Journal of Environment & Biotechnology Research

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Response surface optimization of Cu(II) biosorption onto *Candida tropicalis* immobilized strontium alginate beads by Box-Behnken experimental design

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

A study was conducted to examine the Cu(II) removal from aqueous solution by immobilized *Candida tropicalis* in strontium alginate beads using three factor, three-level Box-Behnken experimental design in lab-scale batch study. Prior to the statistical analysis, the *Candida tropicalis* immobilization was performed using strontium chloride and sodium alginate. A second-order polynomial regression equation was developed to predict Cu(II) removal efficiency as the response using three independent variables, Adsorbent dosage (C_{ads}) ranging from 2.0 to 6.0 g/L, initial pH of the solution (pH_o) ranging from 2.0 to 6.0 and initial Cu(II) ion concentration (C_o) ranging from 250 to 750 mg/L were coded as x_1, x_2 and x_3 at three levels (-1, 0 and 1). At 95 % confidence limits (α = 0.05), the significance of the independent variables and their interactions were examined using Analysis of Variance (ANOVA). By solving the quadratic regression model, the optimum values of the selected variables (C_{ads} = 6.0 g/L, pH_o = 5.24 and C_o = 530.05 mg/L) were obtained with the maximum Cu(II) removal efficiency of 98.35 % and the results were validated experimentally.

KEYWORDS

Biosorption; Box-Behnken experimental design; *Candida tropicalis*; Cu(II) removal

1. INTRODUCTION

In the context of rapid industrialization scenario, copper is one of the desirable materials of choice for the engineers and technologists to exploit in varied applications. Having the quality of thermal conductivity and resistance to corrosion, copper finds its place in many of the areas including electrical and electronic applications, construction applications, chemical and marine fields, wood preservatives, anti-fouling agents in paints and as a trace nutrient in livestock feeds. Hence the exposure of copper and its ions, Cu(II) in humans is highly incidental and it is likely to be beneficial only at its low levels. Even at slightly higher levels of Cu(II) ions cause adverse effects on health might cause chest pains, vomiting and irritation of the eyes and nose. The major man-made releases of copper are from coal-fired power stations, metal production, waste incinerators, sewage treatment processes and from the application of agricultural chemicals. Copper leaching from household plumbing systems finds the prime route of entry into the drinking water (Nithya et al., 2018). In soil system, the excess copper is highly toxic to the soil microbe by disrupting their nutrient-cycling and inhibiting the metabolic action of essential nutrients such as nitrogen and phosphorous. By concerning all these, the Environmental Protection Agency (EPA) and Indian Standards set 1.3 mg/L and 2.0 mg/L as the Maximum Contaminant Level Goals (MCLG) for drinking water. Hence, the removal of Cu(II) ions from the environment is of high-priority to meet the quality standards of drinking water. Several remediation strategies were developed to remove the dissolved metal ions which include chemical precipitation, ion exchange, electrochemical treatment, membrane technologies and evaporative recovery (Padma et al., 2003). The use of expensive equipment, monitoring devices and energy intensive nature of the traditional methods, alternate technologies to clean up the metal

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Received: 01-02-2019
Revised: 15-03-2019
Accepted: 19-03-2019
Available online: 01-04-2019

ions from the aqueous solutions are continuously researched (Aksu et al., 1999). The use of materials of biological origin, which may be bacteria, fungi, yeast, algae and molds, referred as biosorption has given significant attention as alternate remediation process (Madrid and Carmen, 2003). Among the thousands of biosorbents, marine algae (e.g. *Sargassum natans*), bacteria (e.g. *Bacillus subtilis*), fungi (e.g. *Rhizopus arrhizus*), yeast (e.g. *Saccharomyces cerevisiae*) and alginate were proved as prominent and effective candidate in copper removal process (Wang and Chen, 2006; Karthika et al., 2010). Of these biomaterials, biopolymer (alginate), has been widely used for the removal of heavy metals and organic dyes from wastewater (Papageorgiou et al., 2009; Ngah and Fatinathan, 2008). Alginate has higher affinity to metal ions and forms complexes between its carboxyl groups and metal ions. The use of non-viable biomass renders the metal-sequestering property which provides the ease of approach in maintaining the integrity of the system. The present study focused on the preparation of a novel adsorbent by immobilizing the non-viable cells of *Candida tropicalis* onto the strontium alginate beads and was used for batch adsorption studies. The classical method of batch adsorption studies involves changing one independent variable while keeping the other factors at a fixed level is extremely time consuming and expensive process (Ghorbani et al., 2008). In order to overcome, the prepared novel adsorbent was used to design and optimize the Cu(II) ions removal capability in aqueous solution with the using three level, three factor Box-Behnken design. Furthermore, the main and the quadratic interaction effects of the selected independent variables were also examined.

2. MATERIALS AND METHODS

2.1. Reagents

The microorganism *C. tropicalis* (MTCC 2795) was purchased from Microbial Type Culture Collection, Pune and was stored at 277 K in a nutrient medium containing malt extract 3 g/L, yeast extract 3 g/L, peptone 3 g/L, glucose 10 g/L, agar 20 g/L. The pH of the medium was adjusted to 6.20. The anhydrous $CuSO_4$ (molecular weight=159.609 g/mol, Purity>99%) was purchased from Himedia, was used as copper source. Anhydrous $SrCl_2$ (Molecular weight 158.53 g/mol), sodium alginate (molecular weight = 216 g/mol) were procured from Himedia, being used to prepare carrier beads. Distilled water obtained from a distillation unit (AQDD-50P-DB, Infusil India) was used throughout the study. All the chemicals used for culture medium were of analytical grade and without further purification.

2.2. Preparation of non-viable biomass

C. tropicalis was deactivated by heating in an oven at 353 K for 24 h (Schiewer and Volesky, 1995). The dead biomass was further treated with ethanol solution for 20 min at room temperature. The samples are centrifuged at 4000 rpm for 20 min to remove the excess ethanol. The biomass was rinsed twice or thrice with distilled water to remove the adsorbed nutrients and allowed to dry at 343 K for 12 h (Goksungur et al., 2005). The dried biomass were ground and screened through a 100 mesh sieve and stocked for the further use.

2.3. Preparation of C.tropicalis immobilized strontium alginate beads

The grounded non-viable biomass of about 250 g was suspended in 3% sodium alginate solution. The biomass dispersed in sodium alginate solution was vigorously stirred with a mechanical stirrer for 2 h. The suspension was transferred drop wise through a needle into 0.05 M $SrCl_2$ solution (crosslinking agent), and spherical alginate beads were formed instantaneously. The flow rate was set as 50 mL/min. The immobilized beads were allowed to cure in $SrCl_2$ solution for 6 h to ensure complete gelation reaction.

Table 1. Details of the independent variables and their studied range.

Factors / Variables	Coded Levels		
	-1	0	+1
Adsorbent dosage (C_{ads}, g/L)	2.0	4.0	6.0
Initial pH of the solution (pH_0)	2.0	4.0	6.0
Initial Cu(II) concentration (C_o, mg/L)	250	500	750

2.4. Batch adsorption studies

A designed set of batch adsorption studies for Cu(II) removal using *C. tropicalis* immobilized strontium alginate beads were carried out in a thermostatic

Table 2. Box-Behnken design matrix with actual and coded levels of the three independent variables

Run	Adsorbent dosage (C_{ads}, g/L)		Initial pH of the solution (pH_o)		Initial Cu(II) concentration (C_o, mg/L)		Cu(II) removal efficiency (%)
	x_1 (coded)	x_1 (actual)	x_2 (coded)	x_2 (actual)	x_3 (coded)	x_3 (actual)	Y*
1	0	4	-1	2	-1	250	62.35
2	0	4	0	4	0	500	93.25
3	1	6	0	4	1	750	94.72
4	0	4	0	4	0	500	92.82
5	1	6	1	6	0	500	97.62
6	-1	2	-1	2	0	500	38.92
7	0	4	1	6	-1	250	91.52
8	1	6	-1	2	0	500	86.25
9	0	4	0	4	0	500	93.12
10	0	4	0	4	0	500	92.82
11	0	4	-1	2	1	750	88.72
12	-1	2	0	4	1	750	39.25
13	0	4	1	6	1	750	88.25
14	1	6	0	4	-1	250	62.63
15	-1	2	0	4	-1	250	24.32
16	-1	2	1	6	0	500	55.28
17	0	4	0	4	0	500	93.42

Y*, average Cu(II) removal efficiency of triplicate batch studies.

shaker. A stock solution of 1000 mg/L of Cu(II) ions was first prepared by dissolving the analytical grade of anhydrous $CuSO_4$. Then the working solution were prepared from 250 mg/L to 750 mg/L of Cu(II) ions by diluting appropriate amounts of $CuSO_4$ stock solution with distilled water. The experimental ranges for the selected variables, adsorbent dosage (Cads), initial pH of the solution (pH_0) and the initial Cu(II) ion concentration (C_0) were 2.0 to 6.0 g/L, 2.0 to 6.0 and 250 mg/L to 750 mg/L, respectively (Table 1). The ranges of the independent variables were considered based on the previous finding (Zhu et al., 2014). A series of lab-scale shake flask studies were carried out to determine the Cu(II) adsorption capacity using the three independent variables. All the experiments were conducted at 301 K for 6 h at 150 rpm. Samples were taken at defined time and the samples were separated through centrifugation at 4000 rpm (REMI) before analysis. The concentration of Cu(II) ions were analyzed through Atomic Absorption Spectrometer (WPX 450 Systronics AAS) using air-acetylene flame and a hollow cathode lamp.

2.5. Statistical analysis

Design Expert (Trial version 7.0.0, Stat-Ease, Inc. USA) software package was used for Design of Experiments (Box-Behnken design) and Graphpad online interface was used to calculate the Mann-Whitney U test and Chi-Square statistics.

3. RESULTS AND DISCUSSION

3.1. Determination of regression equation

The following second-order polynomial equation was obtained to explain Cu(II) removal efficiency by applying the multiple regression analysis on the design

Table 3. Test of Significance of the Model using ANOVA.

Source	Sum of squares	df	Mean square	F- Value	Prob > F
Model	9051.65	9	1005.74	95.1	< 0.0001*
x_1	4206.74	1	4206.74	397.77	< 0.0001*
x_2	398.04	1	398.04	37.64	0.0005*
x_3	614.6	1	614.6	58.11	0.0001*
$x_1 x_2$	6.23	1	6.23	0.59	0.468
$x_1 x_3$	73.62	1	73.62	6.96	0.0335*
$x_2 x_3$	219.63	1	219.63	20.77	0.0026*
x_1^2	2743.1	1	2743.1	259.37	< 0.0001*
x_2^2	16.11	1	16.11	1.52	0.257
x_3^2	640.3	1	640.3	60.54	0.0001*
Residual	74.03	7	10.58		
Lack of Fit	73.75	3	24.58	349.79	Not significant
Pure Error	0.28	4	0.07		
Total	9125.68	16			

*p-values < 0.05 were considered to be significant.

Table 4. Chi – Square test of observed and predicted values

Run	Coded Factors			Cu(II) removal efficiency (%)		$\chi^2 = \sum(O_i-E_i)^2/E_i$
	x_1	x_2	x_3	Observed	Predicted	
1	0	-1	-1	62.35	59.88	0.101885438
2	0	0	0	93.25	93.09	0.000275003
3	1	0	1	94.72	90.84	0.165724351
4	0	0	0	92.82	93.09	0.000783113
5	1	1	0	97.62	98.26	0.004168532
6	-1	-1	0	38.92	38.30	0.010036554
7	0	1	-1	91.52	88.80	0.083315315
8	1	-1	0	86.25	86.66	0.001939765
9	0	0	0	93.12	93.09	9.66806E-06
10	0	0	0	92.82	93.09	0.000783113
11	0	-1	1	88.72	91.46	0.082086158
12	-1	0	1	39.25	36.40	0.223145604
13	0	1	1	88.25	90.74	0.06832819
14	1	0	-1	62.63	65.50	0.125754198
15	-1	0	-1	24.32	28.22	0.538979447
16	-1	1	0	55.28	54.90	0.002630237
17	0	0	0	93.42	93.09	0.001169836
					χ^2_{cal}	1.411015

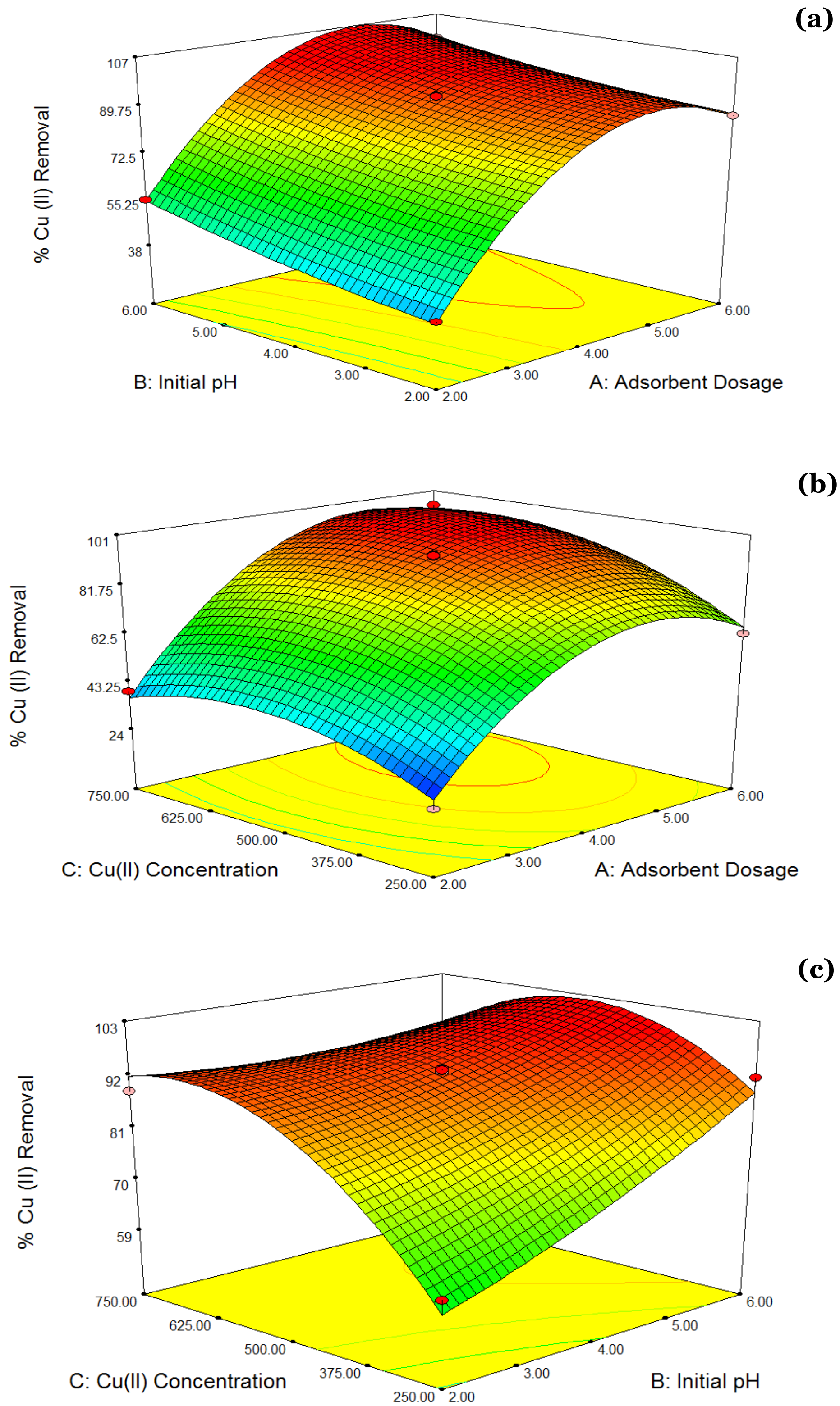

Figure 1. Surface plots showing the effects of the mutual interactions between the two independent variables while the other variable is kept at its center point.

Table 5. Multiple regression results and significance of the components for the quadratic model eavy metals concentration in the tissues of the fish species studied in mg/kg of the dry matter

Factor	Parameter	Estimate	Effect	Standard error	t ratio	p value
Intercept	β_0	93.09				
x_1	β_1	22.93	45.86	1.15	39.87826	0.0160*
x_2	β_2	7.05	14.1	1.15	12.26087	0.0418*
x_3	β_3	8.77	17.54	1.15	15.25217	0.0417*
$x_1 x_2$	β_{12}	-1.25	-2.5	1.63	-1.53374	0.3678
$x_1 x_3$	β_{13}	4.29	8.58	1.63	5.263804	0.1195
$x_2 x_3$	β_{23}	-7.41	-14.82	1.63	-9.09202	0.0697
x_1^2	β_1^2	-25.52	-51.04	1.58	-32.3038	0.0197*
x_2^2	β_2^2	1.96	3.92	1.58	2.481013	0.2439
x_3^2	β_3^2	-12.33	-24.66	1.58	-15.6076	0.0497*

matrix and the response given in the Table 2.

$$Y = 93.09 + 22.93x_1 + 7.05x_2 + 8.377x_3 - 1.25x_1x_2 + 4.29x_1x_3 - 7.41\, x_2x_3 - 25.52\, x_1^2 + 1.96\, x_2^2 - 12.33\, x_3^2 \quad (1)$$

where Y is the predicted Cu(II) removal efficiency, x_1, x_2 and x_3 are the coded terms of the three independent variables, C_{ads}, pH_o and C_o, respectively. The regression equation was used to obtain the optimum values for the screened independent variables. The ANOVA is essential to test the significance of the model as stated by Sen and Swaminathan (2004), and the model was analyzed to determine the significance of the fit with the experimental data. From the Table 3, the ANOVA showed that the quadratic model was highly significant as evident from the Fisher's F-test, $F_{model} = 95.10$ with a probability value of $P_{model} < 0.0001$. Also the calculated F value ($F_{cal} = 95.10$) was found to be greater than the tabulated F value ($F_{0.05, 9, 7} = 3.68$) indicating that the model is highly significant as similarly reported by Liu et al. (2004).

The goodness of fit of the model was checked by the regression coefficient, ($R^2 = 0.9919$) and the value of adjusted coefficient ($R^2_{adj} = 0.9815$) was also high, showing a high significance of the model, as reported by others (Adinarayana and Ellaiah, 2002; Thirunavukkarasu and Nithya, 2011). The significant difference between the expected response and the observed data was checked by the Chi-square (χ^2) test. From the Table 4, the calculated chi-square value (χ^2 cal = 1.411) was found to be less than that of the tabulated value (χ^2 tab, 0.05, 16 = 26.296), indicating that there was no statistical difference between the observed data and the predicted response. Hence the alternative hypothesis (H_a) was rejected at 95% confidence level.

3.2. Determination of the interaction effects of model components on Cu(II) removal efficiency

Student's t-test and p-values were determined to check the significance of each coefficient and their interaction effects. The main effects of the adsorbent dosage (C_{ads}, x_1), initial pH of the solution (pH_o, x_2) and initial Cu(II) ion concentration (C_o, x_3) was found to be more significant than that of their quadratic effects as shown in the Table 5. These results suggest that the selected variables are highly correlated with the Cu(II) removal efficiency. Our results were supported by Can et al. (2006) for the removal of Ni (II) ions from the aqueous solution by *P. slyvestris*. The Cu(II) removal efficiency, measured for the designed set of experiments, showed wide variations from 24.32% to 97.62% (Table 2). This clearly indicates that the variables screened for the study were strongly affecting the removal efficiency.

3.3. Surface plots and optimization studies for maximizing Cu(II) removal efficiency

Adinarayana and Ellaiah (2002) stated that surface plots are more helpful in understanding the main and interaction effects among the screened variables. Also by analyzing the surface plots as shown in Figures 1a, 1b and 1c, the optimal values of the test variables in coded units were found as x_1=1.0, x_2 = 1.31 and x_3=1.06 with the corresponding Y= 98.35%. The actual values were then calculated as C_{ads} = 6.0 g/L, pH_o= 5.24 and C_o=530.05 mg/L by substituting in the model equation.

Table 6. Validation of the proposed regression model

Run	x_1	x_2	x_3	Observed	Predicted
1	1	1	0.478	97.52	97.96
2	1	-1	-1	54.36	54.25
3	-1	0.111	-0.304	42.65	43.45
4	1	1	1	97.32	91.19
5	-1	0.111	-0.739	3236	36.44
6	-1	0.111	0.478	51.32	44.33
7	-0.143	0.111	-0.304	91.33	86.87
8	-0.143	0.111	0.478	94.23	90.62
9	1	0.111	0.478	94.65	94.02
10	1	-1	0.478	86.32	93.44
Mann Whitney Statistic (U Score)					35 ($U_{critic, <0.05}$=23)
Two-tailed Z- Score					1.0961, p=0.27134

3.4. Validation of the regression model

In order to verify the validity of the developed regression model, experiments were carried out randomly as done by Yetilmezsoy et al. (2009) and the observed responses were compared with the predicted results (Table 6).

A non-parametric Mann-Whitney U-test was performed to test the significant difference between the two groups. The obtained U score is greater than that of the U_{critic} at 5% confidence level, indicating that there is no noticeable difference between the experimental and the predicted response. The U-test results were supported by the two-tailed Z-ratio, Z_{cal} = 1.0961, with the probability value of p=0.2734. Thus, the proposed regression model gives a satisfactory fit to the experimental data at 95% certainty.

4. CONCLUSIONS

The optimum values for the selected variables were found to be 6.0 g/L (x_1=1.0) for adsorbent dosage, 5.24 (x_2 = 1.31) for the initial pH of the solution and 530.05 mg/L (x_3=1.06) for the initial Cu(II) concentration with a predicted Cu(II) removal efficiency of about 98.35% which was higher than the observed removal in the initial 17 experiments. The validity of the developed regression model was analyzed with the various test statistics and found that the model equation were in very good agreement with the observed responses. (R_2 = 0.9919, R^2_{adj} = 0.9815, χ^2_{cal}= 1.411 < χ^2_{tab}). Findings of the study reported that the main effects of the adsorbent dosage (C_{ads}, x_1), initial pH of the solution (pH_o, x_2) and initial Cu(II) ion concentration (C_o, x_3) was found to be more significant than that of their quadratic effects on Cu(II) removal efficiency. Also the theoretical adsorption capability of the model was verified by set of randomly designed experiments and it clearly confirmed that the model equation fits well with the experimental response at 95% certainty.

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