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Article

Evaluating Scatchard and Differential Equilibrium Functions to Study the Binding Properties of Cu(II) to the Surface of Mixed Species of Lyophilized *Spirulina* (Cyanobacteria)

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A ligação de Cu(II) à superfície de espécies mistas da microalga *Spirulina* foi estudada em pH 6.0 por titulação potenciométrica monitorada com eletrodo íon-seletivo para Cu(II). Três materiais foram estudados: a suspensão completa da alga, a água de lavagem obtida por centrifugação da suspensão completa e a suspensão composta por células lavadas com água. Os métodos de Scatchard e de Funções Diferenciais de Equilíbrio (*DEF*) foram usados para tratamento dos dados de titulação. Os gráficos de Scatchard possibilitaram a determinação de duas classes de sítios de complexação, sendo que os valores de log K' para os sítios mais fortes variaram entre 7,3 e 7,9. Para os sítios mais fracos os valores de log K' foram determinados entre 3,5 e 3,9. As concentrações totais dos sítios de complexação foram 1,6±0,1; 1,5±0,5 e 0,92±0,08 mmol g^{-1} para a suspensão total, água de lavagem e células lavadas, respectivamente. O método *DEF* revelou uma variação linear dos valores de log K_{DEF} em função do log θ (θ = grau de ocupação dos sítios de complexação), sendo que os valores de log K_{DEF} decresceram de 9 para 4 em conseqüência do aumento de log θ de –2,5 para –0,25. Os graus de heterogeneidade determinados por *DEF* ficaram na faixa entre 0,4 e 0,5 para os três materiais estudados.

The binding of Cu(II) to the surface of mixed species of *Spirulina* was studied at pH 6.0 by potentiometric titration monitored with a copper ion selective electrode. Three materials were studied: the total suspension of alga, the washing water that resulted from centrifugation of the total suspension, and the water-washed cells. The Scatchard method and the Differential Equilibrium Functions (*DEF*) were used for the treatment of the titration data. The Scatchard plots determined two classes of binding sites, with log K' values for stronger sites varying between 7.3 and 7.9. For the weaker sites the log K' values ranged between 3.5 and 3.9. The total concentration of binding sites were 1.6±0.1, 1.5±0.5 and 0.92±0.08 mmol g^{-1} for the total suspension, washing water, and washed cells, respectively. The *DEF* approach revealed a linear variation of log K_{DEF} as a function of log θ (θ = degree of site occupation), with log K_{DEF} decreasing from 9 to 4 as a consequence of increasing log θ from –2.5 to -0.25. The degree of site heterogeneity determined by the *DEF* approach was between 0.4 and 0.5 for the three materials studied.

Keywords: adsorption, complexation, copper, *Spirulina*, potentiometry

Introduction

The binding of metal ions to microorganisms is a phenomenon that has strong influence on the transport of heavy metal cations in aquatic environments. This phenomenon has also been explored for treatment of natural and waste waters, as well as for remediation of polluted areas.[1-5]

Cell surfaces of algae and bacteria are characterized by strong chemical heterogeneity of binding sites, consisting of sulfonic, carboxylic, phosphate, imidazolic, amine and phenolic groups. Sulfur-containing groups, which have especially high affinity for heavy metal cations, are also present in the cell walls, but at lower abundance in comparison to the previous mentioned groups. All these groups are bound to a polymeric network of polysaccharides, cellulose, phospholipids, hemicellulose, proteins and amino acids that compose the cellular wall.[6]

The chemical heterogeneity of binding sites leads to a distribution of affinities of the cell surface for the metal cations. The affinity depends on the total metal loading, or the degree of site occupation. Thus, the binding sites with greater affinity are occupied first, that is, at lower concentrations of the metal cation.[6]

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The interpretation of a titration curve of a cell surface by metal cations is difficult since no inflection points mark the saturation of the binding sites. The determination of binding constants and binding capacities is usually made using the linearized adsorption isotherms of Langmuir or Freundlich, including the linear Scatchard plots.[7] The major limitation of these treatments is that the results are average stability constants that reflect only partially the actual distribution of affinities of the binding sites as a function of the metal loading. To overcome this limitation, a continuous differential equilibrium function has been proposed to interpret the metal binding properties of metal cations to humic substances.[8-10] This method was recently applied to study the thermodynamic of Cd(II) uptake by *Chlorella marina*.[11] When this approach is applied, no a priori assumptions are made, so that effects of the chemical heterogeneity, electrostatic interactions and conformational changes can be evaluated performing the titrations under different conditions of pH, ionic strength and initial metal loading.

This paper presents an evaluation of the Scatchard plots[7] and the continuous differential equilibrium function[6] to study the binding properties of Cu(II) to the surface of non-viable lyophilized mixed species of *Spirulina* (Cyanobacteria), as well as the soluble fraction of the alga suspension. This material was chosen due to its commercial availability and its well-controlled source, making it a suitable material for evaluation of the Scatchard plots and differential equilibrium functions. The thermodynamic parameters determined were used to model the titration curve, that is, to predict the free Cu(II) concentration as a function of the total loading of Cu(II) in the titration medium.

Experimental

Apparatus

Potentiometric measurements were made with two Metrohm 654 pH-meters (precision of 0.1 mV or 0.001 units of pH). For acid-base titration and pH measurements during complexometric titrations, a Mettler Toledo HA405-60-88G-S7/120 - Ag/AgCl combination glass electrode was used. For complexometric titrations both pH-meters were used; one of them was used for monitoring the pH, which was kept at pH 6.0 ± 0.1 with the aid of the above mentioned combination glass electrode. The other pH-meter was used with a Cu(II) ion selective electrode (ISE-Orion 9429) and a Mettler-Toledo double junction Ag/AgCl reference electrode model 405NS-S7/80.

All titrations were performed at 25.0±0.1 °C using an Ética 521D thermostat. A Gilmont GS 4200A microburette (capacity of 2.5 mL and resolution of 0.1 μL) and a Gilmont GS 1200A microburette (capacity of 2 mL and resolution of 2 μL) were used for titrant addition and pH corrections.

Flame atomic absorption determinations of the total dissolved Cu(II) concentration were performed with a model 703 Perkin-Elmer spectrophotometer.

Reagents and sample preparation

De-ionized water obtained from a Millipore Milli-Q system was used to prepare all solutions and alga suspensions. All chemicals were of analytical grade and used without further purification. Copper stock solution, as $Cu(NO_3)_2$, with concentration of 1.574×10^{-2} mol L^{-1} (1000 mg L^{-1}), was prepared diluting the content of an ampoule of atomic absorption standard Normex (Carlo Erba) to 1.0 L. Working solutions were prepared by properly diluting this stock solution in medium of 0.020 mol L^{-1} KNO_3.

Mixed species of lyophilized *Spirulina* (Cyanobacteria) was purchased from Sigma (Lot 29F40201). A stock 6.25 g L^{-1} suspension was prepared by weighing 0.3125 g (±0.1 mg) of alga and transferring the material to a 50.00 mL volumetric flask with deionized water.

Equilibrium time for adsorption

To 250 mL of a 1.00 g L^{-1} *Spirulina* suspension prepared in medium of 0.020 mol L^{-1} KNO_3 at pH 6.0±0.1, Cu(II) solution was added providing total initial Cu(II) concentrations of 1.2×10^{-3} or 1.0×10^{-4} mol L^{-1}. The suspension was continuously homogenized in a horizontal shaker and aliquots of 10 mL of the suspension were withdrawn at intervals of 0, 5, 10, 15 and 30 min. The aliquot was centrifuged for 5 min at 3.000 rpm, and the solution filtered through 0.45 mm cellulose acetate membranes that had been previously washed with deionized water. Determinations of the total dissolved Cu concentration in these solutions were performed by flame atomic absorption spectrophotometry.

Titrations of Spirulina *with Cu(II)*

Adequate volumes of stock 0.0050 mol L^{-1} Cu(II) were transferred to twelve polypropylene centrifuge tubes (50 mL capacity) to provide total Cu(II) concentrations between 2.0×10^{-6} and 1.0×10^{-3} mol L^{-1} after dilution to a total volume of 25.00 mL. For titration of the total suspension, 4.00 mL aliquots of the 6.25 g L^{-1} homogenized *Spirulina* stock suspension were transferred to the tubes

providing a concentration of 1.00 g L^{-1} after dilution to 25.00 mL. Before completing this volume, the ionic strength was adjusted to 0.020 mol L^{-1} with KNO_3 and the pH adjusted to 6.0±0.1 by adding small amounts of 2 x 10^{-3} mol L^{-1} NaOH or HNO_3. To minimize variation of ionic strength during the experiments, these NaOH or HNO_3 solutions were also prepared in 0.020 mol L^{-1} KNO_3. This procedure was used because most buffers are also complexing agents for Cu(II), which would increase the complexity of calculations due to the competition between the buffer and *Spirulina* materials being studied.

All tubes were maintained immersed in a thermostatic water bath controlled at 25.0±0.1 °C. After 30 min of equilibration time, the Cu-ISE and the reference electrode were immersed in the suspensions for measurement of the potential in each tube. For the tubes containing total Cu(II) concentration between $2x10^{-6}$ and $1x10^{-5}$ mol L^{-1}, an additional time of about 30-60 min was necessary for stabilization of the potentiometric signal. This additional time decreased as the total Cu(II) concentration increased in the titration medium. The potential measurement was made only when the potential drift was < 0.555 mV min^{-1}.

For titration of the washed cells and the washing water, the 6.25 g L^{-1} stock suspension was centrifuged at 3 000 rpm for 20 min. After separation of the supernatant, the residue was re-suspended in de-ionized water inside a 50.00 mL volumetric flask, resulting the material here named washed cells. For titration of the washed cells the procedure was the same described for the total suspension.

The solution resulting from the previous centrifugation was named washing water. This solution was titrated with Cu(II) using a procedure similar to described for total and washed cells. For this, 4.00 mL aliquots of the solution were transferred to 50 mL tubes, adjusting the total Cu(II) concentration between 2.0 and 1000 μmol L^{-1} after dilution to a final volume of 25.00 mL (with previous adjust of ionic strength and pH). The results of these titrations were expressed as g L^{-1} of the original suspension, that is, the concentrations of soluble species that resulted in a 1.00 g L^{-1} *Spirulina* suspension. No detectable Cu(II) concentrations were verified in the washing water (The limits of detection and determination were 0.01 and 1.2 μmol L^{-1}, respectively).

The calibration of the electrode system was performed in terms of concentration titrating a 0.02 mol L^{-1} KNO_3 solution kept at pH 6.0±0.1 and 25.0±0.1 °C with Cu(II) solution in the same medium, but in absence of *Spirulina*.

Acid-base titrations

The calibration of the glass electrode was performed in terms of H^+ concentrations instead activities just before the titrations of *Spirulina* suspension.[12-14]

Fifty milliliters of a 1.00 g L^{-1} *Spirulina* suspension in medium of 0.020 mol L^{-1} KNO_3 were transferred to a water-jacketed titration flask. Next, 2.50 mL of 0.10 mol L^{-1} HNO_3 (also in medium of 0.020 mol L^{-1} KNO_3) were added to the suspension in order to protonate all ionizable sites on the surface of the cells. The titration flask was closed after the introduction of the combination glass electrode, the burette tip and two small tubes that allow the circulation of CO_2-free nitrogen inside the flask. The temperature was kept at 25.0±0.1 °C circulating thermostated water through the external jacket of the titration flask. The titration was performed adding small increments of the standard NaOH solution to provide increases of approximately 0.1 units of pH (~10mV). The pH range covered in the titration was between 2.5 and 11. The potential measurement was made only when the potential drift was < 0.555 mV min^{-1}, as indicated in the control panel of the pH-meter.

Modeling

Proton binding characterization

Treatment of the acid-base potentiometric titration data was performed by non-linear regression fitting as previously described.[13, 14] This treatment assumes a discrete site model[14, 15] which allows the determination of the stoichiometry and pKa of the ionizable sites on the surface of the *Spirulina* cells.[13]

The free metal and bound metal cation concentration

The concentration of the free Cu(II) is computed from the equation:

$$E = A + S \log [Cu(II)] \quad (1)$$

where E is the measured potential of the electrochemical cell, A is a constant that includes the potential of the reference electrode, the standard reduction potential of Cu(II), the liquid junction potential and the activity coefficient of Cu(II) in the conditions of ionic medium in which the experiments were performed. S is given by the expression for which the terms have their usual meaning, assuming the theoretical value of 29.6 mV at 25 °C.

The terms A and S of equation 1 were determined by titration of a 0.02 mol L^{-1} KNO_3 solution, at pH 6.0±0.1, with solutions of Cu(II) in the same ionic medium. The experimental value of S was 28.6 mV, indicating the nernstian behavior of the selective electrode in the ionic

medium studied. The experimental values of A and S were further used together with the measured potentials and the equation 1 to compute [*Cu(II)*] during the tritrations of the *Spirulina* suspensions. Due to the nernstian behavior of the electrode, [*Cu(II)*] was assumed as the free unhydrolyzed Cu(II) concentration and no metal hydrolysis was considered in the calculations.

The experimental concentration of the bound Cu(II), [*CuL*], was obtained from the mass balance: [*CuL*] = $C_{Cu(II)}$ – [*Cu*(II)], where C_{Cu} is the total concentration of Cu(II) added to the titration cell.

The discrete site model – Scatchard plots and the Complexing Capacity Parameter

In order to evaluate the total number of complexing sites, the use of the discrete site model proposed by Scatchard[7] to study the interactions between ions and small molecules with macromolecules has been quite extensive. This model assumes that only a small number of different kinds of complexing sites are present in the macromolecule and that there is no interaction among them. The charge on the macromolecules should be constant (experiment performed at constant pH). It is also assumed that only complexes with 1:1 stoichiometry are formed.[6, 7] The computation of the total concentration of binding sites, $[L_j]_t$ and the average equilibrium constants, K_j, are obtained from the equation:

$$\frac{[CuL]}{[Cu(II)]} = K_j[L_j]_t - K_j[CuL] \quad (2)$$

where [*CuL*] is the concentration of complexed metal. A plot of [CuL_j] / [*Cu*(II)] vs. [*CuL*] is linear with the slope giving the negative of the average equilibrium constant. The total concentration of the complexing site *j*, $[L_j]_t$, is obtained dividing the linear coefficient by the slope. When *j* = 2 the equation 2 is no longer linear and the parameters are calculated by nonlinear fitting, or, if K_1 and K_2 are significantly different, two linear regions can be depicted, allowing graphical evaluation of *K* and $[L_j]_t$.[6]

The Differential Equilibrium Function (DEF)

For computation of K_{DEF}, the equation 3 was used:[16]

$$K_{DEF} = -\frac{\alpha^2}{C_{Cu(II)}}\left[\frac{1}{1+(\alpha-1)\left(\frac{d\ln C_{Cu(II)}}{d\ln\alpha}\right)}\right] \quad (3)$$

where $\alpha = C_{Cu(II)}/[Cu(II)]$. The differential term was obtained by fitting a polynomial function to the data plotted as $\ln C_{Cu(II)}$ versus $\ln\alpha$ using Microsoft Excel® or Microcal Origin® software.

The degree of site occupation, θ, is computed as the ratio $[CuL]/C_{L,}$ where C_L is the total concentration of ionizable sites that are de-protonated at pH 6.0. This concentration was obtained from the acid-base titration curves. The heterogeneity parameter was determined from the slope of the log θ vs. log K_{DEF} plot, which shows a linear relationship according to the Freundlich isotherm:

$$\log\theta = \Gamma \log K^0_{DEF} - \Gamma \log K_{DEF} \quad (9)$$

where Γ is the heterogeneity parameter (with values between 0 and 1, being 1 for a single ligand and 0 for a totally heterogeneous ligand) and K^0_{DEF} is the value of K_{DEF} when = 1.[8, 16]

Results and Discussion

Acid-base characterization

The chemical heterogeneity of the *Spirulina* surface was first studied by the acid-base characterization (Figure 1), which shows the discrete site distribution spectra of the three materials studied. These spectra show the pKa value of the ionizable sites and their abundance (in mmol per gram of alga), determined with the potentiometric titration

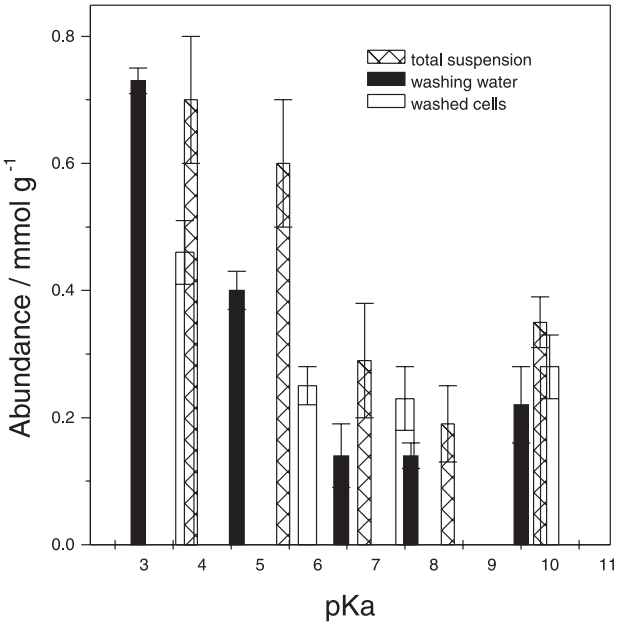

Figure 1. Distribution and abundance of ionizable sites in the three *Spirulina* materials studied determined from treatment of acid-base potentiometric titration data by a discrete site distribution model. The results are an average of three experiments performed in ionic medium of 0.020 mol L^{-1} KNO_3 at 25.0±0.1 °C.

data using a nonlinear multi-parametric fitting described in previous papers.[13, 14] The two classes of ionizable sites with pKa < 7 can be attributed to carboxylic groups, corresponding to almost 50% of the total of ionizable sites. Carboxylic groups are bound to polysaccharides and hemicellulose, which are among the major components of the cell walls. In the case of the washing waters, the classes with pKa < 6.5 may be assigned to carboxylic acids, amino-acids and proteins, as well as polysaccharides that are adhered to the surface, or liberated from the cell interior.

In the total suspension and washing water, a site was characterized with pKa in the range between 6.4 and 7.5. This species may be attributed to phosphate since this anion was determined at concentration of (2.4±0.3) x 10^{-4} mol L^{-1} in the washing water. It is also known that phospholipids are among the constituents of the cell walls of microalgae.[11] Additionally, Cyanobacteria accumulates highly polymerized polyphosphate granules that serve as reserve of phosphate for the cell. When the cell is dried, these polyphosphate granules remains, but the cellular membrane is too sensitive to resist. When the lyophilized *Spirulina* is suspended in water at pH 6.0 these polyphosphate granules dissolve, explaining the high concentration of phosphate found in the washing water. It is interesting to notice that ionizable groups with pKa between 6.5 and 7.9 were not observed in the washed cells (Figure 1), giving support to the hypothesis that these ionizable species are due to inorganic soluble phosphate.

The classes of sites with pKa > 8 may be assigned to amine or phenolic groups in all materials. The method used for the acid-base characterization has no capability to distinguish between the contributions of these species due to the proximity of their pKa values.[13, 14]

From the results of the acid-base characterization, it is possible to compute the concentration of de-protonated sites at pH 6, which resulted 1.22, 1.15, and 0.61 mmol g^{-1} for the total alga suspension, the washed cells, and the washing waters, respectively. The heterogeneity and concentrations of ionizable sites found in this work for *Spirulina* are of the same magnitude reported for other species of algae.[13, 17-20]

Equilibrium time with Cu(II)

Experiments performed with 1.0 g L^{-1} suspensions of *Spirulina*, using initial Cu(II) concentrations of 0.1 and 1.2 mmol L^{-1}, led to similar results (Fig 2), with the adsorption equilibrium reached after 15 minutes. The steep profile of the curve at the lower times indicates that the adsorption is fast, implying the suitability of this material to treat dilute heavy metal solutions, as observed for other biosorbents.[5, 21] No evidences of de-sorption of Cu(II) in these suspensions were observed in a period of 48 h. Also, after this period of 48 h, no significant increase of Cu(II) adsorption was observed. To assure reproducible experimental conditions for the adsorption experiments, an equilibrium time of 40 min was adopted before to perform the measurements.

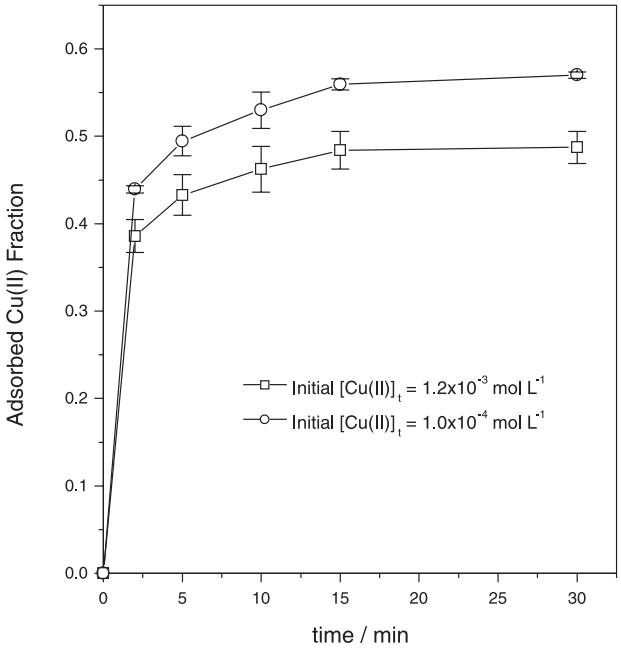

Figure 2. Adsorption of Cu(II) in a 1.0 g L^{-1} total suspension of *Sprirulina* determined by flame atomic absorption spectrophotometry. Results are the average of three experiments performed in ionic medium of 0.020 mol L^{-1} KNO_3 at 25.0±0.1 °C and pH 6.0±0.1.

Adsorption isotherms

The adsorption isotherms for Cu(II) and the total alga suspension, the washing water and the washed cells at pH 6 and ionic medium of 0.02 mol L^{-1} KNO_3 are shown in Figure 3. The isotherms of the total suspension are rather steep at the lower concentrations of Cu(II), suggesting the suitability of this material to treat dilute solutions. On the other hand, the adsorption isotherm for the washing water was similar to that obtained for the total suspension (first points of isotherm shown in the insert of Figure 3), implying that although the cells retain significant amounts of Cu(II), the washing water components maintain part of the Cu(II) in solution as a complex. This is an inconvenient since the biosorbent should be immobilized to avoid the transport of Cu(II), or other heavy metal cations, to outside the treatment site.[5]

The slope of the adsorption isotherm for the washed cells was similar to the other materials for the first three

points of the isotherms (total Cu(II) concentration between 2.0 and 6.0 μmol L^{-1}) as shown in the insert of Figure 3. However, for higher loadings of Cu(II) the slope of the adsorption isotherm are significantly smaller than for the whole suspension or the washing water, denoting a narrower range of concentration for which the washed cells are suitable to remove Cu(II).

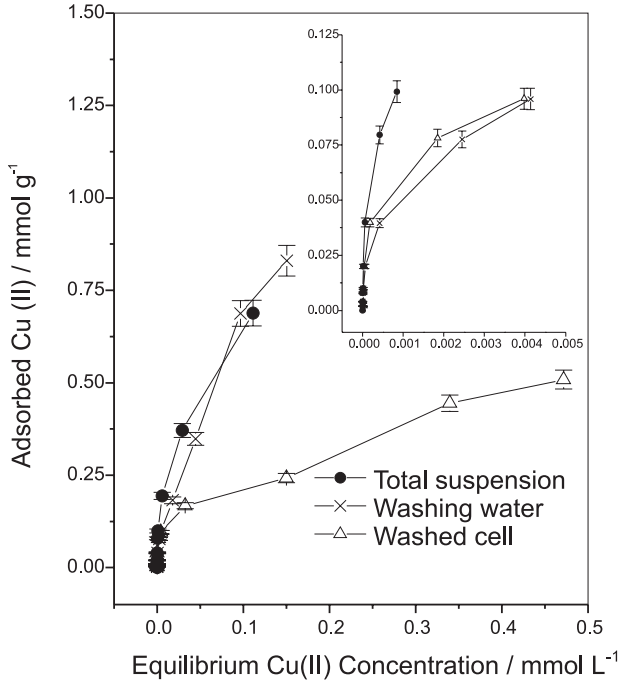

Figure 3. Adsorption isotherms of Cu(II) with the *Spirulina* materials determined by potentiometric titration monitored with Cu(II) ion-selective electrode for total Cu(II) concentrations between 2.0 x 10^{-6} and 1.0 x 10^{-3} mol L^{-1}. The insert shows a zoom for total Cu(II) concentrations between 2.0 x 10^{-6} and 4.0 x 10^{-5} mol L^{-1}. The titrations were performed in triplicate in ionic medium of 0.020 mol L^{-1} KNO_3 at 25.0±0.1 °C and pH 6.0±0.1.

Scatchard plots

Table 1 presents the values of the conditional stabilities (log K') and the complexing capacity (total concentration of binding sites, $[L]_t$) determined by the linear Scatchard plots (Figure 4A). Two classes of binding sites were determined for the three materials studied. The sites forming stronger complexes are represented as j = 1 in Table 1. These sites are probably composed of phosphate and/or amine groups. The complexing sites represented by j = 2 form the less stable complexes, with probable binding to carboxylic groups, the major component of ionizable sites on algae surfaces.

The log K' values for the most stable complexes formed with the three materials studied ranged from 7.3 to 7.9.

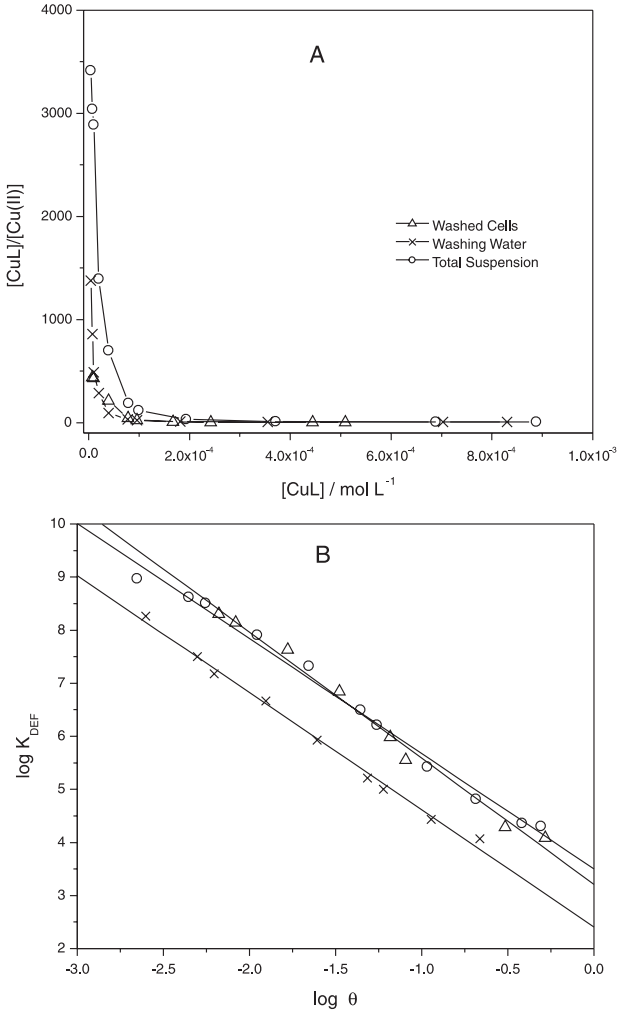

Figure 4. Scatchard (A) and *DEF* (B) plots describing the binding of Cu(II) to the *Spirulina* materials. The experimental conditions are described in the caption of Figure 3.

Table 1. Scatchard results for complexation between Cu(II) and *Spirulina* materials in ionic medium of 0.020 mol L^{-1} KNO_3, pH 6.0±0.1 and 25.0±0.1 °C. Results correspond to average of three experiments

Material	j=1		j=2		$\sum_{j=1}^{2} [L]_t$
	log K'	$[L]_t$ (mmol g^{-1})	log K'	$[L]_t$ (mmol g^{-1})	(mmol g^{-1})
Total suspension	7.9 ± 0.2	0.039 ± 0.004	3.9 ± 0.2	1.56 ± 0.10	1.6 ± 0.1
Washing water	7.6 ± 0.3	0.022 ± 0.002	3.6 ± 0.1	1.48 ± 0.10	1.5 ± 0.1
Washed Cells	7.3 ± 0.2	0.057 ± 0.009	3.5 ± 0.3	0.86 ± 0.09	0.92 ± 0.08

The one-way ANOVA statistical test shows the variations in the log K' values may be explained by random experimental errors, rather than by a systematic fixed effect factor, that is, the differences observed are not statistically significant.[27] The same observation is valid for the less stable class of binding sites, for which the log K' values ranged between 3.5 and 3.9 (Table 1). The complexing capacity of the stronger class of binding sites corresponds to only 2.4% of the total capacity (Table 1). For the washing water and the washed cells, the ratio between the strongest binding sites and their total concentration was approximately the same as observed for the total cell suspension. The complexation capacities of the total suspension and the washing water were of the same magnitude (Table 1), while for the washed cells this capacity decreased approximately 41 and 45% in comparison to the capacities of the washing water and total suspension, respectively. This suggests that in the total suspension, part of the material that is washed from the cells, composing the complexing material of the washing water, is not available for complexation. It seems that part of this material is weakly associated to the cell surface, explaining the fact that the complexing capacity of the total suspension is smaller than the sum of the complexing capacities of the washed cells and washing water. The total complexing capacity and log K' values found in this work are consistent with data in the literature reported for other biosorbents.[20-24]

The difference between the log K' for the stronger and weaker binding sites was larger than 4 units, causing a marked break in the Scatchard plots (Figure 4A). This facilitates the differentiation of the binding parameters between the two classes of binding sites, leading to unambiguous values of log K' and $[L]_t$, even with the graphical approach used in this work. On the other hand, since the Scatchard plot is a model that translates the actual continuous distribution of complexing sites to discrete sites, the log K' values (Table 1) should be thought as average equilibrium values.

The parameters log K' and $[L]_t$ can be easily introduced in general-purpose speciation programs such as MINEQL+[25,26] to compute $[Cu(II)]$, $[CuL_1]$ and $[CuL_2]$ in function of the total concentrations of Cu(II) and L in the medium. Distribution of Cu species computed using the MINEQL+ software are shown as the log C vs. log $[Cu(II)]_t$ plots (Figure 5), where the experimental data were also plotted for comparison purposes.

The differential equilibrium functions

The log θ range studied involved more than two orders of magnitude, varying from –2.8 to –0.25, for which the log K_{DEF} values decreased from 9.3 to 4.0 (Figure 4B). This decrease of log K_{DEF} with the increase of the log θ may be explained by the fact that the sites with stronger affinity by Cu(II) are occupied first, resulting higher log K_{DEF} values. Table 2 presents the values of K^0_{DEF} for the three materials studied, as well as the heterogeneity parameters, which were between 0.4 and 0.5, denoting an elevated degree of heterogeneity[8-11,16] for both, the cell surfaces and the soluble complexants. As mentioned before, this heterogeneity is due to the presence of amine, phenolic, phosphate and carboxylic groups in the studied materials. The literature data on binding of metals to algal surfaces using differential equilibrium functions is very limited. Usually, these studies report results for natural organic matter in waters (humic substances), and using anodic stripping voltammetry (ASV) for measurement of the labile fraction of the metal cation.[8-10,16] Rollemberg *et al.*[11] reported the thermodynamics of uptake of cadmium by *Chlorella marina* using the DEF approach and the ASV technique. Despite the differences of experimental conditions and measurement techniques, the log K_{DEF} and Γ values obtained for the *Spirulina* are of the same order of magnitude reported in the literature[8-11,16] for other materials. This suggests that these values are governed by the nature of the metal cation and by the chemical nature of the binding sites, which have some similarity between algal materials and humic substances.

From the differential equilibrium functions, the log K values can be computed for each log θ value, that is, for each $[Cu(II)]_t$ and $[L]_t$ at the pH studied. The free concentrations of Cu(II) and CuL were computed by the MINEQL+ software (Figure 5).

Modeling evaluation

From Figure 5, it is clear that the log K' and $[L]_t$ values

Table 2. Results of differential equilibrium functions as a function of log θ for the three materials studied

Materials	log θ range	log K_{DEF} range	log $K_{DEF}{}^0$	Γ
Total suspension	-2.5 to –0.25	9.3 to 4.3	3.26 ± 0.06	0.44 ± 0.01
Washing water	-2.5 to –0.25	8.2 to 4.1	2.38 ± 0.02	0.49 ± 0.03
Washed cells	-2.2 to –0.5	8.3 to 4.1	3.07 ± 0.06	0.42 ± 0.01

determined from Scatchard plots were more appropriate than the *DEF* functions to represent the experimental data at low total concentrations of Cu(II), a situation in which the [*Cu(II)*] computed from the *DEF* functions were significantly underestimated in relation to the experimental data.

At the intermediate range of the titration curve, the simulated Cu(II) concentrations computed from Scatchard parameters presented an inflection that was not followed by the experimental data (Figures 5A and 5B) of the total suspension and the washing water. The absence of such inflection in the experimental data suggests the existence of binding sites buffering the free Cu(II) concentration in the range of log $[Cu(II)]_t > -4.5$, which are not detected by the Scatchard model. It is interesting to notice that these complexing sites occur probably in the soluble fraction of the studied material, since both, simulated and experimental data for the washed cells presented inflection in the log *C* vs log$[Cu(II)]_t$ plot in the range of log$[Cu(II)]_t$ between –3.5 and – 4.5 (Figure 5C).

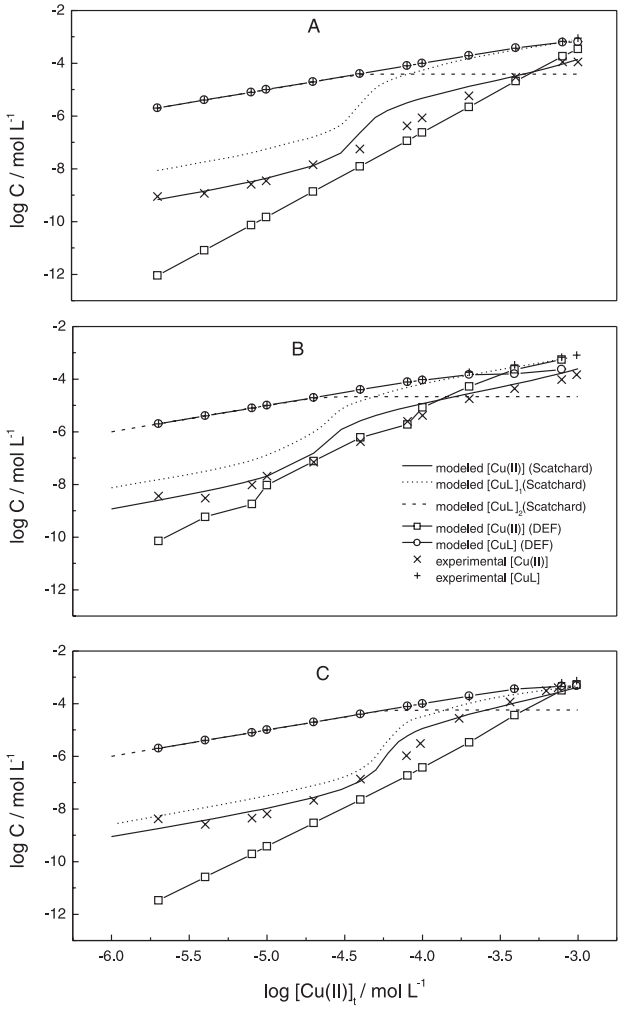

Figure 5. Modeling of Cu(II) species in the titration medium of the total suspension of *Spirulina* (A), the washing water (B), and the washed cells (C). Experimental conditions are described in caption of Figure 3.

The *DEF* approach failed to predict the free Cu(II) concentration at low loadings of Cu(II) for all materials studied. For the washing water and the total suspension, the predicted [Cu(II)] switched toward the experimental value with the increase of the total Cu(II) (Figures 5A and 5B) concentration. In the case of the washed cells, the predicted [*Cu(II)*] showed a similar trend but not the inflection in the range of log $[Cu(II)]_t$ between – 4.5 and –3.5. An explanation for the failure of *DEF* to predict the free Cu(II) concentrations is not clear at this time, but it can be due to inappropriate weight attributed to total concentration of ligand in the computation of simulated [*Cu(II)*]. One of the main advantages of the *DEF* reported in the literature[6, 8-10] is that it is not necessary to know the ligand concentration to compute the log K_{DEF} values since this concentration was constant during the titration. Computation of Γ and log θ can be made using the concentration of alga expressed as g L^{-1} or mol L^{-1}, but the concentration of binding sites (in mol L^{-1}) has to be known for appropriate modeling. In the present work the use of the total concentration of ionizable sites determined by acid-base characterization, or the total concentration of deprotonated ionizable sites at pH 6.0, led to underestimated values of log [*Cu(II)*] at low log$[Cu(II)]_t$.

Conclusions

This study demonstrated that the lyophilized mixed species of *Spirulina* display strong heterogeneity of surface ionizable sites, with strong affinity for Cu(II) at low loadings of the cation. The adsorption isotherms and the equilibrium time plots were quite steep under low loadings of Cu(II), suggesting the adequacy of this material to treat dilute aqueous wastes containing Cu(II).

The modeling study demonstrated that the log K' and $[L]_t$ determined by the Scatchard plots, although being averaged values, were appropriate to predict the free Cu(II) at low and high loadings of the cation. At the intermediate range of the titration, these parameters failed to model the system, suggesting that additional binding sites, should be included to properly model the titration curve in the whole range of log$[Cu(II)]_t$ studied, indicating that more complex data treatment like multi-site Langmuir isotherms should be used.[25] The *DEF* approach, although attributing a log K' value for each log θ, failed to model the titration curve with the concentrations of binding sites determined by acid-base characterization.

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