

## **An Assessment of Cadmium Removal from Simulated Waste Water Using Leftover Biomass of Water Hyacinth Immobilized via *Emericella nidulans***

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### **Authors' contributions**

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aims:** To study Cadmium removal from simulated waste water using water hyacinth biomass immobilized with *Emericella nidulans*.

**Place and Duration of Study:** Department of Environmental Science and Engineering, Guru Jambheshwar University of Science and Technology, Hisar. June 2012 and July 2013.

**Methodology:** The spent biomass left after enzyme production was immobilized with *Emericella nidulans* and dried at 50°C. Furthermore, the dried biomass was utilized for the removal of heavy metals i.e. cadmium, chromium, copper and nickel. Response surface methodology (RSM) was applied for screening and optimization of process parameters for heavy metal removal.

**Results:** Previously, production of enzyme from cellulosic biomass was done with the help of

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fungus *Emericella nidulans* using solid state fermentation method. In the present study, the leftover water hyacinth biomass was further assessed to be used as biosorbent for the removal of heavy metals. To accomplish the same, four heavy metals viz. cadmium (Cd), nickel (Ni), chromium (Cr) and copper (Cu) were screened out. It was revealed that the removal efficiency was maximum for Cd, followed by Cu, Ni and Cr, respectively. Percent removal was 97.2, 96.3, 95 and 94.6 for Cd, Cu, Ni and Cr, respectively. Furthermore, different process parameters were optimized using one factor at a time and box behnken design (BBD) of RSM. From the optimization studies it was concluded that removal efficiency was maximum at 40°C and pH 6. Optimum values of initial metal concentration, adsorbent dose and contact time were found to be 118 mg/L, 1% and 142 min.

**Conclusion:** The spent biomass immobilized with *Emericella nidulans* left after enzyme production effectively removes cadmium from simulated waste water.

**Keywords:** Cadmium; spent biomass; water hyacinth; immobilized; RSM.

## 1. INTRODUCTION

Beyond a certain range, most of the heavy metals such as copper, cadmium, nickel, chromium, zinc and lead are toxic for living organisms. These heavy metals are introduced to the environment from the different sources such as hospitals, smelting, metal plating, cadmium plated steel scrap waste recycling, pigment manufacture, ceramics, metallurgical alloying, natural deposit's erosion, mining, metal refineries effluent, waste batteries overspill and refining of non-ferrous metals, phosphate fertilizers manufacturing waste, printing and photographic industry [1-7]. The wastewater mostly contains residual cadmium from these industries and its economic removal from the effluent is a tough task. Cadmium ( $Cd^{2+}$ ) is one of the noxious elements which are hazardous to living organisms. It cannot be biodegraded and because of anthropogenic activities its concentration is progressively increasing in the environment [8]. Cadmium is repetitively dispersed among three main environmental components: air, water, and soil [9,3]. Cadmium ions being non biodegradable keep on bioaccumulating in the living organisms and cause various disorders [10-11]. Cadmium enters body mainly through the oral route and primarily affects the kidney [6]. The World Health Organization (WHO), US Environmental Protection agency and the directive from EU, set a maximum limit of 0.005 mg/L for Cd(II) in domestic water supplies [9,12]. Conventional physicochemical methods like precipitation, membrane separation, filtration, ion exchange, oxidation-reduction and electrochemical treatment are normally associated with the disadvantage of being expensive for the removal of metals from dilute concentrations [3].

Currently, bio-based technologies such as use of microbes and plants (namely bioremediation and phytoremediation) are being encouraged to deal with heavy metal problem and thus for detoxification of industrial effluents [3,13-14]. Various agricultural byproducts including rice bran [15], sugarcane bagasse pith [16], neem oil cake [17], coconut shell carbon [18], orange peel [19], citrus peels [20], rice straw [21], *Tectona grandis* [22], spent immobilized biomass [23] etc. have been used to deal with the removal of heavy metals from wastewater and has provided noteworthy results. In the present study, the efficiency of using cellulosic waste of water hyacinth immobilized with *Emericella nidulans* left after enzyme production has been assessed as biosorbent for the removal of heavy metals.

## 2. MATERIALS AND METHODS

### 2.1 Biosorbent Preparation

*Emericella nidulans* was grown on microwave alkali treated water hyacinth biomass for cellulolytic enzyme production [24]. The spent biomass resulting from the solid state fermentation process was used as biosorbent. First of all, the resulting biomass was dried at 50°C and then the dried biomass was utilized for cadmium removal.

### 2.2 Heavy Metal Removal Screening

Preliminary screening experiments were carried out with four heavy metals to get an idea of removal efficiency with Cr, Cd, Cu and Ni. The immobilized and dried biomass is then used as biosorbent to find out the heavy metal which is removed with maximum efficiency. Removal experiments were carried out in flasks of 150 ml

**Table 1. Independent variables with selected experimental range in the box behnken design experiment for cadmium removal**

Code	Parameters	Low level (-1)	Middle level (0)	High level (+1)
A	Adsorbent dose (%)	1	3	5
B	Initial metal concentration (mg/L)	100	300	500
C	Time (min)	60	120	180

capacity. Simulated waste water with heavy metals concentration of 100 mg/l and biosorbent dose of 1 g were put in a shaker at 30°C temperature. The samples were withdrawn for analysis after specific time, centrifuged at 10,000 rpm in triplicates and quantified by Atomic Absorption Spectrophotometer (AAS) (Shimadzu AA-6300, Japan).

### 2.3 Optimization of Cadmium Removal by OFAT and Statistical Methodology

#### 2.3.1 pH and temperature optimization using OFAT (one factor at one time)

Experiments for biosorption were carried out in 150 ml flasks by varying pH (2-8) and temperature (25-50°C) for contact period of 24 h with cadmium metal concentration of 100 mg/L. The percentage of removal and adsorption capacity on spent immobilized biomass of water hyacinth was calculated by the following equations [24]:

$$\text{Percent removal (\%)} = (C_0 - C_e) \div C_0 \times 100$$

$$\text{Adsorption capacity } \left(\frac{\text{mg}}{\text{g}}\right) q_e = (C_0 - C_e) \times V \div M$$

Where, initial cadmium concentration (mg/L) is denoted by  $C_0$  and the residual cadmium concentration (mg/L) is denoted by  $C_e$ . Amount of cadmium metal biosorbed on biosorbent (mg/g) is denoted by  $q_e$ , volume (L) of solution by  $V$  and the mass of adsorbent (g) by  $M$  in the equation.

#### 2.3.2 RSM approach

RSM was used to find out the key effects of different parameters on removal of cadmium by using spent lignocellulosic biomass immobilized with fungus. Adsorbent biomass doses (A), initial heavy metal concentration (B) and time (C) were chosen as independent parameters. BBD was employed for designing the experiments. The data was found to be fitted to 2<sup>nd</sup> order polynomial model and regression coefficients

were also obtained using the same modeling approach [25-28]. All independent variables with selected range for Cd removal have been illustrated in Table 1 above.

Basic form of polynomial quadratic equation fitted in the model for the evaluation of the response with each independent variable:

$$Y (\% \text{ removal}) = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC$$

## 3. RESULTS AND DISCUSSION

Results of screening study have been presented below in Table 2. Four heavy metals were screened and it was found that cadmium (Cd) was maximum removed, followed by copper (Cu), nickel (Ni) and chromium (Cr), respectively. Percent removal for the individual metals was 97.2, 96.3, 95 and 94.6 for Cd, Cu, Ni and Cr, respectively (Table 2).

**Table 2. Screening of heavy metals for % removal using immobilized biomass**

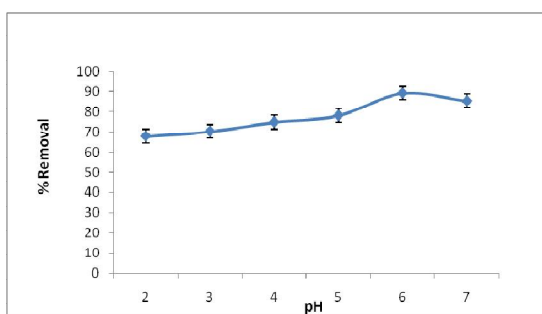
Heavy metal	% removal
Cr(VI)	94.6
Cd(II)	97.2
Cu(II)	96.3
Ni(II)	95

### 3.1 Optimization of pH

The effect of pH on cadmium removal was studied in the pH range of 2.0 - 7.0 because Cd(II) precipitated as cadmium hydroxide at higher pH leading to removal of cadmium ions from solution [29]. An Initial cadmium concentration of 100 mg/L was taken in the flasks with an adsorbent dose of 1% and was put in a shaker at 30±2°C for a contact period of 24 h. The changes in percent biosorption of cadmium with pH have been presented in Fig. 1. The removal of cadmium ions onto spent immobilized biomass was found to increase with increase in pH till optimum pH, past which removal was found to decrease because of the

lesser availability of adsorption sites and in accordance with solubility product for metal ion.

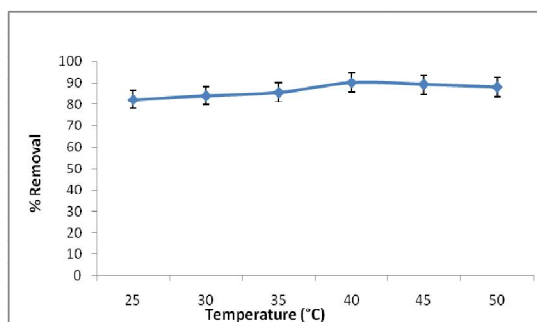
At lower pH, adsorbent surface and adsorbate species both are mainly positively charged, consequently, electrostatic repulsion exists between them [12]. So, solution's pH has been found as a key parameter that controlling the process of metals removal on surface of adsorbent. The metal ion adsorption also rely on the properties of adsorbent surface and distribution of adsorbate species ions in aqueous medium, which in turn depends on the pH of aqueous medium. It also affects the metal ions solubility, degree of ionization of adsorbent and contradictory functional groups concentration on the adsorbent [30]. From the Fig. 1, it is clear that maximum removal i.e. 89.2% was at pH 6 optimum for cadmium. Similar results have been reported by many researchers [12,29,31].



**Fig. 1. Effect of pH on percentage removal of cadmium by water hyacinth biomass immobilized via *Emericella nidulans***

### 3.2 Optimization of Temperature

The effect of temperature on cadmium removal was studied and results have been shown in Fig. 2. Temperature has a straight control over the adsorbed metals amount. In the present study, adsorption experiments were carried out in the range of 25-50°C temperature. The increase in percent removal corresponding with temperature indicated that several chemical interactions were also active during the adsorption course [12]. The cadmium ions adsorption by adsorbent of spent immobilized biomass increased from 82.3 to 90.2% with the raise in temperature from 25°C to 40°C. Furthermore, increase in percent removal with temperature may also be attributed to increase in active sites due to bond breakage at higher temperature [32]. As adsorption process is endothermic by nature, the amount of cadmium ions adsorbed at equilibrium increased with the mounting temperature.



**Fig. 2. Temperature effect on percentage removal of cadmium by water hyacinth immobilized via *Emericella nidulans***

### 3.3 Optimization of Parameters by Box-Behnken Design (BBD)

Chief factors were standardized by design of experiment (DOE) provided by Design Expert software version 9. On the basis of various combinations of DOE, 17 experiments were conducted and reactions were formulated and analyzed. The actual and predicted results have been tabulated in Table 3.

### 3.4 Statistical Analysis

For the justification of the adequacy of model, ANOVA (analysis of variance) was carried out. The results of 2<sup>nd</sup> order polynomial model of RSM in the ANOVA form have been presented in Table 4. The developed model quality was evaluated on the basis of correlation coefficient ( $R^2$ ) and standard deviation. From the data shown in Table 4, it was observed that the model was found to be significant at 5% confidence level ( $P$  values  $< 0.05$ ). The value of  $R^2$  was close to unity and a smaller standard deviation also stands for the accuracy of responses. For the good fitness of any model correlation coefficient must have a minimum value of 0.80 [33]. The correlation coefficient ( $R^2 = 0.965$ ) of the study was superior to 0.80 which indicated that only 3.5% of the whole dissimilarity was not explained by the empirical model for cadmium removal.

A model F-value is 21.79 as shown in ANOVA table of the quadratic model for cadmium removal that also indicating the model significance. As shown in Table 4, values of "probability  $>F$ " were less than 0.05 thus pointing towards the significance of model terms. In the present study, A, B, C, AC,  $A^2$  and  $C^2$  were significant model terms whereas AB, BC and  $B^2$  were insignificant model terms that have limited

influence. The adequate precision ratio was 18.47 as shown in table 5, which again was an adequate signal towards the model significance. It should be noted that adequate precision value of greater than 4 is desirable and confirmed that the predicted model could be used to navigate the space as defined by BBD. The coefficient of

variance (CV) expressed in % was found to be 4.83. It was calculated as the ratio of the standard error of the estimate to the mean value of observed responses. It is a sign of reproducibility of a model and a model is usually considered reproducible if its CV is not more than 10% [2,34].

**Table 3. Design of experiments and results of BBD for cadmium removal**

Run	A:Initial metal concentration Mg/L	B:Adsorbent dose %	C:Contact time Min	Removal (Actual) %	Removal (Predicted) %
1	300	3	120	80.9	85.10
2	500	1	120	75.05	71.83
3	100	3	60	60.58	59.18
4	100	1	120	99.9	99.53
5	300	3	120	82.91	85.10
6	300	3	120	88.87	85.10
7	300	3	120	83.48	85.10
8	500	3	60	68.21	69.67
9	100	3	180	98.1	96.64
10	300	5	60	71.3	69.47
11	300	3	120	89.34	85.10
12	300	1	180	84	85.83
13	500	5	120	69.76	70.13
14	300	1	60	73.8	75.56
15	100	5	120	79.21	82.44
16	500	3	180	44.74	46.14
17	300	5	180	74.89	73.13

**Table 4. ANOVA for the response surface quadratic model**

Source	Sum of squares	Df	Mean square	F value	p-value Prob > F	
Model	2785.26	9	309.47	21.79	0.0003	Significant
A-Initial metal concentration	800.60	1	800.60	56.38	0.0001	
B-Adsorbent dose	176.63	1	176.63	12.44	0.0096	
C-Contact time	96.88	1	96.88	6.82	0.0348	
AB	59.29	1	59.29	4.18	0.0803	
AC	929.95	1	929.95	65.49	< 0.0001	
BC	10.92	1	10.92	0.77	0.4095	
A <sup>2</sup>	156.93	1	156.93	11.05	0.0127	
B <sup>2</sup>	16.59	1	16.59	1.17	0.3156	
C <sup>2</sup>	517.61	1	517.61	36.45	0.0005	
Residual	99.40	7	14.20			
Lack of fit	42.15	3	14.05	0.98	0.4853	Not significant
Pure error	57.25	4	14.31			
Cor total	2884.66	16				

**Table 5. ANOVA of response surface for Box Behnken design**

Statistical determinants	Value	Statistical determinants	Value
Std. dev.	3.77	R <sup>2</sup>	0.9655
Mean	77.94	Adj R <sup>2</sup>	0.9212
C.V. %	4.83	Pred R <sup>2</sup>	0.7352
PRESS	763.87	Adeq precision	18.475

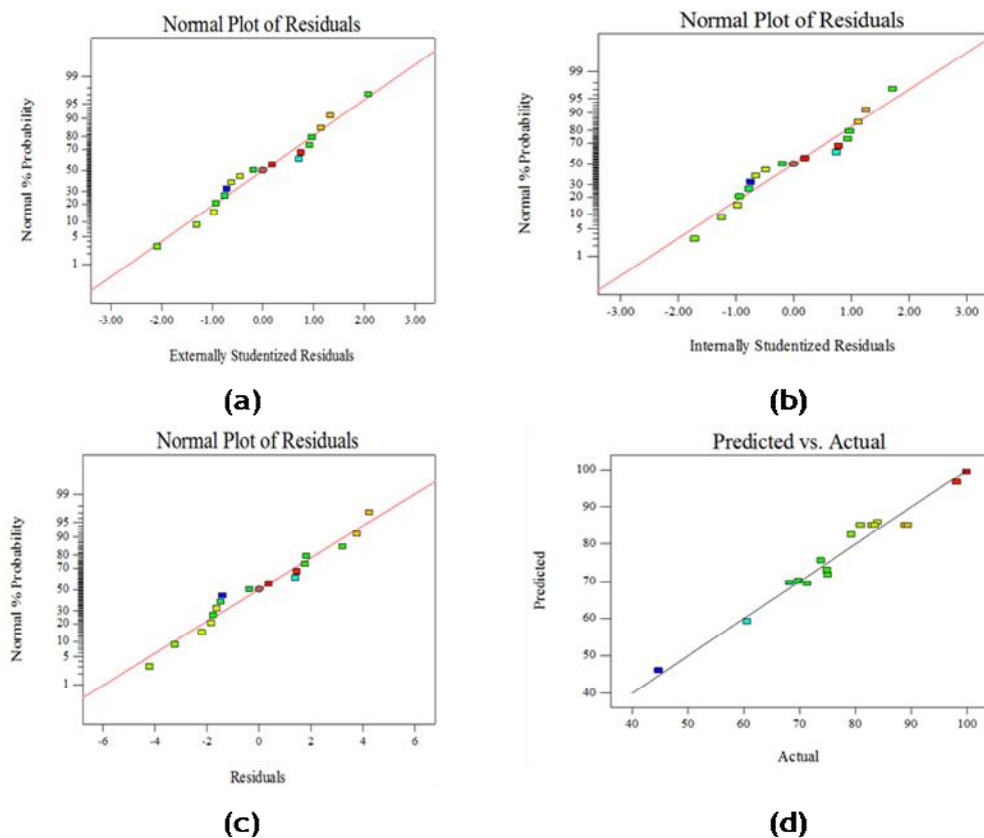
It is also important to confirm, whether or not the model gives satisfactory approximation to the real system. For this, analysis of diagnostic plots as provided by design expert (version 9) software (normal probability plots of the studentized residuals, predicted versus actual response plots) was performed. The normal probability plots of the studentized residuals for cadmium removal are shown in (Figs. 3a-c) which indicates that residuals follow a normal distribution as points follow a straight line. Another diagnostic plot (Fig. 3d) of predicted versus actual response also helps to check adequacy of model. Plot shown in (Figs. 3a-d) have adequate harmony with in real data and data from the model.

The natural logarithm (ln) of the residuals sum of square (SS) against  $\lambda$  was found to be one. A sudden dip thus representing a minimum was observed in the region of the best optimum value i.e. 0.92 (Fig. 4a). The data did not require any transformation [35]. The minimum and maximum confidence interval values were found to be 0.6 and 3.06 respectively as shown by the model.

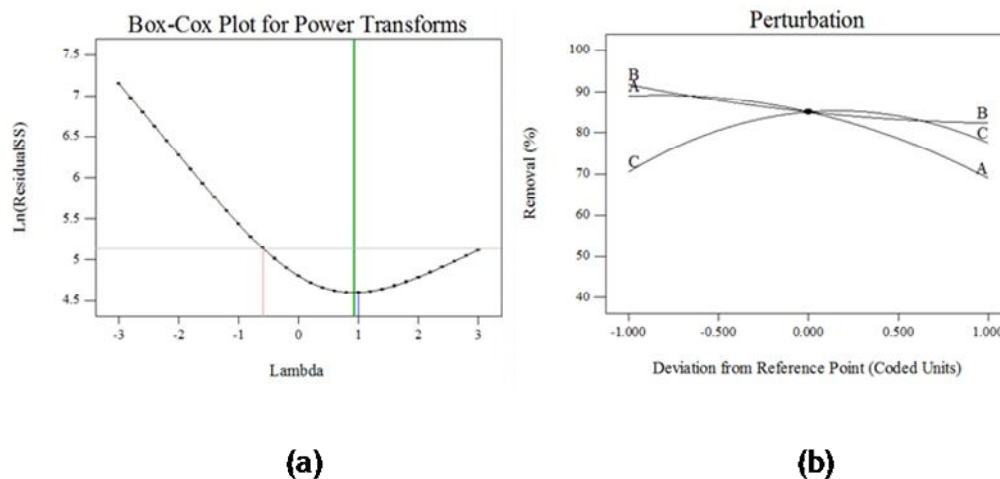
The final regression models, in term of their coded parameters have been expressed by the 2<sup>nd</sup> order polynomial equation as given below:

$$Y = 85.10 - 10.00 A - 4.70 B + 3.48 C - 3.85 AB - 15.25 AC - 1.65 BC - 6.11 A^2 + 1.98 B^2 - 11.09 C^2$$

The perturbation plots showed the variation of cadmium removal with the deviation of each factor from the selected reference point when other factors were kept constant at the reference point. Perturbation curve was plotted with vertical axis showing cadmium removal and horizontal axis showing preparation conditions in terms of A, B and C as shown in Fig. 4b. It can be seen that all the factors imparted significant quadratic impact. However, initial metal ion concentration (A) was most significant factor that contributed to cadmium removal and had the most pronounced quadratic effect. The adsorbent dose (B) and contact time (C) showed least prominent change but still showed significant quadratic effect.



**Fig. 3. Diagnostic plots: (a-c) Normal distribution probability plots of the studentized residuals; (d) Predicted versus actual**



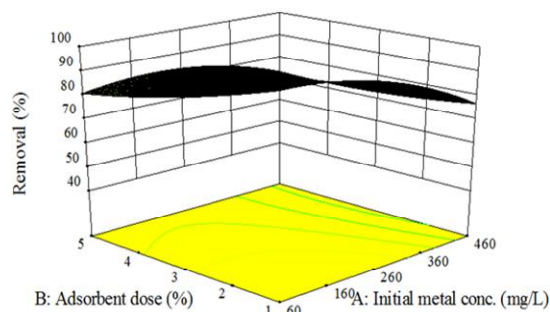
**Fig. 4. Represents (a) Box-Cox plot for power transformations (b) Perturbation plot showing the effect of all factors on removal of heavy metal**

The 3D surface plot and contour plot were also used for determining the interactive effects of factors combination like adsorbent dosage, contact time and initial metal concentration on the percent removal of cadmium.

**3.5 Interactive Effect of Adsorbent Dose and Initial Metal Concentration on Cadmium Removal by Spent Immobilized Biomass**

Fig. 5 illustrates the percent adsorption of cadmium. Maximum adsorption was found at small initial concentration of cadmium using little adsorbent selected for the experimental range. In the present study, efficiency of metal ions is also affected by the initial metal concentration, as the concentration increases removal decreases at optimized pH 6. The removal percentage of cadmium ions relay on initial concentration of cadmium because at lower concentration, high energy sites were present for the metal ion adsorption and as the metal concentration increases, these sites were saturated, resulting in a decrease of adsorption quantity [12,36]. Similar study of cadmium adsorption onto pumice showed the sharp increase in removal percentage with initial cadmium concentration from 95 to 105 mg/L and then did not change with further increasing in initial cadmium concentration. At the lower cadmium concentration, more adsorption sites were present for the cadmium ions to be adsorbed [37-39]. As a result, amount of adsorbed cadmium ions also increases but with further increase of

metal concentration, active sites on adsorbent surface became saturated thus decrease the adsorption amount.

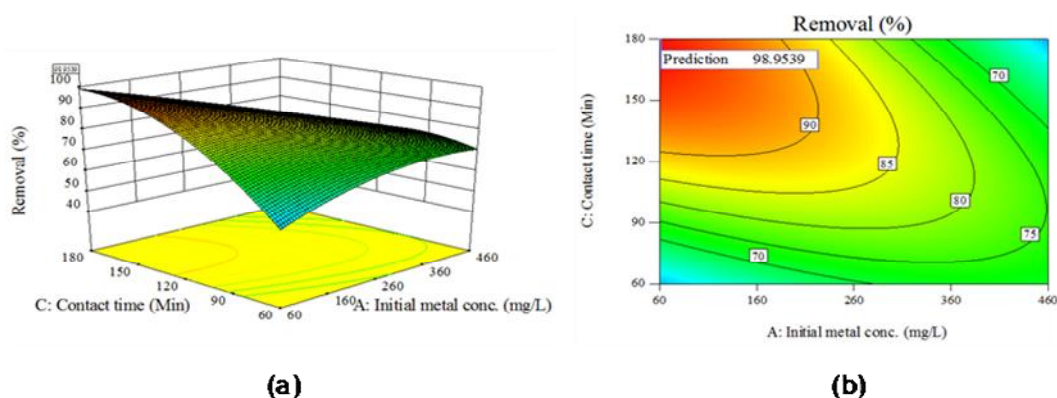


**Fig. 5. Response surface plots showing the effect of adsorbent dose and initial cadmium concentration**

**3.6 Effect of Initial Metal Concentration and Contact Time on Cadmium Removal by Spent Immobilized Biomass**

The initial metal concentration and contact time were also having significance in the percent removal heavy metals. Experiments were performed as provided by model with selected parameters in selected range and results were displayed in Fig. 6b. The maximum percent removal of cadmium was 98.9% by spent immobilized fungal biomass at 180 min of contact time and initial metal concentration of 60 mg/L (Fig. 6b).

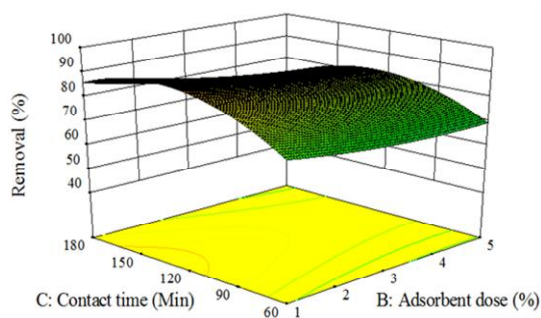




**Fig. 6. Showing the effect of initial metal concentration and contact time on heavy metal removal (a) Response surface plots (b) Desirability graph**

### 3.7 Effect of Contact Time and Adsorbent Dose on Cadmium Removal by Spent Immobilized Biomass

Fig. 7 represents the effect of adsorbent dose and contact time on percent removal which was achieved maximum i.e. 92.3%, at adsorbent concentration of 1% and 135 min of contact time. It was found that increase in adsorbent dose more than 1% has not shown any positive improvement in the removal of cadmium. Generally, the enhancement in the removal of cadmium ions with raise in adsorbent dose was found which may be due to the increase in adsorption surface area with more active surface sites and saturation was a result of no availability of cadmium ions for adsorption. But in present study, lowest adsorbent dose 1% is sufficient for higher percent cadmium removal.



**Fig. 7. Response surface plots showing the effect of contact time and adsorbent dose on cadmium removal**

Thus it can be concluded that adsorbent dose, contact time and initial metal concentration have significant effect on cadmium removal. Increase in contact time and decrease in initial cadmium concentration enhance cadmium

removal. Similar findings were reported by various researchers, as they also found augmentation in removal with adsorbent dose [40-42]. The minimum initial metal concentration ensued increase of the mass transfer driving force, so, the rate of cadmium moved from the aqueous medium to the particle surface increases [43]. From the observation response surface plots it was found that contact time favours the adsorption towards higher level, initial metal concentration towards lower level and adsorbent dose more favourable in lower level. The suggested optimized parameters by the model were Initial metal concentration 118 mg/L, adsorbent dose 1% and contact time 142 min with highest desirability.

### 4. CONCLUSION

In the present study, screening and optimization for heavy metal removal was studied. Experimental results obtained during screening revealed the maximum removal of Cadmium. The interaction between different variables for cadmium removal was studied using box behnken design. Spent immobilized biomass of water hyacinth left after enzyme production proved an effective biosorbent as the efficiency of Cadmium removal was more than 99%.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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