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Optimization of Cu(II) biosorption onto *Ascophyllum nodosum* by factorial design methodology

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ABSTRACT

A Box–Behnken factorial design coupled with surface response methodology was used to evaluate the effects of temperature, pH and initial concentration in the Cu(II) sorption process onto the marine macro-algae *Ascophyllum nodosum*. The effect of the operating variables on metal uptake capacity was studied in a batch system and a mathematical model showing the influence of each variable and their interactions was obtained. Study ranges were 10–40 °C for temperature, 3.0–5.0 for pH and 50–150 mg L^{-1} for initial Cu(II) concentration. Within these ranges, the biosorption capacity is slightly dependent on temperature but markedly increases with pH and initial concentration of Cu(II). The uptake capacities predicted by the model are in good agreement with the experimental values. Maximum biosorption capacity of Cu(II) by *A. nodosum* is 70 mg g^{-1} and corresponds to the following values of those variables: temperature = 40 °C, pH = 5.0 and initial Cu(II) concentration = 150 mg L^{-1}.

1. Introduction

Heavy metals are major pollutants of some ground and surface waters and are often present in industrial or urban wastewaters. Classical physicochemical techniques for the removal of heavy metals, such as precipitation and ion-exchange processes, are often ineffective and/or very expensive when used for the reduction of heavy metal ions at low concentrations [1,2]. Considerable research has been carried out in developing cost-effective techniques for the removal of heavy metals. One of the promising techniques for the removal of metals is the use of biological materials (living or nonliving organisms and their derivates) as biosorbents. The economy of environmental remediation dictates that the biomass must come from nature or even be a waste material [3]. The main advantages of the biosorption process over traditional techniques are the high effluent quality it generates, its terms of operation under a broad range of service conditions and its cost-effectiveness [4]. The application of biosorption is particularly important as a refining technique where metal concentrations in wastewater range from 1 to 100 mg L^{-1}. These levels can be lowered to drinking water standards. Seaweeds present advantages for biosorption because their macroscopic structures offer a convenient basis for the production of biosorbent particles suitable for sorption process applications [3]. From all algal biomass available, marine algae are considered to be the most useful as biosorbents due to their abundance in the sea [5]. Brown algae of the order *Laminariales* and *Fucales* (division *Phaeophyta*) are the most important groups of algae for biosorption because of the abundance of polysaccharides and extracellular polymers on the cell wall matrix. The alginate polysaccharide is mainly responsible for the natural ion-exchange capacity of the brown algae [4]. It has been reported that *Sargassum* sp. biomass exhibits a high cadmium binding capacity, superior to some organic and inorganic sorbents [6].

Biosorption capacity may be significantly influenced by some operating conditions: pH, temperature, initial concentration of metal and biomass, agitation, particle size, presence of other ions in solution and contact time. The pH value of solution strongly influences not only the functional groups dissociation at the biomass surface, but also the metals chemistry in solution: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation and metals speciation [7]. The majority of the metal binding groups on algae such as carboxyl, hydroxyl, sulphate and amine is pH-dependent. Low and high affinity functional groups are involved in sorption at high and low concentrations of metal ions, respectively [8]. Thus, the optimum pH for the removal of metals is related to the pK_a of these functional groups. High pH values can cause the precipitation of metals. As regards temperature, contrary results for sorption of heavy metals by algae have been obtained. Increased biosorption of heavy metals with increasing temperature

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has been ascribed to cause rupture that perhaps enhances the number of active sites involved in metal sorption or the affinity of sites for metal ions respectively [8]. However, the temperature effect seems to be contradictory, as reported by several authors: it can increase [9,10], decrease [6] or keep unaffected the algae ability for metal sorption [11–13]. The effects of various factors on the biosorption process have been studied extensively. Although most existing studies have concentrated on individual effects, it would be useful for the understanding of the complexity of systems to know the interactive effects of the factors.

Allen and Yu [14] studied "low-cost response surface methods", based on central composite and Box–Behnken designs that typically require half the experimental runs of standard response surface methods. These researchers concluded that the proposed methods offer attractive alternatives when the experimenter selects few factors to use standard response surface methods and would like the ability to perform a relatively small number of runs. A number of scientists have used this type of methodology in their studies [15–27]. However, there are not many studies concerning the application of this methodology to biosorption of metals [28–31] and even less when the biosorbents are marine algae [1,5,32].

The effect of some operating variables – temperature, pH and initial concentration of Cu(II) – on the sorption process onto the marine macro-algae *Ascophyllum nodosum* was studied by using a Box–Behnken factorial design method, which gives a mathematical model that shows the influence of each variable and their interactions.

2. Materials and methods

Samples of marine macro-algae *A. nodosum* were collected in February 2004 at the coast of the Atlantic sea in the north of Portugal. *A. nodosum* is a large, common, brown seaweed that forms egg-shaped bladders in long, flattened fronds. The fronds are olive-brown in colour and typically between 0.5 and 2 m in length. This species attaches to rocks and boulders on the middle shore, from estuaries to moderately exposed coasts. *Ascophyllum* is used for alginate production, as an organic fertilizer or for the manufacture of high-quality meals for animal or human consumption.

Seaweeds were washed with distilled water to remove part of the existing salt and dried at room temperature. Then, they were powdered using a centrifugal mill (Retsch, ZM 100 (Germany)) and sieved (Retsch, AS 200 (Germany)) to get a fraction with uniform particle size of 0.5–1.0 mm.

Analytical grade salt, $CuCl_2{\cdot}H_2O$, was employed for the preparation of Cu(II) solutions.

For batch biosorption experiments, weighed amounts (100 mg) of algae were added to Erlenmeyer flasks containing 100 mL of metal aqueous solution and the pH was adjusted (WTW, 538 pH meter (Germany)) to the required value by using NaOH or HCl solutions. Before starting the study an experiment was performed to check the influence of compounds eventually released by algae on the solution pH. An amount of 2.00 g of biomass was placed in 500 mL of distilled water and suspended by shaking at 500 rpm and 25 °C. The pH of the solution (WTW, 538 pH meter (Germany)) gradually increased for 1 h (the pre-established contact time). It was then adjusted to 4.0 by adding HCl solution, remaining almost constant over time. The increase in pH was also observed for the algae *Sagassum fluitants* [33] and *A. nodosus* [10], which was attributed to the affinity of H^+ to binding sites of components of the cytoplasm and the simultaneous release of other ions present in the algae (K^+, Mg^{2+}, Ca^{2+}).

The flasks were agitated on a rotary shaker (Velp Scientifica, Multi-stirrer (Italy)) for 2 h. The pH of the solutions was measured and re-adjusted after 2 h and the agitation proceeded for 30 min more. The suspensions were filtered (cellulose acetate membrane filters, Albet-AC-045-25-BL (Spain)) and the copper content in the filtrates was determined by atomic absorption spectrometry (GBC, 932 Plus AAS, Australia) with air–acetylene flame. The interference of K, Mg and Ca eventually leached from the algae into the solution is negligible. Biosorption capacities (Q, mg g^{-1}) were calculated by mass balance from the initial and final metal concentrations in solution.

Table 1
Experimental range of the factors in terms of actual and coded forms.

Factor	Code levels		
	−1	0	1
Temperature (°C)—T	10	25	40
pH	3.0	4.0	5.0
Initial concentration (mg L^{-1})—C	50	100	150

3. Experimental design

Response surface methodology (RSM) is an experimental technique designed to find the optimal response within specific ranges of pre-established factors, through a second-order equation. In industrial applications, RSM designs involve a small number of factors, because the required number of runs increases dramatically with the number of factors. Box–Behnken design was chosen to study the effects of temperature (T, °C), pH and metal initial concentration (C, mg L^{-1}) on Cu(II) biosorption by the marine macro-algae *A. nodosum*. Each factor was studied at three different levels (−1, 0, +1). The inclusion of centre points offers a more precise estimate of the experimental error and provides a measure for the adequacy of the model by comparing the lack of fit of the model prediction with the experimental results. It also enables the determination of the significance of linear, interaction and quadratic effects. The need for a model-independent estimate of the random variation means that replicate measurements made under identical experimental conditions are required to carry out a lack of fit test [34]. So, some of the experimental runs had to be replicated (repetitions which are subject to all the sources of error that affect runs made at different experimental conditions) [35]. The minimum and maximum values of the investigated factors and the correspondence between real and coded forms are listed in Table 1.

The variables values were selected taking into account the existing studies, the typical effluents characteristics and operational costs. Given the fact that the biosorption process may be used as a polishing treatment, after metal precipitation as hydroxide, the resulting solutions have relatively high pH values. In fact, lime precipitation has been found as one of the most effective means to treat effluents with a metal concentration higher than 1000 mg L^{-1} [36].

Using a statistical analysis computer software package [36] a regression analysis was carried out to determine the coefficients of a second-degree polynomial of the form:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=i}^{k} \beta_{ij} x_i x_j \qquad (1)$$

where y is the predicted response, β_0 is the intercept, β_i, β_{ij} are the coefficients of the linear, square and interaction effects and x_i, x_j are the coded variables.

The performance of the model was evaluated by analysis of variance (ANOVA), which included the Fisher's F-test (overall model significance), its associated probability $p > F$, the determination coefficient R^2 and the lack of fit. The Student's t-value for the estimated coefficients and the associated probabilities $p > |t|$ was also applied. The second-order polynomial models were represented as

Table 2
Results from Box–Behnken design for Cu(II) uptake (mg g^{-1}).

No.	T	pH	C	$Q_{\text{experimental}}$	$Q_{\text{predicted}}$
1	−1	−1	0	40.3	42.2
2	−1	1	0	54.0	54.3
3	1	−1	0	46.6	47.3
4	1	1	0	59.2	56.8
5	−1	0	−1	35.5	35.1
6	−1	0	1	56.7	57.1
7	1	0	−1	38.4	38.0
8	1	0	1	61.7	61.9
9	0	−1	−1	32.8	31.7
10	0	−1	1	55.1	52.2
11	0	1	−1	38.7	40.1
12	0	1	1	66.3	65.5
13	0	0	0	48.7	46.5
14	0	0	0	46.2	46.5
15	0	0	0	43.9	46.5
16	−1	−1	0	41.4	42.2
17	−1	1	0	56.7	54.3
18	1	−1	0	45.9	47.3
19	1	1	0	57.1	56.8
20	−1	0	−1	35.8	35.1
21	−1	0	1	57.2	57.1
22	1	0	−1	38.1	38.0
23	1	0	1	61.1	61.9
24	0	−1	−1	32.4	31.7
25	0	−1	1	52.6	52.2
26	0	1	−1	38.4	40.1
27	0	1	1	63.0	65.5
28	0	0	0	48.0	46.5
29	0	0	0	46.8	46.5
30	0	0	0	45.3	46.5

response surface plots that show the effect of two variables on the response, whilst keeping constant the third variable.

4. Results and discussion

In order to test three levels for three experimental factors with a Box–Behnken design, 15 runs were required in duplicate, three of which corresponding to all three factors at their central levels. The values of the experimental and predicted responses Q, for each run, are shown in Table 2.

The summary of fit is presented in Table 3, where R^2 estimates the proportion of the variation in the response around the mean that can be accounted by the model and is not due to random error. $R^2 = 1$ occurs when there is a perfect fit (the errors are all zero) and $R^2 = 0$ means that the fit predicts the response no better than the overall mean of response. The obtained value for the correlation between the actual and predicted response ($R^2 = 0.9791$) indicates that the accuracy of polynomial model was high. The coefficient R^2 adj. (0.9696) is more suitable for comparing models with different numbers of independent variables [24]. The root mean square error (1.699) estimates the standard deviation of the random error. The mean of response (48.11) means the overall mean of the response values and is an important statistical parameter for comparing different models.

ANOVA results are shown in Table 4. The three sources of variation are model, error and C. Total (corrected total sum of squares). DF stands for the degrees of freedom associated with each source of variation. The sum of squares indicates the associated sum of squares for each source of variation and the mean square is calculated by dividing the sum of squares by DF. The F ratio (model mean square divided by the error mean square) tests the hypothesis that all the regression parameters (except the intercept) are zero. Prob. > F is the probability of obtaining a greater F-value by chance alone, if the specified model fits no better than the overall response mean. Significance probabilities of 0.05 or less are often considered to evidence that there is at least one significant regression factor in the model.

Table 3
Summary of fit.

R^2	0.9791
R^2 adj.	0.9696
Root mean square error	1.699
Mean of response	48.11

Table 4
Analysis of variance.

Source	DF	Sum of squares	Mean square	F ratio	Prob. > F
Model	9	2698.8989	299.878	103.9420	<0.0001
Error	20	57.7010	2.885		
Corrected total	29	2756.5999			

Table 5
Lack of fit.

Source	DF	Sum of squares	Mean square	F ratio	Prob. > F
Lack of fit	3	25.896250	8.63208	4.6139	0.0154
Pure error	17	31.804733	1.87087		
Total error	20	57.700983			
				Max. Rsq	0.9885

A high F-value (103.942) and a very low probability (Prob. > F) < 0.0001, as presented in Table 4, indicate that the model adequately predicts the experimental results.

The results of the lack of fit test are presented in Table 5. The pure error is the measured error for the replicates, i.e., the fraction of the sample error that cannot be explained or predicted no matter which form of model is used. The difference between the residual error from the model and the pure error is called lack of fit error. The F ratio indicates whether the variation due to the lack of fit is small enough to be accepted as a negligible fraction of the pure error. Prob. > F is the probability of obtaining a greater F-value by chance alone, if the variation due to lack of fit variance and the pure error are the same. This means that an insignificant proportion of error is explained by lack of fit. The F-value obtained (4.6139) and the value of Prob. > F inferior to 0.05 (0.0154) indicate a good adherence of the model to the experimental results at 95% confidence level.

The lack of fit is significant because Prob. > F is below the 0.05 level. However, the major deviation between the measured and

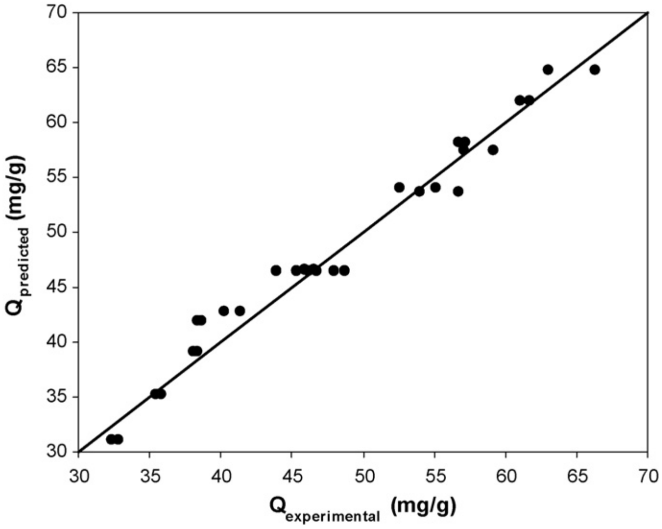

Fig. 1. Parity plot comparing the Cu(II) uptake data with the model predictions by Eq. (3).

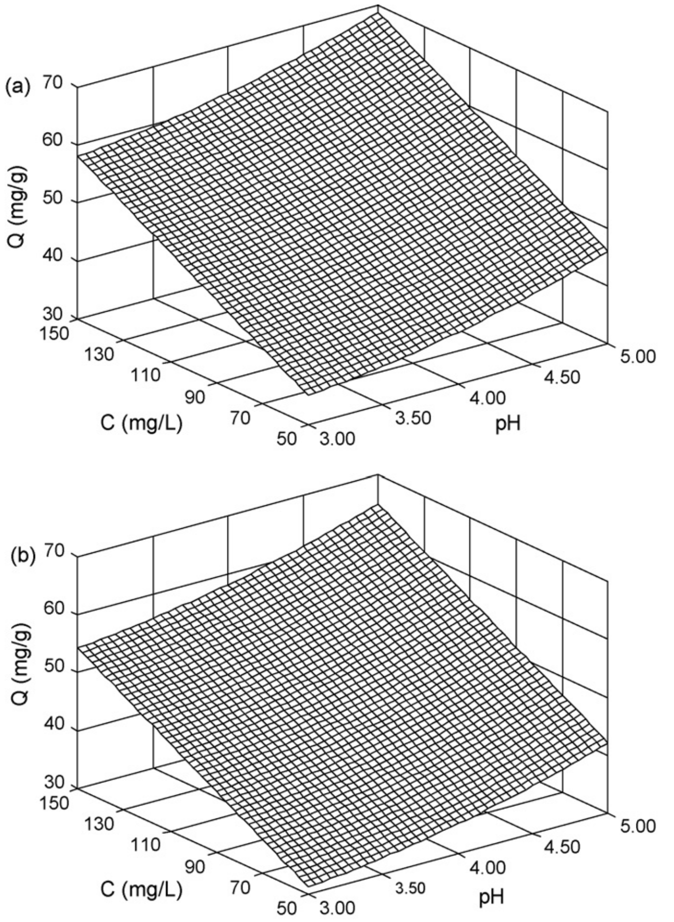

Fig. 2. Response surface plot showing the effects of initial concentration and pH on biosorption of Cu(II) at 40 °C (a) and 10 °C (b).

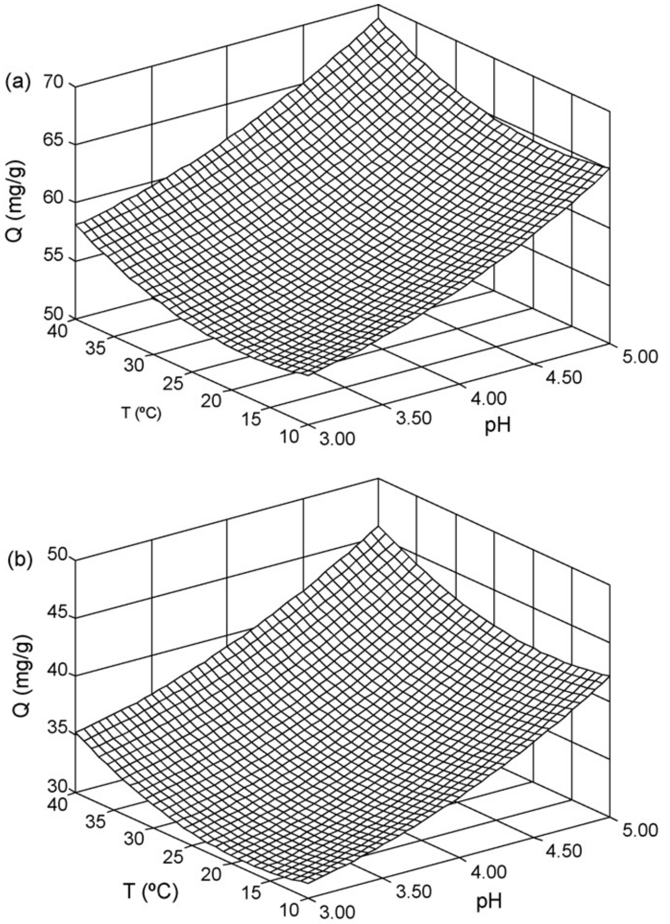

Fig. 3. Response surface plot showing the effects of temperature and pH on biosorption of Cu(II) at initial concentration 150 mg L^{-1} (a) and 50 mg L^{-1} (b).

modeled results is only 0.6%. So, for the present study we assumed that an adequate model was obtained.

The estimates of the coefficients of Eq. (1) are given in Table 6, as well as the values of the standard error, t-ratio and Prob. > |t|. The mathematical model of the factorial design is then given by:

$$Q = 46.46 + 1.90\left(\frac{T-25}{15}\right) + 5.40(\text{pH}-4) + 11.47\left(\frac{C-100}{50}\right) - 0.63\left[\left(\frac{T-25}{15}\right)(\text{pH}-4)\right] + 0.47\left[\left(\frac{T-25}{15}\right)\left(\frac{C-100}{50}\right)\right] + 1.21\left[(\text{pH}-4)\left(\frac{C-100}{50}\right)\right] + 2.16\left[\left(\frac{T-25}{15}\right)\left(\frac{T-25}{15}\right)\right] + 1.51[(\text{pH}-4)(\text{pH}-4)] - 0.59\left[\left(\frac{C-100}{50}\right)\left(\frac{C-100}{50}\right)\right] \tag{2}$$

Table 6
Parameter estimates for Cu(II) uptake by *A. nodosum*.

	Estimate	Std. error	t-Ratio	$P > \|t\|$
Intercept	46.46	0.6934	67.0	<0.0001
T	1.903	0.4246	4.48	0.0002
pH	5.401	0.4246	12.7	<0.0001
C	11.47	0.4246	27.0	<0.0001
T–pH	−0.6338	0.6005	−1.06	0.3004
T–C	0.4663	0.6003	0.78	0.4466
pH–C	1.214	0.6003	2.02	0.0569
T–T	2.161	0.6251	3.46	0.0025
pH–pH	1.514	0.6251	2.42	0.0251
C–C	−0.5914	0.6251	−0.95	0.3553

Prob. > |t| less than 0.05 is often considered as significant evidence that the coefficient is not zero. So, the linear effects of temperature (T), pH and metal initial concentration (C) and the quadratic effects of T and pH are significant. Considering that the quadratic effect of C and the effect of the interactions have no statistical significance and can be discarded, the model is then represented by:

$$Q = 46.46 + 1.90\left(\frac{T-25}{15}\right) + 5.40(\text{pH}-4) + 11.47\left(\frac{C-100}{50}\right) + 2.16\left[\left(\frac{T-25}{15}\right)\left(\frac{T-25}{15}\right)\right] + 1.51[(\text{pH}-4)(\text{pH}-4)] \tag{3}$$

The comparison of the model predictions calculated from Eq. (3) vs. the experimental responses is given in the parity plot of Fig. 1. Maximum absolute deviations using this simplified equation are around 8–9% and the average deviation is only 0.7%.

The response surface plots presented in Figs. 2–4 were obtained by keeping one parameter constant while varying the other two. When keeping the temperature constant (40 °C) copper uptake significantly increases with metal concentration but a smaller increase with pH is observed. The biosorption is highly dependent on the amount of metal initially present in solution, increasing with the initial concentration [2,12,37]. The biosorption capacity decreases with pH, which is explained by the greater affinity of the proton by the binding sites at the algal particles surface when compared with the metal ion. Oppositely, at higher pH, where copper concentration is much greater than H$^+$ concentration, the metal uptake increases. Similar results have been obtained by other authors [37–41], that found an optimal pH range of 4–5 [2,42–48]. At this pH range, some

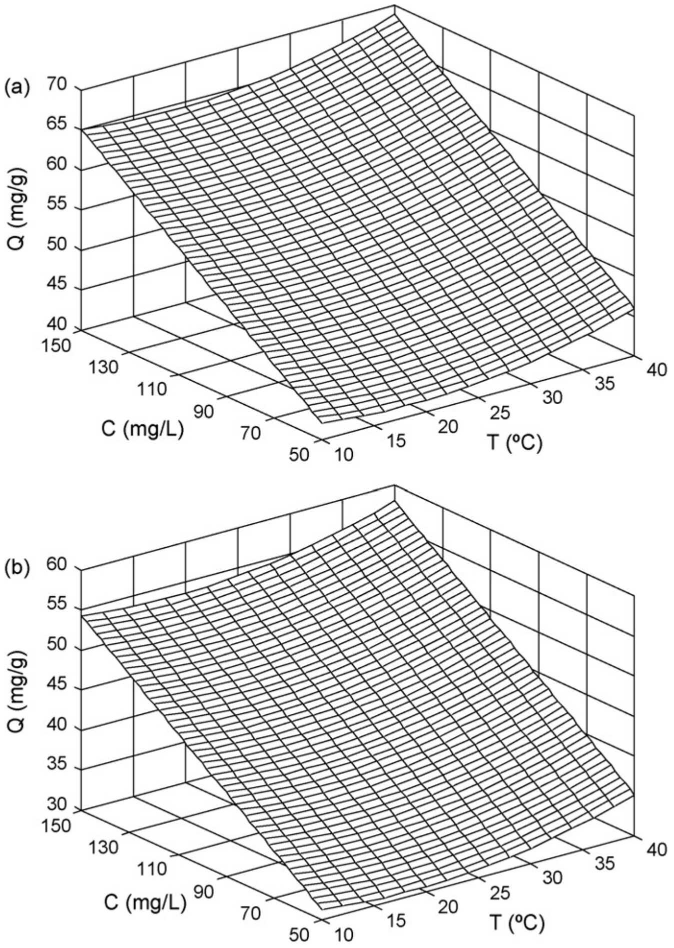

Fig. 4. Response surface plot showing the effects of initial concentration and temperature on biosorption of Cu(II) at pH 5.00 (a) and pH 3.00 (b).

functional groups in the algae surface, e.g. carboxyl and sulfate, become negatively charged, increasing the electrostatic interactions with the metal cation [45].

The best results were achieved at the higher levels of pH (5.00) and initial concentration (150 mg L^{-1}).

Temperature variation between 10 and 20 °C practically has no influence on the metal uptake capacity, but it slightly increases for temperature above 20 °C. The maximum biosorption capacity occurred at 40 °C. Several studies have indicated the same effect of temperature [9,11–13,39,41,44]. Antunes et al. [49] concluded that increasing temperature (in the range 25–55 °C) only favours copper biosorption by *Sargassum* sp. for high initial metal concentrations (above 500 mg L^{-1}). In contrast, Cruz et al. [6] observed, for the same algae, that the cadmium uptake slightly decreased with an increase in temperature for initial metal concentrations above 390 mg L^{-1}. Cordero et al. [37] reported a negligible effect of temperature on cadmium sorption by *Fucus spiralis* in the range 15–45 °C, with an increase of only 6%. For this reason, the process is usually conducted at room temperature, between 20 and 30 °C.

Surface response methodology is a useful tool to help in designing full-scale plants to operate within the pre-established range of the studied variables. Wastewaters from metal electroplating, metal finishing industries (spent baths and rinsing waters) and integrated circuits manufacture are usually acid, with a temperature close to air temperature, after screening and homogenization, and a copper concentration not exceeding 150 mg L^{-1}. The model does not predict the response for the variables which values are outside of the studied ranges but those values are not expected to occur, at least in most situations. The effect of the presence of other contaminants on metal biosorption was not studied but a reduction in the uptake capacity can occur. However, certain industrial effluents, for example from integrated circuits manufacture, mainly contain acid and cooper, and other constituents are in small quantities.

5. Conclusions

Box–Behnken design proved to be an efficient method for testing the effect of operating conditions and their interactions on copper uptake by the marine algae *A. nodosum*, allowing to significantly reducing the number of experiments. The selected variables – temperature, pH and initial concentration of Cu(II) – showed to influence the biosorption process, but their importance varies according to the sequence: initial concentration > pH > temperature. The higher uptake capacity was about 70 mg g^{-1} and corresponds to the following conditions: pH = 5.0, initial Cu(II) concentration = 150 mg L^{-1} and temperature = 40 °C. The equation describing the relation between the response and the variables allows identifying the statistically significant variables and evaluating quantitatively the effect of each one on the uptake capacity and the interactions eventually existent between two variables. Binary interactions of Cu concentration with temperature and pH were identified.

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