Kinetic, isotherm and thermodynamic aspects of Cu2+ biosorption onto Rosa damascena leaf as a low-cost biosorbent: Optimization of process variables by Response Surface Methodology

Mustafa A. Fawzy (mafawzy@tu.edu.sa)
Taif University
Hatim M. Al-Yasi
Taif University
Tarek M. Galal
Taif University
Reham Z. Hamza
Taif University
Esmat F. Ali
Taif University
Tharwat G. Abdelkader
Taif University

Research Article

Keywords:

Posted Date: December 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1120602/v1

Kinetic, isotherm and thermodynamic aspects of Cu^{2+} biosorption onto *Rosa damascena* leaf as a low-cost biosorbent: Optimization of process variables by Response Surface Methodology

Mustafa A. Fawzy*, Hatim M. Al-Yasi, Tarek M. Galal, Reham Z. Hamza, Esmat F. Ali, Tharwat G. Abdelkader

Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.

*Corresponding author, Email: mafawzy@tu.edu.sa, Phone (+9665594732248), (+201002931424)

Abstract

In this study, the *Rosa damascena* leaf powder was evaluated as a biosorbent for copper removal from aqueous solutions. Optimized conditions of 4.0 g/L biosorbent dosage, pH of 5.5 and initial copper concentration of 55 mg/L obtained by Response Surface Methodology were employed for Cu^{2+} biosorption by *R. damascena* leaves and up to 88.7 % Cu^{2+} was removed. The biosorption data were well fitted to the pseudo-second order and Elovich kinetic models. The Langmuir and Dubinin-Radushkevich isotherm models were also best fit the experimental data showing monolayer isotherm with q_{max} value of 25.13 mg/g obtained at optimum conditions. Thermodynamic parameters showed the spontaneity, feasibility and exothermic nature of adsorption. Scanning electron microscopy, Energy-Dispersive X-Ray, and Fourier transform infrared spectroscopy were used to characterize the biosorbent before and after Cu^{2+} biosorption, revealing outstanding structural characteristics and high surface functional groups availability. In addition, immobilized *R. damascena* leaves adsorbed 90.7 % of copper from aqueous solution, which is greater than free biosorbent (85.3 %). It can be concluded that *R. damascena* might be employed as a low-cost biosorbent for removing heavy metals from aqueous solutions.

Keywords: *Rosa damascena* leaves; Box–Behnken design; Copper; Isotherms; Kinetics; Thermodynamics.

Introduction

With the development of agricultural, industrial activities, and other human activity, various types of generated heavy metals have been released into water, and water resources have been severely contaminated[1]. Heavy metal pollution has been a hot topic for a long time because of its difficulty in removal and high toxicity[2]. Copper and its composites are the most frequent heavy metal contaminants in the

environment, according to the Environmental Protection Agency[3]. Wastewater from mining companies, tanneries, metal plating plants and refineries are the most prevalent sources of copper[4].

Copper is a trace metal that is needed as a catalyst by numerous enzymes in living organisms. High quantities of copper, on the other hand, may be highly poisonous and cause major health concerns. Extreme ingestion of copper causes liver accumulation, which can lead to anemia, gastrointestinal issues, and renal difficulties[5]. Several strategies for heavy metals removal from wastewaters have been developed in recent years. Some of these approaches include solvent extraction, membrane filtration, chemical reduction and precipitation, coagulation and ion exchange. The primary drawbacks of these procedures include the need for significant chemical amounts, residual metal solubility, costly capital investment, high operating costs, and the formation of a huge quantity of sludge[6]. Adsorption is a potential processing approach in terms of cost and efficiency for treatment of wastewater[7]. In recent research, a variety of typical agricultural waste products, microorganisms, algae, and natural polymers have been stated as excellent biosorbents for heavy metal removal[8-10]. Different parts of plants and their wastes are available and affordable when compared to chemically modified products. As a result, the majority of adsorption research has focused on untreated plant products and wastes[11-12].

Roses are cultivated as significant shrubs all over the world. These are decorative plants that are also utilized in medicine, fragrances, and scented products. Rose water, rose oil, and rose waste biomass are the three products of rose distillation[13]. Rose oil is made after distilling rose petals that is a blend of essential volatile oils. Rose oil is produced from two principal species of rose: Firstly, *Rosa damascena* which is commonly grown in China, India, Russia, Turkey, Spain, Italy, Bulgaria, and Saudi

Arabia. *Rosa centifolia*, which is more widely cultivated in France, Morocco, and Egypt, is the second species. Roses are widely grown in Saudi Arabia's governorates of Riyadh, Tabuk, Yanbu, and Taif. The Taif's rose is a widely distributed and cultivated plant in Saudi Arabia's cold and moderate regions. Because of the soil temperature and climate, the Taif's rose has a high-quality fragrance. Rose flowers biomass as a biosorbent after distillation has been examined in a number of studies[13-16].

Yet, to the best of our knowledge, no reports of Rose leaves being used as waste biomass for biosorption of heavy metals have been available.

As a result, *Rosa damascena* leaf powder was employed as a biosorbent to examine its efficiency in removing copper ions from aqueous solutions. A Response Surface Methodology (RSM) was used in the present study to optimize the examined response, providing significant advantages such as time, effort, and resource savings. Three independent factors were optimized, including biosorbent dosage, pH, and initial Cu^{2+} concentration, with the goal of maximizing copper removal by *Rosa damascena* leaf powder. Furthermore, to assess the efficiency of immobilized *Rosa damascena* biomass as a Cu^{2+} ion biosorbent. Kinetic, equilibrium, and thermodynamic investigations were carried out to study more about the biosorbent nature and the biosorption process. The biosorbent was characterized by SEM, EDX and FTIR techniques.

Results and Discussion

Box–Behnken Experimental Design

A Box–Behnken design was utilized for decreasing the number of tests and predicting the best conditions for copper removal by *R. damascena* leaf powder. Seventeen experiments were carried out using a BBD with varied combinations of three

parameters at three levels in order to maximize removal of Cu^{2+} ions from aqueous solution. Table 1 illustrates the actual and predicted removal percentages of Cu^{2+} for the 17 runs of the employed design matrix. The results show that the copper removal percentage varies greatly depending on the independent parameters. The copper removal percentage by *R. damascena* leaves varied between 44.25 % and 87.68 %. In run 4, with a biosorbent dosage of 5 g/L, pH of 6, and initial Cu^{2+} concentration of 60 mg/L, the maximum copper removal was obtained with a value of 87.68 %. Based on the Box–Behnken design results, a second order polynomial equation was created to characterize the relationship between the independent parameters and the response, and the final model generated by backward elimination of insignificant parameters is as follows (Eq. 4):

$$Cu^{2+}\text{ removal percentage} = 57.2 + 10.54A + 5.13B - 4.28C + 5.71A^2 \quad (4)$$

Where: A, B and C: the biosorbent dosage, pH and initial concentration of copper ions, respectively.

Table 2 summarizes the results of the analysis of variance (ANOVA) for the established model. The model was highly statistically significant, as evidenced by the high F-value (10.26) and low p-value (< 0.001). Furthermore, the F-value of lack of fit was 1.69, suggesting that lack of fit is not significant (as the p value is larger than 0.05) in comparison to the real pure error, and so the model validity may be affirmed[17].

The determination coefficient (R^2) and adjusted R^2 were used to assess the model's fit. The R^2 value ranges between 0 and 1.0, with values around 1.0 indicates that the model is more accurate. However, in certain conditions, a larger R^2 value indicates that the model has a large number of insignificant variables, which expects poor response. As a result, the adjusted R^2 was developed, which adjusts the value of R^2

based on the number of variables and sample size in the model. The high value of R^2 (0.87; Table 2) in this investigation implies that the actual and expected values are well correlated, and the model can explain 87.0 % of the variability in the response. The adjusted R^2 of 0.80 agrees rather well with the R^2 value of 0.87, indicating that the model is valid. The actual and expected results were highly correlated, with the adjusted R^2 value being high and close to the predicted R^2 value (Table 2).

Moreover, the value of variation coefficient as an estimate for standard error was less than 10%, indicating that the model was reproducible[18]. The signal-to-noise ratio is defined by adequate precision. In this investigation, a ratio of 9.8 (higher than 4) was found to be sufficient signal[19]. As a result, the model may be utilized to explore the design space. So, biosorption studies may be conducted using the model.

Effect of interactive variables

In order to understand the interaction impacts of the factors on the investigated response, 3-D response surface plots were made using the second-order equation (4) (Fig. 1). Each plot depicts the impact of two independent factors on the response within the examined ranges, while all other factors are kept constant.

In Fig. (1a), 3-D plots depict the reciprocal interaction between biosorbent dosage and pH on Cu^{2+} removal by *R. damascena* leaves biomass. The data revealed that raising the biosorbent dosage improved the Cu^{2+} biosorption by *R. damascena* leaves. ANOVA findings also demonstrated that biosorbent dosage was significant and had a positive linear influence on the efficiency of copper removal (p= 0.0003; Table 3). As the biosorbent dosage is increased, the number of binding sites on the surface of *R. damascena* leaves rises, resulting in a higher percentage of copper removal[20]. On the other hand, the elimination of copper by the *R. damascena* leaves is pH-dependent as well. The pH exhibited a significant positive linear influence on removal of copper by

R. damascena leaves, according to the ANOVA data (Table 3). When the pH was raised from 2.0 to 6.0, biosorption of Cu^{2+} increased. Whereas, the copper biosorption onto *R. damascena* leaves was minimal at lower pH levels. However, in the pH 6.0 range, the maximum removal efficiency was recorded. These findings are clarified by the fact that the surface charge of the biomass is positive at lower pH levels, which makes cation biosorption difficult. Meanwhile, H^+ ions compete with copper ions for active site space, leading to reduced biosorption process. The competitive impact of H^+ ions and electrostatic repulsions between cations and surface sites reduce as pH rises. As a result, metal biosorption rises[21]. Fawzy[22] stated that, pH 5.0 was shown to be the most effective for copper removal by *Codium vermilara*.

Fig. 1b depicts the mutual impacts of biosorbent dosage and initial copper concentration on the effectiveness of copper removal by *R. damascena* leaves.

When the biosorbent dosage was increased from 1 to 5 g/L, the copper removal efficiency increased. More binding sites on the surface of *R. damascena* leaves become available to the copper ions as the biosorbent dosage increases, resulting in enhanced removal efficiency. At 5 g/L of biosorbent dosage, the optimal removal effectiveness of 79 % could be achieved. Generally, higher biosorbent dosage and lower copper concentrations improved biosorption process[23].

As a result, raising the concentration of copper ions had a significant negative impact on Cu^{2+} ions removal (Table 3). Because more copper ions in the solution connected with the binding sites at lower copper concentrations, biosorption of Cu^{2+} ions gradually increased; however, as concentrations of copper increased, biosorption reduced due to biosorbent site saturation and a large number of ions competing for the residual binding sites in the biosorbent.

The joint influence of pH and initial Cu^{2+} concentrations on the metal ions removal was also investigated in the pH range of 2- 6 and initial copper concentrations of 30-90 mg/L as shown in Fig. 1c. The findings revealed that the removal of copper ions decreases as the pH reduces. The ANOVA analysis revealed that biosorbent dosage was the most statistically significant factor that influence the removal of copper (p= 0.0003), followed by pH (p= 0.03) and initial copper concentration (p< 0.05; Table 3).

Validation of the optimized variables

The goal of the optimization was to find the optimal values for independent parameters for Cu^{2+} ions elimination by *R. damascena* leaf powder. In terms of copper removal efficiency, the aim was to maximize copper removal efficiency to achieve the maximum optimal rate of Cu^{2+} removal. The average value for Cu^{2+} removal efficiency was compared to the expected value in triplicate experiments conducted under optimized conditions. With biosorbent dosage of 4.0 g/L, pH of 5.5, and initial copper concentration of 55 mg/L, the highest expected Cu^{2+} elimination by *R. damascena* biomass could be achieved. The experimentally observed value of copper removal efficiency (88.7 %) was found to be in accordance with the expected value (87.4 %) presented by the design expert software, implying that the optimized conditions were rational and trustworthy.

Kinetic models

The impact of contact time on the Cu^{2+} ions biosorption was used to evaluate the kinetics process. Copper biosorption was examined under optimal conditions of 4.0 g/L biosorbent dosage, pH 5.5, and initial Cu^{2+} concentration of 55 mg/L by altering biosorption time from 0 to 150 minutes (Fig. 2a). In the first 30 minutes, the rate of Cu^{2+} ion elimination was obviously fast. But, after reaching equilibrium, the biosorption efficiency increased to a steady level, and within 90 minutes, over 85.5 %

of the total metal had been eliminated. The rate of adsorption and desorption are now in dynamic equilibrium, and no biosorption progress was seen after this optimal equilibrium duration[24]. Because copper ions came in contact with unoccupied surface biosorption sites, biosorption of copper was initially quicker; however, as the equilibrium reached 90 minutes, the biosorption sites became saturated, and no further biosorption was allowed[25].

Various kinetic models might be used to explain the mechanism and rate of metal ion sorption[26]. The biosorption kinetics of copper ions on *R. damascena* leaf biomass was studied using pseudo-first order, pseudo-second order, Elvoish, and Intra-particle diffusion models.

Pseudo-first order kinetic model

This model suggests adsorption of one adsorbate molecule onto one active site of the biosorbent. It is expressed as follows:

$$Log\ (q_e - q_t) = Log\ q_e - \frac{K_1\ t}{2.303} \qquad (5)$$

Where q_e and q_t (mg/g): the quantity of copper ions absorbed by the biomass of *R. damascena* at equilibrium and at any time, respectively, and K_1 (min^{-1}): the pseudo-first order model's rate constant.

The constants of K_1 and q_e were estimated from the slope and intercept of plotting log ($q_e - q_t$) vs. time, respectively (Fig. 2b). The high value of determination coefficient (R^2= 0.980; Table 4), indicating that the experimental results accurately fit the pseudo-first order model for describing copper ion biosorption kinetics. However, the difference between the copper ions quantity biosorbed onto *R. damascena* surface estimated by experiments ($q_{e\ exp.}$; 17.1 mg/g) and the modeled value ($q_{e\ calc.}$; 13.4 mg/g) were larger. It suggests to the fact that the biosorption process included both copper ions and biosorbent[27]. As a result, it is indicated that the pseudo-first order

model is unable to describe the experimental data of Cu^{2+} biosorption onto the *R. damascena* biomass. The sorption kinetics of various metal ions onto various adsorbents has been described with similar findings[15, 28-29].

The rate constant of pseudo-first order (K_1= 0.029 min^{-1}; Table 4), is not a quantifiable value for clarifying the rapid equilibrium for biosorption of Cu^{2+} ions onto *R. damascena* leaves surface reported within 30 minutes. As a result, this model was shown to be inadequate to accurately explain the order of copper biosorption by *R. damascena* biomass.

Pseudo-second order kinetic model

The pseudo-second order kinetic model states that chemical adsorption, which includes the exchange or sharing of electrons between the adsorbate and the adsorbent, controls the process of adsorption. This model can be identified as follows

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \qquad (6)$$

Where K_2: is the pseudo-second order model's rate constant (g/mg min), which may be used to calculate the initial rate of biosorption (h; g/mg min).

$$h = K_2 q_e^2 \qquad (7)$$

The kinetic constants of K_2 and q_e were calculated from the intercept and slope of the t/q_t against t plot, respectively, (Fig. 2c).

The rate constant of pseudo-second order (K_2) and initial rate of adsorption (h) were 0.005 and 1.55 g/mg min, respectively (Table 4). At the beginning of the biosorption process, the h value was in good accordance with rapid biosorption of copper ions. Since the determination coefficient was determined to be 0.983, plots of pseudo-second order model showed strong linearity with the experimental data (Table 4). As well as, the experimental q_e (17.1 mg/g) is near to the calculated q_e (18.6 mg/g). Therefore, pseudo-second order was chosen as the best fit model, demonstrating

significant interactions between the adsorbate and the adsorbent, indicating the presence of copper chemisorption on the surface of *R. damascena* leaves. Several results support the pseudo-second order model for adsorption of copper ions by different biosorbents, including adsorption of Cu^{2+} onto activated rubber wood sawdust[30], *Tectona grandis* leaf[31], sour orange residue[32], banana trunk fiber[33], sulfur-modified bamboo powder[34], *Platanus orientalis* leaf powder[35].

Elovich model

The Elovich model was utilized to explain the kinetics of chemical adsorption of a gas by solid adsorbents, but it was proven to be effective in various types of adsorption. The Elovich model can be represented by the following equation:

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \qquad (8)$$

Where, α (mg/g min): the initial rate of sorption process, and β (g/mg): the constant of desorption.

To study the mechanism of Cu^{2+} biosorption, the experimental data were fitted to the Elovich kinetic model (Fig. 2d). By plotting q_t versus *lnt*, the Elovich constants (α and β) were determined from intercept and slope, respectively. The extent of chemisorption is proportional to the value of α. The high value of Elovich constant (α= 1.3 g/mg min; Table 4), implying chemisorption is the rate-limiting stage and approving the pseudo-second order type of biosorption.

The lower the value of Elovich constant β, the lower chemisorption activation energy, implying quick adsorption[36]. In the current investigation, the β value is fairly low (0.303 g/mg), suggesting a reduced activation energy of chemical adsorption. In addition, the high value of determination coefficient (R^2= 0.971), showing that the experimental data fits well the Elovich kinetic model.

Intra-particle diffusion model

The intra-particle diffusion kinetic model is related with the adsorbate diffusion to the inner pores as the rate-controlling step which is represented by eq. 9:

$$q_t = K_i t^{1/2} + C_i \qquad (9)$$

Where K_i denotes the intra-particle diffusion rate constant (mg/g $min^{0.5}$) and C_i denotes the intercept.

The biosorption of Cu^{2+} ions on *R. damascena* leaf surface was studied using an intra-particle diffusion kinetic model, which had a low R^2 value (0.80; Table 4). The linear plot indicated in Fig. 2e does not pass through the origin, proposing that the intra-particle model is not the rate-limiting stage during the process of biosorption. Fig. 2e also exhibited the multilinearity of the plot, which has two steps. All of the points of experimental data found on two straight lines, with the first line indicating copper ions biosorption on the most available empty sites or the external surface (external diffusion) on the *R. damascena* leaf surface, and the second steep line indicating copper ions biosorption on internal pores (intra-particle diffusion)[37].

Equilibrium Isotherms

At a certain experimental conditions, the adsorption isotherm represents the equilibrium correlation between the amounts of ions adsorbed by the biosorbent and the metal ions concentration in solution[38].

Biosorption equilibrium isotherms were performed under optimized conditions obtained by BBD. Langmuir, Freundlich, Temkin and Dubinin-Radushkevich isotherm models were developed to describe and estimate the experimental data of copper biosorption.

Langmuir model

This model implies that metal ions are adsorped by monolayer adsorption on a homogeneous surface with no interaction between the adsorbed metal ions. The Langmuir model is represented in linear form as follows:

$$\frac{C_{eq}}{q_e} = \frac{1}{q_{max}b} + \frac{C_{eq}}{q_{max}} \qquad (10)$$

Where q_{max}: the maximal sorption quantity (mg/g) required to produce full monolayer coverage on *R. damascena's* surface at a high equilibrium ion concentration (C_{eq}; mg/L), and b: the constant of Langmuir model, which is associated with binding site affinity[39].

The high value of R^2 (0.979; Table 5) indicated that Langmuir isotherm offers a suitable model of the biosorption process, which is based on homogeneous biosorption of copper ions by *R. damascena* leaf surface.

When C_{eq}/q_e was plotted versus C_{eq}, a straight line was formed, and the slope and intercept were used to determine the q_{max} and b values, respectively (Fig. 3a). The greater value of Langmuir constant (b= 0.095 L/mg), suggesting a stronger interaction with the functional groups of *R. damascena* leaf surface. Furthermore, *R. damascena* biomass had a maximum biosorption capacity (q_{max}) of 25.13 mg/g. *Rosa damascena* leaves are a very good biosorbent when compared to the capacity of other biosorbents occurred in the literature for copper removal. For example, dried marine green macroalga *Caulerpa lentillifera* has 5.57 mg/g[40], lentil shell has 8.98 mg/g[41], 14.4 mg/g for *Codium vermilara*[22] and 23.14 mg/g for dried bael flowers[29].

A dimensionless separation factor (R_L) may be used to determine the shape and favorability of the biosorption process, which can be computed using Eq. (11):

$$R_L = 1/(1 + bC_0) \qquad (11)$$

Where C_o: the metal ion concentration (mg/L). The type of Langmuir isotherm was determined by the R_L value, which was either unfavorable ($R_L > 1$), linear ($R_L = 1$),

irreversible ($R_L = 0$) or favorable ($0 < R_L < 1$)[35]. The value of R_L between 0 and 1 indicate that the adsorption is favorable. In the current study, the R_L value was determined to be 0.062–0.201, showing that copper biosorption by the leaves of *R. damascena* is favorable (Table 5).

Freundlich isotherm model

The adsorption of ions on an energetically heterogeneous surface is described by the Freundlich isotherm model. The following equation represents the linearized Freundlich model (12):

$$Ln\ q_e = Ln\ K_f + \frac{1}{n}\ Ln\ C_{eq} \qquad (12)$$

Where K_f: the Freundlich isotherm constant which reflects the sorption capacity, and n: the Freundlich constant correlated to the adsorption intensity.

The intercept and slope of the plotting of $ln\ q_e$ against lnC_{eq} are used to calculate the K_f and $1/n$ values, respectively (Fig. 3b). The greater the K_f value, the more biosorbent may be loaded. In addition, the adsorption is favorable when the $1/n$ value is between 0.1 and 1.0[42]. In this study, the value of $1/n$ was lower than 1.0 (0.313; Table 5), indicating that biosorption of copper ions by *R. damascena* leaves is favorable. The low value of determination coefficient (R^2= 0.735) suggested that Freundlich model is not appropriate for describing the experimental data of biosorption process (Table 5).

Temkin model

The adsorption in the Temkin model is supposed to be represented by a uniform distribution of binding energies up to the maximal binding energy, as shown in the following equation[43].

$$q_e = B\ lnA + B\ lnC_{eq} \qquad (13)$$

$$B = \frac{RT}{b} \qquad (14)$$

Where *A* (L/mg): represents the equilibrium binding constant, *b* (J/mol): the constant of Temkin isotherm model, and *B* (J/mol): the heat of sorption constant.

The Temkin model constants (*A* and *b*) were determined using the intercept and slope of the q_e versus lnC_{eq} plotting (Fig. 3c). The high *b* value (551.4 J/mol; Table 5) indicates that the adsorbate and biosorbent surface interact strongly[44]. The Temkin model fails to explain the results reported for copper biosorption by *R. damascena* leaves, with copper exhibiting low value of R^2 (0.807; Table 5). This demonstrates that biosorption of Cu^{2+} is exothermic in nature, with the adsorption heat of the molecules increasing linearly as the biosorbent surface is covered[45].

Dubinin–Radushkevich model (D–R)

The D–R model describes the chemical or physical nature of biosorption process, as well as the mean sorption energy of the process. D–R model is calculated from the following Equations:

$$Lnq_e = lnq_0 - \beta\varepsilon^2 \qquad (15)$$

$$\varepsilon = RT\, ln(1 + \frac{1}{C_{eq}}) \qquad (16)$$

$$E = \sqrt{1/2\beta} \qquad (17)$$

Where q_o: is the theoretical maximum capacity (mg/g), β: is the constant of D–R model associated with mean free energy (mol^2/J^2), ε: Polanyi potential, *T* (K): the absolute temperature, *R* (8.314 J/mol K): the gas constant, and *E* (kJ/mol): the mean adsorption energy.

Table 5 shows the values of the D–R model parameters. The mean adsorption energy of the system (*E*) was determined using the parameter β (eq. 17). In addition, the chemical and physical characteristics of the adsorption process may be assessed by the amount of mean adsorption energy.

Physical sorption is defined as a value of E less than 8 kJ/mol, whereas chemical sorption is defined as a value of 8 to 16 kJ/mol[25]. The value of E (9.13 k/J mol) indicates that *R. damascena* removed copper ions mostly by chemisorption. This is also consistent with predictions from pseudo-second order and Elovich kinetic models.

From the previous data, it is concluded that, the Langmuir and D–R isotherm models may best describe the experimental data of copper ions biosorption onto *R. damascena* leaf surface, according to a comparison of R^2 values (0.926; Fig. 3d; Table 5).

Thermodynamic studies

Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) are all thermodynamic parameters that describe the spontaneity of a biphasic adsorption process[46].

The following equations demonstrate the relationship between the thermodynamic parameters and absolute temperature (T)[47].

$$\Delta G = \Delta H - T\,\Delta S \qquad (18)$$

$$\Delta G = -RT\,lnK_C \qquad (19)$$

$$LnK_C = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \qquad (20)$$

Where *Kc*: the equilibrium thermodynamic constant.

At the experimental temperatures, the values of ΔG were negative (Table 6), indicating that the biosorption process was feasible and spontaneous[47]. Furthermore, the negative value of ΔG indicated that the concentration of copper ions on the *R. damascena* leaf was significantly higher than that in the aqueous solution[48]. Also, its values revealed that as the temperature rose, so did the spontaneity.

The changes of enthalpy and entropy were evaluated from the slope and intercept of the *ln K* versus *1/T* plot, respectively (Fig. 4).

The negative ΔH value of 21.7 K/J mol for copper biosorption by *R. damascena* leaves indicates that biosorption process was exothermic. In addition, the positive ΔS value indicated that biosorption of copper ions onto *R. damascena* leaf surface occurred as a result of randomization at the adsorbate-biosorbent interface[49].

Characterization of *R. damascena* leaf surface

Scanning electron microscopy (SEM)

The morphology of the *R. damascena* leaf surface before and after Cu^{2+} biosorption was examined by SEM (Fig. 5). SEM micrographs demonstrated that the surface morphology of the *R. damascena* leaf before and after Cu^{2+} biosorption was different. The SEM micrographs of the *R. damascena* leaf surface before copper biosorption revealed a rough surface with substantial porosity (Fig. 5a). This rough flaky surface allows copper ions to adhere more easily, improving the process of biosorption. The biosorbent's porosity also helps it interact with the adsorbate more quickly[50]. On the other hand, the SEM images after biosorption of copper revealed a flatter biosorbent surface, appearance of discrete lumps and vanishing of big spaces (Fig. 5b). These morphological alterations verified the interaction of copper ions with functional groups on the *R. damascena* leaf surface[22].

Energy dispersive X-ray spectroscopy (EDX)

The EDX analysis was used to determine the adsorbent surface's composition as well as to confirm the presence of copper ions on the *R. damascena* leaf surface. Figure 6 displays the EDX spectra of *R. damascena* biomass. The EDX spectra showed that the *R. damascena* leaves consist mostly of C and O, with traces of additional elements including Na, Mg, Cl, K, Si and Ca that were exchanged or disappeared during the process of biosorption (Fig. 6a, b). This shows that biosorption of Cu^{2+} ions was caused by ion exchange. After biosorption, the EDX spectra of *R. damascena* biomass

exhibit an additional Cu^{2+} peak (1.09 %) on the *R. damascena* leaf surface, demonstrating that the biomass of *R. damascena* is participated in biosorption of Cu^{2+} ions from solution (Fig. 6b). In this regard, El-Naggar et al.[51] observed that the distinctive copper peak appeared following contact with copper.

Analysis of Fourier transform infrared spectra (FT-IR)

The functional groups found on the surface of biosorbent biomass play a significant role in the process of adsorption. Heavy metal biosorption has been related to various functional groups such as sulfonate, sulfhydryl, amine, carboxyl, hydroxyl, carbonyl, and others[52]. Figure 7a, b displays the FT-IR spectra of *R. damascena* leaves before and after copper biosorption.

The presence of wide absorption peak at around 3421–3425 cm^{-1} is allocated to O–H stretching of hydroxyl radicals of polysaccharides or water[53], and to N-H stretching of proteins (amide A)[54]. The functional groups such as O–H and N–H are commonly present in natural cellulose and proteins found in plant cell walls[55]. The O–H stretching vibration of the carboxylic acid might be represented by the bands at 2921 cm^{-1} and 2922 cm^{-1} [55]. This refers to the existence of an acidic group in the biosorbent cell wall, such as -COOH, which serves as a hyper chemical group for the adsorption of various multivalent metal ions[56]. Absorption bands at 2852 cm^{-1} and 2853 cm^{-1} are allocated to stretching of C–H, more specifically to C−H stretching vibrations of lipids[57]. The C=O stretching of amide I, which is related with proteins, is shown by the absorption peak at around 1654 cm^{-1}[58]. After copper biosorption onto the *R. damascena* leaf surface, the appearance of new absorption bands at 1546 cm^{-1} and 1460 cm^{-1} might be attributed to C=O stretching vibration in different carboxylic and amide (I, II) groups, respectively[59]. The protein band spectrum identified at 1240 cm^{-1} on the leaf surface was caused by the P=O asymmetric stretching vibration[60]. The

absorption peak at about 1160 cm^{-1} detected only on the *R. damascena* leaf surface following copper biosorption is related to C–O–C stretching of polysaccharides from carbohydrates[53]. Furthermore, after copper biosorption, the peak at 878 cm^{-1} shifts to 893 cm^{-1}, indicating binding of copper ions to the amine group on the leaf surface. The band found only at 670 and 593 cm^{-1} onto the *R. damascena* leaf surface after copper biosorption may be associated with the compounds of organic halide[52]. From the Fig. 7, it can be observed that the *R. damascena* leaf biomass included several functional chemical groups such as carbonyl, acids, phosphate, amides, hydroxyl, halides, carboxyl, and amine groups. They might compensate the biosorption of copper ions onto *R. damascena* leaf surface from the aqueous solution.

Copper removal by immobilized *R. damascena* biomass

The results in Fig. 8 showed that the Ca-alginate immobilized *R. damascena* leaves removed 90.7 % of copper ions after 120 minutes under optimized conditions obtained by BBD such as biosorbent dosage (4 g/L), pH (5.5) and initial copper concentration (55 mg/L), which was higher than the removal shown using non-immobilized biosorbent (85.3 %). Various studies stated that immobilized biosorbent has the ability to use a straightforward approach for recovering and removing heavy metals from wastewater, in comparison to free biosorbent[61-62]. In this regard, Ansari et al.[15] reported that immobilized rose waste is more effective than free biomass at absorbing Pb^{2+} from aqueous solutions. Ca-alginate immobilized *Fucus vesiculosus* is also an effective biosorbent for copper, lead, and cadmium according to Mata et al.[63], with sometimes greater biosorption efficacy than free alga or even alginate alone. According to Davis et al.[64], the metal ions affinity for alginate is proportional to the quantity of guluronic acid and other uronic acids present. These acids are responsible for biosorption of heavy metals since they include the majority of the carboxyl groups

in alginate. Furthermore, the “egg-box” structure of the gels, as well as crosslinking between carboxyl groups and metal ions have been linked to alginate's metal selectivity. This selectivity is determined by the stereochemical environment created by the structure of the gel. Therefore, *R. damascena* immobilized in Ca-alginate has a great potential for adsorption of heavy metals from wastewater.

Conclusions

The Box–Behnken experimental design has been shown to be an effective method for maximizing the removal of copper ions from solutions using the *R. damascena* leaf by decreasing the number of experimental tests, expecting the highest efficiency conditions, and keeping a good accuracy of the predicted response. The maximum removal percentage of Cu^{2+} ions (88.7%) was reached under optimal conditions of biosorbent dose of 4.0 g/L, pH of 5.5, and initial copper content of 55 mg/L. The pseudo-second order and Elovich kinetic models were best fit to the experimental data. Furthermore, the equilibrium isotherm studies demonstrated that the D–R and Langmuir isotherm models could better describe Cu^{2+} biosorption than the Freundlich and Temkin models with a highest monolayer biosorption capacity of 25.13 mg/g, suggesting the chemical interaction between the metal ions and biosorbent. The thermodynamics parameters such as Gibbs free energy and enthalpy, as well as entropy, showed that the biosorption process is spontaneous and exothermic. As a result of the biosorption process, SEM and EDX tests indicated noticeable alterations in the properties of the *R. damascena* leaf surface. In addition, FT-IR investigations revealed the existence of functional groups in the *R. damascena* leaves biomass such as carbonyl, acids, phosphate, amide, hydroxyl, halide, carboxyl, and amine groups, all of which are likely to be involved in biosorption of copper ions. Immobilization was shown to be a promising method for producing efficient adsorbents, which might

be used to sequester metal ions from wastewater. Therefore, *R. damascena* leaves can be used as a low-cost biosorbent to remove copper ions from aqueous solutions.

Materials and methods

Preparation of biosorbent

Rosa damascena Miller var. *trigintipetala* Dieck was collected from Taif rose farms at Al-Shafa highland, Taif region, Saudi Arabia. Voucher specimens were deposited and identified by staff members of the herbarium of Taif University, Taif, Saudi Arabia. The leaves of the rose plant were removed off the plant, washed under running water to eliminate any impurities or pollutants, and then dried at room temperature for two weeks. For further investigation, the leaves were crushed into fine powder and kept in an airtight container.

Preparation of copper solutions

About 3.93 g copper sulphate ($CuSO_4.5H_2O$) was dissolved in 1000 mL distilled water to prepare the Cu^{2+} stock solution. All of the chemicals used in this investigation were analytical grade and obtained from Sigma-Aldrich, including $CuSO_4.5H_2O$, HCl, NaOH, Na-alginate and $CaCl_2$.

Optimization of Cu^{2+} removal by Box–Behnken statistical design (BBD)

Response Surface Methodology is a multivariable optimization approach that fits experimental results to a second-order equation to determine the optimal response of a process that is a function of numerous independent factors. Designing an experimental matrix, developing a mathematical model, and optimizing the response are the three key processes of RSM[65]. Experiments were developed to determine the optimal copper biosorption onto *R. damascena* leaves using Box–Behnken Design of RSM (Stat-Ease Inc., Minneapolis, USA). The design includes 17 runs with three independent factors such as biosorbent dosage (1, 3, 5 g/L), pH (2, 4, 6) and initial

copper concentration (30, 60, 90 mg/L), at three coded levels (−1, 0, +1) (Table 1). The following equation is a second-order polynomial equation concerning the independent factors and the dependent response.

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

Where Y: is the expected response, β_0: is the intercept term, β_i, β_{ii} and β_{ij}: are the linear, quadratic, and interaction impacts, respectively, X_i and X_j: are the independent variables, and ε: is the error.

Statistical analysis of the data

It has been stated that three-dimensional response surface plots may be used to analyze the principal and interaction impacts of two parameters while keeping all other variables constant. The regression model was used to produce the 3D response surface plots for the copper removal percentage, by keeping one factor at the center level. The experimental design and statistical analysis were done in Design Expert version 7. Multiple regression analysis and ANOVA were used to assess the experimental data, and the significance was determined at probability levels using the F test ($p \leq 0.05$).

Biosorption experiments

The tests were carried out in 250 mL conical flasks with 100 mL of copper solution, which were agitated at 170 rpm in a shaker. All of the tests were achieved at a temperature of 25 °C and a contact period of 90 minutes. Deionized water was used to prepare the solutions, and the pH was adjusted using 0.1 M HCl or 0.1 M NaOH.

Each experiment was repeated three times, with the average results provided. The biosorbent was removed from the solutions at the end of the biosorption procedure by centrifugation for 5 minutes at 4000 rpm.

The concentration of copper in the filtrate was determined by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) model Perkin Elmer Optima 2000 DV. The following equation is used to calculate the biosorption of copper ions onto *R. damascena* leaf biomass (q_e; mg/g).

$$q_e = \frac{V\,(C_i - C_{eq})}{W} \qquad (2)$$

Where C_i and C_{eq} (mg/L): the copper ion concentrations before and after the equilibrium contact time, V (mL): the volume of copper solution, and W (g): the weight of *R. damascena* leaf powder.

The removal percentage of Cu^{2+} ions by *R. damascena* leaves was calculated by the following Eq. (3):

$$\text{Removal}\ (\%) = \frac{(C_i - C_{eq})}{C_i} \times 100 \qquad (3)$$

The optimized conditions obtained from BBD were used to determine the kinetic biosorption models at various time periods (0- 150 min), as well as to estimate isothermal models at varied initial Cu^{2+} concentrations (30, 60, 90, 120 and 150 mg/L) and contact time of 90 minutes at 25 ˚C. Thermodynamic investigations of Cu^{2+} ion biosorption were also examined, with the biosorption process being tested under optimal conditions at different temperatures (25, 35, and 45 ˚C).

Characterization of *R. damascena* leaves

A scanning electron microscope (SEM– JEOL JSM-6510 L.V operated at 30 KV) with energy–dispersive X–ray spectroscopy (EDX, JEOL JEM-2100 (HRTEM) operated at a voltage of 200 KV) was used to examine the surface morphology of the *R. damascena* leaves. The functional groups on the *R. damascena* leaf surface were determined using Fourier transform infrared radiation (FTIR, Thermo Fisher Scientific model FT-IR is 10, USA) before and after biosorption process.

Biosorbent immobilization

Rosa damascena leaf was immobilized for 30 minutes at 60 °C with continual stirring by dissolving 4 g of Na-alginate in 100 mL distilled water[66]. In a Na-alginate solution, 4 g/L of *R. damascena* leaf powder was added. This mixture was then put into a 2 % $CaCl_2$ solution using a 3 mL syringe for formation of beads. For full gelation, the spherical beads (3 mm) were maintained for 2 hours in a 2 % calcium chloride solution. The beads were then rinsed in distilled water to eliminate any excess $CaCl_2$ and kept in the refrigerator until they were needed again. The biosorption experiment was performed as previously described utilizing immobilized *R. damascena* leaves and non-immobilized biosorbent as a control under optimum conditions obtained by BBD at a temperature of 25 °C and various time intervals (0- 150 min).

All methods were performed in accordance with relevant guidelines and regulations.

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Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number 1-441-129.

Author Contributions

M.A.F.: Investigation, formal analysis, writing-original draft. H.M.A., T.M.G.: Writing—original draft, supervision, project administration, funding acquisition, writing—review & editing. M.A.F., R.Z.H., T.G.A.: Methodology, supervision, writing—review & editing. E.F.A.: Methodology, writing—review & editing.

Competing interests

The authors declare no competing interests.

Corresponding author

Correspondence to Mustafa A. Fawzy.

Figure captions:

Fig. 1. 3-D response surface plots for Cu^{2+} removal percentage showing the interaction influences of (a) biosorbent dosage and pH, (b) biosorbent dosage and initial copper concentration, and (c) pH and initial copper concentration.

Fig. 2. (a) Influence of contact time, (b) Pseudo-first order plot, (c) Pseudo-second order plot, (d) Elovich plot, and (e) Intra-particle diffusion plot for the biosorption of Cu^{2+} ions onto *R. damascena* leaf.

Fig. 3. Biosorption isotherms of copper ions onto *R. damascena* leaf including (a) Langmuir, (b) Freundlich, (c) Temkin, and (d) Dubinin–Radushkevich models.

Fig. 4. Plot of *1/T* against *lnK* for copper ions biosorption onto *R. damascena*.

Fig. 5. SEM images of *R. damascena* leaf (a) before, and (b) after Cd^{2+} biosorption.

Fig. 6. EDX images of *R. damascena* leaf (a) before, and (b) after Cd^{2+} biosorption.

Fig. 7. FTIR spectra of *R. damascena* leaf (a) before, and (b) after Cd^{2+} biosorption.

Fig. 8. Copper ions removal by Ca-alginate immobilized *R. damascena*.

Figures

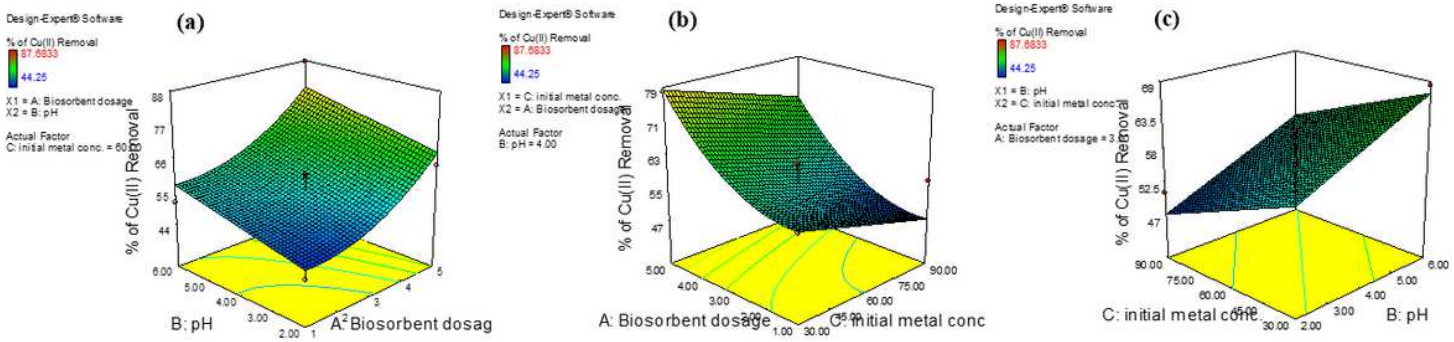


Figure 1

3-D response surface plots for Cu2+ removal percentage showing the interaction influences of (a) biosorbent dosage and pH, (b) biosorbent dosage and initial copper concentration, and (c) pH and initial copper concentration.

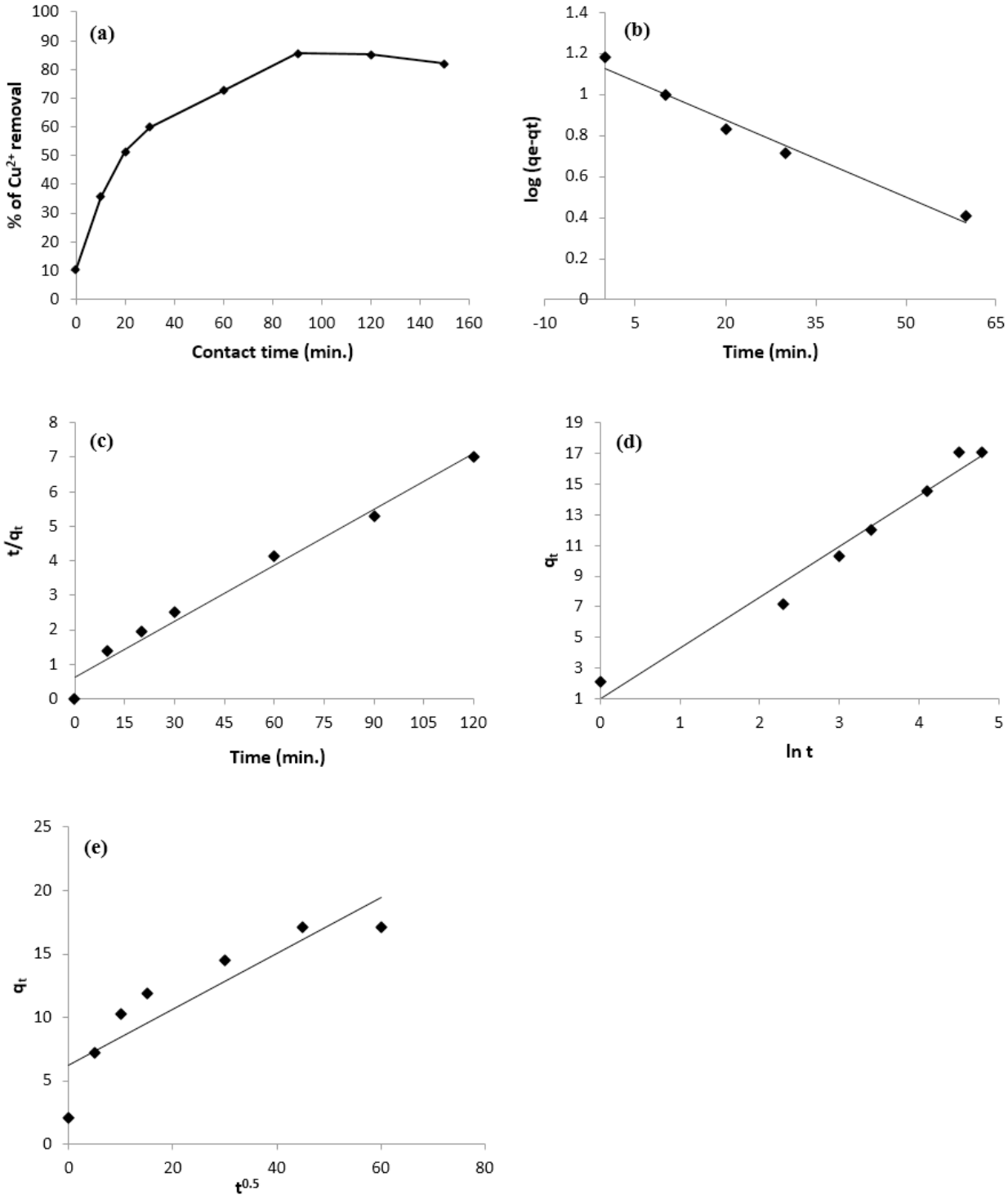


Figure 2

(a) Influence of contact time, (b) Pseudo-first order plot, (c) Pseudo-second order plot, (d) Elovich plot, and (e) Intra-particle diffusion plot for the biosorption of Cu2+ ions onto R. damascena leaf.

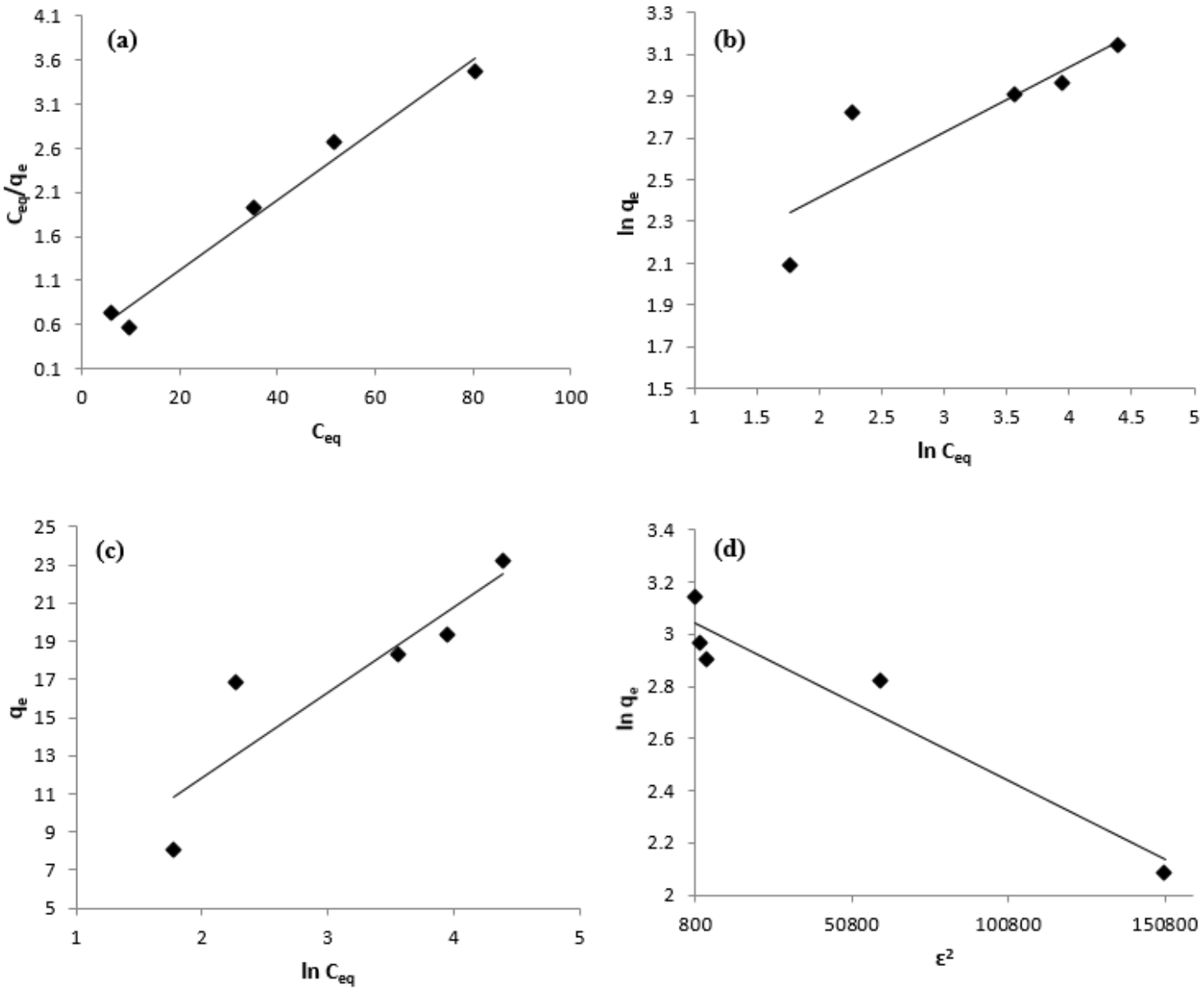


Figure 3

Biosorption isotherms of copper ions onto R. damascena leaf including (a) Langmuir, (b) Freundlich, (c) Temkin, and (d) Dubinin–Radushkevich models.

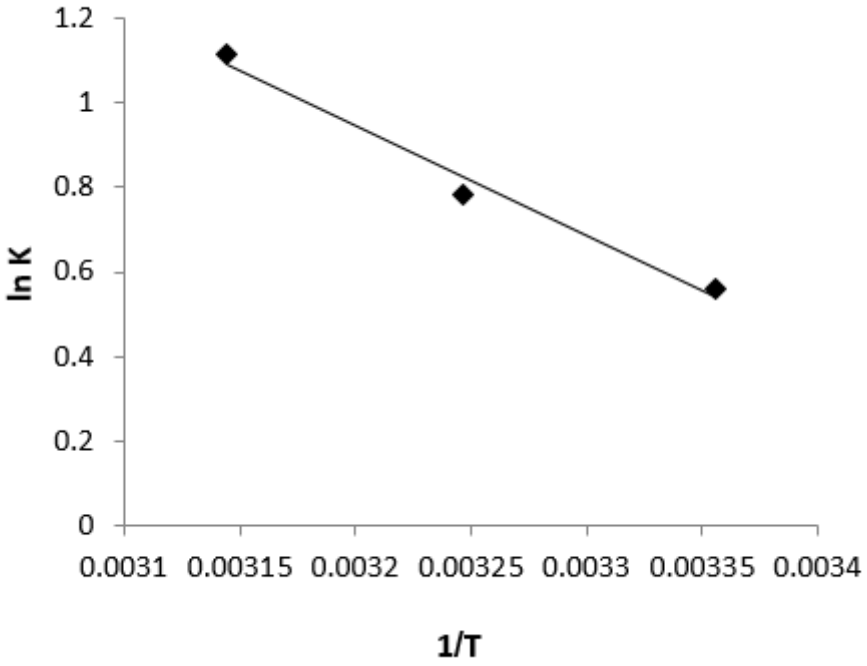


Figure 4

Plot of 1/T against lnK for copper ions biosorption onto R. damascena.

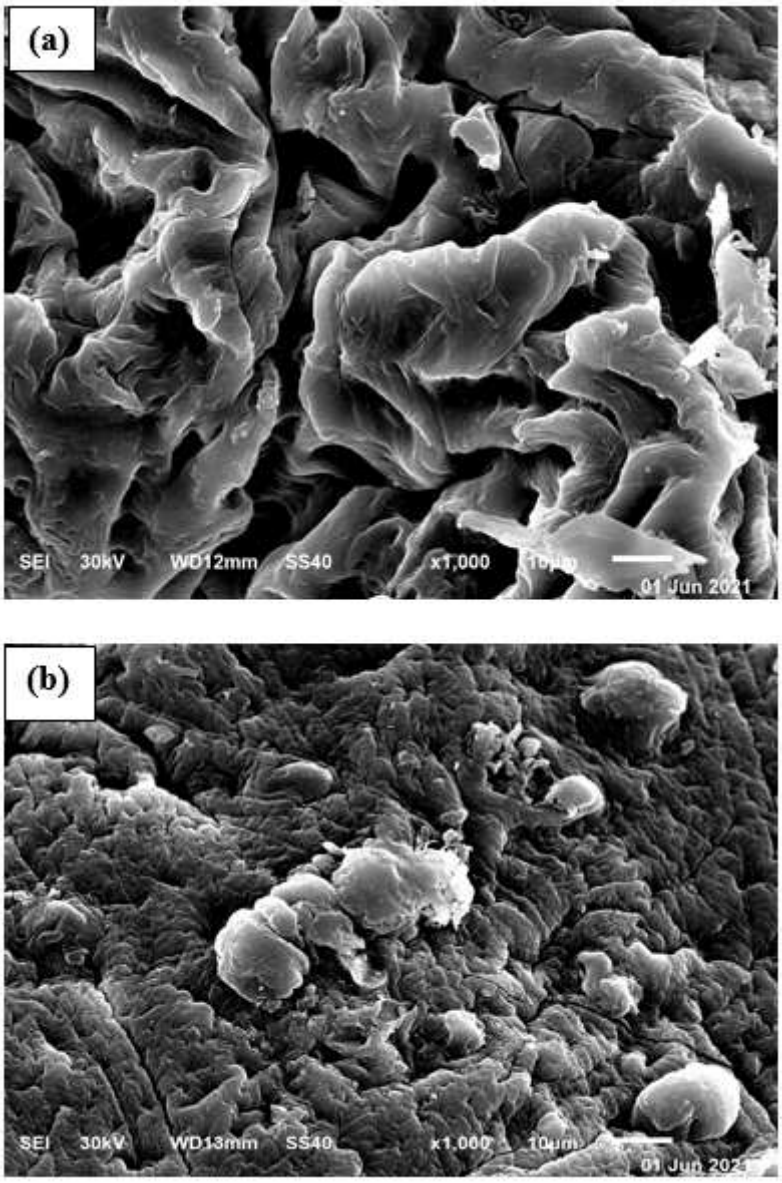
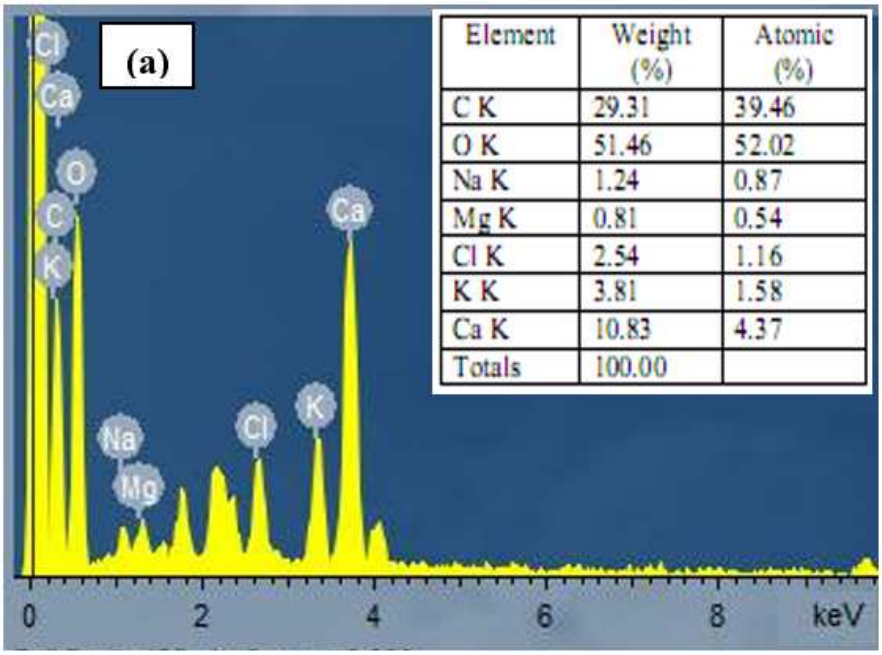
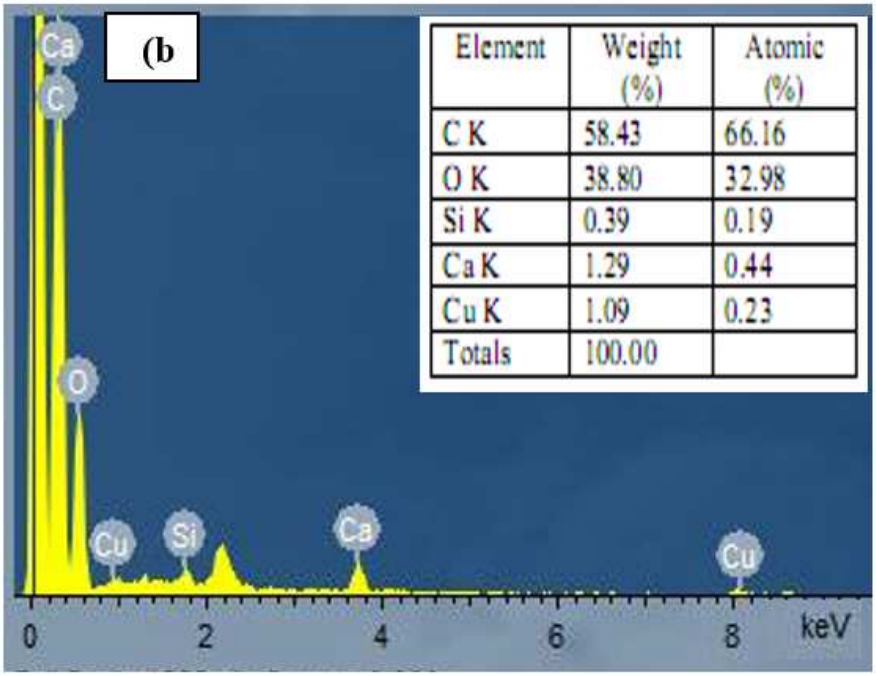


Figure 5

SEM images of R. damascena leaf (a) before, and (b) after Cd2+ biosorption.



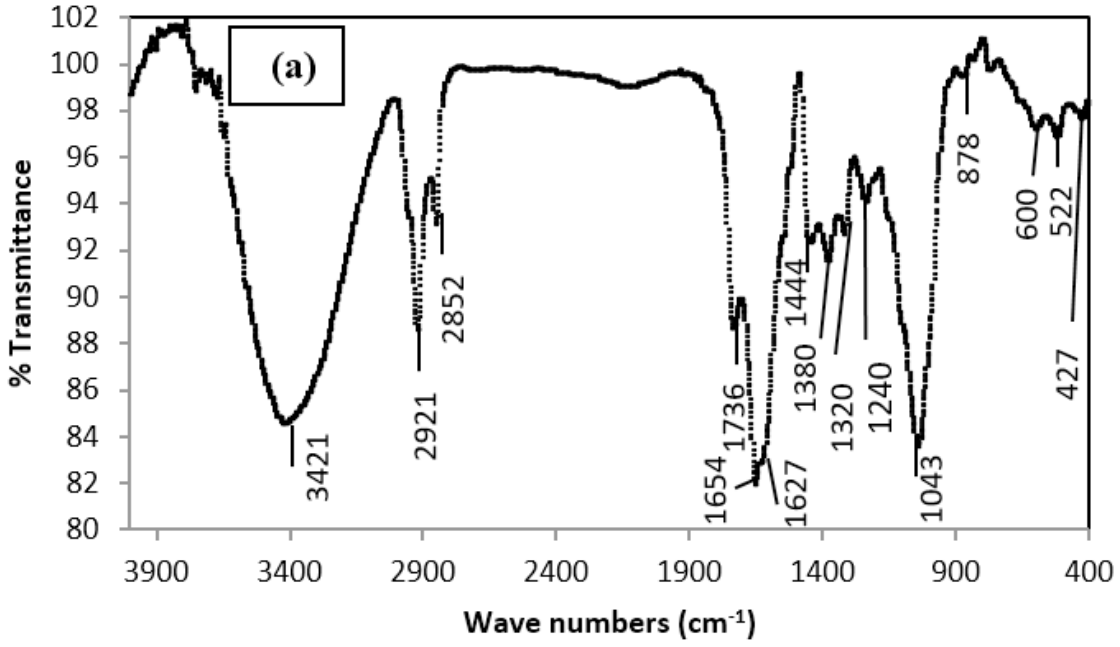
Element	Weight (%)	Atomic (%)
C K	29.31	39.46
O K	51.46	52.02
Na K	1.24	0.87
Mg K	0.81	0.54
Cl K	2.54	1.16
K K	3.81	1.58
Ca K	10.83	4.37
Totals	100.00	



Element	Weight (%)	Atomic (%)
C K	58.43	66.16
O K	38.80	32.98
Si K	0.39	0.19
Ca K	1.29	0.44
Cu K	1.09	0.23
Totals	100.00	

Figure 6

EDX images of R. damascena leaf (a) before, and (b) after Cd2+ biosorption.



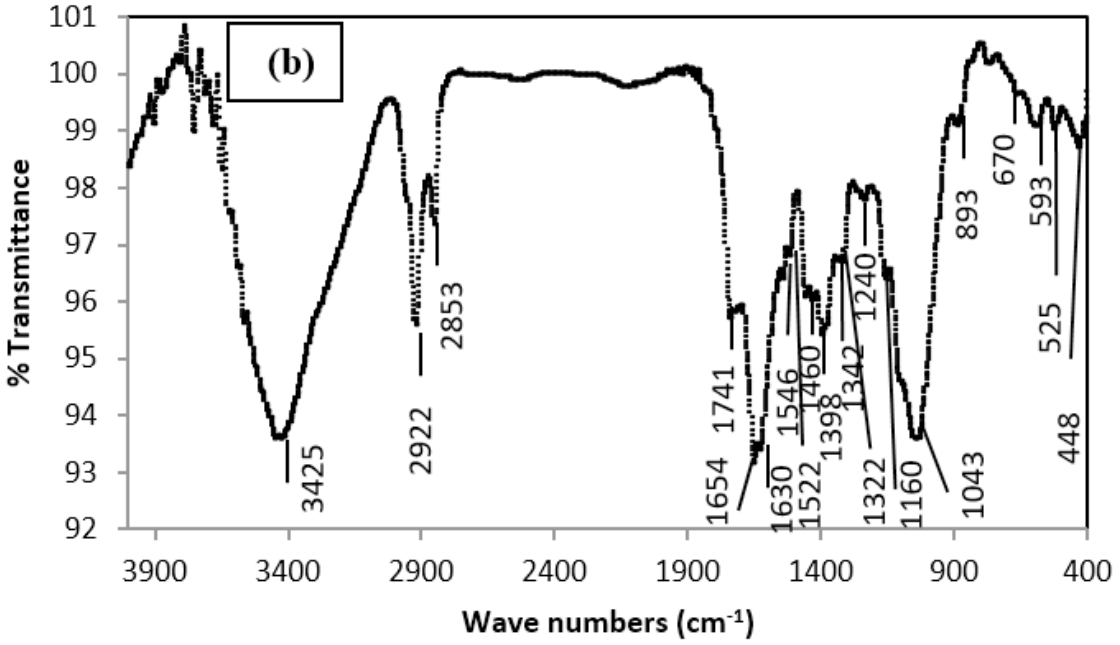


Figure 7

FTIR spectra of R. damascena leaf (a) before, and (b) after Cd2+ biosorption.

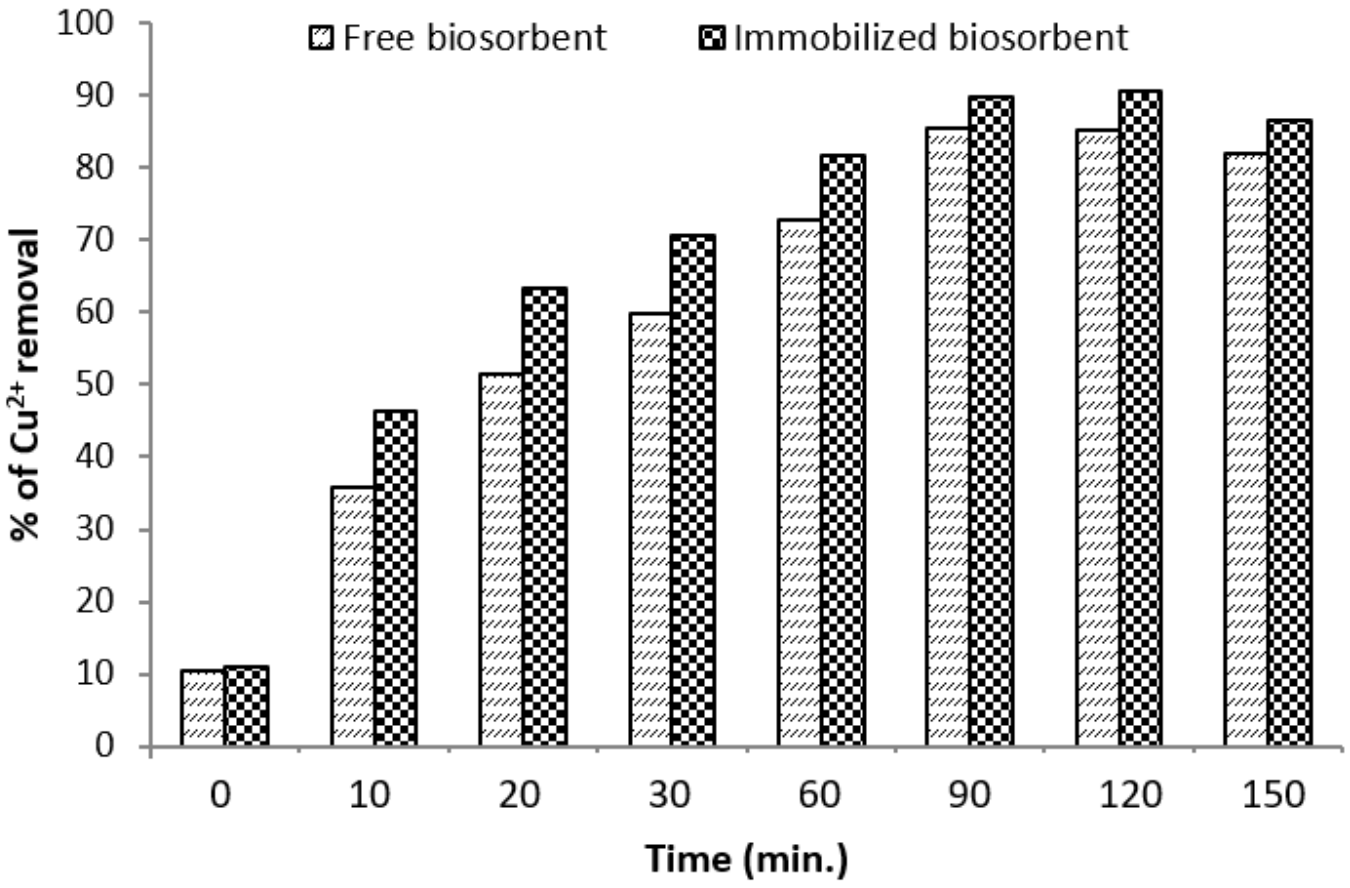


Figure 8

Copper ions removal by Ca-alginate immobilized R. damascena.