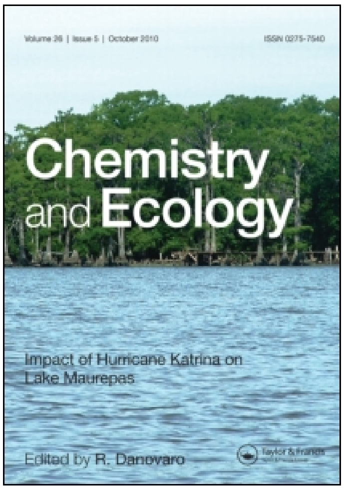

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Biosorption of copper, zinc, cadmium and nickel by *Chlorella vulgaris*

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The sorption capacity of the microalga, *Chlorella vulgaris*, was investigated using different metals (Cu, Zn, Cd and Ni), in both monometallic and bimetallic solutions. The final metal concentrations were significantly low. In the case of copper, an acid pretreatment (at pH 3) of the biomass was required to avoid an excessive increase in pH and the subsequent precipitation of metal during tests. This pretreatment was not necessary for the rest of the metals. The study of the influence of pH led to a greater metal uptake at a higher pH, suggesting a clear competition between metal cations and protons during the biosorption process. The biomass concentration was also a relevant variable, and the best sorption capacities were achieved at the lowest biomass concentration. pH also had a great influence on the elution of the metal retained by the biomass. The best recovery yields were obtained for the lower pH of the eluent solution. Sorption isotherms were well fitted to the Langmuir model, for both single-metal and two-metal systems. In both cases, the biomass showed a greater affinity for Cd.

Keywords: Biosorption; Copper; Zinc; Cadmium; Nickel; *Chlorella vulgaris*

1. Introduction

Industrial processes for extracting metals or, more generally, all processes involving metals in their productive cycle generate significant amounts of effluents contaminated with metal cations. The environmental damage produced by this type of effluents is a worrying problem that has resulted in the promulgation of strict regulations by national agencies to guarantee the quality of the liquids being disposed of.

Some metals (Hg, Pb and Cd) are especially toxic and very harmful for the cycle of some living beings. Other metals, although they are essential elements, can be very harmful at concentrations above certain limits, as is the case for Fe, Zn, Cu and Ni [1]. Most have a great affinity for sulphur atoms of enzymes and ability to form bonds with carboxylic and amine groups of proteins, thereby inhibiting their biological functions. Some are able

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to link to cell membranes, thus hindering transport processes through the cell wall. Additionally, heavy metals can either precipitate phosphate-type biocompounds or catalyse their decomposition [2].

Considering the harmful effects that heavy metals exert on the environment, their removal from sewage or, at least, the reduction in their concentration to the limits allowed by current strict regulations must be accomplished. Chemical methods for metal removal have a limited effectiveness and can be extremely expensive when the metal concentration target is very low.

Biosorption and bioaccumulation of heavy metals by different biomasses (such as algae, fungi and bacteria), dead or alive, have received much attention in recent years because of their potential use in the treatment of sewage loaded with heavy metals [3]. Biosorption equilibria are conditioned by several chemical variables, among which pH, biomass concentration and the coexistence of several cations in solution play an important role. The biosorption and the subsequent desorption process have been optimized for a great variety of biomass in monometallic systems [4–6]; however, there are few studies on multimetallic systems [7], in spite of the fact that real solutions contain more than one metal.

This study concerns the biosorption of a green alga (*Chlorella vulgaris*), whose biosorptive capacity has been the subject of several investigations in single-metal systems [8, 9] but not in multi-component systems.

In this case, four metals have been tested: Cu, Zn, Cd and Ni. The influence of variables controlling both the sorption and desorption process has first been considered followed by the sorption isotherms for both single-metal and two-metal systems. Two mathematical models have been used in this study: Freundlich and Langmuir. The Matlab program was an indispensable tool for representing three-dimensional sorption isotherms surfaces in two-metal systems and in calculating the corresponding kinetic constants for predicting the influence of a metal on the sorption capacity of another one.

2. Materials and methods

2.1 *Biomass*

Most of the experimental part was carried out with *Chlorella vulgaris* alga obtained from a culture collection. The original strain CCAP 211/12 was supplied by CCAP (Culture Collection of Algae and Protozoa) of The Institute of Freshwater Ecology, Cumbria, UK.

Chlorella vulgaris is a unicellular green alga, mainly marine, able to grow in freshwater and abundant in coastal waters. Its cell size is around 3–10 μm, and it multiplies by spores. Its rigid wall consists of 22.6% cellulosic material, and the rest is formed by saccharides and unknown substances. The alga was grown in Jaworski culture medium (JM) recommended by CCAP [10].

Cells were harvested from the culture by centrifugation. The biomass pellets collected were then washed with distilled water and centrifuged again. Then, cells were dried at 50–60 °C for 24 h, disaggregated and screened through a 0.1 mm mesh.

Additionally, several comparative tests were carried out with different photosynthetic micro-organisms: *Microcystis aeruginosa*, a prokaryote belonging to the cyanobacteria group, also known as blue-green algae; *Spirogyra insignis*, a freshwater eukaryotic green alga and *Tetraselmis suecica*, also a unicellular eukaryotic green alga but with a marine origin. In contrast to *Chlorella vulgaris*, there are no bibliographic records for these three types of algae in terms of their use as biosorbents; nevertheless, they were tested based on their good growth characteristics.

2.2 *Dissolutions*

Experiments were performed using synthetic single-metal solutions of Cu^{2+}, Zn^{2+}, Cd^{2+} and Ni^{2+} prepared from chemical reactants of analytical grade: $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}H_2O$, $CdSO_4{\cdot}8/3H_2O$ and $NiSO_4{\cdot}7H_2O$, respectively. Tested concentrations varied according to each case.

2.3 *Experimental procedure*

Biosorption tests were carried out in 100 mL Erlenmeyer flasks placed on a magnetic stirring plate. Each flask contained the solution and sufficient biomass. Periodically, 1 mL of sample was removed for analysis, and the pH was measured. After centrifugation at 5000 rpm to separate the biomass, the samples were analysed by atomic absorption spectrophotometry.

The amount of adsorbed metal was calculated from the following expression:

$$q = V(C_i - C_e)/S, \tag{1}$$

where q is the metal uptake (in mg or mmol/g of biomass); C_i and C_e are the metal concentrations before and after adsorption, respectively; and S is the mass of biosorbent used.

Two-metal experiments were carried out by adding the biomass (1 g/l) to solutions containing both metals. All the possible binary metal combinations were tested for concentrations of 0, 10, 30, 60 and 250 mg/l, at pH 5.

Desorption tests were performed with dry biomass previously loaded with metal. The biomass concentration used was 1 g per litre of eluent. The experimental procedure followed in these tests was the same as in the adsorption tests.

3. Results and discussion

3.1 *Previous experiments*

A series of previous experiments were carried out to fix the starting conditions. Thus, *C. vulgaris* was first tested with copper dissolutions, and subsequently its biosorbent efficiency was compared with the other three types of mentioned algae.

For these tests, the copper concentration was 25, 50, 100 and 200 mg/l (0.39, 0.78, 1.56 and 3.12 mmol/l, respectively) and the biomass concentration was 1 g/l. Figure 1 shows the results of both metal concentration and pH evolution. It is clear that when copper concentration was equal to or lower than 100 mg/l (1.56 mmol/l), the pH was higher than the metal precipitation pH (around 5.5); thus, the decrease in copper concentration was mainly due to its chemical precipitation and, to a lesser degree, to a sorption phenomenon. Nevertheless, at 1.56 mmol Cu^{2+}/l, during the first minutes, the pH was lower than that for metal precipitation, and the initial decrease in copper concentration would be associated with a sorption process.

In the experiment with 200 mg Cu^{2+}/l (3.12 mmol/l), the pH was always below 5.5, thus ensuring that the decrease in copper concentration was only due to a sorption phenomenon. This fact suggests a clear competition between Cu^{2+} and protons for the active sites of the biomass and accounts for the incomplete metal sorption.

In an attempt to overcome the difficulties in quantifying the metal uptake, tests were performed with buffer solutions of $KHC_8O_4H_4$/NaOH, adjusting the pH to around 4.5. The results showed a negligible copper sorption, thus justifying the preference of biomass for proton exchange.

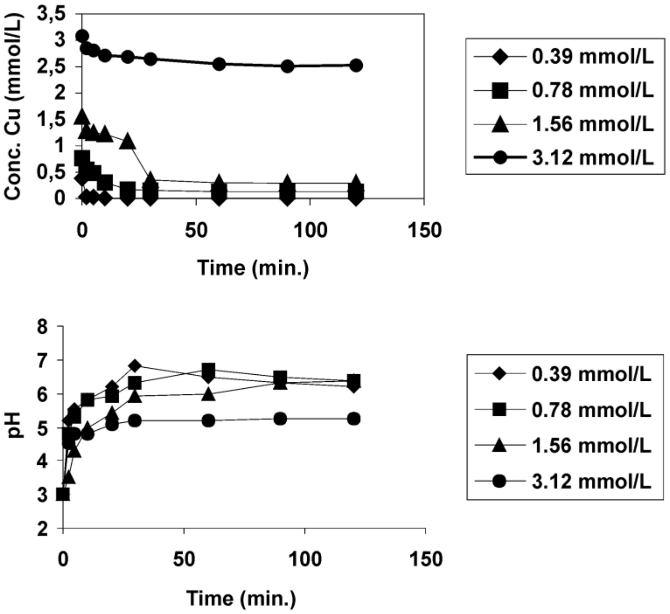


Figure 1. Copper concentration and pH evolution versus time.

Another possible alternative would be the pretreatment of the biomass with acid [11]. This modus operandi would produce the previous fixation of protons on the biomass, and later on, the metal sorption capacity could be evaluated without taking into account chemical precipitation. Additionally, the exchange between protons and copper ions could be measured.

The biomass pretreatment pH was tested with sulphuric acid at three different initial values: 1.5, 2 and 3, with 100 mL of sample and 1 g/l of biomass. The percentage of adsorbed protons was practically the same in the three cases (table 1). When copper uptake was tested with pretreated biomass, starting with 0.78 mmol of metal/l, the results showed that sorption capacity increased with pH, and metal precipitation did not occur (figure 2). Thus, acid pretreatment of the biomass at pH 3 is advisable in the case of copper.

Parallel, a comparative sorption study carried out with the other three types of algae at the same copper concentration (0.78 mmol/l), same pH (4.5) and same biomass concentration (1 g/l) justified the later use of *C. vulgaris* (figure 3). This was based on the best sorption capacity of this alga, though the improvement with respect to *Spirogyra* was very small and, moreover, due to its ample environmental growth conditions.

3.2 *Influence of pH*

This variable was investigated for the four metals in experiments at room temperature, with a metal concentration of 50 mg/l (0.78 mmol Cu^{2+}/l, 0.76 mmol Zn^{2+}/l, 0.86 mmol Ni^{2+}/l and 0.44 mmol Cd^{2+}/l) and with 1 g/l of biomass which, in the case of copper, was subjected

Table 1. Concentration of protons adsorbed by *C. vulgaris*.

$pH_{initial}$	1.50	2.00	3.00
pH_{final}	3.45	3.95	5.05
Percentage adsorbed	99.00	98.88	99.11

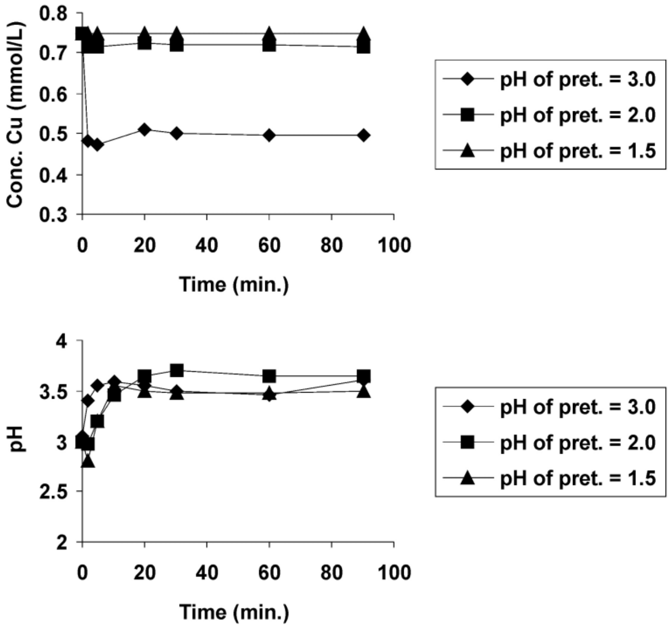


Figure 2. Copper uptake using biomass with an acid pretreatment.

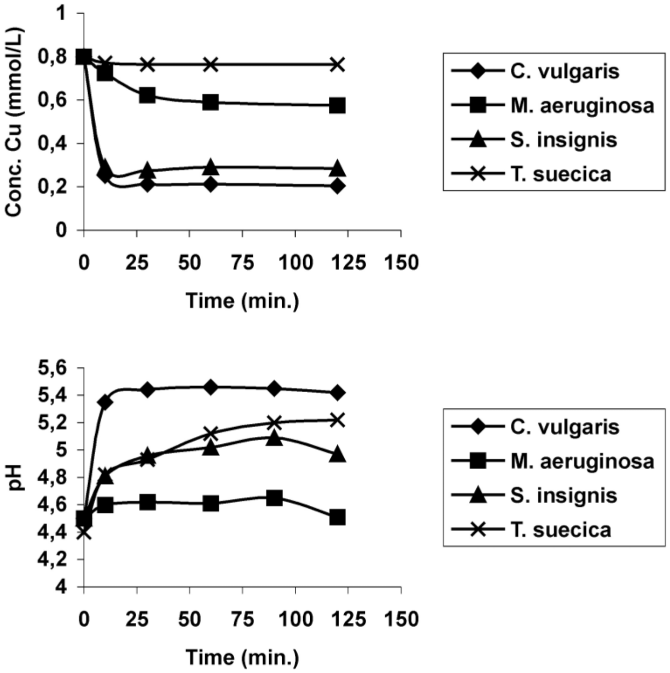


Figure 3. Copper concentration and pH evolution versus time for four types of biomass.

Table 2. Sorption capacity as a function of pH.

Element	pH	q (mmol/g biomass)
Cu	1.5	0.047
	3.0	0.130
	4.0	0.201
	5.0	0.252
Zn	1.5	0.008
	3.0	0.115
	5.0	0.268
	7.0	0.229
Cd	2.0	0.023
	4.0	0.254
	6.0	0.292
	8.0	0.314
Ni	1.5	0.061
	3.0	0.123
	5.0	0.342
	7.0	0.317

to an acid pretreatment at pH 3. Previous protonation of the biomass was not necessary with the other three metals, since the pH was always lower than the precipitation pH.

pH values tested were chosen, taking into account the solubility diagrams corresponding to each metal. There was a marked influence of the initial pH value on the sorption capacity (q) of each metal (table 2). At low pH values, the metal sorption capacity of the biomass was practically negligible. In contrast, sorption capacity increased with increasing initial pH. The highest q values were recorded at the highest pH values, except for zinc and nickel where q values were slightly greater at pH 5 than at pH 7, though in both cases, such value was much greater than at pH 3.

These results indicate that an increase in pH has a positive effect on metal uptake, since the competition between protons and metal cations for the active sites of the biomass decreases. For instance, see figure 4 for the case of Cd (the results for the rest of the metals were similar and, therefore, they are not shown). On the other hand, the possible presence of bulky cations, of the type $MeOH^+$, at pH values close to chemical precipitation, seems to be of little importance.

A general trend was observed for the four metals and the pH stabilized after a few minutes of starting each test (figure 4). Such behaviour would be conditioned by the functional groups attached to the alga cell wall (carboxylic, hydroxil, etc.). Each group has an acid constant (pK_a) which determines its equilibrium state with the corresponding conjugated base. Thus, when the biomass is in contact with a medium at a pH lower than pK_a, the equilibrium shifts towards the formation of the acid species with consumption of protons, increasing, in turn, the pH value. In the opposite case, when the pH of the medium is above pK_a, the equilibrium shifts towards the formation of the basic species with an increase in protons in solution, and therefore decreasing the pH value [12].

3.3 *Influence of biomass concentration*

This variable can significantly affect the sorption process. The sorption capacity increased with decreasing cell density, since the distance between cells was greater. In this way, literature studies [13] indicate that electrostatic interactions between cells play an important role in the process, and that the shell effect produced on the external layer, when cell concentration is

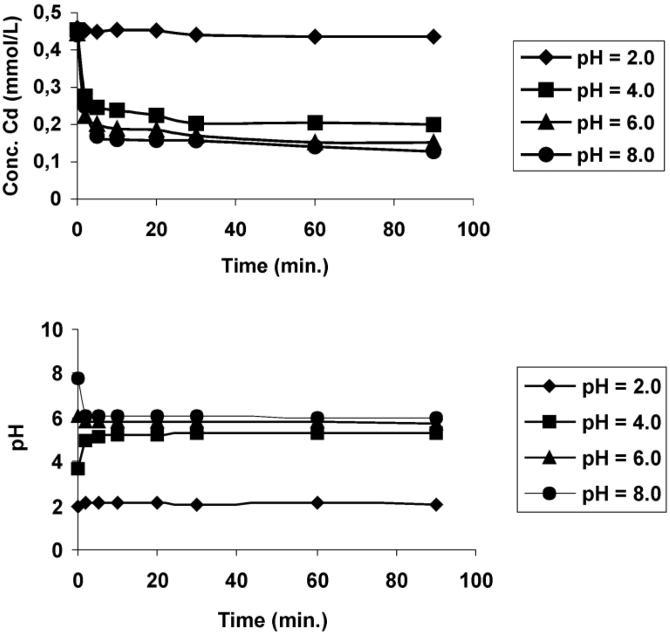


Figure 4. Influence of initial pH on Cd sorption.

high, masks active sites. Nevertheless, a high biomass density is synonymous with a higher number of active sites and, in turn, of a high metal recovery.

The study was carried out for each metal at three different biomass concentrations (1, 2 and 3 g/l) and, as previously, the biomass was acid-pretreated in the case of copper. The initial concentration of each metal was maintained constant at 50 mg/l. The test pH was 5 for copper, zinc and nickel and 8 for cadmium.

Figure 5 shows the sorption yields and the maxium sorption capacity (q) for each metal versus biomass concentration. Two criteria could be used to determine the best operational conditions: (1) the decrease in the metal amount in solution, which would be applicable when biomass availability is easy, and (2) the maximum sorption capacity. In the latter case, the result would be a higher metal recovery per mass of biosorbent; such criterion would be adequate when the biomass availability was a serious concern. According to this, the results obtained are in agreement with bibliographic data which support the idea that a decrease in biomass concentration improves sorption capacity. Thus, for Cd and Zn, this value was approximately double with 1 than with 3 g of biomass/l. That difference was less marked for the case of nickel. On the contrary, copper was the only metal for which the maximum sorption capacity was obtained not for the lowest concentration, but for 2 g of biomass/l. Such behaviour could be explained through the blockage of the active sites by protons, resulting from the previous treatment of the biomass.

Finally, as expected, an increase in biomass concentration decreased the equilibrium metal concentration and, in turn, increased the percentage of metal removal from solution.

3.4 *Desorption experiments: Influence of pH*

Several different types of eluents have been proposed in the literature for the recovery of the metal retained by the biomass [14,15]. In most cases, diluted solutions of organic or inorganic

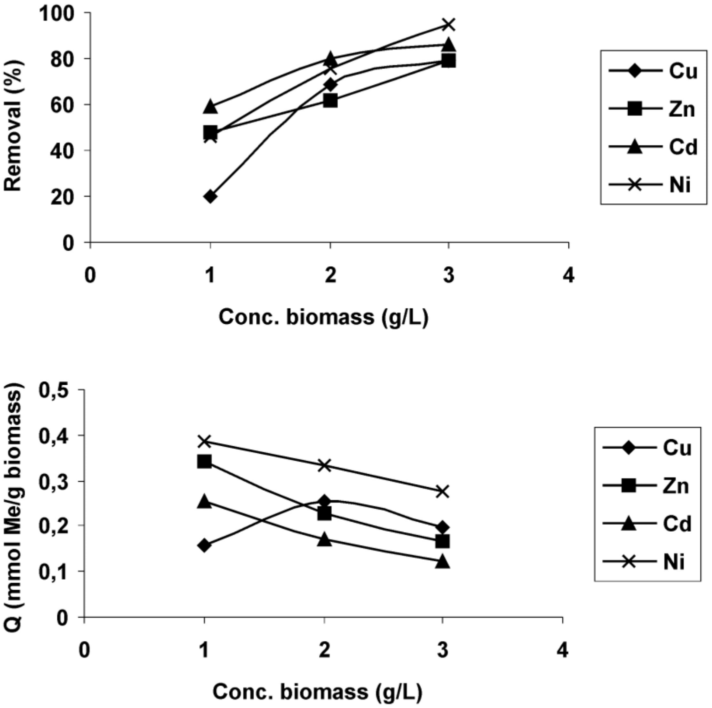


Figure 5. Influence of biomass concentration.

acids have been used. In the present work, diluted low-cost solutions of acetic, sulphuric and hydrochloric acid have been employed.

The most significant variable is the eluent concentration or, for the case under study, the pH. This study was carried out using loaded metal biomass and setting the pH value at 2, 3 and 4. The results obtained are shown in table 3.

In all cases, a decrease in pH increased the percentage of metal recovery, as a result of a higher concentration of protons in the medium, which favoured its exchange with the metal cations retained by the biomass.

Similarly, copper recovery with the three different eluents reached the lowest percentages, less than 25% at the lowest pH. This could be related to a stronger bond between cations and active sites of the algal cell wall, since the previous copper adsorption took place on biomass sites that were not occupied during the acid pretreatment.

In the case of Zn, Cd and Ni, the recovery was higher at low pH, and this was independent of the type of eluent used. At pH 4, desorption percentages were remarkably lower for the four metals (especially for Cu and Cd), slightly higher for Zn and even better for Ni. In this case, the hydrochloric acid had the best performance, with an improvement with respect to other

Table 3. Metal desorption percentage (%) with several acids.

	H_2SO_4				HCl				CH_3COOH			
pH	Cu	Zn	Cd	Ni	Cu	Zn	Cd	Ni	Cu	Zn	Cd	Ni
2.0	24.9	87.0	96.9	90.4	22.8	83.0	98.6	86.6	25.0	86.7	96.0	91.7
3.0	16.3	81.7	83.7	98.1	12.7	83.2	92.3	92.6	15.1	79.7	80.1	92.4
4.0	3.2	19.6	11.4	40.3	8.0	35.1	20.1	39.0	2.9	20.0	11.1	32.1

eluents of 62.5% for Cu, 75.6% for Zn and 50.0% for Cd. However, no improvement could be seen in the case of Ni.

3.5 *Sorption isotherms*

3.5.1 Single-metal systems. Sorption isotherms in single-metal systems were evaluated, defining concentrations in millimoles. This allowed an estequiometric comparison between sorption capacities for each metal in spite of their different initial concentration, which varied between 10 and 250 mg/l. For each metal, the pH and biomass concentration were chosen at its optimum value.

Figure 6 shows sorption capacity (q) versus equilibrium concentration (C_e). An increase in the latter parameter increased the sorption capacity until the limit was reached. Such a value represents the maximum metal sorption capacity by the biomass, that is, when all active

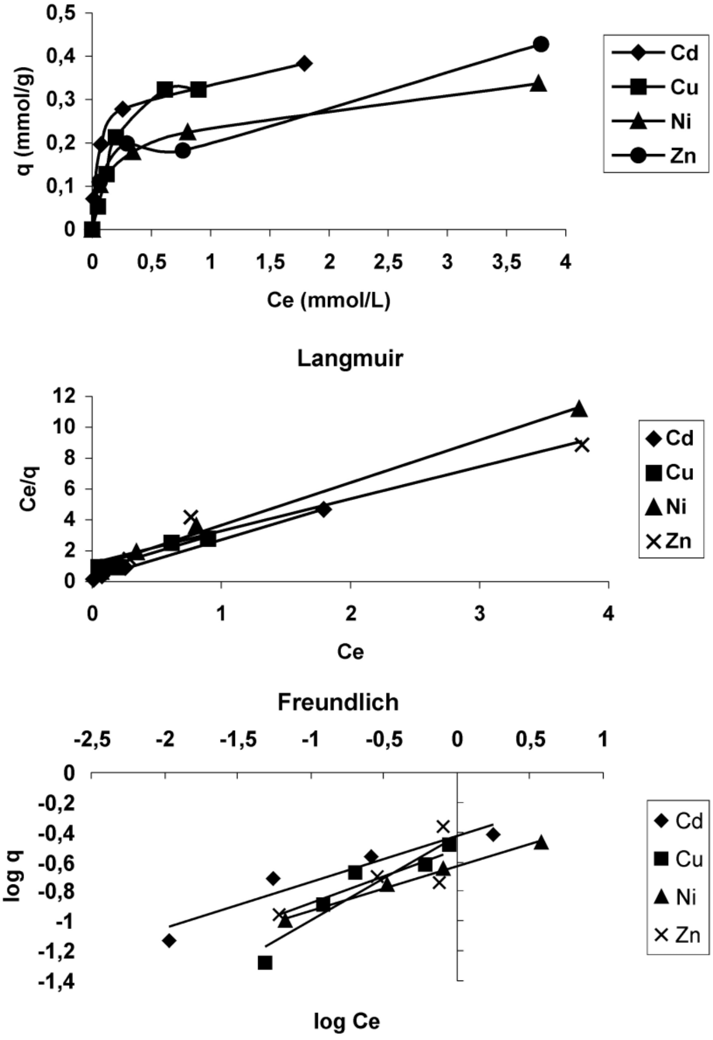


Figure 6. Sorption isotherms of Cu, Cd, Ni and Zn.

sites have been occupied. At low metal equilibrium concentrations, small changes in this value are indicative of a change in sorption capacity; by contrast, at high metal equilibrium concentrations, metal uptake is independent of the equilibrium concentration. These results agree with bibliographic data [16].

The affinity between the biomass and the different metals was quantified by adjusting the obtained sorption values to a Langmuir isotherm:

$$q = bC_e q_{\max}/(1 + bC_e), \tag{2}$$

where q is the maximum sorption capacity and b the Langmuir constant, which establishes the relationship between sorption and desorption rate.

In addition, a Freundlich isotherm was also used:

$$q = K_e C_e^a, \tag{3}$$

where K_e and a are constants.

Parameters defined by both models were evaluated from experimental data of C_e and q, by representing their linear expressions defined by the following equations, respectively (figure 6):

$$C_e/q = (C_e/q_{\max}) + (1 + bq_{\max}) \tag{4}$$

$$\log q = \log K_e + a \log C_e. \tag{5}$$

Linear regressions for both models are collated in table 4. The Langmuir model fitted very well for the four metals, with regression coefficients above 0.95, especially in the case of Cd and Ni. The Freundlich model fitted less accurately for Cd and Ni, not as well for Cu and poorly for Zn. Langmuir parameters, $q_{\max}$ and b, are also shown in table 4. Since there is a close relationship between a maximum b value and a higher affinity between adsorbent and adsorbate [17], *Chlorella vulgaris* clearly shows a higher affinity for Cd, followed by Ni and Zn, and in a lesser proportion for Cu. Nevertheless, the maximum sorption capacities were very similar for the four metals.

3.5.2 Two-metal systems. Three different binary-metal combinations were tested: Cd–Zn, Cd–Ni and Zn–Ni. Copper was excluded from this study due to the necessity of pretreating the biomass, which did not apply for the rest of metals.

Equilibria of two-metal sorption can be studied by the construction of surfaces representing sorption isotherms in three dimensions. Such surfaces can be obtained representing equilibrium concentrations of the two metals or of both metals at the same time along the x and y axes. In this sense, MATLAB software was an indispensable tool to carry out this task.

Several models were evaluated [18] in order to choose the equation that best fitted the experimental data in a three-dimensional representation. The model chosen was a Langmuir type of binary order. Such a model, represented by the following equations, assumes an

Table 4. Sorption constants in single-metal systems.

	Langmuir			Freundlich
Metal	$q_{\max}$ (mmol/g)	b (l/mmol)	R^2	R^2
Cu	0.40	3.69	0.95	0.89
Cd	0.40	12.89	1.00	0.90
Ni	0.36	3.07	0.99	0.99
Zn	0.48	0.58	0.95	0.63

Table 5. Langmuir sorption constants in two-metal systems.

System	K_1 (mmol/l)	K_2 (mmol/l)	$Q_{\max}$ (mmol/g)
Cd–Zn	Cd: 0.10	Zn: 0.16	0.36
Cd–Ni	Cd: 0.06	Ni: 0.20	0.30
Zn–Ni	Zn: 0.05	Ni: 0.18	0.27

equilibrium between the two metallic species, M_1 and M_2, and the corresponding species that result from its sorption by the biomass B, B-M_1 and B-M_2, respectively:

$$\mathrm{B} + \mathrm{M}_1 \rightleftarrows \mathrm{B} - \mathrm{M}_1 \quad K_1 = k_{-1}/k_1 \tag{6}$$

$$\mathrm{B} + \mathrm{M}_2 \rightleftarrows \mathrm{B} - \mathrm{M}_2 \quad K_2 = k_{-2}/k_2 \tag{7}$$

$$q(\mathrm{M}_1) = \frac{q_{\max} C_e[\mathrm{M}_1]/K_1}{1 + C_e[\mathrm{M}_1]/K_1 + C_e[\mathrm{M}_2]/K_2}. \tag{8}$$

The three parameters derived from the application of this model are collated in table 5 for the three systems tested. From a comparison of K_1 and K_2 values, there is a clear affinity of the biomass for Cd due to its lower value of K_1 for both systems, Cd–Zn and Cd–Ni; this corroborates the results obtained in single-metal systems. On the other hand, Zn uptake was better than Ni uptake, though in single-metal systems, there was an important difference between the sorption capacities of these two metals with respect to Cd. The maximum sorption capacity was very likely for all systems, similarly to single-metal systems.

Equation (8) can be represented using sorption isotherms surfaces in three dimensions, as shown in figure 7 for the Cd–Zn system, in figure 8 for the Cd–Ni system and in figure 9 for the Zn–Ni system.

Since these surfaces are a representation of the equilibrium in the presence of both metals, its evolution represents the variation of the sorption capacity of each metal for different concentrations of the other metal.

In this sense, for the Cd–Zn system, the presence of Cd reveals a sharp decrease in Zn sorption capacity (figure 7). Similarly, there was a marked decrease in Ni sorption capacity in

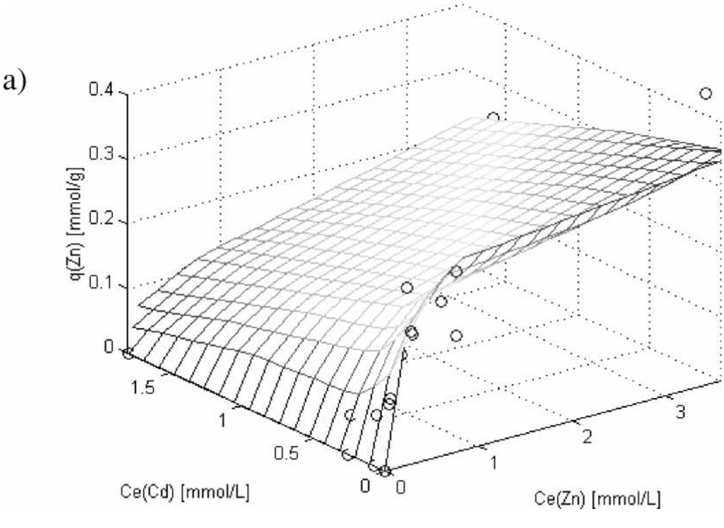


Figure 7. Sorption isotherm surface of: (a) Zn in the presence of Cd and (b) Cd in the presence of Zn.

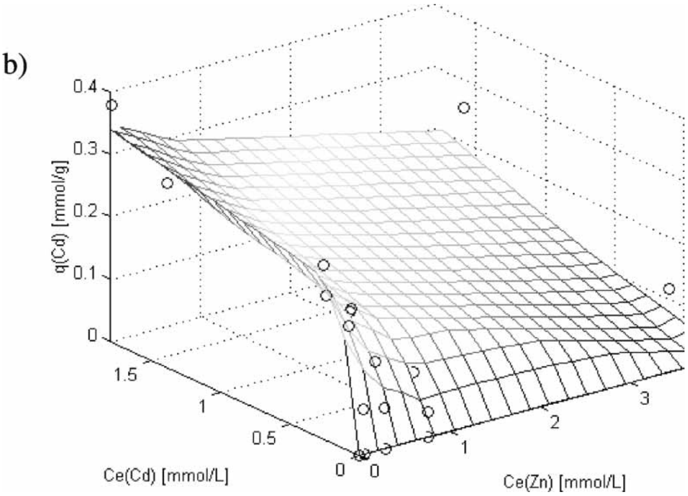
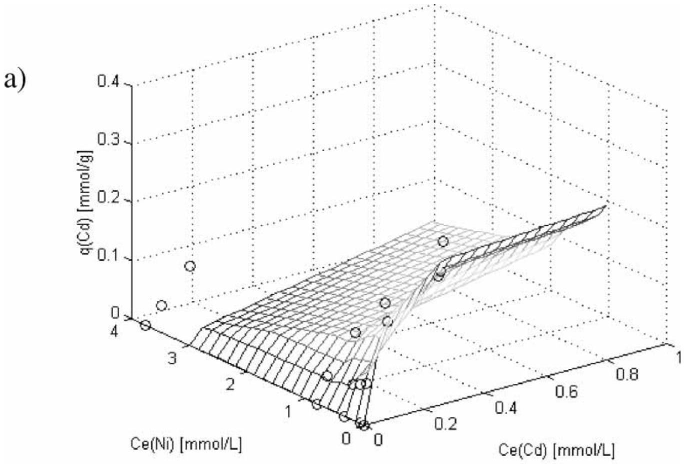


Figure 7. *continued.*



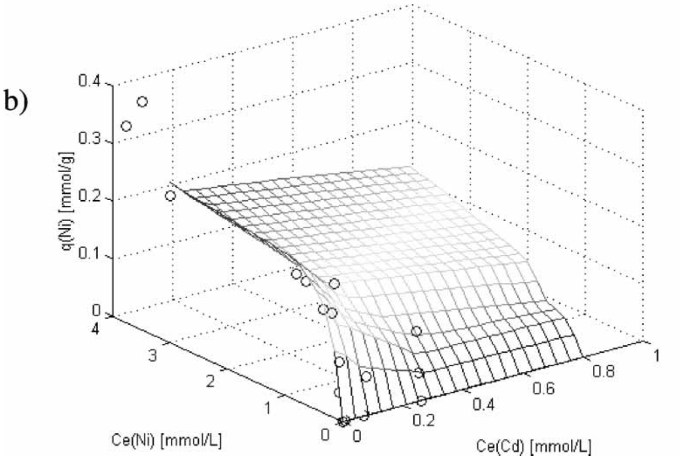


Figure 8. Sorption isotherm surface of: (a) Cd in the presence of Ni and (b) Ni in the presence of Cd.

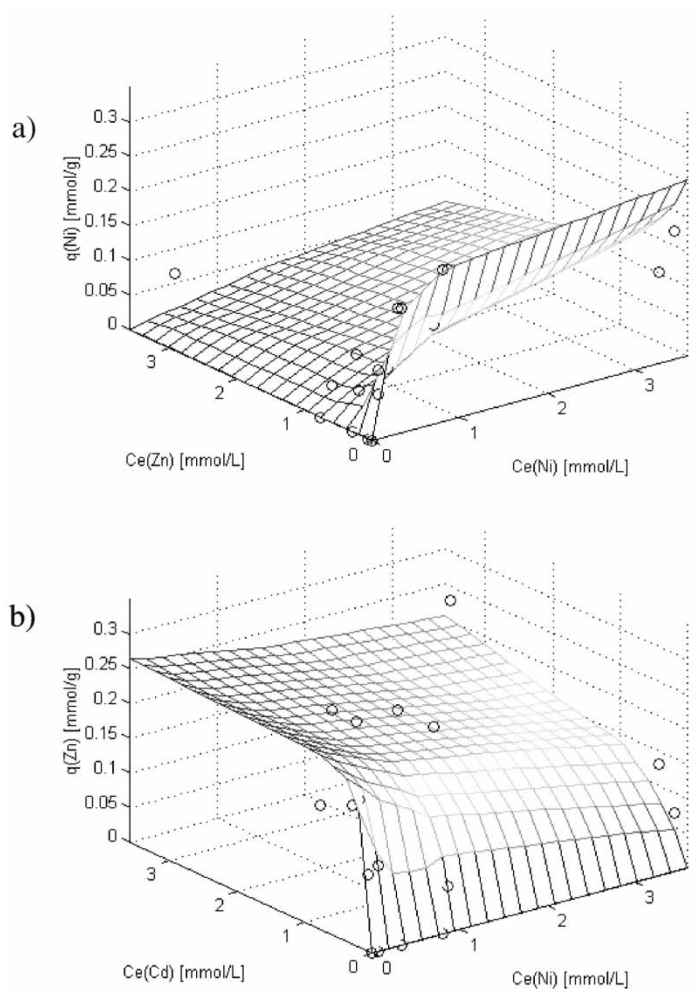


Figure 9. Sorption isotherm surface of: (a) Ni in the presence of Zn and (b) Zn in the presence of Ni.

the presence of Cd (figure 8). For both cases, the results agree with *K* values, greater for Zn and Ni than for Cd.

In the case of Zn–Ni system, there was a higher decrease on Ni sorption capacity in the presence of Zn than viceversa (figure 9). This would confirm that the biomass presents a sligthly higher affinity for Zn than for Ni, which is shown from the comparative study of parameters in table 5.

4. Conclusions

Microalga *C. vulgaris* was very efficient in metal recovery from solutions, achieving reductions in metal concentration up to very low levels. Its use was advantageous compared with other types of similar biomass. However, in the case of copper, it was necessary to carry out an acid biomass pretreatment at pH 3 to quantify the sorption process, without taking into account chemical precipitation.

pH was an important factor in the sorption process due to competition between protons and metal cations for the active sites of the biomass. As a general trend, the sorption levels increased with pH.

Biomass concentration was another main factor in the sorption process. The best sorption capacities were achieved at the lowest biomass concentration, when the electrostatic interactions between cells decreased.

pH also had a great influence on the elution of the metal retained by the biomass. The best recovery yields were obtained for a lower-pH eluent solution.

Sorption isotherms in single-metal systems have shown a similar trend for the four metals tested, as deduced from the representation of sorption capacity (q) versus equilibrium concentration (C_e). Experimental data fitted the Langmuir model better than the Freundlich model. Constant values of the former model have demonstrated a much higher affinity of the biomass for Cd than for the rest of the metals tested.

In two-metal systems, using three-dimensional sorption isotherms, it was possible to quantify the influence of the one metal concentration on the other metal sorption. The biomass affinity was determined using the Langmuir model, and again, the biomass showed a higher affinity for Cd.

Acknowledgement

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