Expert 7.0 software[20]. Dose of bio-sorbent: agitation time: pH of the solution varied over the following ranges 0.5-2.5 g L^{-1}: 20-60 min: 2-6, respectively as shown in the Table-1.

TABLE-1
FACTORS USED FOR THE REMOVALOF
Cr(III) USING *Thespesia populnea*

Variable	Range
Bio-sorbent concentration (g L^{-1})	0.5-2.5
Agitation time (min)	20-60
Initial pH	2-6

Using a statistical analysis used by Ozer *et al.*[20], response surface methodology by regression analysis was carried out to determine the coefficients of a second-degree polynomial of the form:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=i}^{k} \beta_{ij} x_i x_j \qquad (3)$$

where: y is the predicted response, b_0 is the intercept, b_i, b_{ij} are the coefficients of the linear, square and interaction effects and x_i, x_j are the coded variables. The performance of the model was evaluated by analysis of variance (ANOVA), which included the Fisher's F-test (overall model significance), its associated probability $p > F$, the determination coefficient R^2 and the lack of fit. The larger the F-values and *p*-values, the more significant is the corresponding coefficient. The second-order polynomial models were represented as response surface plots that show the effect of two variables on the response, whilst keeping constant the third variable[20].

RESULTS AND DISCUSSION

In this study, biomass of *Thespesia populnea* (0.250 and 0.710 mm particle size) was used for the removal of chromium ions from the effluent of tannery industry. Three parameters (initial pH. biomass concentration and agitation time) were employed in the optimization study using response surface methodology.

Response surface methodology for optimization of Cr(III) biosorption: Earlier information and understanding of the process and its variables under study are necessary for achieving a more practical model. On the basis of previous literature reports on biosorption study the range and level of variables were used in the present experimental design[22-24]. Response surface methodology with central composite design was used to analyze the optimum condition of higher biosorption efficiency for the selected variables. The experiments for Cr(III) removal were carried out with 2^3 factorial designs as per central composite design and the maximum metal uptake rate obtained. The predicted and actual percentage removals after biosorption using 0.250 and 0.710 mm particle sizes with 20 experiments in replicate are shown in Tables 2 and 3, respectively. The application of response surface methodology for the removal of chromium using 0.250 and 0.710 mm particle sizes of biomass expressed in the following regression equations.

Eqns. 4 and 5, respectively, are empirical relationship between metal uptake (q) and tested variables within the coded unit, using 0.250 and 0.710 mm particle sizes of bio-sorbent.

$$q = 34.13 - 0.27A^2 - 4.03B^2 - 0.003C^2 + 7.72A + 20.35B + 1.04C - 2.00AB - 0.13AC - 0.08BC \qquad (4)$$

$$q = 90.16 + 0.21A^2 + 1.79B^2 + 0.004C^2 - 6.85A - 19.20B + 0.12C + 2.52AB - 0.06AC + 0.06BC \qquad (5)$$

where: q is the response, *i.e.*, metal uptake and A, B and C are the coded values of the main effects initial pH, bio-sorbent dose concentration and agitation time, respectively. Whereas the variables AB, AC and BC represent the interaction effect of initial pH bio-sorbent dose concentration, initial pH agitation time and bio-sorbent dose concentration agitation time, respectively. A^2, B^2 and C^2 are the measures of the square effect of variables initial pH, bio-sorbent dose concentration and agitation time, respectively. The importance of the individual coefficient in the eqns. 4 and 5 was established by F-values and P-value. The F-value is the test for comparison of the curvature against the residual variance. If this difference is similar, *i.e.*, a ratio of nearly one, the curvature is less likely to be significant. *p*-value is the probability of seeing the observed F-value if the null hypothesis is true. Small probability values call for rejection of the hypothesis that the curvature is not significant. The actual and the predicted Cr(III) capacity of the biosorbent are shown in Fig. 1 (for the 0.250 mm particle size) and in Fig. 2 (for the 0.710 mm particle size). Actual values are the measured response data for a particular run and the predicted values are evaluated using the approximating functions.

TABLE-2
EXPERIMENTAL DESIGN BASED ON CENTRAL COMPOSITE DESIGN (CCD)
FOR THE REMOVAL OF Cr(III) (0.25 mm PARTICLE SIZE)

Run	Independent values			Responses		
	pH	Biomass concentration (g L^{-1})	Agitation time (min)	Removal (%)	Predicted	Residual
1	2.81	0.91	28.11	80.30	78.4646	1.8354
2	5.19	0.91	28.11	79.29	78.8174	0.4726
3	2.81	2.91	28.11	78.34	79.087	-0.747
4	5.19	2.1	28.11	74.04	73.7799	0.2601
5	2.81	0.91	51.9	86.52	87.2105	-0.69
6	5.19	0.91	51.9	80.57	80.2533	0.3167
7	2.81	2.1	51.9	84.80	85.7029	-0.903
8	5.19	2.1	51.9	70.82	73.0858	-2.266
9	2.00	1.5	40	85.96	85.8672	0.0928
10	6.00	1.5	40	76.07	75.5542	0.5158
11	4.00	0.5	40	79.14	80.4976	-1.358
12	4.00	2.5	40	76.96	74.9938	1.9662
13	4.00	1.5	20	75.90	77.1903	-1.29
14	4.00	1.5	60	85.86	83.9611	1.8989
15	4.00	1.5	40	82.55	81.7741	0.7759
16	4.00	1.5	40	81.80	81.7741	0.0259
17	4.00	1.5	40	82.14	81.7741	0.3659
18	4.00	1.5	40	81.37	81.7741	-0.404
19	4.00	1.5	40	81.05	81.7741	-0.724
20	4.00	1.5	40	81.63	81.7741	-0.144

TABLE-3
EXPERIMENTAL DESIGN BASED ON CENTRAL COMPOSITE DESIGN (CCD)
FOR THE REMOVAL OF Cr(III) (0.71 mm PARTICLE SIZE)

Run	Independent values			Responses		
	pH	Biomass concentration (g L^{-1})	Agitation time (min)	Removal (%)	Predicted	Residual
1	2.81	0.91	28.11	64.20	64.8605	-0.66
2	5.19	0.91	28.11	55.20	55.7592	-0.559
3	2.81	2.91	28.11	62.91	63.461	-0.551
4	5.19	2.1	28.11	57.09	54.3597	2.7303
5	2.81	0.91	51.9	76.27	72.6673	3.6027
6	5.19	0.91	51.9	59.9	63.566	-3.666
7	2.81	2.1	51.9	72.86	71.2678	1.5922
8	5.19	2.1	51.9	67.55	62.1666	5.3835
9	2.00	1.5	40	70.22	71.1667	-0.947
10	6.00	1.5	40	54.97	55.8603	-0.89
11	4.00	0.5	40	67.83	64.6903	3.1397
12	4.00	2.5	40	59.27	62.3367	-3.067
13	4.00	1.5	20	58.74	56.9488	1.7912
14	4.00	1.5	60	68.33	70.0782	-1.748
15	4.00	1.5	40	61.95	63.5135	-1.564
16	4.00	1.5	40	63.47	63.5135	-0.044
17	4.00	1.5	40	63.07	63.5135	-0.444
18	4.00	1.5	40	62.28	63.5135	-1.234
19	4.00	1.5	40	62.61	63.5135	-0.904
20	4.00	1.5	40	61.55	63.5135	-1.964

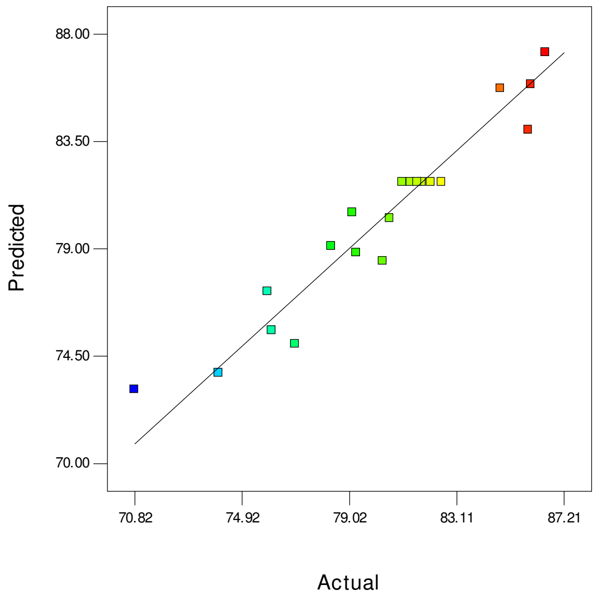


Fig. 1. Parity plot comparing actual Cr(III) uptake with the predicted values (using 0.250 mm particle size)

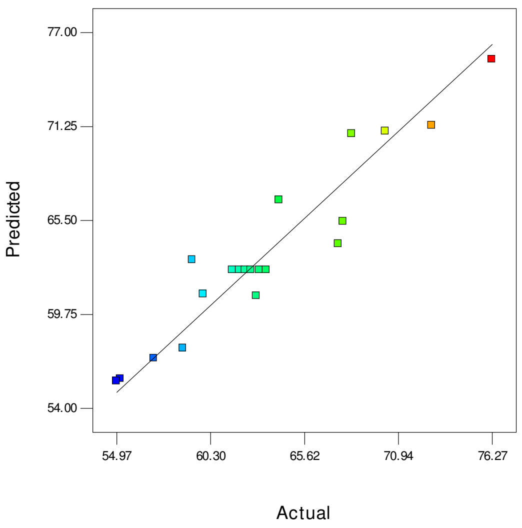


Fig. 2. Parity plot comparing actual Cr(III) uptake with the predicted values (using 0.710 mm particle size)

TABLE-4
ANOVA RESULTS OF THE QUADRATIC MODELS
OF FINAL Cr(III) UPTAKE CAPACITY OF BIO-SORBENT
(0.250 mm PARTICLE SIZE)

SOV[1]	SS[2]	df[3]	MS[4]	F-value	P-value
Model	296.3	9	32.9	14.05	0.0001
Residual	23.44	10	2.34	–	–
Lack of fit	22.00	5	4.40	15.25	0.0048
Pure error	1.443	5	0.289	–	–
Cor total	319.8	19	–	–	–

[1]SOV = source of variations; [2]SS = sum of squares; [3]df = degree of freedom; [4]MS = mean sum of square.

The results of second-order response surface model in the form of analysis of variance (ANOVA) for chromium (at 0.250 and 0.710 mm) are shown in Tables 4 and 5, respectively. The statistical significance of the model equation was evaluated by the F-test ANOVA. The significance of each coefficient was determined by F-values and *p*-values. The results indicate a small probability value ($p < 0.0001$), which signifies that the individual terms in the predicted model have significant effect.

Optimization of experimental parameters: The pH of the solution and biomass loading play a significant function in heavy metal biosorption, moreover the combination of both effects greatly influence in Cr(III) ion removal. In Fig. 3a showed that the percentage of the removal of Cr(III) ions increased with the increase in solution pH from 2.81-3.41. The biosorption efficiency lowers, as the pH of solution increased more than 4.0, which correlates with previous investigations[25].

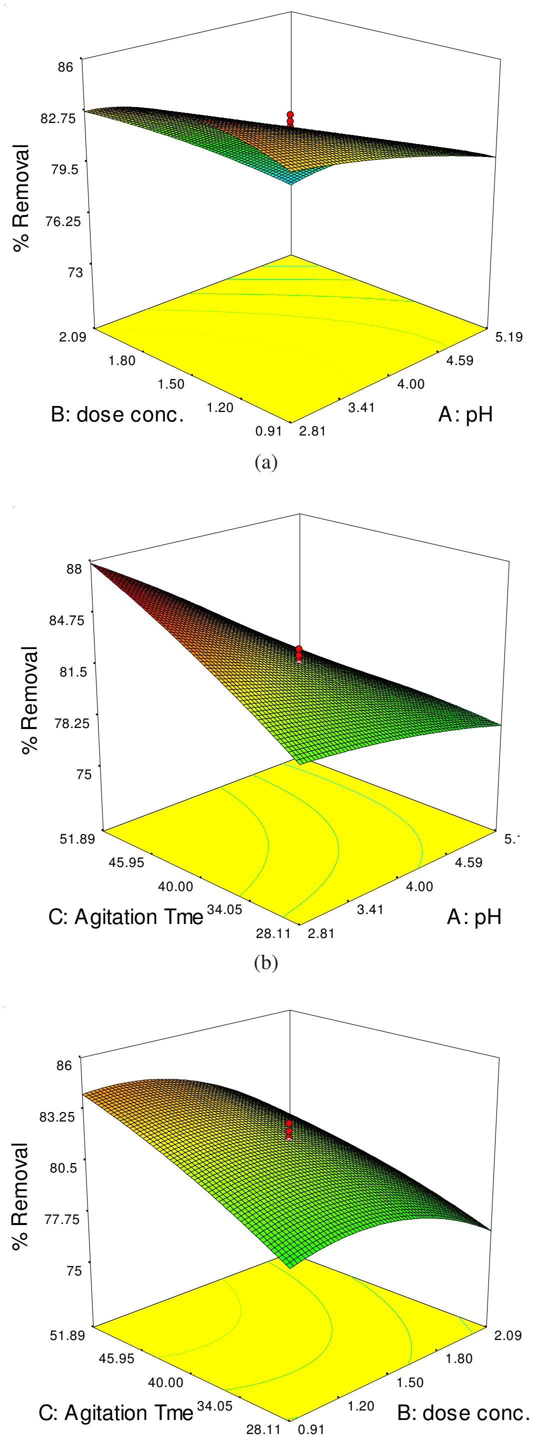


Fig. 3. Effect of (a) pH and biomass loading; (b) pH and agitation time; (c) dose concentration and agitation time on Cr(III) removal using biomass of 0.250 mm particle size

Vilar and Botelho[26] suggested that a possible reason is that increase in the pH caused precipitation of metal ions and thus lowered the biosorption efficiency.

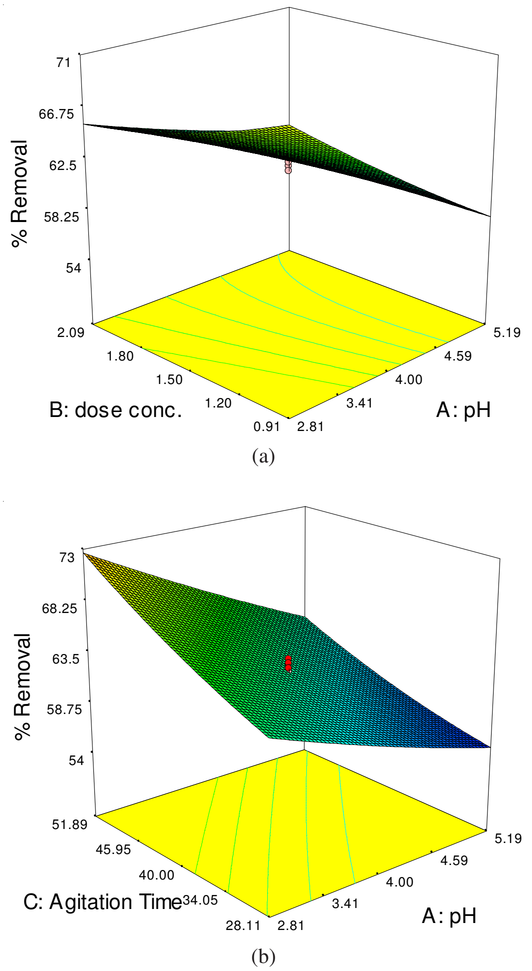
TABLE-5
ANOVA RESULTS OF THE QUADRATIC MODELS
OF FINAL Cr(III) UPTAKE CAPACITY OF BIO-SORBENT
(0.710 mm PARTICLE SIZE)

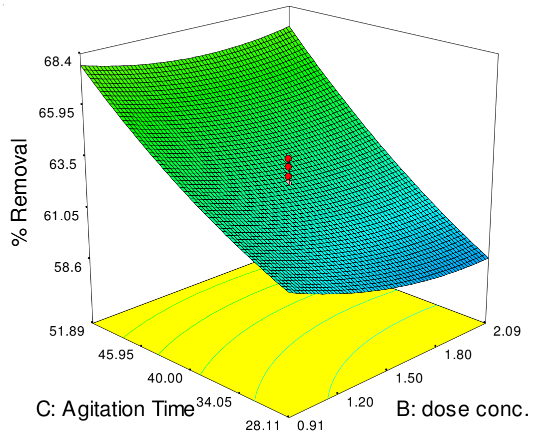
SOV[1]	SS[2]	df[3]	MS[4]	F-Value	*p*-Value
Model	541.4	9	60.2	10.25	0.0006
Residual	58.67	10	5.87	–	–
Lack of fit	56.14	5	11.2	22.18	0.002
Pure error	2.530	5	0.506	–	–
Cor total	600.1	19	–	–	–

[1]SOV = source of variations; [2]SS = sum of squares; [3]df = degree of freedom; [4]MS = mean sum of square.

The interaction between agitation time and solution pH is shown in Fig. 3b. It is observed from Fig. 3b that the efficiency of the removal of Cr(III) ion increases with increase in agitation time. Whereas, increase in the pH also showed some influence on the removal of Cr(III) ions. The interaction between agitation time and biomass loading is illustrated in Fig. 3c. It shows that for a higher biomass loading *i.e.*, more than 1.5 g L^{-1}, the percentage of the removal of Cr(III) ions was more than 70 %. Similar findings were also reported[27,28] stating that increase in biomass loading will increase the removal of lead ion. The reason being the availability of more binding sites for the biosorption within the bio-sorbent. Fig. 3c also showed an increase in Cr(III) removal with the increase of agitation time.

Fig. 4a illustrates that the percentage of Cr(III) ions removal decreased with the increase in solution pH and biomass dose concentration. The possible reason is that increase in the pH may cause precipitation of metal ions thus lower the biosorption efficiency[26]. Fig. 4b shows the interaction between agitation time and solution pH. It is observed from data that efficiency of Cr(III) ions removal increases with the increase in the agitation time and decreases with increase in the pH, again these results correlate with previous researchers[26,27].





(c)

Fig. 4. Effect of (a) pH and biomass loading; (b) pH and agitation time; (c) dose concentration and agitation time on Cr (III) removal using biomass of 0.710 mm particle size

The interaction in Fig. 4c shows that for a biomass loading less than 1.7 g L^{-1}, the percentage of Cr(III) ions removal was low, however this increased with biomass dose till 2.09 g L^{-1}. It was also noted that Cr(III) ions removal increased with the increase in the agitation time until an equilibrium was achieved[28-31].

Conclusion

The present investigation was carried out to investigate the feasibility of using *Thespesia populnea* as a biosorbent for Cr(III) removal from wastewater. The effect of four operational parameters (pH; biomass dose; biomass particle size; and agitation time) was evaluated by using response surface methodological approach involving central composite design (CCD). The model predicted maximum reduction capacity of 86 % at the best identified conditions of pH = 2.81, biomass dose concentration of 0.91 g L^{-1}, particle size of 0.250 mm, agitation time of 52 min. The analysis of variance revealed that the proposed quadratic polynomial model showed good agreement with the experimental data.

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