

Response Surface Methodology for Optimization of Cd (II) Biosorption by *Cystoseria myricaas*

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Abstract

In this study, biosorption of cadmium by *Cystoseria myricaas* as brown algae was investigated. Heavy metals, such as cadmium Cd (II), which are often present in industrial wastewaters, are dangerous to the aquatic ecosystem and pose a risk to human health as well. The experiment was conducted under conditions of varying pH, temperature, sorbent dosage, metal concentration, and contact time. The number of experiments was calculated by software Minitab. The design package Minitab software, a statistical program package, was used for regression analysis of the data obtained and estimation of the coefficient of the regression equation. The optimum condition was calculated by response surface methodology (RSM). The optimum conditions for removal of cadmium ion were obtained by theoretical equations. The response surfaces plots and contour plots were drawn using MATLAB.

Keywords: Cadmium, Response Surface Methodology, Biosorption, *Cystoseria myricaas*

1. Background

The increase in the levels of toxic heavy metals in aquatic plants and animals is an effect of anthropogenic activities, particularly industrial activities (1). One of the most common environmental threats is heavy metal pollution of surface and ground water because the dissolved toxic ions can eventually reach the top of the food chain and thus become a risk factor for human health. The presence of heavy metals in aqueous effluents is a major environmental problem because of their toxicity to humans and other life forms. Heavy metals, such as Cd (II), are often present in industrial.

Wastewaters, are perilous to the aquatic ecosystem, and pose a risk to human health (2-4). Cadmium is used in a wide variety of industries, including metal plating, mining, and ceramics. Cadmium toxicity can cause renal dysfunction, hypertension, hepatic injury, lung damage, and teratogenic effects (5).

To combat the toxicity and persistence of heavy metal toxins in the environment, many technologies have been implemented, such as conventional types, biosorption, and nanotechnology. Conventional physiochemical methods for eliminating heavy metals from waste streams include chemical reduction, electrochemical treatment, ion exchange, precipitation, and evaporative recovery (6). One technology for removing heavy metals from industrial wastewater is biosorption. It is effective in decreasing the

concentration of heavy metal ions to significantly low levels and implements the use of inexpensive bio-sorbent materials, such as algae derived from fermentation industries as a by-product or from other industries as a waste material or a bio-sorbent. A useful technique for development and optimization of the biosorption process is RSM (6). This process comprises three main steps: experimental design, modeling, and optimization. This model provides relatively few combinations of variables for the determination of complex response functions. In a biosorption study, the efficacy of micro algae (*Cystoseria myricaas*) in removing cadmium and another heavy metal from an aqueous solution was examined Kumar et al. 2009). The uptake rate of *C. myricaas* when in contact with heavy metals (cadmium) was measured under experimental conditions to determine the uptake and impact of heavy metals on the clearance rate.

2. Materials and Methods

2.1. Preparation of Biomass

Cystoseria myricaas was collected from the Persian Gulf on Bushehr Island. It was washed several times with distilled water to remove dirt. The clean alga was dried in an oven at 60 °C for 24 h and powdered to maximize the surface of absorption. The dry biomass was chopped and sieved and finally used for biosorption measurements (7).

2.2. Dissolutions

Experiments were performed using synthetic single-metal solutions of Cd (II) prepared from chemical reactants of analytical grade, $\text{CdNO}_3 \cdot 4\text{H}_2\text{O}$. Concentrations varied in each case. Different initial pH was adjusted with 0.1N HCl and 0.1N NaOH (8).

2.3. Analytical Methods

First, the chemical solution and bio-sorbent were in contact in specific time and then filtered. The concentration of chemical was determined using a Varian SpectrAA.200 atomic absorption spectrometer.

2.4. Mechanisms of Interactions with Metal Species

2.4.1. Ion Exchange

“Ion exchange” refers to a class of mechanisms in which adsorbing metal ions take the place of other species already associated with the sorbent surface. These entities may be metal ions such as Na^+ , Ca^{2+} , or the proton, etc. Let us suppose that a given bio-sorbent material has been prepared by equilibration in either NaOH solution or NaCl brine. In such cases, it is reasonable to expect that acidic sites on the substrate will be mainly associated with Na ions. If the Ni (II) ion, for instance, is then introduced to the system, it may have a higher affinity for acidic sites in comparison to a monovalent action. The resulting competition will lead to a net desorption of Na^+ from surface sites and a net uptake of Ni (II).

2.4.2. Chemical Complexation

The concept of chemical complexation assumes a site-specific interaction between particular kinds of metal ions and functional groups at the sorbate surface. Based on such concepts, some authors (9) propose that chemical complexation, rather than ion exchange, accounts for the adsorbed amounts as a function of solution concentrations.

2.4.2.1. Metal Specificity

Some of the most cogent evidence in support of a chemical complexation concept of metal ion-sorption consists in a dependency of molar adsorption capacities on the identity of the tested metal ion (10). According to theories of chemical complexation, differences in the ability of a surface site to bind different metals are often attributed to matching the radius of the metal ion, as well as the symmetry (octahedral, etc.) of its valence electron orbitals, to the positions of the surface-bound atoms (e.g., carboxylate groups) at the site of absorption.

2.4.2.2. Hard and Soft Ions

The concept of hard and soft ions has been used effectively to explain why certain metal ions tend to have a greater affinity for certain types of sorbent surfaces (11). “Soft” ions have relatively loosely-held, polarizable outer electrons, so that greater contributions of covalent character can be expected in their interaction with surface sites. Examples are lead and mercury. By contrast, “hard” ions have more closely-held, less polarizable outer electrons, so that their interactions are more simply dominated by electrostatic factors. Nickel is a prime example. In some cases, the molar amounts of adsorbed ions have been shown to be related to the ionic radius of the metal (12). Soft ions thus favor sites containing S, C, and P atoms, whereas hard ions favor sites with O and N atoms.

2.5. Modeling

Optimization of biosorption of heavy metals by the classical method involves changing one independent variable, while maintaining all others at a fixed level. It is extremely time consuming and expensive to perform biosorption on a large number of variables. To overcome this difficulty, experimental factorial design and response methodology can be employed (13).

Optimum conditions for the biosorption of Cd (II) by *C. myricaas* were determined by means of response surface methodology (RSM). Five variables, including pH (A), temperature (B), *C. myricaas* dosages (C), heavy metal concentration (D), and contact time (E) are studied. The independent variables used in this study were coded according to Equation 1:

$$x_i = \left(x_i - \frac{x_0}{\Delta x} \right) \quad (1)$$

Where x_i is the dimensionless coded value of the i th independent variable, X_0 is the value of X_i at the center point, and Δx is the step change value. The following second-order polynomial model Equation 2 is used to explain the behavior of the system:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} x_i x_j \quad (2)$$

Where Y is the predicted response; X_1, X_2, \dots, X_k are the input variables, which affect the response Y ; $X_{i2}, X_{j2}, \dots, X_{k2}$ are the square effects; $X_i X_j, X_i X_k, X_j X_k$ are the interaction effects; β_0 is the intercept term; β_i ($i = 1, 2, \dots, k$) is the linear effect; β_{ii} ($i = 1, 2, \dots, k$) is the squared effect; β_{ij} ($i = 1, 2, \dots, k; j = 1, 2, \dots, k$) is the interaction effect and e_i is the error (14, 15).

The optimum values of the selected variables were obtained by solving the regression equation. Each of the parameters was coded at five levels: -1.5, -1, 0, +1 and +1.5. The

series and levels of individual variables are presented in [Table 1](#).

3. Results

3.1. Statistical Analysis

32 experiments were selected from initial lists of response surface methodology to obtain observed data. Subsequently, theoretical values were obtained for the best conditions for removal of cadmium by equation Equation 3:

$$Y = 72.3172 - 0.6140 \times A + - 0.2757 \times B + 0.1587 \times C + 3.4066 \times D + 0.8713 \times E - 3.9006 \times A \times A - 1.5354 \times B \times B + 2.2089 \times C \times C + 3.2927 \times D \times D + 0.9190 \times E \times E + 0.3563 \times A \times B + 0.1507 \times A \times C - 1.0006 \times A \times D - 1.3013 \times A \times E + 0.3813 \times B \times C + 0.1614 \times B \times D + 0.1987 \times B \times E + 0.9744 \times C \times D - 0.0763 \times C \times E + 0.0884 \times D \times E \quad (3)$$

The second order response surface model for optimum values obtained from the results of multiple linear regressions is shown in [Table 2](#). The significance of model terms included in the regression equations were estimated by the F-test ([Table 2](#)).

The associated P value is used to judge whether the F value is large enough to be considered statistically significant. Results were considered significant at the level of $P < 0.05$. Thus, C, E, A2, B2, C2, D2, E2, AB, AC, AD, BC, BD, BE, CD, CE, and DE are significant: they are effective means of removing cadmium Equation 4 ([16](#)).

$$Y = 72.3172 - 0.6140 \times A + 3.4066 \times D + 0.8713 \times E - 3.9006 \times A \times A - 1.5354 \times B \times B + 2.2089 \times C \times C + 3.2927 \times D \times D + 0.9190 \times E \times E + 0.3563 \times A \times B + 0.1507 \times A \times C - 1.0006 \times A \times D - 1.3013 \times A \times E + 0.3813 \times B \times C + 0.9744 \times C \times D \quad (4)$$

3.2. Residual Plots for Data Observed

The histogram curve was employed for the investigation of distribution of error. The histogram curve resembles a bell-shape, which shows that the second-order polynomial model is correct. We can use this mathematical function, i.e., equation 4, to predict nickel biosorption for other conditions ([Figure 1](#)).

3.3. Response Surfaces and Contour Plots

Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels is helpful in understanding both the main and the interaction effects of these two factors. The response surface curves for the removal of cadmium have been drawn in this study. Effects of PH and temperature °C at time of absorption are

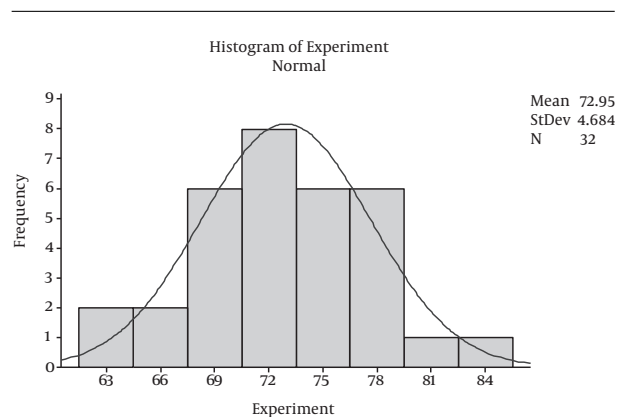


Figure 1. Residual Plots for Observed

illustrated in [Figure 2](#). The combined effect of pH and temperature on removal efficiency of Cd (II) at constant adsorbent dosage (5 g/L) and contact time (45 min) and initial concentration (100 mg/L) are illustrated in [Figure 2](#). The percentage removal of Cd (II) ion increased with increases in temperature from 20 to 45°C. Intensity of Cd (II) uptake or increases in temperature may be related to disturbed bonds between cell walls and actions ([17, 18](#)).

Maximum uptake was observed at pH = 3 ([Figure 2](#)), in which the algae cells could develop a net negative charge. Hence, the strong pH dependence of Cd (II) biosorption observed in this study could be attributed to more pronounced electrostatic attraction occurring between the bio-sorbents and the metal ions at a higher pH. It has also been reported that the solution pH may vary during the process of heavy metal biosorption, indicating the involvement of the hydrogen ion in the process of heavy metal biosorption. The effect of pH solution on biosorption are similar to those of weakly-acidic cation exchange resins. pH solution can be related to a number of mechanisms, such as metal speciation in solution, changes of ionic forms of the functional groups involved in biosorption, and the competition effects of hydrogen ions. At solutions with a very low pH, the concentration of the hydrogen ion is high, and can directly compete with the heavy metal ions. The protonation of the binding sites or the ionized functional groups on the cell wall make the binding site inaccessible to heavy metal cations. The increase in heavy metal uptake has been attributed to reduced solubility and metal precipitation.

The effect of adsorbent dose and concentration metal on Cd(II) removal at constant pH (3.00) and contact time (45 min) and constant temperature 40°C is shown in [Figure 3](#). Percentage of Cd (II) ion removal increased with increases in adsorbent dose. Such a trend is mostly at

Table 1. The Experimental Ranges and Levels of Independent Variables for Cadmium Uptake

Independent Variable	Range and Levels				
	-1.5	-1	0	1	1.5
PH (A)	1	2	3	4	5
Temperature (B) °C	20	30	40	50	60
Sorbent dosage (C), g/L	3	4	5	6	7
Cadmium concentration (D), mg/L	50	75	100	125	150
Contact time (E), min	15	30	45	60	75

Table 2. Analysis of Variance (ANOVA) for Response Surface Quadratic Mode^a

Term	Coef	SE Coef	T	P
Constant	72.3172	0.2117	341.574	0.0
A	-0.6140	0.1378	-4.455	0.001
B	-0.2757	0.1378	-2.001	0.071
C	0.1587	0.1378	1.151	0.274
D	3.4066	0.1378	24.720	0.0
E	0.8713	0.1378	6.323	0.0
A*A	-3.9006	0.1842	-21.172	0.0
B*B	-1.5354	0.1842	-8.334	0.0
C*C	2.2089	0.1842	11.989	0.0
D*D	3.2927	0.1842	17.872	0.0
E*E	0.9190	0.1842	4.988	0.0
A*B	0.3563	0.1560	2.284	0.043
A*C	0.1507	0.1560	0.966	0.355
A*D	-1.0006	0.1560	-6.414	0.0
A*E	-1.3013	0.1560	-8.343	0.0
B*C	0.3813	0.1560	2.445	0.033
B*D	0.1614	0.1560	1.035	0.323
B*E	0.1987	0.1560	1.274	0.229
C*D	0.9744	0.1560	6.247	0.0
C*E	-0.0763	0.1560	-0.489	0.634
D*E	0.0884	0.1560	0.566	0.582

^aR-Sq = 99.37%, R-Sq (adj) = 98.23%.

tributed to an increase in the sorptive surface area and the availability of more active binding sites on the surface of the adsorbent. At concentrations of 150 mg/L and adsorbent doses of 7 g/L, algae removed 88.21% of Cd (II) from the aqueous solution.

Figure 4 shows the interaction between initial concentration and contact time at constant pH (3.00) and adsorbent dose (5 g) and constant temperature 40°C. After

75 minutes, initial concentrations of 150 mg/L removed 87.03% of Cd (II). As illustrated in Figure 4, adsorption of Cd (II) ion increased with increases in contact time. The fast adsorption rate at the initial stage may be explained by an increased availability in the number of active binding sites on the adsorbent surface.

The effect of PH and time on Cd (II) removal at constant temperature 45°C and initial concentration 100 mg/L and

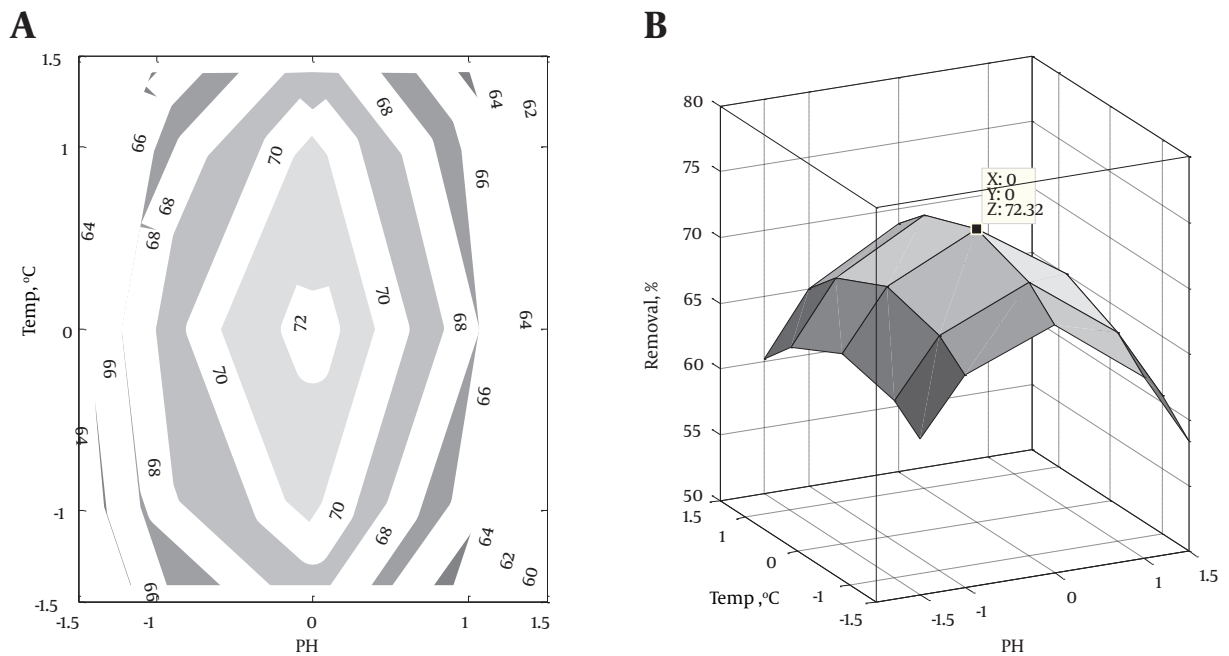


Figure 2. Effect of the Interaction Between pH and Temperature on Cd (II) Removal a Contour Plot b Response Surface Plot

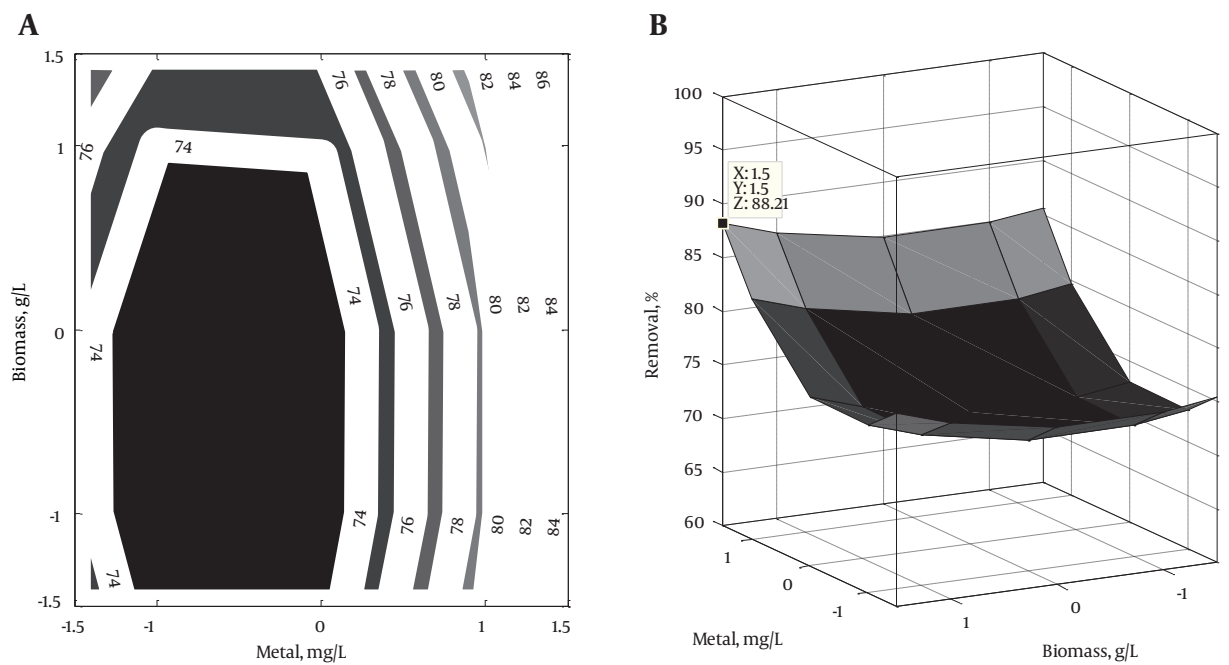


Figure 3. Effect of the Interaction Between Biomass and Initial Concentration on Cd(II) Removal a Contour Plot b Response Surface Plot

biomass 5g/L is shown in Figure 5. At 75 minute and PH 3, removed 78.27% of Cd (II).

The interaction between biomass and temperature on

Cd (II) removal is presented in Figure 6. It was observed that a sharp increase in Cd (II) ion removal occurred when the biomass value of the solutions changed from 5 to 7.

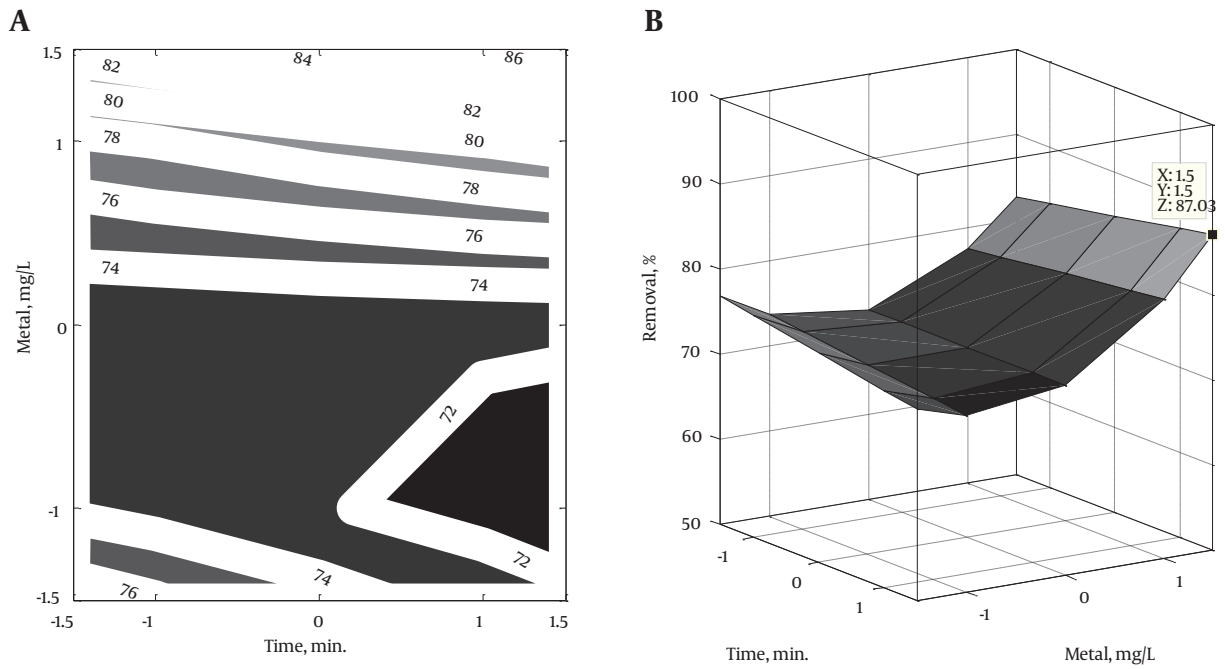


Figure 4. Effect of the Interaction Between Time and Initial Concentration on Cd (II) Removal a Contour Plot b Response Surface Plot

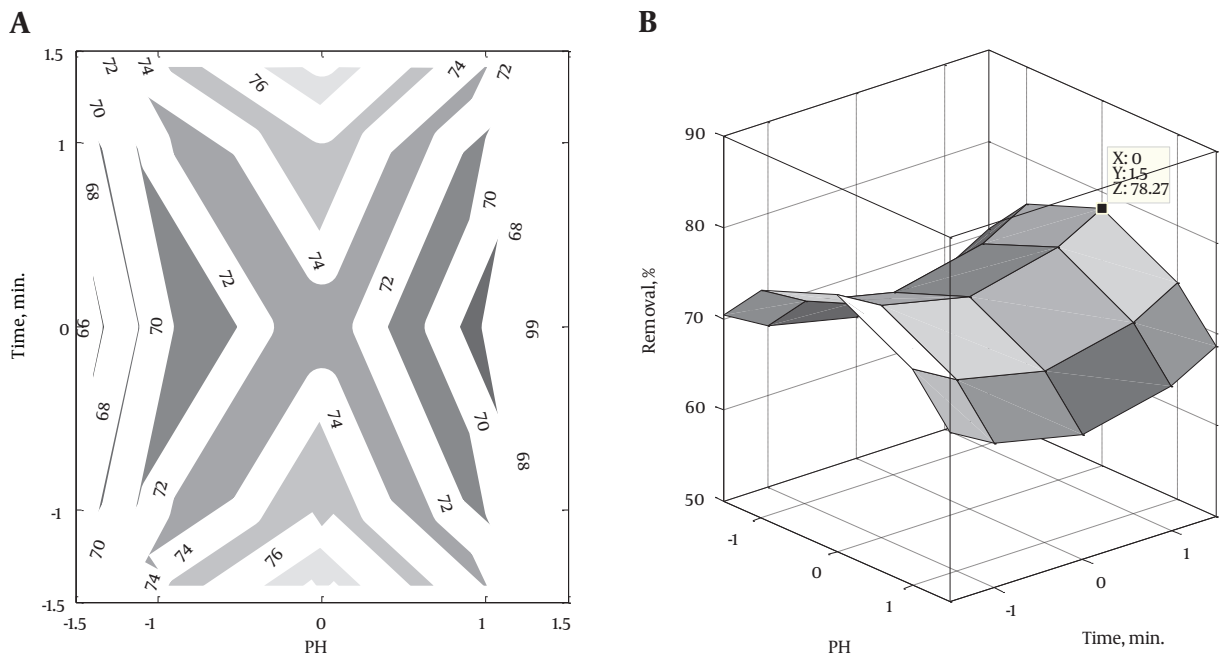


Figure 5. Effect of the Interaction Between PH and Time on Cd (II) Removal a Contour Plot b Response Surface Plot

The maximum adsorption of Cd (II) ions are obtained at biomass 7g/L and a temperature of 40°C (19, 20).

3.4. Fourier Transforms Infrared Spectroscopy

The functional groups contained in the samples were identified using FTIR technique. Cell walls were observed

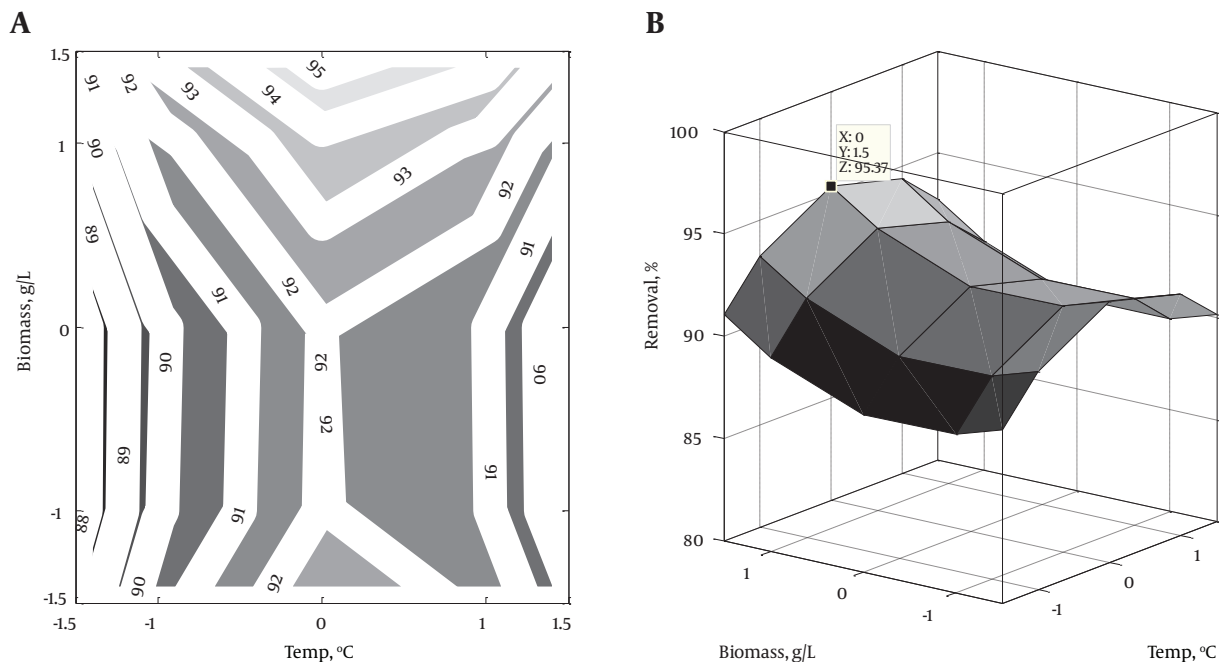


Figure 6. Effect of the Interaction Between Biomass and Temperature on Cd (II) Removal a Contour Plot b Response Surface Plot

to contain functional groups: The 3435 cm^{-1} as a result of -NH_2 and -OH stretching's, -CH at 2962 cm^{-1} , $\text{C}=\text{O}$ at 1631 cm^{-1} , (amide II) at 1431 cm^{-1} , SO_3 at 1262 cm^{-1} and in end C-O at 1029 cm^{-1} (Figure 7). The decrease in functional groups in *C. myricaas* is the result of biosorption of heavy metals by processes such as complex, exchange ion, physical biosorption, and form precipitated.

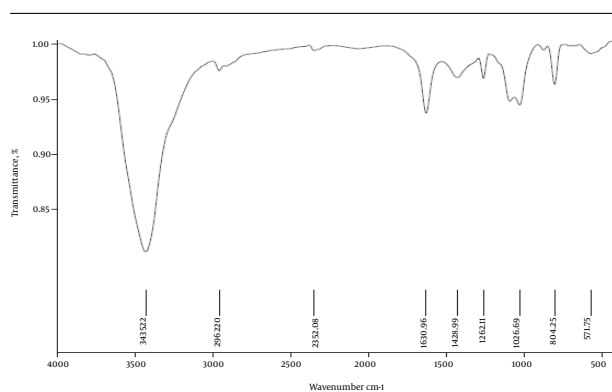


Figure 7. FTIR Spectra of Biomass (*Cystoseria myricaas* Algae)

The ten electrons in orbital d Cd (II) replace the (d^{10}) as a result of complex $[\text{Cd}(\text{H}_2\text{O})]^{2+}$. According to crystal field theory (CFT) and valence theory, these can be sorpted by

chemical complexation on algae.

Thus, the exchange of kinetic ligand H_2O with functional groups on surface algae during the process of chemical complexation is high.

A kind of substitution reaction in chemical complexation for biosorption is dissociative (D) oniony and/or interchange of kind dissociative (I_d) that functional groups were replaced with H_2O of $[\text{Cd}(\text{H}_2\text{O})_6]^{2+}$.

In the dissociative mechanism, the $\text{Cd-H}_2\text{O}$ bond is broken before the entering group y attaches (y = functional groups of algae). The coordination number of Ni (II) decreased by one during the transition state. This mechanism is denoted as D corresponding to the notation S_{Ni} used for substitution at a carbon center. In dissociative mechanisms, the rate-determining step involves bond breaking and the energy required for this determines the activation energy.

The interchange mechanism lies between these extremes, as it involves the synchronous weakening of the $\text{Cd-H}_2\text{O}$ bond and attachment of y . It is denoted as I and corresponds to the notation S_{Ni2} used in organic chemistry. If bond weakening makes a larger contribution to the energy of the transition state, then the interchange mechanism is labeled I_d .

Cadmium (II) ion is the softest, most stable chemical complexation metal ion formed with carbon from the lig-

ands CO, RNC and metals S and P, all of which are soft.

4. Discussion

The correlation relation (R^2) was calculated to be 99.37. This value indicated that more than 99% of observed data was compatible with the data predicted by the model and only less than .63% of the total variations were unexplained by the model. The adjusted R^2 value corrects the R^2 value for the sample size and for the number of terms in the model. Further, the value of the adjusted R^2 (98.23%) was suitable to advocate a high significance of the model. The experiment was conducted in variable conditions, such as pH, temperature, sorbent dosage, metal concentration and contact time. In this study, 32 experiments for finding optimum were conducted (21).

Cystoseria myricaas was used to remove nickel ion in solution. The effects of pH, temperature, biomass dosages, nickel concentration and contact time were investigated. The optimum conditions with respect to all variables were determined using response surface methodology (RSM). A second-order regression model was used to analyze the data. The optimum conditions for removing nickel can be obtained by insertion of the independent variables in our model. According to second-order polynomial models, optimum conditions for removing Cd (II) from aqueous solutions include: initial Cd (II) concentration-150 mg/L, pH-3.00, adsorbent dose-7 g/L, and contact time of 75 minutes. Under these conditions, the maximum removal of Cd(II) was 95.37% (22, 23).

References

1. Tichaona N, Olindah H. Equilibrium Isotherm Analysis of the Biosorption of Zn 2 Ions by Acid Treated Zea Mays Leaf Powder. *Int J Eng-Tec*. 1963;**6**(2):128-39.
2. Al-Mamun M, Poostforush M, Mukul SA, Subhan MA. Isotherm and Kinetics of As (III) Uptake from Aqueous Solution by Cinnamomum zeylanicum. *Res J Chemical Scie*. 2013;**2231**:606X.
3. Yoonaiwong W, Kaewsarn P, Reanprayoon P. Biosorption of lead and cadmium ions by non-living aquatic macrophyte, *Utricularia aurea*. *Sustain Environ Res*. 2011;**21**(6):369-74.
4. Davis TA, Volesky B, Vieira RHFS. Sargassum seaweed as biosorbent for heavy metals. *Water Res*. 2000;**34**(17):4270-8.
5. Lodeiro P, Barriada JL, Herrero R, Sastre de Vicente ME. The marine macroalga *Cystoseira baccata* as biosorbent for cadmium(II) and lead(II) removal: kinetic and equilibrium studies. *Environ Pollut*. 2006;**142**(2):264-73. doi: [10.1016/j.envpol.2005.10.001](https://doi.org/10.1016/j.envpol.2005.10.001). [PubMed: [16360252](https://pubmed.ncbi.nlm.nih.gov/16360252/)].
6. Kiran B, Thanasekaran K. Copper biosorption on *Lyngbya putealis*: application of response surface methodology (RSM). *Int Biodeterior Biodegrad*. 2011;**65**(6):840-5.
7. Takagi M, Yoshida T. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. *J Biosci Bioeng*. 2006;**101**(3):223-6. doi: [10.1263/jbb.101.223](https://doi.org/10.1263/jbb.101.223). [PubMed: [16716922](https://pubmed.ncbi.nlm.nih.gov/16716922/)].
8. da Cruz C. A. A. , Barros MUG, Bezerra JHC, da Silva JWA, Moreira RL, Farias WRL. Growth of the microalgae *Tetraselmis tetraathele* and nitrate depletion in culture medium Guillard f/2 and Conway-doi: [10.4025/actascibiolsci.v35i2.13971](https://doi.org/10.4025/actascibiolsci.v35i2.13971). *Acta Sci Biol*. 2012;**35**(2):163-8.
9. Valix M, Cheung WH, Zhang K. Role of heteroatoms in activated carbon for removal of hexavalent chromium from wastewaters. *J Hazard Mater*. 2006;**135**(1-3):395-405. doi: [10.1016/j.jhazmat.2005.11.077](https://doi.org/10.1016/j.jhazmat.2005.11.077). [PubMed: [16423455](https://pubmed.ncbi.nlm.nih.gov/16423455/)].
10. Qadeer R, Hanif I, Hanif J. Effect of different cations on the adsorption of Dy3+, Gd3+, Eu3+ and Sm3+ ions on activated charcoal from aqueous solutions. *Sci Technol*. 1996;**13**(1):27-9.
11. Avery SV, Tobin JM. Mechanism of adsorption of hard and soft metal ions to *Saccharomyces cerevisiae* and influence of hard and soft anions. *Appl Environ Microb*. 1993;**59**(9):2851-6.
12. Chen C, Wang JL. Characteristics of Zn2+ biosorption by *Saccharomyces cerevisiae*. *Biomed Environ Sci*. 2007;**20**(6):478-82. [PubMed: [18348406](https://pubmed.ncbi.nlm.nih.gov/18348406/)].
13. Korbahti BK. Response surface optimization of electrochemical treatment of textile dye wastewater. *J Hazard Mater*. 2007;**145**(1-2):277-86. doi: [10.1016/j.jhazmat.2006.11.031](https://doi.org/10.1016/j.jhazmat.2006.11.031). [PubMed: [17184910](https://pubmed.ncbi.nlm.nih.gov/17184910/)].
14. Rajeshkannan R, Rajamohan N, Rajasimman M. Removal of malachite green from aqueous solution by sorption on *Hydrilla verticillata* biomass using response surface methodology. *Front Chem Engin China*. 2009;**3**(2):146-54.
15. Saha PD, Chakraborty S, Das S. Optimization of hazardous crystal violet by chemically treated rice husk: Using central composite response surface methodology. *Arch Environ Sci*. 2012;**6**:57-61.
16. Rajasimman M, Murugaiyan K. Sorption of nickel by *Hypnea valentiae*: application of response surface methodology. *Int J Civil Environ Eng*. 2011;**3**(1):7-12.
17. Saleem M, Pirzada T, Qadeer R. Sorption of acid violet 17 and direct red 80 dyes on cotton fiber from aqueous solutions. *Colloids Surf A, Physicochem Eng Asp*. 2007;**292**(2):246-50.
18. Khezami L, Capart R. Removal of chromium(VI) from aqueous solution by activated carbons: kinetic and equilibrium studies. *J Hazard Mater*. 2005;**123**(1-3):223-31. doi: [10.1016/j.jhazmat.2005.04.012](https://doi.org/10.1016/j.jhazmat.2005.04.012). [PubMed: [15913888](https://pubmed.ncbi.nlm.nih.gov/15913888/)].
19. Özer A, Özer D. Comparative study of the biosorption of Pb (II), Ni (II) and Cr (VI) ions onto *S. cerevisiae*: determination of biosorption heats. *J Hazard Mater*. 2003;**100**(1):219-29.
20. Sarı A, Tuzen M, Uluözülü ÖD, Soylak M. Biosorption of Pb (II) and Ni (II) from aqueous solution by lichen (*Cladonia furcata*) biomass. *Biochem Eng J*. 2007;**37**(2):151-8.
21. Hamsaveni DR, Prapulla SG, Divakar S. Response surface methodological approach for the synthesis of isobutyl isobutyrate. *Process Biochemistry*. 2001;**36**(11):1103-9.
22. Faryal R, Hameed A. Isolation and characterization of various fungal strains from textile effluent for their use in bioremediation. *Pak A J*. 2005;**37**(4):1003.
23. Al-Homaidan AA. Heavy metal levels in Saudi Arabian *Spirulina*. *Pakistan J Biol Sci*. 2006;**9**(14):2693-5.