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ORIGINAL RESEARCH PAPER

Optimization of chromium(VI) biosorption using gooseberry seeds by response surface methodology

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ABSTRACT: The current investigation presents the role of gooseberry (*Phyllanthus acidus*) seeds as an effective biosorbent for remediating chromium(VI)), a toxic heavy metal pollutant commonly found in effluents from tanneries and relevant industries. Biosorption was affected by pH, temperature and initial metal concentration. Furthermore, there is a need to understand the holistic effect of all variables to ascertain the best possible conditions for adsorption, therefore, these factors were considered and a total of 17 trials were run according to the Box Behnken design. Quadratic model had maximum R^2 value (0.9984) and larger F value (1109.92). From the Analysis Of Variance table and R^2 value, quadratic model was predicted to be the significant model with the best fit to the generated experimental data. The optimal parameters obtained from the contour plot for the maximum removal of chromium(VI) were initial metal concentration of 60 mg/L, pH value of 2, and temperature of 27°C. Under these conditions, maximum removal of 92% was obtained. Thus this biosorbent substantially eliminates chromium(VI) under optimized conditions, enabling its use in larger scale.

KEYWORDS: *Analysis Of Variance (ANOVA); Biosorbent; Box behnken design; Chromium(VI); Gooseberry seed; Optimal.*

INTRODUCTION

Heavy metals such as chromium, nickel, cadmium, copper etc., are discharged at significant levels in to aquatic and agricultural ecosystems due to rapid industrialization, posing a serious threat to human health and environment (Das *et al*., 2007). Further many anthropogenic activities involving mining, tanning and electroplating industrial generate effluents containing chromium as a lead toxic pollutant in the effluents from such industries (Raji and Anirudhan 1998; Cheung and Gu, 2007). Due to its carcinogenic and toxicological consequences, it leads to lung cancer, liver damage, ulceration of septum, pulmonary congestion, weakened immune response and death (Rao and Prabakhar 2011). The acceptable and permissible limits in discharges according to United States Environmental Protection Agency (USEPA) are 0.1 and 0.05 mg/L Cr(VI) in surface and portable water respectively (EPA,1990). Thus, it necessitates the need to reduce the level of Cr(VI) before environmental discharge.

Common technique for residual heavy metal removal from waste and waste water includes many methods either involving chemical via, chemical precipitation, coagulation or physical separation methods via, electrochemical, floatation, ion exchange, membrane filtration and oxidation-reduction methods. However certain factors such as energy requirements, operational complexities, operational cost and sludge deposition should be taken into consideration while selecting a method for the treatment of waste water at large scale (Kundu and Gupta 2005). This is particularly essential for eventual application in industries which

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handle waste in large scale (Goyal *et al.,* 2011). The most economically viable technique for the sequestration of heavy metal points towards adsorption using bio resource materials especially waste material from agriculture residues (Cheung and Gu 2007; Salman *et al*., 2014) and plants (Yu and Gu 2007; Yu *et al.,* 2007).

Agricultural waste residues are considered as important source of biosorbents due to the presence of certain functional groups such as hydroxyl, carboxyl, amino, ester, sulphydryl, carbonyl and phosphor group (Aravind *et al*., 2015). Agricultural waste residues such as rice husk, wheat bran, orange peel, watermelon shell, pineapple peel, lemon residues had been extensively studied in past for their adsorption efficiency (George *et al*., 2013). Bioresource material showing high adsorption capacity and metal selectivity had been suggested as suitable biosorbent for heavy metal sequestration (Anupam *et al*., 2011; Nguyen *et al.,* 2013). Further, Gooseberry seed (*Phyllanthus acidus*), a novel biosorbent was examined by varying one factor at a time (OFAT) for Cr (VI) binding efficiency (Aravind *et al*., 2015), with a scope to optimize the parameters and to understand the interactions between the factor impacting overall adsorption efficiency.

Response Surface Methodology (RSM) could be employed as a statistical tool to optimize and study interactions (Ariffin *et al*., 2008).RSM, a fractional factorial design involves a set of designed experiment to obtain optimal response. In addition the relative significance of various factors involved in complex interactions can be evaluated (Sahu *et al.,* 2009). Nowadays, these techniques are widely employed for optimization of number of processes including adsorption studies (Rene *et al*., 2007; Özdemer *et al.,*2011). In the present work, influence of operating parameters such as pH, contact time, adsorbent dosage and initial metal concentration for the removal of Cr(VI) onto Gooseberry seed powder were investigated in batch mode. Process optimization has been carried out by employing BBD of RSM for the search of optimum parameter for the maximum removal of Cr (VI).The results reported in this article were part of work done at Coimbatore, India, from the period December 2014 to April 2015.

MATERIALS AND METHODS

Biosorbent preparation from gooseberry seed

Gooseberry seeds were collected from Coimbatore, Southern part of Tamilnadu. The seeds were washed under running tap water to remove the dirt and other particulates. It was subjected to drying at 40°C for 24 h. Then the seeds were finely ground and sieved. The finely powdered biosorbent was subjected to washing. After washing with distilled water, the mixture was filtered and dried at 50°C for a period of 1 h. The dried powder was stored in an air tight container to prevent moisture. The dried powder was used as a biosorbent for all the experiment.

Preparation of synthetic metal solution

Stock solution of 1000 mg/L was prepared using Potassium dichromate crystals. pH of the solution was adjusted using 0.5N NaOH or H_2SO_4. Fresh dilutions of 100mg/l were prepared from the stock solutions for each study.

Table 1: Experimental design matrix with response

		Factor 1	Factor 2	Factor 3	Response 1
SD	Run	A: pH	B: Temperature (°C)	C: Initial metal concentration (mg/L)	Removal percent (%)
5	1	2	42	20	89.89
6	2	6	42	20	16.66
9	3	4	27	20	30.71
16	4	4	42	60	23.08
8	5	6	42	100	9.09
4	6	6	57	60	7.69
15	7	4	42	60	25
1	8	2	27	60	91.26
2	9	6	27	60	14.28
12	10	4	57	100	20
17	11	4	42	60	22.22
7	12	2	42	100	86.27
14	13	4	42	60	25
10	14	4	57	20	26.47
11	15	4	27	100	27.27
13	16	4	42	60	23.07
3	17	2	57	60	81.18

Chromium analysis

Chromium was estimated using 1, 5 Diphenyl carbazide as a complexing agent spectrophotometrically. Different standards containing less than 100 mg/L (20, 40, 60, 80, and 100) were prepared and maintained at pH less than 2. To 10ml of standard, 0.2ml of diphenyl carbazide was added as a complexing agent. The solution was incubated until red violet color was developed. The absorbance was measured spectrophotometrically at 540nm. A blank was prepared for Cr (VI) analysis. The amount of chromium present in the sample was determined from calibrated curve according to the standard method (American Public Health Association (APHA)., 2005).

Removal efficiency

The percentage removal of chromium (R %) was determined using the equation 1:

$$\text{Removal efficiency (R \%)} = \frac{C_i - C_0}{C_i} \times 100 \quad (1)$$

C_i and C_0 represents the initial and final concentration of chromium metal in mg/L.

Experimental design

RSM is a collection of mathematical and statistical techniques employed to optimize the operating parameters for the maximum removal of Cr(VI). The process parameters affecting the removal of Cr(VI) were studied by means of a three level BBD. Experimental design was created using Design Expert 9.0.3.1 software. The main objective behind design of experiments is to optimize a response and to determine the relationship between a response (output variable) and the interactive effects of independent variables (input variables) (Montgomery 1997; Lee *et al*., 2000).

The variable input parameters were pH values in the range of 2-6, initial metal concentrations of 20-100 mg/L and temperature in the range of 27-57° C. The three independent variables were designated as A (pH), B (Temperature), C (Initial metal concentration) respectively for statistical analysis. Three basic steps involved in optimization process are to perform statistically designed experiment, to determine the coefficient estimate, to analyze the response and to check the adequate model (Wang *et al*., 2010; Arulkumar *et al*., 2012).

A total of 17 trials were run in order to optimize the parameter at which the maximum removal was obtained. Investigations over different tests such as sequential model sum of squares, lack of fit tests, model summary statistics helps in selecting the best model for describing the relationship between the response and other influencing independent variable. Regression analysis and Analysis Of Variance response were also employed to analyze the result.

In order to fit the generated experimental data and to identify the relevant model terms, the most widely used second order polynomial equation can be represented as equation 2:

$$Y = \beta_o + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_{ii}^2 + \Sigma\beta_{ij} X_i X_j + \epsilon \quad (2)$$

Where Y is the predicted response (the percentage removal of Cr(VI)),β_0 is the constant coefficient, β_i is the linear coefficient of the input factor X_i, β_{ii} is the ith quadratic coefficient of the input factor X_i,β_{ij} is the different interaction coefficient between the input factor X_i and X_j and ϵ is the error of the model (Box and Behnken, 1960). For this study, the independent variables were coded as A, B and C and thus, the equation can be represented as equation 3:

$$Y = \beta_O + \beta_i A + \beta_i B + \beta_i C + \beta_{ii} A^2 + \beta_{ii} B^2 + \beta_{ii} C^2 + \beta_{ij} AB + \beta_{ij} AC + \beta_{ij} BC \quad (3)$$

Table 2: Selection of adequate model for Cr(VI) removal

Source	Sum Squares	Df	Mean Square	F Value	p-value Prob.> F
Mean vs Total	22549.08	1	22549.08	-	-
Linear vs Mean	11471.01	3	3823.67	18.83	< 0.0001
2FI vs Linear	9.24	3	3.08	0.012	0.9981
Quadratic vs 2FI	2621.39	3	873.80	618.98	< 0.0001
Cubic vs Quadratic	3.53	3	1.18	0.74	0.5801
Residual	6.35	4	1.59	-	-
Total	36660.60	17	2156.51	-	-

RESULTS AND DISCUSSION

The usability, biosorption characteristics of goose berry seeds have already been reported in a preliminary work (Aravind *et al.,* 2015). Optimization by classical method involving One Factor at a Time (OFAT) is time consuming and expensive. Besides this, number of trials required will be large, making the full factorial design very complex. In order to overcome such difficulties experimental BBD can be employed for the optimization of various parameters for the biosorption of various heavy metals (Ravikumar *et al*., 2005; Ferreira *et al*., 2007).

Experiments were performed according to the design created using design expert software to identify the optimum combination of parameters influencing the biosorption of Cr(VI). From the response, it had been concluded that maximum removal of 91.26% was attained at pH 2, with an initial metal concentration of 60 mg/L at 27°C (Table1).

Selection of adequate model for Cr(VI) removal

A system or process with several variables is likely to be influenced by several external as well as internal parameters and low order interactions. Investigations on linear, cubic, two factor interaction and quadratic model were done to select the statistically significant model for determining the relationship between the response and the input (independent variables). From the sequential model sum of squares, it can be seen that p value is lower than 0.001 and larger F value (618.98) for quadratic model (Table 2). Lack of fit tests for quadratic model is found to be not significant with p value of 0.58 (Table 3). Comparable results were obtained by Das *et al.,* (2013), where F value was found to be 0.98. From the model summary statistics, it can be predicted the quadratic model had maximum predicted and adjusted R^2 value. From the above results, it had been concluded that quadratic model provide an excellent explanation for the relationship between response and independent variables.

Regression analysis

Regression analysis was performed to fit the response. Regression model developed represents responses as a function of pH (A), Temperature (B) and Initial metal concentration (C). Relationship between response and input variables is represented by the equation 4 in terms of coded factor.

$$Y = 23.674 - 37.61A - 3.5225B - 26375C + 0.8725AB - 0.9875AC - 0.775BC + 24.6467A^2 + 0.28175B^2 + 2.15675C^2 \quad (4)$$

The equation aids in determining the effect of individual variables or combination of several variables over the response (removal percent). Positive coefficient values indicate the positive interaction of factors and its impact on biosorption process where as the negative coefficient values points to the detrimental and interfering effect of the parameters on overall adsorption. From the equation, it is clear that all individual factors (pH, temperature and initial metal concentration) had competed with each other and impeded Cr(VI) removal, whereas the interaction of pH with temperature and interaction of pH, temperature and metal concentration with themselves favored better and effective sorption. Similar results were obtained with *Borasus flabellifer* coir powder, where pH and initial metal concentration was found to have detrimental effect on response (Krishna *et al*., 2013).

Table 3: Analysis of variance

Source	Sum Squares	Df	Mean Square	F Value	p-value Prob.> F
Model	14101.64	9	1566.85	1109.92*	< 0.0001
A-pH	11316.10	1	11316.10	8016.04	< 0.0001
B-Temperature	99.26	1	99.26	70.32	< 0.0001
C-Initial metal concentration	55.65	1	55.65	39.42	0.0004
AB	3.05	1	3.05	2.16	0.1854
AC	3.90	1	3.90	2.76	0.1404
BC	2.30	1	2.30	1.63	0.2430
A^2	2557.74	1	2557.74	1811.84	< 0.0001
B^2	0.33	1	0.33	0.24	0.6414
C^2	19.59	1	19.59	13.87	0.0074
Residual	9.88	7	1.41	-	-
Lack of fit	3.53	3	1.18	0.74	0.5801#
Pure error	6.35	4	1.59	-	-
Correlation (total)	14111.52	16		-	-

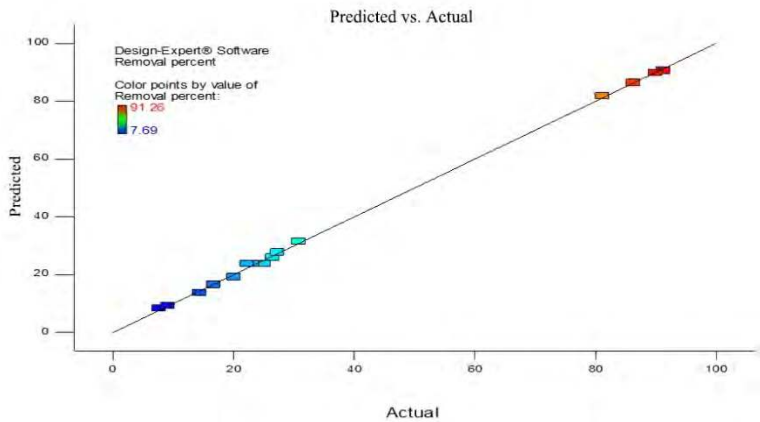

Fig. 1: The actual versus predicted value

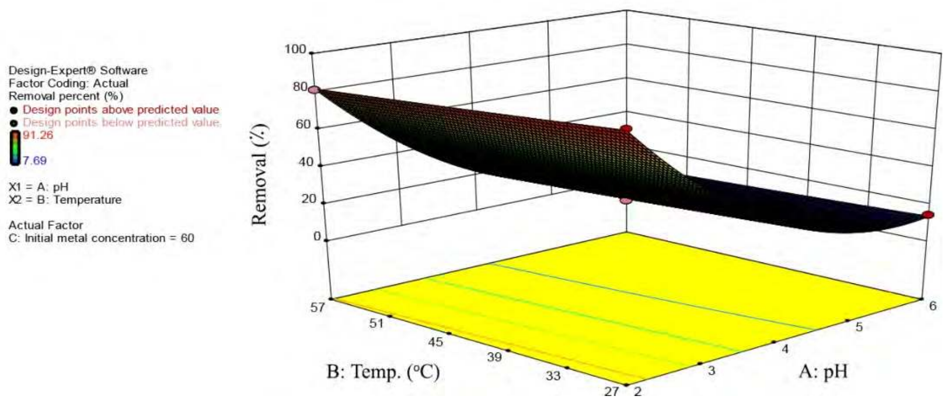

Fig. 2: Influence of pH and temperature on removal percent

ANOVA for response surface quadratic model

ANOVA table (Table 3) suggests whether the equation is adequate to describe the relationship between response and other independent variable. The model can be considered as statistically significant, if the value of p is lower than 0.05 with a larger F value (Hamsaveni *et al.,* 2001). From the ANOVA table (Table 3), it is observed that the quadratic model fitted well with the data generated and can be considered as statistically significant, since the F value is very large(1109.92) and p value is lesser than 0.0001. In this case A, B, C,A^2, and C^2 were considered to be the significant model terms, whereas other terms are not listed as significant factors since their p values were greater than 0.1. The Coefficient of Variance (CV) is the ratio of standard error of estimate to the mean value and considered reproducible once it is not greater than 10%. In our study, CV obtained was 3.26%. Adequate precision value measures signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 90.276 indicated an adequate signal. The actual factor is represented as equation 5.

$$Y = 217.10386 - 68.57937A - 0.380B - 0.12529C + 0.029083AB - 0.012344\,AC - 1.2625 \times 10^{-3}BC + 6.169\,A^2 + 1.25222 \times 10^{-3}\,B^2 + 1.34797 \times 10^{-3}\,C \quad (5)$$

Diagnostic plot

The generated data were analyzed to determine the correlation between the actual and predicted values as shown in the Fig. 1. From the plot, it is observed that the data points are distributed near the straight line indicating that the quadratic model could be employed as the significant model for predicting response over the independent input variables.

Optimization

Each contour plot gives the graphical representation of influence of interactive effects of individual parameter over the response. The contour plot may be rising ridges, saddle point, elliptical or circular plot. Circular or elliptical plot indicates that there exists a significant interaction between the operating parameters (Kanmani *et al*., 2013). The maxima formed by the x and y coordinate on the rising ridges in the response contour plots determine the optimum values of the parameter. From the Fig. 2, it is observed that removal percent increases from 7.69 to 91.26 with the decreasing values of pH, temperature and initial metal concentration. Although 100% removal of Cr(VI) ions was achieved by electrochemical methods (Fu and Wang., 2011), the current investigation is better in terms of energy efficiency and economy and the Cr(VI) ions after adsorption are well within the USEPA range. The best optimal parameters determined from the studies of contour plot for the biosorption of Cr(VI) were at pH 2, with an in initial metal concentration of 60 mg/L at 27°C (Figs. 2, 3 and 4).

Validation

The results obtained from RSM based experimental trials were validated by carrying out an independent run at a maximum pH of 2 with an initial metal concentration of 60 mg/L at 27°C. A maximum removal of 91.26 percent was attained which validated the design.

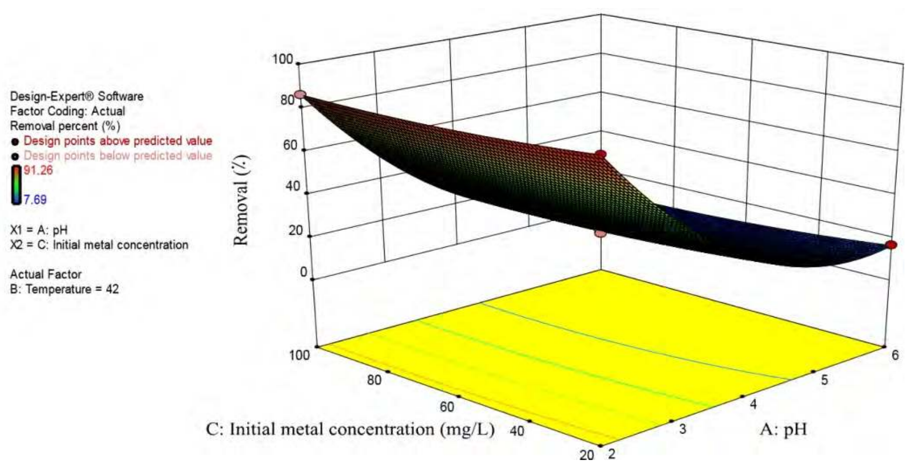

Fig. 3: Influence of pH and initial metal concentration on removal percent

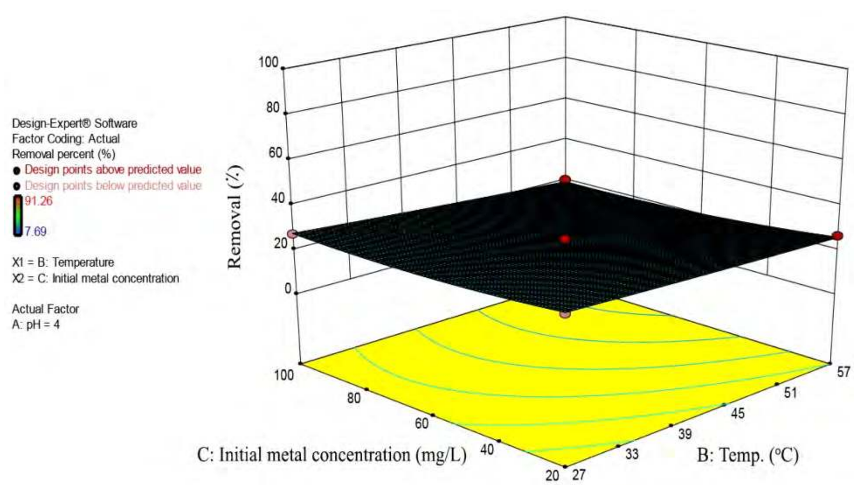

Fig. 4: Influence of temperature and initial metal concentration on removal percent

CONCLUSION

The sorbent prepared from gooseberry seeds appears to be a promising biosorbent for the removal of Cr(VI) ion from aqueous solution. Maximum removal was obtained at an acidic pH of 2 and equilibrium was attained within 30 minutes. The influence of process parameters in adsorptive removal of Cr(VI) was studied using RSM. From the ANOVA table (Table 3) and R^2 value, it had been concluded that quadratic model is the best model to describe the relationship between response and input variables. To conclude, the sorbent prepared from gooseberry seeds could be employed as a locally available alternative for the effective removal of Cr(VI) ions from industrial effluents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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