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# Biosorption of copper(II) ions on *Enteromorpha prolifera*: Application of response surface methodology (RSM)

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ABSTRACT

In this study, the biosorption of copper(II) ions on *Enteromorpha prolifera*, a green seaweed, was investigated in a batch system. The effects of operating parameters such as initial pH, temperature, initial metal ion concentration and biosorbent concentration on the copper(II) biosorption were analysed using response surface methodology (RSM). The proposed quadratic model for central composite design (CCD) fitted very well to the experimental data that it could be used to navigate the design space according to ANOVA results. The optimum biosorption conditions were determined as initial pH 4.0, temperature 25 °C, biosorbent concentration 1.2 g/L and initial copper(II) ion concentration 200 mg/L. The Langmuir and Freundlich isotherm models were applied to the equilibrium data at different temperatures and initial pH values. The maximum monolayer coverage capacity of *E. prolifera* for copper(II) ions was found to be 57.14 mg/g at 25 °C and initial pH 4.0 indicating that the optimum biosorption temperature and initial pH. The external and intraparticle diffusion models were also applied to biosorption data of copper(II) ions with *E. prolifera* and it was found that both the external diffusion as well as intraparticle diffusion contribute to the actual biosorption process. The pseudo-second order kinetic model described the copper(II) biosorption process with a good fitting. The thermodynamics of copper(II) biosorption on *E. prolifera* indicated its spontaneous and exothermic nature.

## 1. Introduction

Copper(II) is known to be one of the heavy metals most toxic to living organisms and it is one of the more widespread heavy metal contaminants of the environment [1]. For example, continued inhalation of copper-containing sprays is linked with an increase in lung cancer among exposed workers [2]. The World Health Organization (WHO) recommended a maximum acceptable concentration of copper(II) in drinking water of 1.5 mg/L. It is essential that potable waters be given some treatment to remove copper before domestic supply [3]. The potential sources of copper in industrial effluents include metal cleaning and plating baths, pulp, paper board mills, wood pulp production, the fertilizer industry, etc [4]. The conventional methods of copper(II) removal from wastewaters are precipitation, ion-exchange, electrolysis, adsorption, etc. These methods require high capital and operating costs, and may result in large volumes of solid wastes. In addition, there are also limitations concerning their use, especially for treating a large volume of dilute metal concentrations [5]. Therefore, there is a growing interest in the search of low-cost, easily available and environment friendly biological materials suitable for the efficient removal of heavy metal ions [6].

Biosorption is an alternative technology to remove heavy metals from dilute aqueous solutions using inactive and dead biomass [7]. Biosorption uses cheaper materials such as biosorbents and biosorptive process is generally rapid and is suitable for the extraction of metal ions from large volumes of water [5]. Biosorption is either metabolism, such as physical or chemical adsorption onto the cell wall, or metabolism related, such as transport, internal compartmentalization, and extra cellular precipitation by metabolites [8]. The physico-chemical phenomenon of metal biosorption, based on adsorption, ion-exchange, complexation and/or microprecipitation, is relatively rapid and can be reversible. Therefore, the biosorption is a process separation and can be explained with the adsorption principles. The characteristics of the adsorption behaviour is generally understood in terms of both equilibrium and adsorption kinetics.

Recent investigations by various groups have shown that selected species of seaweeds possess impressive adsorption capacities for a range of heavy metal ions and dye anions but no information are available for copper(II) ions from aqueous solutions by inactivated *Enteromorpha prolifera*, a green seaweed, in the

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**Nomenclature**

| | |
|---|---|
| $A$ | cross-sectional area of metal ion ($m^2$) |
| $C_{ad,eq}$ | the amount of copper(II) ion (mg) biosorbed on the biosorbent per L of the solution at equilibrium (mg/L) |
| $C_{eq}$ | metal ion concentration remaining in solution at equilibrium (mg/L) |
| $C_0$ | initial metal ion concentration (mg/L) |
| $D$ | effective diffusivity of solutes within the particle |
| $e_i$ | error in the model, $i = 1–4$ |
| $\Delta G$ | free energy change (kJ/mol) |
| $\Delta H$ | enthalpy change (kJ/mol) |
| $k_2$ | pseudo-second order rate constant (g/mg min) |
| $K_a$ | constant related to the affinity of the binding sites (L/mg) |
| $K_F$ | adsorption capacity |
| $K_i$ | intraparticle rate constant (mg/g $min^{1/2}$) |
| $M$ | molecular weight of metal |
| $n_F$ | adsorption intensity |
| $n$ | number of experiments |
| $N$ | Avogadro number |
| $p$ | number of model parameters |
| $q_{eq}$ | the amount of biosorbed metal per unit weight of biosorbent at equilibrium (mg/g) |
| $q_{eq,exp}$ | experimental amount of biosorbed metal per unit weight of biosorbent at equilibrium (mg/g) |
| $q_{eq,cal}$ | calculated amount of biosorbed metal per unit weight of biosorbent at equilibrium (mg/g) from the pseudo-second order kinetic model |
| $q_{max}$ | maximum monolayer coverage capacity of biosorbent (mg/g) |
| $q_f$ | amount of biosorbed metal on the surface of the biosorbent at the end of biosorption (mg/g) |
| $R$ | the universal gas constant, 8.314 (J/mol K) |
| $R^2$ | correlation coefficient |
| $R^2_{adj}$ | adjusted correlation coefficient |
| $S$ | specific surface area ($m^2$/g biosorbent) |
| $\Delta S$ | entropy change (kJ/mol K) |
| $T$ | absolute temperature (K) |
| $t$ | time (min) |
| $X_0$ | biosorbent concentration (g/L) |
| $x_i$ | independent variable, $i = 1–4$ |
| $y_i$ | response, $i = 1–4$ |

*Greek letters*

| | |
|---|---|
| $\beta_0$, $\beta_i$ and $\beta_{ij}$ | linear and quadratic interaction coefficients, $i$ and $j = 1–4$ |
| $\beta_L$ | external diffusion constant (cm/min) |
| $\eta$ | response |
| $\sigma^2$ | residual mean square from ANOVA table |

literature. Seaweeds are a widely available source of biomass as over two million tonnes are either harvested from the oceans or cultured annually for food or phycocolloid production, especially in the Asia-Pasific region [9]. Seaweeds are suitable for human and animal feed, as well as for fertilizer, fungicides, herbicides, and phycocolloids (alginate, carrageenan and agar) [10]. The green algae genus *Enteromorpha* has great potential for commercial exploitation because of its abundant and varied chemical composition, and quality and concentration of basic nutrients for other living organisms [10]. Aguilera-Morales et al. were reported that the main constituents in *Enteromorpha* spp. were minerals, protein and ether extract and hemicellulose was dominant in the cellular wall of algae [10]. *Enteromorpha* spp. contains 9–14% protein, 2–3.6% ether extract, 32–36% ash, polyunsaturated fatty acids *n*-3 and *n*-4:10.4 and 10.9/100 g of total fatty acids, respectively [11]. Christ et al. reported that cell wall of *E. prolifera* was rich in sulphated polysaccarides which are strong ion-exchangers [12].

The design and efficient operation of adsorption processes require equilibrium data for use in kinetic and mass transfer models. These models can then be used to predict the performance of the adsorption contact processes under a range of operating conditions [13]. The purpose of this study was to investigate the biosorption characteristics of copper(II) ions on *E. prolifera*. The effects of operating parameters such as initial pH, temperature, initial metal ion and biosorbent concentrations on the biosorption of copper(II) ions on *E. prolifera* were analysed using RSM. In this study, response surface design was selected because it provides a reasonable distribution of data points throughout the region of interest, allows model adequacy including lack of fit, allows designs of higher order to be built up sequentially, provides an internal estimate of error. Response surface designs also do not require a large number of runs and do not require too many levels of the independent variables [14,15].

## 2. Materials and methods

### 2.1. Preparation of biosorbent solution

*E. prolifera*, a kind of green seaweed collected from Mediterranean cost in Mersin, Turkey was used as a biosorbent. For the biosorption studies, the harvested fresh cells were rinsed with tap water, washed several times with distilled water and then inactivated in an oven at 105 °C for 24 h. After that, a given amount (10 g) of inactivated dried *E. prolifera* was suspended in 1 L double-distilled water, homogenizing in a commercial blender (Waring). Necessary dilutions were made from the stock biosorbent solution to prepare solutions in the range of concentrations 0.5–2.0 g/L.

### 2.2. Copper(II) solution

A stock solution of copper(II) (1.0 g/L) was prepared by dissolving weighed amount of $CuCl_2$ in 1 L of distilled water. Necessary dilutions were made from the stock solution to prepare solutions in the range of concentrations 25–300 mg/L. The initial pH of each solution was adjusted to the required value with concentrated and diluted HCl and NaOH solutions before mixing the biosorbent suspension.

### 2.3. Batch biosorption studies

Batch biosorption experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of biosorption solution. The biosorbent solution (10 mL), except for the studies of biosorbent concentration effect, was mixed with 90 mL of the desired initial copper(II) concentration and initial pH in Erlenmeyer flasks. The flasks were agitated on a shaker at constant temperature for 2 h ample time for biosorption equilibrium. Samples were taken before mixing the biosorbent suspension and copper bearing solution, then at pre-determined time intervals for the residual copper(II) concentration in the solution. Samples were filtered by using Whatman # 1 filter paper and the copper(II) ion concentration remaining in supernatant was analysed. Experiments were repeated for different initial pH, initial copper(II) concentration, temperature and biosorbent concentration values.

### 2.4. Copper(II) analysis

The concentration of copper(II) ion remaining in the biosorption medium was determined spectrophotometrically according to a previous report [7]. 0.2 mL of 1% (w/v) sodium diethyl dithiocarbamate solution, and 20 mL of 1.5N NH3 solution was added to the sample (1 mL) containing lower than 60 mg/L of copper(II) ions and diluted to 25 mL with distilled water. The absorbance of the yellow-brown colored solution was read at 460 nm. The biosorbed metal ion amount (mg/g) at equilibrium were computed as follows, respectively:

$$q_{\text{eq}}\ (\text{mg/g}) = \frac{C_0 - C_{\text{eq}}}{X_0} \tag{1}$$

where $C_0$ and $C_{\text{eq}}$ are the metal ion concentration at initial time $t = 0$ and equilibrium (mg/L), respectively and $X_0$ is the biosorbent concentration (g/L).

### 2.5. Experimental design and optimization

The response surface methodology (RSM) is essentially a particular set of mathematical and statistical methods for designing experiments, building models, evaluating the effects of variables, and searching optimum conditions of variables to predict targeted responses. RSM is an important branch of experimental design and a critical technology in developing new processes, optimizing their performance, and improving design and formulation of new products. Its greatest applications particularly have been in situations where a large number of variables influencing the system feature [14,15].

In the present study, central composite design (CCD) was used for the RSM in the experimental design, which is well suited for fitting a quadratic surface and usually works well for the process optimization. The CCD is an effective design that is ideal for sequential experimentation and allows a reasonable amount of information for testing lack of fit while not involving an unusually large number of design points [14,15]. Therefore, face centered CCD with four factors was applied using Design-Expert® 7.1 with the bounds of the factors of initial copper(II) concentration: 25–300 mg/L, biosorbent concentration: 0.5–2.0 g/L, initial pH: 3.0–5.0 and temperature: 20–30 °C as shown in Table 1.

The four factor designed experiments were conducted with three replications at the design center to evaluate the pure error and were carried in randomized order as required in many design procedures. Performance of the process was evaluated by analyzing the response of copper(II) biosorption capacity of biosorbent. These parameters obtained at the end of 120 min biosorption time were attributed as “final”.

In the optimization process, the responses can be simply related to chosen factors by linear or quadratic models. A quadratic model, which also includes the linear model, is given as

$$\eta = \beta_0 + \sum_{j=1}^{k}\beta_j x_j + \sum_{j=1}^{k}\beta_{jj}x_j^2 + \sum_{i}\sum_{<j=2}^{k}\beta_{ij}x_i x_j + e_i \tag{2}$$

The success of the RSM depends on the approximation of $\eta$ by a low order polynomial in some region of the independent variables. In Eq. (2), $\eta$ is the response, $x_i$ and $x_j$ are variables, $k$ is the number of independent variables (factors), $\beta_0$ is the constant coefficient, $\beta_j$'s, $\beta_{jj}$'s and $\beta_{ij}$'s are interaction coefficients of linear, quadratic and the second-order terms, respectively, and $e_i$ is the error. In the study, copper(II) biosorption capacity of biosorbent was processed for Eq. (2) including ANOVA to obtain the interaction between the process variables and the responses. The quality of the fit of polynomial model was expressed by the coefficient of determination $R^2$ and $R^2_{\text{adj}}$ in Eqs. (3) and (4), respectively. The statistical significance was checked with adequate precision ratio in Eqs. (5) and (6), and by $F$ values and $P$ values:

$$R^2 = 1 - \frac{\text{SS}_{\text{residual}}}{\text{SS}_{\text{model}} + \text{SS}_{\text{residual}}} \tag{3}$$

$$R^2_{\text{adj}} = 1 - \frac{\text{SS}_{\text{residual}}/\text{DF}_{\text{residual}}}{(\text{SS}_{\text{model}} + \text{SS}_{\text{residual}})/(\text{DF}_{\text{model}} + \text{DF}_{\text{residual}})} \tag{4}$$

$$\text{adequate precision} = \frac{\max(\hat{Y}) - \min(\hat{Y})}{\sqrt{\bar{V}(\hat{Y})}} \tag{5}$$

$$\bar{V}(\hat{Y}) = \frac{1}{n}\sum_{1=1}^{n} V(\hat{Y}) = \frac{p\sigma^2}{n} \tag{6}$$

In Eqs. (3)–(6), $SS$ is the sum of squares, DF the degrees of freedom, $P$ the number of model parameters, $\sigma^2$ the residual mean square from ANOVA table, and $n$ is the number of experiments.

**Table 1**
Experimental design of the biosorption of copper(II) ions on *Enteromorpha prolifera*

| Independent variable | Low actual value | High actual value |
|---|---|---|
| Initial copper(II) concentration (mg/L) | 25 | 300 |
| Biosorbent concentration (g/L) | 0.5 | 2.0 |
| Initial pH | 3.0 | 5.0 |
| Temperature (°C) | 20 | 30 |

## 3. Results and discussion

### 3.1. The optimization of biosorption conditions

In the first step of the study, the effects of operating variables such as initial copper(II) concentration, biosorbent concentration, temperature and initial pH on copper(II) biosorption capacity of biosorbent were investigated using RSM according to CCD. In the second step, the main objective was to select the initial pH and temperature in order to achieve optimal copper(II) biosorption capacity of biosorbent at the end of 120 min of biosorption time.

The batch runs were conducted in CCD designed experiments to visualize the effects of independent factors on responses and the results along with the experimental conditions. The experimental results were evaluated and the approximating function of copper(II) biosorption capacity of biosorbent was obtained in Eq. (7) by utilizing Design-Expert® 7.1.

The least squares method was used to estimate the parameters in the approximating polynomial, and then the response surface analysis was done in terms of the fitted surface. The analysis of the fitted surface is approximately equivalent to analysis of the actual system if the fitted surface is an adequate approximation of the true response function [14,15]. In most cases, the second-order model is adequate.

Eq. (7) could be used in predicting the response for the given values of initial copper(II) concentration, biosorbent concentration, temperature and initial pH. The response surface plots of Figs. 5 and 6 were sketched using Eq. (7). This approximating equation of the fitted response surface was also checked for the model adequacy using ANOVA results and the diagnostic plots of Figs. 1–4:

$$\begin{aligned}\hat{y}_{q_f} &= -0.20x_1 + 51.13x_2 + 1.83x_3 + 27.39x_4 + 1.02\times10^{-3}x_1^2\\ &\quad -22.44x_2^2 - 3.75\times10^{-2}x_3^2 - 3.44x_4^2 + 2.06\times10^{-2}x_1x_2\end{aligned}$$

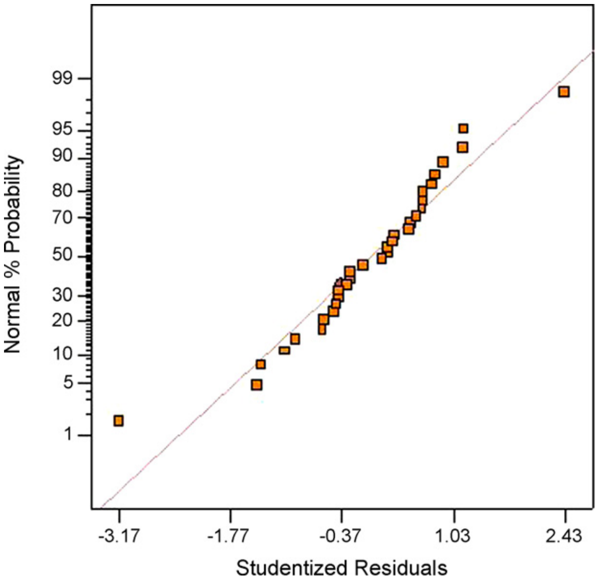

**Fig. 1.** Normal % probability and studentized residual plot for copper(II) uptake capacity of biosorbent.

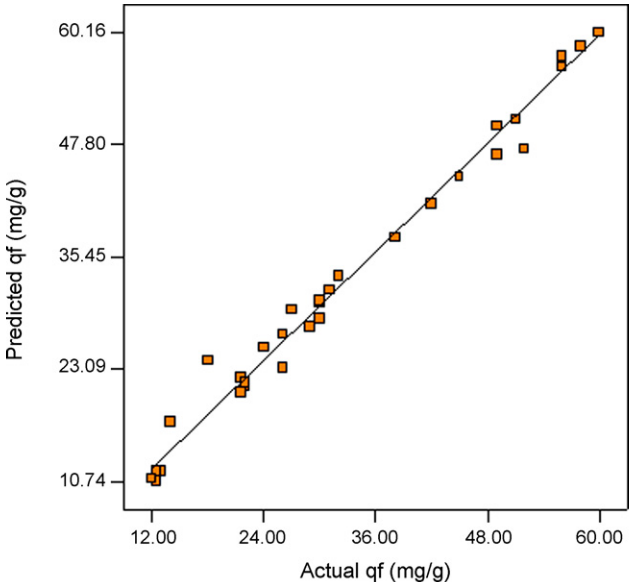

**Fig. 3.** The actual and predicted plot for copper(II) uptake capacity of biosorbent.

$$\begin{aligned}&+1.25\times10^{-4}x_1x_3+6.25\times10^{-4}x_1x_4-1.88\times10^{-2}x_2x_3\\&-9.38\times10^{-2}x_2x_4+6.25\times10^{-3}x_3x_4-67.15\end{aligned}\qquad(7)$$

In Eq. (7), $\hat{y}$ is the response of copper(II) biosorption capacity of biosorbent at the end of 120 min of biosorption time. $x_1$, $x_2$, $x_3$ and $x_4$ are corresponding to independent variables of initial copper(II) concentration, biosorbent concentration, temperature and initial pH, respectively. ANOVA results of this quadratic model presented in Table 2 indicating that it could be used to navigate the design space. The appropriate procedure for testing the equality of several means is the analysis of variance (ANOVA) which has a much wider application [14–18]. It is probably the most useful technique in the field of statistical inference. As can be seen in Table 2, the model F value for copper(II) biosorption capacity of biosorbent was evaluated as 72.87. This value indicated that the quadratic model was significant. Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Therefore, in the quadratic model of copper(II) biosorption capacity of biosorbent, the ratio of 27.989 indicate an adequate signal for the model to be used to navigate the design space. The *P* values less than 0.0500 indicate model terms are significant, whereas the values greater than 0.1000 are not significant. As a result, this quadratic function could be used for the purpose of predicting future responses successfully for copper(II) biosorption on *E. prolifera*.

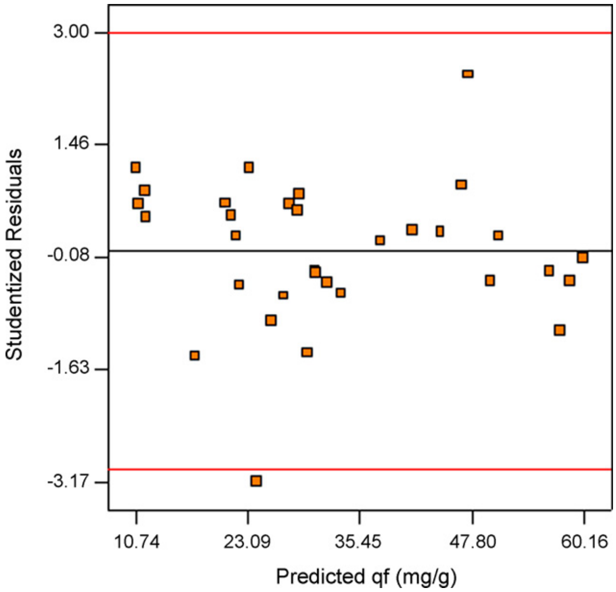

**Fig. 2.** The studentized residuals and predicted response plot for copper(II) uptake capacity of biosorbent.

The optimal temperature and initial pH were determined using Eq. (7) in order to achieve efficient copper(II) biosorption capacity of biosorbent, which is shown as a semi-spherical surface plot in Fig. 5.

The residual plots were examined for the model adequacy checking in Figs. 1–4. The normal % probability and studentized residuals plot is shown in Fig. 1 for copper(II) biosorption capacity of biosorbent. In Fig. 1, residuals show how well the model satisfies the

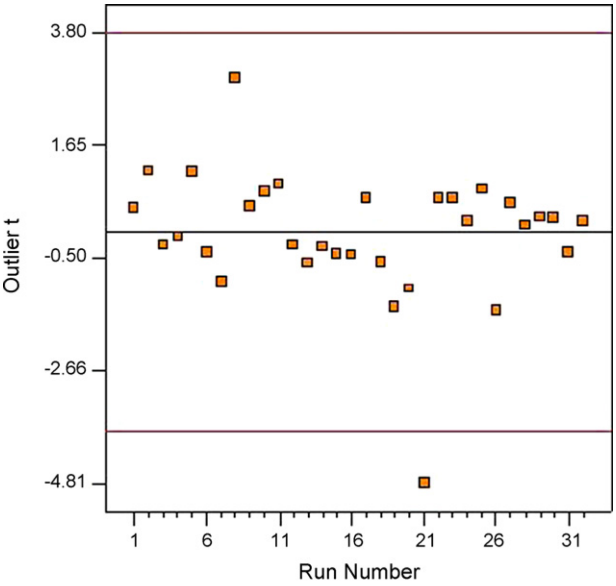

**Fig. 4.** Outlier *t* plot for copper(II) uptake capacity of biosorbent.

**Table 2**
ANOVA results of the quadratic models of final copper(II) uptake capacity of biosorbent

| Source | Final biosorption capacity of biosorbent, $q_f$ (mg/g) | |
|---|---|---|
| | Model | Residual |
| Sum of squares (SS) | 6783.96 | 113.04 |
| Degrees of freedom (DF) | 14 | 17 |
| Mean square (MS) | 484.57 | 6.65 |
| *F* value | 72.87 | |
| *P* value | <0.0001 (significant) | |
| $R^2$ | 0.984 | |
| $R^2_{adj}$ | 0.970 | |
| Adequate precision | 27.989 | |

assumptions of the analysis of variance (ANOVA) where the studentized residuals measure the number of standard deviations separating the actual and predicted values [14–16]. This figure also shows that neither response transformation was needed nor there was any apparent problem with normality.

Fig. 2 shows the studentized residuals and predicted copper(II) uptake capacity of biosorbent. The general impression is that the plot should be a random scatter, suggesting the variance of original observations to be constant for all values of the response. This plot often exhibits a funnel-shaped pattern when the variance of the response depends on the mean level of $\hat{y}$ [14–16]. This also implies that there was no need for transformation of the response variable.

The actual and the predicted copper(II) biosorption capacity of biosorbent is shown in Fig. 3. Actual values are the measured response data for a particular run, and the predicted values are evaluated using the approximating functions generated for the models [14–16]. In designed experiments, $R^2$ is a measure of the reduction amount in the variability of the response obtained by the independent factor variables in the model. However, a large value of $R^2$ does not imply that the regression model fits very well. $R^2$ is always increasing with the addition of variables to the model whether the additional variable is statistically significant or not. Thus it is possible to obtain models having large $R^2$ values that yield poor predictions of new observations or estimates of the mean response [14,15]. Although $R^2$ always increases as adding terms to the model, using an adjusted $R^2$ is preferred as defined in Eq. (4). In general, the adjusted $R^2$ is not always increasing as variables are added to the model. In fact, if unnecessary terms are added, the value of $R^2_{adj}$ often decreases [14,15]. There is a good chance that insignificant terms have been included in the model when $R^2$ and $R^2_{adj}$ differ dramatically [14,15]. In Fig. 3, the values of $R^2$ and $R^2_{adj}$ evaluated as 0.984 and 0.970 for copper(II) biosorption capacity of biosorbent at the end of 120 min. The correlation coefficients showed that the predicted responses well satisfied the calculated copper(II) biosorption capacity of *E. prolifera*. The sum of squares for regression always increases with the addition of a variable to the regression model. Therefore, the sufficiency of the increase in the regression sum of squares must be decided. Furthermore, adding an unnecessary variable to the model can actually increase the mean square error, thereby decreasing the usefulness of the model [14,15].

In designed experiments, the outliers should be carefully examined, because they may represent something as simple as a data recording error or something of more serious concern, such as a region of the independent factor variable space where the fitted model is a poor approximation to the true response surface [14,15].

In Fig. 4, outlier *t* plot for the batch runs of copper(II) biosorption is shown. The outlier *t* is a measure of how many standard deviations the actual value deviates from the predicted value. Most of the standard residuals should lie in the interval of ±3.50 and any observation with a standardized residual outside of this interval is potentially unusual with respect to its observed response [14,15]. In Fig. 4, the outlier *t* values below the interval of ±3.50 indicated that the approximation of the fitted model to the response surface was fairly good with no data recording error. There was only one data obtained below the value of −3.50 which might be resulted by the insignificant terms in the model as well as the nonlinear influence of the investigated parameters on process response [19].

The response surface plots for copper(II) biosorption capacity of *E. prolifera* are shown in Figs. 5 and 6. Fig. 5 shows the effect of temperature and initial pH on copper(II) biosorption capacity of biosorbent. Initial pH and temperature are the most important environmental parameters influencing the biosorption of the heavy metal ions. As can be seen from Fig. 5, the maximum copper(II) biosorption capacity of biosorbent was obtained at initial pH 4.0 and temperature 25 °C. The copper(II) biosorption capacity of biosorbent increased with increasing initial pH up to 4.0 and then decreased with the increase in initial pH at 25 °C temperature. At optimum point, the copper(II) biosorption capacity of

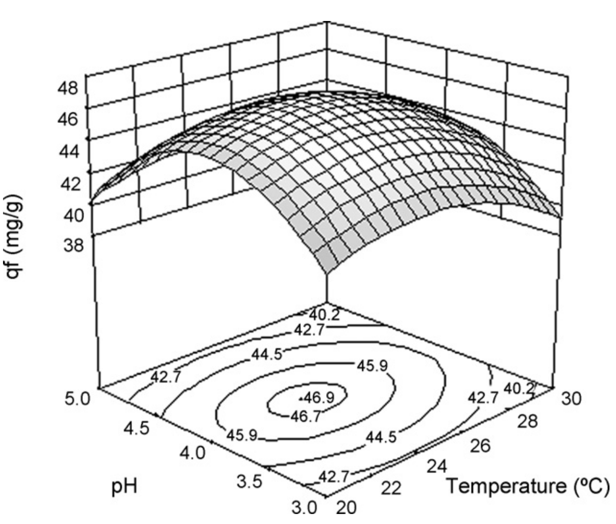

**Fig. 5.** The effect of temperature and pH on final copper(II) uptake capacity of biosorbent ($X_0$: 1.0 g/L; $C_0$: 200 mg/L Cu(II); biosorption time: 120 min, agitation rate 100 rpm).

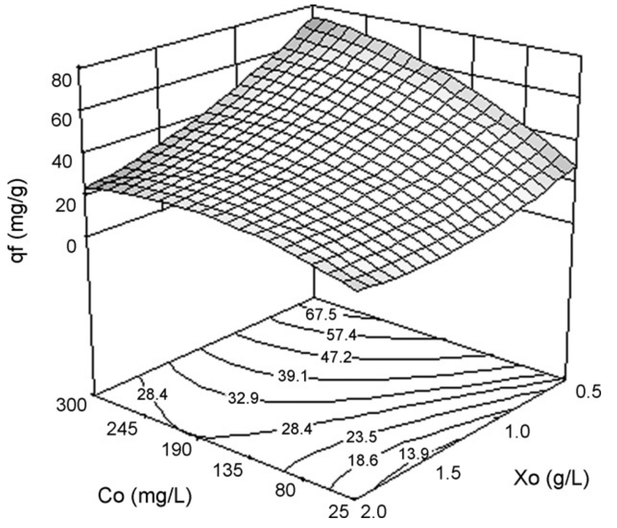

**Fig. 6.** The effect of biosorbent and initial copper(II) concentration on copper(II) biosorption capacity of biosorbent (*T*: 25 °C; pH: 4; biosorption time: 120 min, agitation rate 100 rpm).

biosorbent and biosorbed copper(II) ion concentration with 1.0 g/L biosorbent concentration and 200 mg/L initial copper(II) concentration at the end of 120 min were found as 46.9 mg/g and 46.9 mg/L, respectively. Marine algae contain high content of ionizable groups (carboxyl groups from mannuronic and guluronic acids) on the cell wall polysaccarides, which suggests that the biosorption process could be affected by changes in the solution pH [5]. For the algal biomass, the isoelectric point would be at a pH of 3.0 [20]. At the isoelectric point, electrostatic repulsion between adsorbed molecules is at a minimum. These molecules have a higher structural stability, and therefore a smaller tendency to spread at the interface resulting maximum adsorption at isoelectric point. At lower pH below the isoelectric point, the surface of algae may acquire a positive charge leading to decreased metal cations uptake due to the electrostatic force of repulsion. As the pH of the biosorption process increased, the number of negatively charged sites increases and the number of positively charged sites decrease. So the copper(II) biosorption increases. The metal cations in aqueous solution convert to different hydrolysis products. Elliot and Huang investigated the hydrolysis products of copper as a function of pH [21]. Their results show that the dominant species of copper in the range 3–5 of pH are $Cu^{+2}$ and $CuOH^{+}$, while the copper at above 6.3 occurs as insoluble $Cu(OH)_2$ (s) [21]. In this study, these copper cations at around initial pH 4.0 would be expected to interact more strongly with negatively charged binding sites on the algae. The decrease of the biosorption at increased temperature indicated that the biosorption of copper(II) ions to *E. prolifera* is exothermic in nature. The decrease in adsorption with the rise of temperature may be due to the weakening of adsorptive forces between the active sites of the adsorbents and adsorbate species and also between the adjacent molecules of the adsorbed phase [22]. The exothermic nature of metal ion adsorption/biosorption has also been reported previously for adsorptive removal of copper(II) ions by sugar beet pulp [7] and activated rubber wood sawdust [3].

Fig. 6 shows the effect of initial copper(II) and biosorbent concentration on copper(II) biosorption capacity of biosorbent. As can be seen from Fig. 6, the biosorption capacity of biosorbent increased with the increase in initial copper(II) concentration up to 200 mg/L and then did not change with further increase initial copper(II) concentration resulting the saturation of biosorbent surface for copper(II) ions. The biosorption capacity was decreased with increasing the biosorbent concentration. The decrease in copper(II) biosorption capacity of *E. prolifera* with increasing biosorbent concentration is mainly due to unsaturation of biosorption sites through the biosorption reaction. Another reason may be due to the particle interaction, such as aggregation, resulted from high biosorbent concentration. Such aggregation would be lead to decrease in total surface area of the biosorbent and an increase in diffusional path length [23].

### 3.2. *The modeling of equilibrium depending on temperature and initial pH*

Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. In order to investigate the copper(II) biosorption isotherm, two equilibrium models were analysed. These models are Langmuir and Freundlich isotherms. The Langmuir isotherm theory assumes monolayer coverage of adsorbate over a homogenous adsorbent surface [24]. The well-known expression of the Langmuir model is given by the following equation:

$$q_{eq} = \frac{q_{max} K_a C_{eq}}{1 + K_a C_{eq}} \tag{8}$$

Eq. (8) can be rearranged to the following linear form:

$$\frac{1}{q_{eq}} = \frac{1}{q_{max}} + \frac{1}{(K_a q_{max})} \frac{1}{C_{eq}} \tag{9}$$

where $q_{eq}$ (mg/g) and $C_{eq}$ (mg/L) are the amount of biosorbed metal ion per unit weight of biosorbent and metal ion concentration remaining in solution at equilibrium, respectively. $q_{max}$ is the maximum amount of the metal ion per unit weight of biosorbent to form a complete monolayer on the surface bound at high $C_{eq}$ (mg/L) and $K_a$ is a constant related to the affinity of the binding sites (L/mg). $q_{max}$ represents a practical limiting biosorption capacity when the surface is fully covered with metal ions and it assists in the comparison of biosorption performance, particularly in cases where the biosorbent did not reach its full saturation in experiments.

The Freundlich expression (Eq. (10)) is an exponential equation and therefore, assumes that as the adsorbate concentration increases, the concentration of adsorbate on the adsorbent surface also increases [25]. According to Freundlich model, the amount of adsorbate bound per unit weight of adsorbent at constant temperature is expressed by

$$q_{eq} = K_F C_{eq}^{1/n_F} \tag{10}$$

or, in its linear form

$$\ln q_{eq} = \ln K_F + \frac{1}{n_F} \ln C_{eq} \tag{11}$$

In this equation, $K_F$ and $1/n_F$ are the Freundlich constants characteristic on the system, indicating adsorption capacity and intensity, respectively.

One of the most important factors influencing the biosorption equilibrium is the contact time. The variation of the biosorbed copper(II) amount with contact time at different initial copper(II) ion concentrations was shown in Fig. 7. The data obtained from the biosorption of copper(II) ions on *E. prolifera* showed that a contact time of 60 min was sufficient to achieve equilibrium and the biosorbed copper (II) amounts did not change with further increase in contact time for all of the studied concentrations. Therefore, the uptake amount and copper (II) ion concentration at the end of 60 min are given as the equilibrium values ($q_{eq}$, mg/g and $C_{eq}$, mg/L). The biosorption experiments of copper(II) ions on *E. prolifera* to develop the equilibrium isotherms were studied in the range of 25–200 mg/L of initial metal ion concentrations and the equilibrium values were found for each experimental run. The Langmuir and Freundlich isotherm models were applied to the experimental data at different temperatures and initial pH values. Then, the isotherm model constants were found by using the linear regression method and they were presented in Table 3. As can be seen

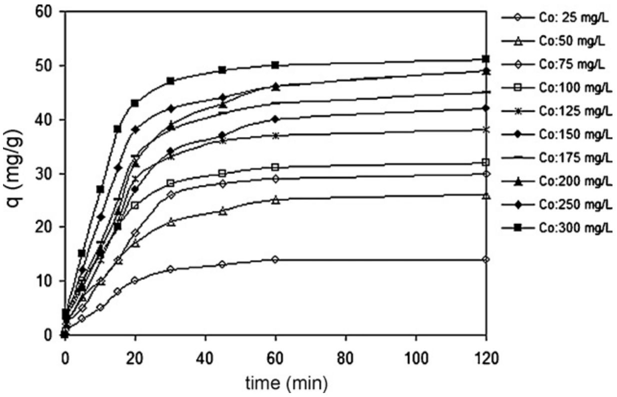

**Fig. 7.** The effect of contact time on copper(II) biosorption (*T*: 25 °C; pH: 4; $X_0$ = 1.0 g/L, agitation rate 100 rpm).

**Table 3**
The constants obtained from the Langmuir and Freundlich isotherm models at different temperatures and initial pH values

| | Temperature (°C) | | | Initial pH | | | |
|---|---|---|---|---|---|---|---|
| | 20 | 25 | 30 | 2.0 | 3.0 | 4.0 | 5.0 |
| Langmuir isotherm model | | | | | | | |
| $q_{\max}$ (mg/g) | 52.35 | 57.14 | 51.81 | 42.37 | 52.08 | 57.14 | 52.63 |
| $K_a$ (L/mg) | 0.0285 | 0.0347 | 0.0261 | 0.0380 | 0.0291 | 0.0347 | 0.0255 |
| $R^2$ | 0.9815 | 0.9978 | 0.9952 | 0.9907 | 0.9881 | 0.9978 | 0.9961 |
| Freundlich isotherm model | | | | | | | |
| $K_F$ | 6.39 | 8.97 | 5.17 | 6.39 | 6.55 | 8.97 | 7.11 |
| $n_F$ | 2.58 | 2.98 | 2.32 | 2.85 | 2.67 | 2.98 | 2.85 |
| $R^2$ | 0.9987 | 0.9950 | 0.9970 | 0.9994 | 0.9845 | 0.9955 | 0.9870 |

from Table 3, the maximum monolayer coverage capacity of *E. prolifera* for copper(II) ions was found as 57.14 mg/g at 25 °C and initial pH 4.0 indicating that the optimum biosorption temperature and initial pH. $K_a$ values calculated from the Langmuir isotherm model decreased with increasing temperature from 25 °C. $K_a$ (L/mg) is a constant related to the affinity of binding sites; therefore higher values of $K_a$ implies strong bonding of copper(II) ions on *E. prolifera.* The experimental and calculated isotherms for the biosorption of copper(II) ions on *E. prolifera* were given in Fig. 8. According to $R^2$ values in Table 3 and Fig. 8, the biosorption data of copper(II) ions on *E. prolifera* fitted very well to the Freundlich isotherm model than the Langmuir isotherm model. The Freundlich type adsorption isotherm is an indication of surface heterogeneity of the adsorbent while Langmuir model hints surface homogeneity of the adsorbent [26]. It is also observed from Table 3 that, the almost all calculated $n_F$ values related to Freundlich isotherm are greater than unity representing favorable biosorption conditions. The maximum monolayer biosorption capacity of *E. prolifera* for copper(II) ions was also noted to be higher than the other previously reported biosorbents and adsorbents (Table 4). Differences of metal uptake are due to the properties of each adsorbent/biosorbent material such as structure, functional groups and surface area.

Monolayer coverage of the surface by the metal ions can be used for the calculation of the specific surface area $S$ according to following equation [4,28]:

$$S = \left(q_{\max}\frac{NA}{M}\right) \tag{12}$$

where $S$ is the specific surface area, m$^2$/g biosorbent; $q_{\max}$ is the monolayer biosorption capacity, g Cu/g biosorbent; $N$ is the Avogadro number, $6.02 \times 10^{23}$; $A$ is the cross-sectional area of metal ion, m$^2$; $M$ is the molecular weight of metal. The molecular weight and the cross sectional area of copper(II) are 63.5 and 1.58 Å$^2$ in a close packed monolayer (Cu(II) radius is 0.71 Å$^2$), respectively [28]. The maximum specific surface area of *E. prolifera* for copper(II) biosorption was calculated as 8.56 m$^2$/g biosorbent. The maximum specific surface areas of the dehydrated wheat bran (DWB) and tree fern for copper adsorption were determined as 7.72 and 1.59 m$^2$/g adsorbent, respectively [4,28]. This showed that the inactivated *E. prolifera* has a larger specific surface area than for the tree fern and DWB.

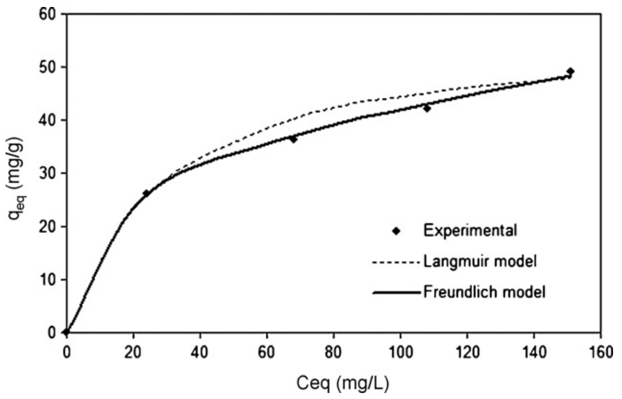

**Fig. 8.** The experimental and predicted isotherms ($T$: 25 °C; pH: 4; $X_0$ = 1.0 g/L, agitation rate 100 rpm).

### 3.3. *Modeling of kinetic of the biosorption of copper (II) ions on E. prolifera*

Several models can be used in order to investigate the mechanism of biosorption and potential rate controlling steps such as mass transport and chemical reaction processes. If the movement of metal ion from the bulk liquid to the liquid film or boundary layer surrounding the biosorbent is ignored, the following sequence of steps can take place in the biosorption process of porous biosorbent: transport of solute ions from the boundary film to the external surface of the biosorbent (film diffusion), transfer of ions from the surface to the intraparticular active sites and uptake of ions by the active sites of biosorbent. The third step being very rapid for adsorption/biosorption of metal ions on adsorbents/biosorbents, does not the rate-limiting step, and therefore either film diffusion or particle diffusion should govern the overall process [42].

**Table 4**
Comparison of copper(II) uptake capacities of different adsorbents/biosorbents

| Adsorbent/biosorbent | $q_{\max}$ (mg/g) | References |
|---|---|---|
| Peat | 16.4 | [27] |
| Tree fern | 10.6 | [28] |
| *Myriophyllum spicatum* | 10.37 | [29] |
| Kaolinite | 11.04 | [30] |
| Herbaceous peat | 4.84 | [31] |
| Groundnut shells | 7.60 | [23] |
| Dye loaded sawdust | 8.07 | |
| *Spirulina sp.* | 6.82 | [32] |
| Dried sugar beet pulp | 31.4 | [7] |
| *Spirogyra* sp. | 133.3 | [33] |
| Waste beer yeast | 1.45 | [34] |
| *Cladonia rangiformis hoffm.* | 7.69 | [35] |
| *Ulva fasciata* sp. | 26.88 | [36] |
| *Aspergillus flavus* | 13.5 | [37] |
| *Tectona gandis 1.f.* | 95.40 | [38] |
| Dried sunflower leaves | 89.37 | [39] |
| *Sargassum* sp. | 72.5 | |
| *Ulva fasciata* | 73.5 | [5] |
| *Padina* sp. | 50.8 | [40] |
| *Ascophyllum nodosum* | 69.2 | |
| *Lessonia flavicans* | 79.4 | |
| *Laminaria japonica* | 76.2 | [41] |
| *Ecklonia maxima* | 77.5 | |
| *Durvillaea potatorum* | 83.2 | |
| *Enteromorpha prolifera* | 57.14 | This study |

The most commonly used technique for identifying the mechanism involved in the adsorption process is by fitting the experimental data in an intraparticle diffusion plot. Intraparticle diffusion model used here refers to the theory proposed by Weber and Morris can be used to assess this opinion [43]:

$$q = f\left(\frac{Dt}{r_p^2}\right)^{1/2} = K_i t^{1/2} \tag{13}$$

where $r_p$ is particle radius, $D$ is the effective diffusivity of solutes within the particle, $q_t$ (mg/g) is the adsorbed metal ion amount at any time and $K_i$ intraparticle rate constant (mg/g min$^{1/2}$). The effect of the intraparticle diffusion on the removal of copper(II) ions with *E. prolifera* from aqueous solution can be determined by applying the Weber–Morris model to the biosorption data of copper(II) ion on *E. prolifera* for different initial metal ion and the biosorbent concentrations. The variation of the biosorbed copper (II) amounts of *E. prolifera* with $t^{1/2}$ was given in Fig. 9 for 50, 100, 150 and 200 mg/L of initial copper(II) ion concentrations. The intraparticle rate constants for copper(II) biosorption were calculated from the slopes of linear regions of curves in Fig. 9 and they were presented in Table 5. It was observed that the biosorbed copper (II) amounts by *E. prolifera* have a multi-linearity that two or more steps occur. The first, sharper portion is the external surface adsorption or instantaneous adsorption stage. The second portion is the gradual adsorption stage, where the intraparticle diffusion is rate-controlled. The third portion is final equilibrium stage where the intraparticle diffusion starts to slow down due to extremely low solute concentrations in the solution. The intraparticle diffusion constants for the studied biosorbent concentrations were also found by using the same procedure and their values were given in Table 5. As can be seen from Table 5, when the initial copper(II) ion concentration increased from 50 to 200 mg/L, the intraparticle rate constants remarkably increased from 2.83 to 4.94 mg/g min$^{1/2}$, indicating that the resistance of copper(II) ions to diffuse into the algae pores became much easier at high initial metal ion concentration. The decrease of the intraparticle rate constants with increase of biosorbent concentration can be explained that it usually results in an increase of the exterior surface, which causes swift decrease of the metal ion concentration in bulk solution and driving force [44].

The high $R^2$ values show that the copper(II) biosorption data fitted very well to the Weber–Morris model. As can be seen from Fig. 9, the linear portions of these curves do not pass through the origin indicating that the biosorption mechanism of copper(II) ions on *E. prolifera* is complex and both the external diffusion as well as intraparticle diffusion contribute to the rate-limiting step.

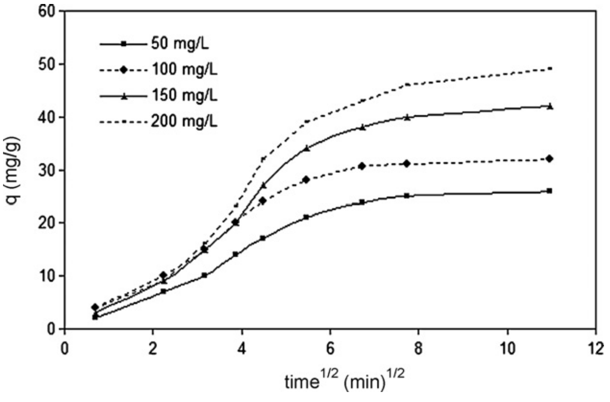

**Fig. 9.** Weber–Morris model (intraparticle diffusion) plots.

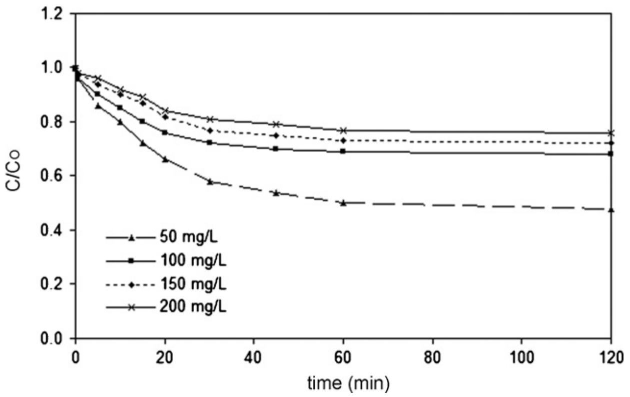

**Fig. 10.** The effect of external diffusion (boundary layer model) on copper(II) biosorption.

Mathews and Weber (M&W) equation defines the external mass transfer effect on the metal ion remove process, by the equation [45]:

$$\frac{C}{C_0} = \exp(\beta_L S t) \tag{14}$$

The values of $\beta_L S$ can be calculated from the slopes at time = 0 of the variation of $C/C_0$ with time, $t$, according to Eq. (14). For the biosorption of copper(II) ions on *E. prolifera*, Mathews and Weber model was applied to the experimental data at the different initial metal ion and biosorbent concentrations. The variation of $C/C_0$ with time, $t$, for initial metal ion concentrations was depicted in Fig. 10. The external mass transfer constants obtained from slopes for initial copper(II) ion and biosorbent concentrations were presented in Table 6. The external mass transfer constants decreased with increasing the initial metal ion concentration and increased with increasing biosorbent concentration. This states that the diffusion of copper(II) ions in the boundary layer reduces with increasing copper(II) ion concentration and decreasing biosorbent concentration. In the other words, the high value of $\beta_L$ suggests that the velocities of mass transfer of all the solutes from solution to the adsorbent phase are quite rapid [42].

Information on the kinetics of metal uptake is required for selecting optimum operating conditions for full-scale batch metal removal processes [9]. The pseudo-second order kinetic model is also based on the adsorption capacity of the solid phase [46]. This model predicts the behaviour over the whole range of adsorption and is in agreement with an adsorption mechanism being the rate-controlling step. In order to quantify the extent of uptake in adsorption kinetics, a pseudo-second order kinetic model can be used. The linear form of the pseudo-second order equation can be represented as [46]

$$\frac{t}{q} = \frac{1}{k_2 q_{eq}^2} + \frac{t}{q_{eq}} \tag{15}$$

where $k_2$ is the rate constant of pseudo-second order kinetic (g/mg min), $q_{eq}$ and $q$ are the biosorbed metal amount per unit weight of biosorbent at equilibrium and any time, $t$, respectively. If the pseudo-second order kinetic model is applicable, the plot of $t/q$ against $t$ of Eq. (15) should give a linear relationship, from which calculated $q_{eq,cal}$ and $k_2$ can be determined from the slope and intercept of the plot.

In this part of the study, the pseudo-second order kinetic model was applied to the experimental data obtained from initial metal ion concentrations and temperatures in order to investigate the biosorption kinetic of copper(II) ions on *E. prolifera*. Fig. 11 shows

**Table 5**
Weber–Morris model parameters

| $C_0$ (mg/L) | $T$ (°C) | $X_0$ (g/L) | $q_{eq,exp}$ (mg/g) | $K_i$ (mg/g min$^{1/2}$) | $R^2$ |
|---|---|---|---|---|---|
| 50 | 25 | 1.0 | 26 | 2.83 | 0.96 |
| 100 | 25 | 1.0 | 32 | 3.58 | 0.97 |
| 150 | 25 | 1.0 | 42 | 4.77 | 0.95 |
| 200 | 25 | 1.0 | 49 | 4.94 | 0.95 |
| $X_0$ (g/L) | $T$ (°C) | $C_0$ (mg/L) | $q_{eq,exp}$ (mg/g) | $K_i$ (mg/g min$^{1/2}$) | $R^2$ |
| 0.5 | 25 | 100 | 52 | 8.44 | 0.94 |
| 1.0 | 25 | 100 | 32 | 3.58 | 0.97 |
| 2.0 | 25 | 100 | 18 | 2.22 | 0.98 |

**Table 6**
External diffusion model parameters ($S$ = 8.56 m$^2$/g biosorbent)

| $C_0$ (mg/L) | $T$ (°C) | $X_0$ (g/L) | $q_{eq,exp}$ (mg/g) | $\beta_L$(cm/min) × $10^5$ | $R^2$ |
|---|---|---|---|---|---|
| 50 | 25 | 1.0 | 26 | 1.90 | 0.97 |
| 100 | 25 | 1.0 | 32 | 1.32 | 0.97 |
| 150 | 25 | 1.0 | 42 | 0.98 | 0.98 |
| 200 | 25 | 1.0 | 49 | 0.86 | 0.98 |
| $X_0$ (g/L) | $T$ (°C) | $C_0$ (mg/L) | $q_{eq,exp}$ (mg/g) | $\beta_L$(cm/min) × $10^5$ | $R^2$ |
| 0.5 | 25 | 100 | 52 | 1.13 | 0.97 |
| 1.0 | 25 | 100 | 32 | 1.32 | 0.97 |
| 2.0 | 25 | 100 | 18 | 1.49 | 0.98 |

the plots of the linearized form of the pseudo-second order kinetic model for the biosorption of copper(II) ions on *E. prolifera* at 50, 100, 150 and 200 mg/L of the initial copper(II) ion concentrations at 30 °C temperature and initial pH 4.0 and the $k_2$, correlation coefficients, $R^2$, experimental ($q_{eq,exp}$) and calculated ($q_{eq,cal}$) uptake values for 50, 100, 150 and 200 mg/L of the initial copper(II) ion concentrations and 20, 25 and 30 °C of temperatures were given in Table 7. As shown in Table 7, the pseudo-second order rate constants were affected by both the initial copper(II) ion concentration and temperature. Increasing the rate constants with increasing the metal ion concentration in solution seems to reduce the diffusion of solute in the boundary layer and to enhance the diffusion in the solid. Also, the experimental equilibrium data for copper(II) ions are in good agreement with those calculated using the pseudo-second order kinetics for the studied parameters. The correlation coefficients obtained from the pseudo-second order rate kinetic model were greater than 0.99 for all of the initial copper(II) ion concentrations and temperatures. This indicates that the biosorption of copper(II) ions on *E. prolifera* follows the pseudo-second order kinetic model.

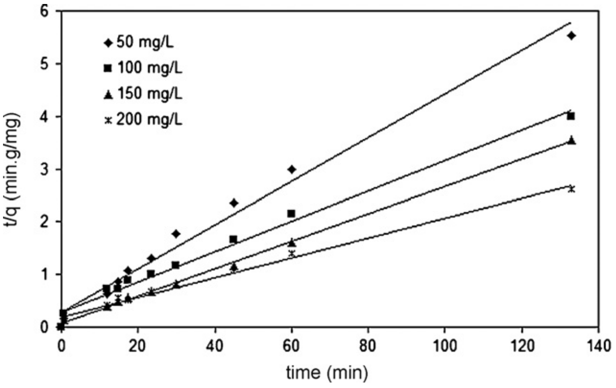

**Fig. 11.** The linear plots of the pseudo-second order kinetic model.

### 3.4. Determination of thermodynamic parameters of the biosorption of copper (II) ions on E. prolifera

Thermodynamic parameters such as enthalpy change ($\Delta H$), free energy change ($\Delta G$) and entropy change ($\Delta S$) were estimated using equilibrium constants changing with temperature. The biosorption process of copper(II) ions can be summarized by the following reversible process which represents a heterogeneous equilibrium [7]:

$$\text{copper(II) in solution} + \text{biosorbent} \Leftrightarrow \text{copper(II)} - \text{biosorbent} \quad (16)$$

The equilibrium constant of biosorption is defined as $K_c = C_{ad,eq}/C_{eq}$. Where $C_{ad,eq}$ (mg/L) is the amount of copper(II) ion (mg) biosorbed on the biosorbent per L of the solution at equilibrium, $C_{eq}$ (mg/L) is the copper(II) ion concentration remaining in solution at equilibrium. If biosorbent concentration is 1.0 g/L, $C_{ad,eq}$ is equal to $q_{eq}$ at a given temperature. For the biosorption of copper(II) ions on *E. prolifera*, the $K_c$ values for 25 and 30 °C were found to be 0.470 and 0.428 at 100 mg/L of initial copper(II) ion concentration, 4.0 initial pH and 1.0 g/L biosorbent concentration, respectively. The $K_c$ value decreased with increasing temperature resulting a shift of the biosorption equilibrium to the left.

**Table 7**
The pseudo-second order kinetic model parameters

| $C_0$ (mg/L) | $T$ (°C) | $q_{eq,exp}$ (mg/g) | $k_2$ (g/mg min) | $q_{eq,cal}$ (mg/g) | $R^2$ |
|---|---|---|---|---|---|
| 50 | 20 | 23 | 0.0061 | 21.10 | 0.9911 |
| 100 | 20 | 31 | 0.0030 | 33.11 | 0.9856 |
| 150 | 20 | 40 | 0.0029 | 38.46 | 0.9903 |
| 200 | 20 | 45 | 0.0020 | 48.78 | 0.9913 |
| 50 | 25 | 26 | 0.0043 | 27.47 | 0.9906 |
| 100 | 25 | 32 | 0.0041 | 33.33 | 0.9920 |
| 150 | 25 | 42 | 0.0021 | 42.73 | 0.9943 |
| 200 | 25 | 49 | 0.0013 | 50.51 | 0.9905 |
| 50 | 30 | 22 | 0.0066 | 22.47 | 0.9926 |
| 100 | 30 | 30 | 0.0037 | 31.80 | 0.9905 |
| 150 | 30 | 39 | 0.0029 | 38.31 | 0.9980 |
| 200 | 30 | 46 | 0.0027 | 48.30 | 0.9924 |

The free energy change of the adsorption reaction is given by the following equation:

$$\Delta G = -RT \ \ln K_c \tag{17}$$

where $\Delta G$ is free energy change, J/mol; $R$ is universal gas constant, 8.314 J/mol K and $T$ is absolute temperature, K. The free energy change indicates the degree of spontaneity of the adsorption process and the higher negative value reflects a more energetically favorable adsorption [44]. According to Eq. (17), the free energy changes for the biosorption of the copper(II) ions on *E. prolifera* were found to be −13.44 and −13.54 kJ/mol at 25 and 30 °C, respectively. The typical range of bonding energy for an ion-exchange mechanism is 1.91–3.82 kcal/mol [47]. It is to be noted that $\Delta G$ values up to 3.82 kcal/mol (20 kJ/mol) are consistent with electrostatic interaction between adsorption sites and the metal ion (physical adsorption) while $\Delta G$ values more negative than 7.62 kcal/mol (40 kJ/mol) involve charge sharing or transfer from the adsorbent surface to the metal ion to form a coordinate bond [48]. The $\Delta G$ values obtained from this study show that the biosorption of copper(II) ions on *E. prolifera* is by the physical adsorption process. The negative values of $\Delta G$ confirm the feasibility of the process and the spontaneous nature of biosorption with a high preference of copper(II) ions on *E. prolifera*.

According to the Van't Hoff equation:

$$\mathrm{Ln}\, K_c = -\left(\frac{\Delta H}{R}\right)\left(\frac{1}{T}\right) + \frac{\Delta S}{R} \tag{18}$$

For the biosorption of copper(II) ions on *E. prolifera*, $\Delta H$ and $\Delta S$ values in the range of temperature 25–30 °C were obtained as −7.65 kJ/mol and 0.0058 kJ/mol K at initial pH 4.0 and initial copper(II) concentration 100 mg/L, respectively. The effect of temperature on the equilibrium constant $K_c$ is determined by the sign of $\Delta H$. The negative value of $\Delta H$ shows that the biosorption of copper(II) ions on *E. prolifera* is exothermic in nature, an increase in $T$ causes a decrease in $K_c$. The positive value of $\Delta S$ reflects the affinity of the algae for copper(II) ions and suggests some structural changes in copper and algae. In addition, the positive $\Delta S$ corresponds to an increase in randomness at the solid/liquid interface during the biosorption of metal ion on biosorbent while low value of $\Delta S$ indicates that no remarkable change on entropy occurs.

## 4. Conclusion

The biosorption of copper(II) ions on *E. prolifera*, a green algae, was investigated in a batch system. The biosorption conditions of copper(II) ions on *E. prolifera* were optimized by using RSM. The relationship between the response and the independent variables was developed via the quadratic approximating function of copper(II) biosorption capacity of biosorbent at the end of 120 min biosorption. The optimum biosorption conditions were determined as initial pH 4.0, temperature 25 °C, biosorbent concentration 1.2 g/L and initial copper(II) concentration 200 mg/L. It was found that the copper(II) biosorption attained to equilibrium after 60 min and this contact time was taken as the equilibrium. The Langmuir and Freundlich isotherm models were applied to the equilibrium data at different temperature and initial pH values. The maximum monolayer coverage capacity of *E. prolifera* for copper(II) ions was found as 57.14 mg/g at optimum temperature and initial pH. The external and intraparticle diffusion models were also applied to biosorption data of copper(II) ions with *E. prolifera* and it was found that both the external diffusion as well as intraparticle diffusion contribute to the actual biosorption process. As a result, a chemical waste such as copper(II) ions can be removed by using a biological waste such as *E. prolifera* which is abundant and cheaply available in nature.

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