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Efficient biosorption of cadmium by *Eucalyptus globulus* fruit biomass using process parameters optimization

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ABSTRACT

BACKGROUND AND OBJECTIVES: Industrial wastewater usually contains metal ions which are hazardous to human and aquatic organisms. Nowadays, the application of inexpensive biomaterials in adsorptive removal of metal ions, such as plant biomass, has been widely considered. In this study, the efficiency of *Eucalyptus globulus* fruit biomass for biological adsorption of cadmium ions from aquatic environments has been evaluated.
METHODS: After drying, the collected biosorbent was ground and powdered. The dried biomass, after screening with particle size of less than 45 micrometers, was used in all experiments. The effects of operating factors, such as biosorbent to cadmium ratio, pH value of the solution and residence time of biomass and metal, on the amount of analyte adsorption were evaluated by response surface methodology. The optimum conditions for maximum metal uptake by *Eucalyptus globulus* fruit biomass were also evaluated using the *Box-Behnken* Design model. Kinetic studies were statistically described to investigate the metal adsorption process.
FINDINGS: Validation experiments showed the accuracy of the model proposed for determining the optimum conditions for the cadmium biosorption process. Based on the experimental data, the values of coefficient of determination, adjusted coefficient and predicted coefficient used in the model were determined as 0.9948, 0.9855 and 0.9245, respectively. Using the model, the maximum cadmium ion adsorption by biomass was obtained at 93.65 percent, biosorbent-to-metal ratio of 9:1, pH value of 6, and contact time of 80 minutes.
CONCLUSION: In the present study, the *Eucalyptus globulus* fruit biomass, under optimal operating conditions, proved to be an efficient sorbent for cadmium uptake from aqueous environments. The results from the experimental data of the adsorption studies were consistent with pseudo-second-order kinetics (maximum capacity of 128.2 milligram per gram), indicating that the chemical adsorption of cadmium on the used biomass occurring in monolayers.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
42	6	5

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INTRODUCTION

Heavy metals enter the environment and water resources through various processes such as battery manufacturing, metal plating, dyeing, etc. (Vilar *et al.,* 2007; Ehzari *et al.,* 2022a; Justus Reymond and Sudalaimuthu, 2023; Sulistyowati *et al.,* 2023; Sabilillah *et al.,* 2023; Sulistyowati *et al.,* 2023). Recent studies have shown that around 1.6 billion people cannot access clean water (Biswas, 2022). This trend is dramatically increasing with the growth of population, showing the necessity of using efficient methods to remove pollutants (Tauqeer *et al.,* 2021). So far, various methods, such as oxidative degradation, reverse osmosis, coagulation, photo-catalysis, membrane separation and ultrafiltration, have been used to purify water and remove pollutants from aquatic environments (Azimi *et al.,* 2019; Samimi and Shahriari-Moghadam, 2020; Janani *et al.,* 2022; Ehzari *et al.,* 2022b). These methods are costly, complex and time-consuming and require high energy consumption and skilled labor. In recent decades, the use of biosorbent has become more common due its high efficiency, simplicity, low cost, and environmental friendliness, compared to the mentioned methods (Abdelfattah *et al.,* 2016; Rafiq *et al.,* 2016; Wang *et al.,* 2021). Low concentrations of heavy metals lead to the production of free oxygen radicals causing cytotoxicity. Therefore, the removal of these contaminants, rather than other water pollutants, has been considered extensively (Islam *et al.,* 2021). Cadmium, as one of the heavy metals, is widely used in various industries. Cadmium ions are very toxic even at low concentrations, causing liver and kidney damage, high blood pressure, increased bone fragility, and decreased red blood cell count (Briffa *et al.,* 2020; Zhang *et al.,* 2020; Pipoyan *et al.,* 2023). Due to toxic properties and bioaccumulation of cadmium, it has been identified as one of the most dangerous metal pollutants by the United States Environmental Protection Agency (Parker *et al.,* 2022). The natural properties of the adsorbent are important in the adsorption processes aided by biological adsorbents. Various adsorbents have been studied and introduced for removing different compounds from aquatic environments (Safari *et al.,* 2019; Samimi and Moeini, 2020; Shourije *et al.,* 2023). Several studies have also been conducted on different parts of eucalyptus tree as an absorbent. For instance, the activated charcoal produced from *Eucalyptus urograndis* wood was used as an adsorbent for copper, cadmium, and nickel ions (de Souza *et al.,* 2023); *E. globulus* leaves were used as an efficient biosorbent for methyl blue dye (Ouldmoumna *et al.,* 2013); *E. camaldulensis* leaves were utilized for the removal of lead ions from aquatic environments (Sabri *et al.,* 2018); *E. globulus* wood was applied for the removal of lead ions (Tejada-Tovar *et al.,* 2021); and *Eucalyptus* leaf ash was used for the removal of cadmium, cobalt, and nickel ions from aqueous systems (Zavarmousavi and Khalegh, 2013). Eucalyptus tree belongs to the Myrtaceae family and includes about nine hundred species and subspecies. This evergreen plant, ranging from shrubs to tall trees, is native to Australia and Tasmania. Among various species of this genus grown in different regions of the world, *E. globulus* is the highly distributed one (Chandorkar *et al.,* 2021). Although numerous studies have been conducted on heavy metals removal by plant adsorbents, no study has investigated the efficiency of *Eucalyptus globulus* fruit for the removal of metal pollutants. The aim of this study was to evaluate the efficiency of *E. globulus* fruit biomass (EFB) for the biological removal of divalent cadmium (Cd(II)) from aqueous environments. In addition, biomass characteristics, optimization of operational factors by Response Surface Methodology (RSM) and Box-Behnken design (BBD), biosorption mechanism, and adsorption kinetics of the biosorbent in the biological adsorption of the analyte were investigated. This study has been carried out in Kermanshah, Iran in 2023.

MATERIALS AND METHODS

Preparation of biomass and the equipment

The *E. globulus* fruit was collected from Zabol city in Sistan and Baluchistan - Iran. The biomass preparation process was carried out according to the study conducted by Samimi and Shahriari-Moghadam (2023). The fruits were washed with deionized water to remove impurities from the surface of the biosorbent and then dried at 35 degrees Celsius (°C) in an oven for 48 hours. The dried adsorbent was ground, powdered, and sieved. Finally, the prepared EFB, with a particle size <45 micrometer (µm), was applied in further studies. Metals measurements were performed using a flame atomic absorption spectrometer (Savant AA model, Australia) and Fourier-transform infrared spectroscopy (FTIR) by a spectrometer (Bruker Ltd., Germany).

Batch adsorption

A certain amount of cadmium sulfate ($CdSO_4.2H_2O$) salt was dissolved in double distilled water and stirred at 100 revolutions per minute (rpm) for 15 min to achieve 1000 milligram per liter (mg/L) of Cd(II) solution. The solutions, with different conditions, required for the experiments were prepared by diluting the stock solution. To evaluate the ability of EFB in Cd(II) adsorption, 100 mg of biosorbent was added to 100 milliliter (mL) of the analyte solution (at 120 rpm) with different concentrations of metal ions. The analyte solution was vortexed and stirred at different times of the experimental design. The EFB-Cd solution was centrifuged (using centrifuge model Z205-A - USA) at 5500 rpm for 15 min to separate any solids from it. The amount of cadmium ions adsorption was indirectly analyzed by determining the residual metal in the supernatant. The kinetics of Cd(II) biosorption by EFB was studied in batch systems at room temperature. Cadmium uptake per gram of EFB and its removal efficiency were calculated using Eqs. 1, 2 and 3 (Samimi and Safari, 2022).

Adsorbate removal efficiency (%):

$$\frac{(C_0 - C_e)}{C_0} \times 100 \tag{1}$$

$$q_e = \frac{(C_0 - C_e)}{M} \times V \tag{2}$$

$$q_t = (C_0 - C_t) \times \frac{V}{M} \tag{3}$$

Where, q_e and q_t are the EFB uptake capacity expressed in milligram per gram (mg/g) at equilibrium and time t (min), respectively; C_0 and C_e are the initial and final cadmium concentrations (mg/g), respectively; M is the EFB dosage (g); and V is the cadmium solution volume (L).

Design of experiments (DOE) and removal optimization

According to the number of operating variables, the Box-Behnken Design (BBD) model was used to determine the conditions of the experimental parameters. Minitab-18 software was used to implement the model. The RSM was used to achieve optimal conditions for the adsorbate removal process. The influence of the *E. globulus fruit* biomass (EFB)-to-cadmium ratio (EC), the potential of hydrogen (pH) of an aqueous solution containing cadmium ions, and the contact time (t) were evaluated as the most important experimental variables for the adsorptive removal of Cd(II). The operating parameters and their variety of levels (coded from -1 to +1) are summarized in Table 1.

The experimental variables were examined at EFB-to-cadmium ratios of 3:1, 6:1 and 9:1, pH values of 2, 4 and 6 and contact times of 10, 45 and 80 min. For this purpose, 15 tests were carried out based on the BBD model (13 tests and 2 duplicate tests to determine the errors). The DOE for operating variables and their results are reported in Table 2. For further statistical analysis of the mathematical model, an analysis of variance (ANOVA) was also performed with a significance level of 5 percent (%).

The full quadratic model for the correlation between the Cd(II) removal percent (CR%) and the response variables are expressed using Eq. 4 (Oliver Paul Nayagam and Prasanna, 2023).

$$CR\% = \alpha_0 + \sum_{i=1}^{3} \alpha_i X_i + \sum_{i=1}^{3} \alpha_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \alpha_{ij} X_{ij} \tag{4}$$

Where, CR% is the response; X_i and X_{ij} are independent variables; and α_0, α_i, α_{ii} and α_{ij} are intercept (offset term), linear, quadratic and interaction terms (regression coefficients), respectively. The F-value used for analyzing the statistical significance of the second-order models was calculated by dividing the mean-square regression value by the mean-square residual, as presented in previous studies (Moghadam and Samimi, 2022).

Table 1: The operational factors and variety of levels

Variables		Rang of levels		
Main factors	Symbol	-1	0	+1
EFB-to-cadmium ratio	EC	3:1	6:1	9:1
The pH value of analyte solution	pH	2	4	6
Contact time (min)	t	10	45	80

RESULTS AND DISCUSSION

Analysis of response variance and process optimization

Based on the BBD model, a least squares quadratic model (regression equation in uncoded units) was derived for the cadmium uptake as explained by Eq. 5 (Samimi and Shahriari-Moghadam, 2018).

$$CR\ \% = 51.63 + 11.265X_{EC} + 8.224X_{pH} + 18.426X_t - 1.74X_{EC}^2 - 2.20X_{pH}^2 - 12.73X_t^2 + 7.24X_{EC}.X_{pH} + 7.02X_{EC}.X_t + 6.52X_{pH}.X_t \quad (5)$$

The model, selected based on the comparison between the measured and predicted amounts of CR%, had coefficient of determination (R^2), adjusted coefficient (R^2_{adj}), and predicted coefficient (R^2_{pred}) values of 0.9948, 0.9855, and 0.9245, respectively. The ANOVA of the quadratic model for cadmium adsorption is presented in Table 3. Based on the results, the total degrees of freedom (DF), the DF for regression, and the DF for residual error were 14, 9, and 5, respectively. The higher the F-value with the p-values less than 0.05, the greater the significance

Table 2: DOE and responses for three independent factors

Run. No.	Manipulated variables			Response
	X_{EC}	X_{pH}	X_t	CR%
1	0	0	0	51.68
2	-1	-1	0	33.21
3	0	0	0	52.84
4	1	-1	0	44.67
5	-1	1	0	36.24
6	-1	0	1	39.11
7	1	0	1	72.28
8	0	0	0	50.37
9	1	0	-1	21.17
10	-1	0	-1	16.08
11	0	1	-1	19.55
12	0	-1	-1	17.20
13	1	1	0	76.64
14	0	-1	1	40.79
15	0	1	1	69.23

Table 3: The ANOVA of the quadratic model for cadmium adsorption

Source	DF	Sum of squares	Mean squares	F-Value	P-Value	Degree of significance
Regression	9	5455.07	606.12	106.40	< 0.001	Significant
Linear	3	4272.46	1424.15	249.99	< 0.001	Significant
X_{EC}	1	1015.20	1015.20	178.20	< 0.001	Significant
X_{pH}	1	541.04	541.04	94.97	< 0.001	Significant
X_t	1	2716.21	2716.21	476.79	< 0.001	Significant
Square	3	605.94	201.98	35.45	0.001	Significant
X_{EC}^2	1	11.13	11.13	1.95	0.221	Not significant
X_{pH}^2	1	17.93	17.93	3.15	0.136	Not significant
X_t^2	1	598.70	598.70	105.09	< 0.001	Significant
2-Way Interaction	3	576.67	192.22	33.74	0.001	Significant
$X_{EC}.X_{pH}$	1	209.38	209.38	36.75	0.002	Significant
$X_{EC}.X_t$	1	197.12	197.12	34.60	0.002	Significant
$X_{pH}.X_t$	1	170.17	170.17	29.87	0.003	Significant
Residual error	5	28.48	5.70			-
Lack-of-Fit	3	25.43	8.48	5.55	0.156	Not significant
Pure Error	2	3.05	1.53	-	-	-
Total	14	5483.56	-	-	-	-

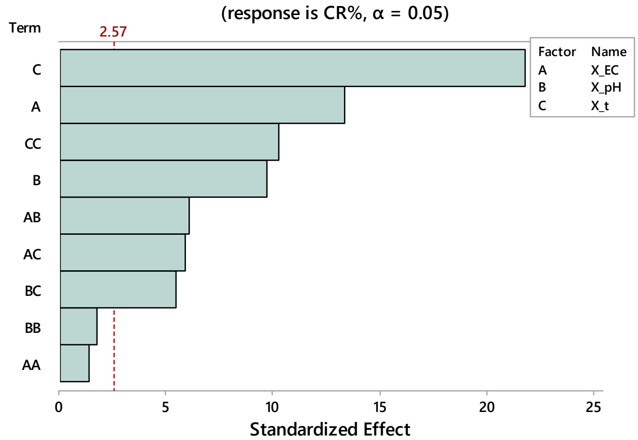

Fig. 1: *Pareto* graphical evaluation of the impact of standardized factors on CR%

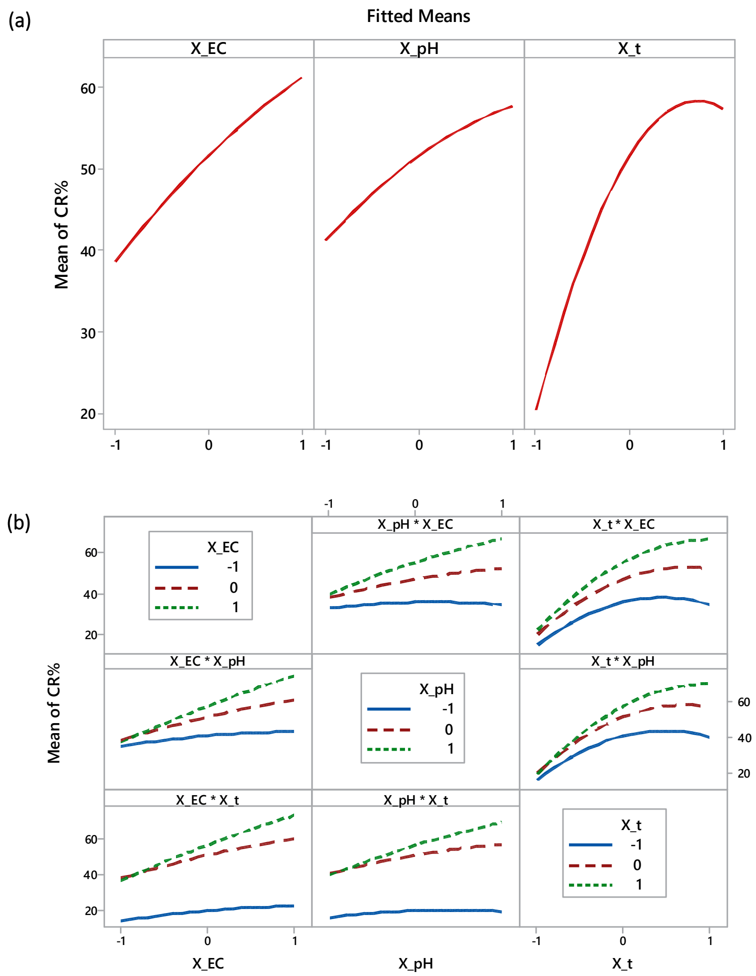

Fig. 2: The mean effects (a) and 2-way interaction diagrams (b) of coded operational parameters on CR%

level of the factors, the coded terms of X_{EC}, X_{pH}, X_t, X_t^2 and the 2-way interaction terms (namely $X_{EC}.X_{pH}$, $X_{EC}.X_t$, and $X_{pH}.X_t$). However, the parameters with p-values greater than 0.05, such as X_{EC}^2 and X_{pH}^2, were not significant.

As shown in the graphical *Pareto* analysis (Fig. 1), all terms except BB and AA (namely X_{EC}^2 and X_{pH}^2) crossed the hypothetical point boundary, confirming their importance as they were further from the vertical line (Samimi *et al.,* 2023a). The analysis result confirmed the data calculated from the regression equation.

The plot of the main effects and the mutual interactions of the encoded operating parameters on the average cadmium removal percentage based on the BBD model are shown in Fig. 2. Obviously, the cadmium uptake was higher at high levels of all operating parameters, and the increasing slope of X_t factor on the removal percentage was more evident. The interaction effects of the parameters also confirmed this trend. Fig. 3 shows the experimental model of CR% in contour plots created by Minitab software tool in different modes. In Fig. 3a, the contour diagram of cadmium sorption was plotted based on X_t and X_{pH} (with the encoded EFB-to-cadmium ratio at the average level: $X_{EC} = 0$). In Fig. 3b, the contour plot of CR% was drawn based on X_t and X_{EC} (with the encoded pH at the average level: $X_{pH} = 0$) and in Fig. 3c it was sketched based on X_{pH} and X_{EC} (with the encoded time at the $X_t = 0$). As shown in Fig. 3a, at the biosorbent-to-metal ratio of 6:1, the CR% increases with the simultaneous increase in contact time and pH value. The results of Fig. 3b at the medium pH level (i.e. pH=4) illustrated that the highest CR% was proportional to the simultaneous increase of the contact time and the

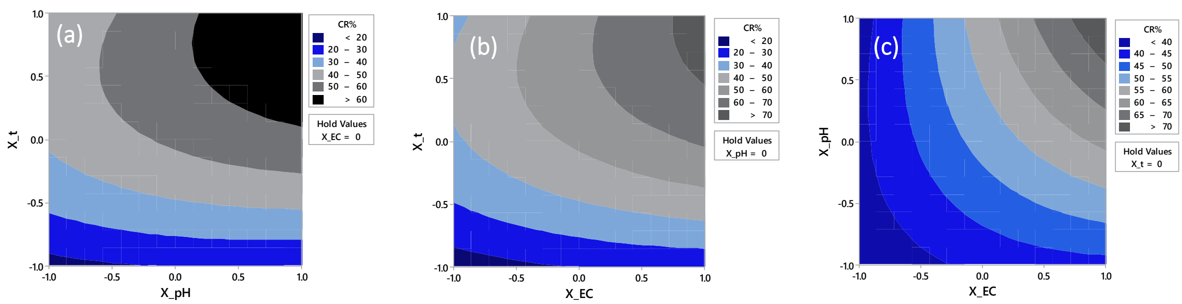

Fig. 3: The contour diagrams of the effect of operational variables on CR% for **a**) versus XpH; **b**) versus ; and **c**) versus .

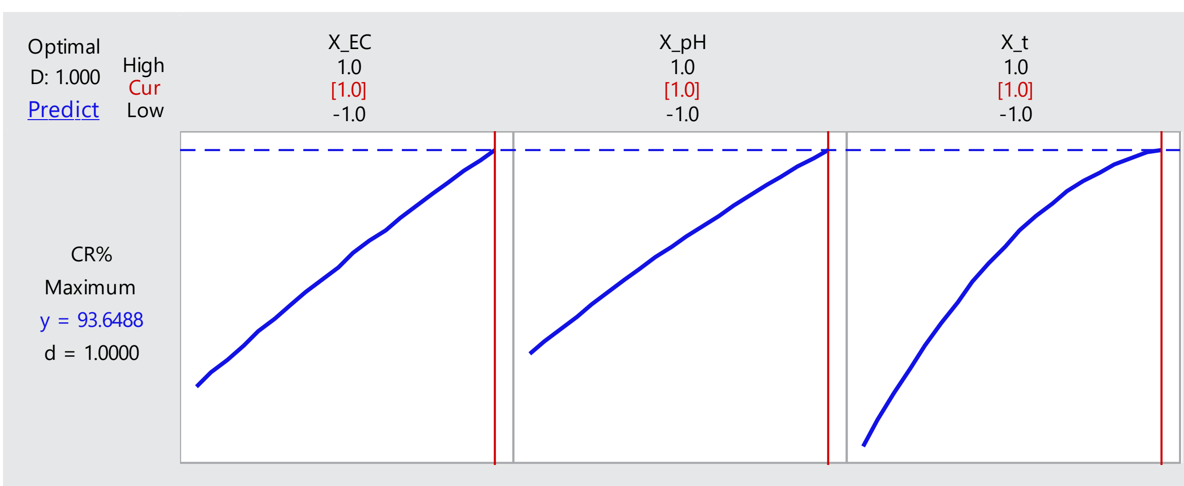

Fig. 4: Optimization diagram of the operating conditions in cadmium biosorption process by EFB

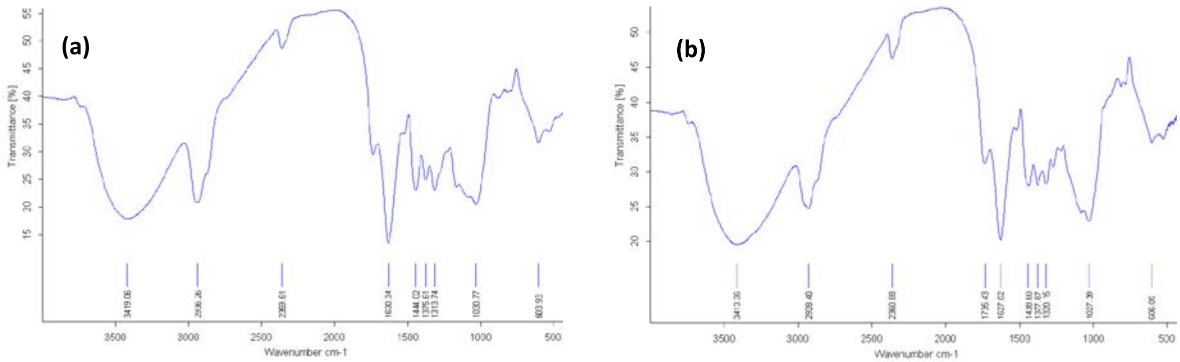

Fig. 5: The FTIR analysis for a) EFB and b) EFB-Cd(II)

Table 4. Constants of PFO, PSO and IDM in the Model

Models	PFO			PSO			IDM	
	q_e	k_1	R^2	q_e	k_2	R^2	k_p	C	R^2
	132.26	0.0514	0.946	128.205	1.85×10^{-4}	0.979	10.15	-3.782	0.969

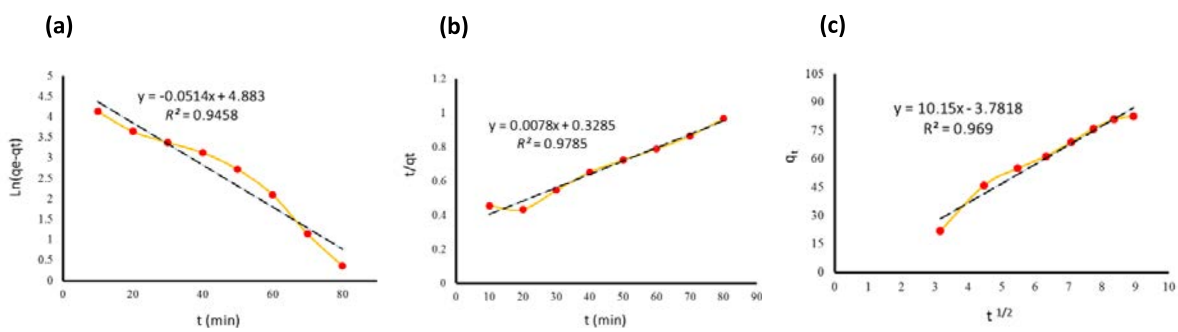

Fig. 6: Kinetics diagram of **a)** PFO, **b)** PSO, and **c)** IDM for Cd(II) biosorption by EFB

Table 5: Adsorption capacities of different biosorbents for the removal of heavy metals

Biosorbent	Analyte	Removal%	q_{max} (mg/g)	Optimal pH	Sources
Banana leaves activated carbon	Copper	83	66.2	5	Darweesh *et al.*, 2022
Corchorus olitorius leaf	Zinc	-	11.63	6	Ali and Bhakta, 2020
Activated *Eupatorium adenophorum*	Cadmium	88.9	45.45	7	Joshi *et al.*, 2022
Activated *Acer oblongum*	Cadmium	96	44.64	7	Joshi *et al.*, 2022
Strain-MS3	Lead	58.69	138.88	6.6	Samimi and Shahriari-Moghadam, 2021
EFB biosorbent	Cadmium	89.57	128.205	6	The current study

EFB-to-cadmium ratio. However, the pH increase in Fig. 3a or the sorbent-to-analyte ratio increase in Fig. 3b at low-time contact did not not considerably affect CR%. According to the direction of the lines and curves, the contour diagram of Fig. 3c at resident time of 45 min also revealed that the CR% increased with a simultaneous increase in pH value and EFB-to-cadmium ratio. However, at low sorbent-to-metal ratio levels, the pH changes did not significantly affect CR%.

Numerical optimization of the BBD was performed to predict the optimal conditions of EFB for achieving the maximum metal sorption. According to the optimization results presented in Fig. 4, the maximum CR% in aqueous environment, obtained at EC of 9:1, pH of 6 and contact time of 80 min, was 93.65%.

As shown in the BBD results (Table 3), the p-value of the *lack-of-fit* term was not significant (0.156), confirming the model's validity (Samimi *et al.,* 2023a). However, based on the experimental validation test under optimal conditions, the removal of cadmium from aqueous environments by EFB was found to be 89.57%. The small difference (<5%) between the value predicted by the model (93.65%) and the actual value (89.57%) demonstrated the high accuracy and validity of the proposed model.

FTIR analysis for biosorbent/metal

The prepared biosorbent was analyzed by FTIR spectroscopy to identify the functional groups of EFB. The FTIR spectrum of the biosorbent is shown in Fig. 5. According to Fig. 5a, the absorption peak at 3419/cm corresponded to the -OH group in EFB. The absorption band at 2936/cm was associated with the stretching vibration of the methyl group (C-H bonds). Other stretching vibration peaks were attributed to the C=O, C-C, and C-O bonds. The above active sites on the EFB surface could affect the adsorption of Cd(II). The shifted peaks after metal biosorption showed that Cd(II) was adsorbed on the surface of EFB (Fig. 5b).

Kinetics of metal biosorption

Cadmium uptake rate by EFB versus contact time were generally evaluated using adsorption kinetics by pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models and intraparticle diffusion mechanism (IDM). The linear forms of the mentioned models are expressed by Eqs. 6, 7 and 8, respectively (Samimi *et al.,* 2023b).

$$\ln\left(q_e - q_t\right) = \ln q_e - k_1 t \quad (6)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (7)$$

$$q_t = k_p t^{\frac{1}{2}} + C \quad (8)$$

Where, k_2 (g/mg/min) and k_1 (1/min) are adsorption rate constants for PSO and PFO kinetics, respectively; and k_p (mg/g/min$^{1/2}$) parameter is IDM constant. All the kinetics experiments were done at pH value of 6. The kinetics diagrams of PFO, PSO and IDM for cadmium adsorption are shown in Fig. 6a to c. As described in Table 4, the higher value of R^2 for PSO kinetics rather than PFO and IDM indicated that the rate-controlling stage in cadmium uptake was the chemical interaction between the EFB functional groups and Cd(II). Comparison of the cadmium uptake capacity obtained in this study and the adsorption capacities of different biosorbents reported in other studies has been presented in Table 5.

CONCLUSION

Cadmium ions, as one of the heavy metals, find their way into the aquatic environments through industrial applications. In the present study, a novel biomass derived from *E. globulus* fruit was evaluated for cadmium uptake from aquatic environments. The results of the regression equation, *Pareto* graphical analysis and contour plots revealed that all the selected experimental parameters, such as biomass-to-metal ratio, pH value of the solution, and contact time, significantly affected the cadmium biosorption by EFB biosorbent. Investigation of the parameters and numerical optimization of the quadratic model were performed to predict the optimum conditions for achieving the maximum cadmium uptake by EFB. The maximum metal uptake (at biosorbent-to-metal ratio of 9:1, pH value of 6 and residence time of 80 min) was determined as 93.65%. The statistical analysis and validation tests revealed the high accuracy of the BBD model in predicting the optimum conditions for cadmium adsorption by EFB. Based on the comparison between the measured and predicted amounts of CR%, coefficients of R^2, R^2_{adj}, and R^2_{pred} in the BBD model were 0.9948, 0.9855, and 0.9245, respectively. According to the results, the cadmium adsorption followed a PSO kinetic model, indicating that the rate-controlling stage in cadmium sorption was the chemical interaction between functional groups in EFB and Cd(II) ions. However, the q_e values calculated by PFO and PSO models (132.36 and 128.2 mg/g, respectively) were obtained from the experiments in optimal conditions based on the BBD model. The results of FTIR analysis, proved the active role of functional groups (e.g. methyl group and hydroxy group) on the EFB surface. The EFB, as a biomass prepared from worthless and unusable fruit for humans, showed a significant potential for the removal of heavy metals from wastewater. The present study illustrated that cadmium could be simply removed from aqueous environments using this naturally occurring adsorbent.

AUTHOR CONTRIBUTIONS

The corresponding author, M. Samimi, managed the project, supervised various stages of the study, wrote the original draft, performed the analysis and validation tests, and reviewed and edited the manuscript.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

%	Percent
°C	Degree Celsius
µm	Micrometre
ANOVA	Analysis of variance
BBD	*Box-Behnken* Design
C_0	Initial cadmium concentrations
Cd(II)	Divalent cadmium
$CdSO_4.2H_2O$	Cadmium sulfate
C_e	Final cadmium concentrations
CR	Cadmium removal
C_t	Cadmium concentrations at time t
DOE	Design of experiments
EC	EFB-to-Cadmium ratio
EFB	*E. globulus* fruit biomass
Eq.	Equation
Fig.	Figure
FTIR	Fourier-transform infrared spectroscopy
g	Gram
IDM	Intraparticle diffusion mechanism
L	Liter
mg	Milligram
mg/g	Milligram per gram
mg/L	Milligram per liter
mL	Milliliter
min	Minute
No.	Number
OD	Optical Density
PFO	pseudo-first-order
pH	Potential of hydrogen
PSO	pseudo-second-order
q_e	Uptake capacity (at equilibrium)
q_t	Uptake capacity (at time t)
R^2	Coefficient of determination
R^2_{adj}	Adjusted R^2
R^2_{pred}	Predicted R^2
rpm	Revolutions per minute

RSM	Response surface methodology
V	Volume
X_{EC}	EFB to cadmium ratio - Coded factor
X_{pH}	pH value - Coded factor
X_t	Contact time - Coded factor
α_0	Offset term
α_i	Linear term
α_{ii}	Quadratic term
α_{ij}	Interaction term

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