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Roberto Herrero, Pablo Lodeiro, Lino J. García-Casal, Teresa Vilariño, Carlos Rey-Castro, Calin David, Pilar Rodríguez

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1 **Full description of copper uptake by algal biomass combining an equilibrium**

2 **NICA model with a kinetic intraparticle diffusion driving force approach**

3 Roberto Herrero<sup>1</sup>, Pablo Lodeiro<sup>1\*</sup>, Lino J. García-Casal<sup>1</sup>, Teresa Vilariño<sup>1</sup>, Carlos Rey-

4 Castro<sup>2</sup>, Calin David<sup>2</sup> and Pilar Rodríguez<sup>1</sup>

5 *<sup>1</sup>Departamento de Química Física e Enxeñería Química I, Universidade da Coruña,*

6 *Alejandro de la Sota 1, 15008 A Coruña, Spain.*

7 *<sup>2</sup> Departament de Química, Universitat de Lleida, Rovira Roure 191, E-25198, Lleida,*

8 *Spain.*

9 \*Corresponding author e-mail address: [plodeiro@udc.es](mailto:plodeiro@udc.es); Tel.: (+34) 981 167000 (ext.

10 2199); Fax: (+34) 981 167065

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26 **Abstract**

27 In this work kinetic and equilibrium studies related to copper binding to the protonated  
28 macroalga *Sargassum muticum* are reported.. An intraparticle-diffusion linear driving  
29 force (LDF) model has been chosen for the quantitative description of the kinetics at  
30 several initial metal concentrations. Copper intraparticle homogeneous diffusion  
31 coefficient ( $D_h$ ) obtained is in the range  $0.2-0.9 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$ . NICA isotherm is  
32 demonstrated to constitute a substantial improvement with respect to a simpler  
33 Langmuir competitive equation. The binding parameters were chosen to provide the  
34 best simultaneous description of the equilibrium experiments. Values of  $\log \tilde{K}_{Cu}$  (4.3),  
35  $n_{Cu}$  (1) and  $p$  (0.31) in NICA isotherm, and  $\log K_{Cu}$  (3.5-5) in Langmuir competitive  
36 model, have been obtained. These parameters have been also used to predict the  
37 competition between copper and cadmium for binding sites. Two acids,  $\text{HNO}_3$  and  $\text{HCl}$ ,  
38 have been tested to evaluate their effectiveness to release copper from the metal-laden  
39 biomass.

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41 **Keywords:** copper; *Sargassum muticum*; kinetics; LDF; equilibrium; NICA.

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52 **1. Introduction**

53 The incidences of different pollution sources, that are harmful to the environment, have  
54 been gradually increased over time due to rapid urbanization and industrialization.  
55 Industrial activities have been recognized as a major contributor to a variety of water  
56 pollution problems. Moreover, their rapid growth has produced an increase in the  
57 volume and toxicity of residues, among which, liquid effluents containing metals are of  
58 special interest.

59 Metals have a high degree of toxicity, which can be harmful for human beings and the  
60 environment. According to EPA (Environmental Protection Agency), copper is an  
61 abundant trace element present in earth's crust and surface waters. Copper is not  
62 regarded as highly toxic, and only at elevated concentrations may become hazardous to  
63 some forms of aquatic life. However, treatment of wastewaters containing these metals  
64 is of importance both for environmental quality and for water reuse. The effluents from  
65 mining, leather, fabricated metal products, and electric equipment represent the most  
66 important sources of copper pollution. The European Pollutant Emission Register  
67 (EPER), which comprises 50 substances that have to be reported by industrial facilities  
68 if their emissions exceed certain threshold values, establishes for copper that the limits  
69 for air, water and land are, respectively, 0.1, 0.05 and 0.05 t. per year (Parliament and  
70 Council, 2006). As an example, these values are ten times higher than those for  
71 cadmium discharges.

72 Although various conventional methods, as ion-exchange, liquid extraction,  
73 precipitation, electrodialysis, etc., could be applied to treat wastewaters, most of these  
74 available physicochemical technologies are expensive or ineffective when they are  
75 applied to metal ions removal at low concentrations. Alternative low-cost technologies

76 are needed to reduce heavy metal concentrations in the environment to acceptable levels  
77 (Srivastava and Majumder, 2008).

78 Biosorption, the passive non-metabolically mediated process of metal binding by dead  
79 biomass, has a great potential to reach these objectives (Lodeiro et al., 2006; Volesky,  
80 2003). Among the advantages offered by this technique are the high purity achieved by  
81 treated wastewaters or the use of inexpensive materials as biosorbents. Waste products  
82 from other industries or natural abundant biomass can be quoted as an example  
83 (Demirbas, 2008). The brown macroalga *Sargassum muticum*, an invasive species in  
84 Europe, has been the biosorbent employed in this work. Its native habitats are Japanese  
85 and Chinese waters, where its presence is much smaller than in the European coasts.  
86 This alga is an alien species that interferes with recreational use of waterways, blocking  
87 propellers and intakes. It is also a fouling organism in oyster beds and a nuisance to  
88 commercial fishermen.

89 The algal cell wall plays an important role in metal binding, due to its high content in  
90 polysaccharides with acid functional groups. In brown algae, the cell wall is mainly  
91 comprised of alginates, which usually constitute about 20-40% of the total dry weight,  
92 in addition to fucoidans (Davis et al., 2003). The carboxyl groups of alginates are likely  
93 to be the main functionalities involved in metal binding reactions because of their  
94 abundance with regard to both carboxyl and amine groups of the proteins.

95 The present work reports a study of Cu adsorption by non-living biomass of the brown  
96 marine macroalgae *Sargassum muticum*. The alga was previously protonated in order to  
97 increase the retention capacity of the raw biomass, and to achieve a further stabilization  
98 of the biomaterial. The process has been analysed through batch experiments with  
99 regard to the influence of initial metal concentration, pH and the presence of cadmium  
100 as competing cation. Both kinetic and equilibrium aspects have been discussed.

101 Desorption process has also been tested. Mathematical models for the quantitative  
102 description of the biosorption process have been employed in order to predict the  
103 dynamics and equilibrium behaviour.

104

## 105 **2. Experimental Methods**

### 106 **Materials**

107 Fresh samples of brown marine alga *Sargassum muticum* were collected from the coast  
108 of A Coruña (NW Spain). The samples were washed extensively with running and  
109 deionised water to removed adhering particles, and oven-dried at 60 °C overnight.

110 Dried samples were then crushed with an analytical mill (IKA A 10) and sieved to a size  
111 range of 0.5-1 mm. After being sieved, the following pre-treatment was performed  
112 (Figueira et al., 2000). The biomass was protonated in 0.1 M HNO<sub>3</sub> (10 g of biomass/L)  
113 for 4 hours at room temperature, washed with deionised water, filtered and dried  
114 overnight at 60 °C. This biomass was stored in polyethylene bottles until use.

### 115 **Chemicals**

116 Analytical grade Cu(NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O, NaNO<sub>3</sub>, HNO<sub>3</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and HCl (Merck),  
117 NaOH (Panreac) were used in this work. Cellulose nitrate membrane filters were  
118 purchased from Whatman and Albet; N<sub>2</sub> C-55 (99.9995 %) was from Carburos  
119 Metálicos.

### 120 **2.1 Methods**

#### 121 **2.1.1 Kinetic studies**

122 A copper ion selective electrode (ISECu, Radiometer Analytica) with a Ag|AgCl  
123 reference electrode, previously calibrated in copper concentration, was employed to  
124 analyse the copper evolution during the kinetic experiments. The experiments were  
125 monitored potentiometrically using a homemade program. This technique allows a great

126 number of experimental points to be obtained easily and quickly without need to  
127 withdrawn solution for the measurements.

128 Experiments were carried out in a glass cell furnished with a thermostated jacket and a  
129 nitrogen stream to remove dissolved O<sub>2</sub> and CO<sub>2</sub>. The ionic strength was adjusted to  
130 0.05 M with NaNO<sub>3</sub>. All measurements were performed at least in duplicate.

131 *Effect of initial metal concentration.* Protonated *S. muticum* samples (0.25 g) were  
132 added to 100 mL of Cu(II) solution at constant temperature ( $25.0 \pm 0.1^\circ\text{C}$ ) and natural  
133 pH (around 3). The initial concentration of tested copper solutions were 0.25, 0.50, 1.00  
134 and 3.00 mmol·L<sup>-1</sup>.

### 135 **2.1.2 Equilibrium studies**

136 All batch equilibrium studies were carried out in 100 mL Erlenmeyer flasks containing  
137 0.1 g of the alga to which 40 mL of Cu(II) solutions were added. The mixtures were  
138 agitated on a rotator shaker at 150 rpm for 3 hours, in order to ensure that equilibrium  
139 was reached. The solution pH was adjusted by using HNO<sub>3</sub> or NaOH during the  
140 equilibrium period; from these additions and the quantity of Cu(NO<sub>3</sub>)<sub>2</sub> in solution, the  
141 ionic strength was calculated for each sample. The experiments were performed at room  
142 temperature. The algal biomass was removed by filtration through a 0.45 μm membrane  
143 filter. In all equilibrium experiments the presence of organic matter was avoided by UV-  
144 digestion of the aliquots for 75 minutes at 90°C (705 UV Digester, Metrohm);  
145 afterwards aliquots were analyzed for Cu(II) released into solution by differential pulse  
146 anodic stripping voltammetry (DPASV) using a 757 VA Computrace (Metrohm). All  
147 batch experiments were carried out at least in duplicate.

148 *Influence of pH on metal biosorption.* The effect of solution pH was studied by using  
149 eight Cu(II) solutions (40 mL, 2.22 mmol·L<sup>-1</sup>) added to flasks containing 0.1 g of dry

150 biomass. The solution pH was adjusted to 1.5, 2.5, 3, 3.5, 4.0, 4.5, 5.0 and 5.5 by  
151 addition of HNO<sub>3</sub> or NaOH solutions.

152 *Adsorption isotherms.* 40 mL of nine Cu(II) solutions of several concentrations (0.1,  
153 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.5 and 5.0 mmol·L<sup>-1</sup>) were placed in Erlenmeyer flasks  
154 containing 0.1 g of alga. The solution pH was adjusted to 4.0 by addition of NaOH  
155 solution.

156 *Effect of Cd(II) ions on Cu(II) biosorption.* The competition effect of cadmium ion was  
157 tested through batch sorption experiments carried out with 0.1 g of protonated *S.*  
158 *muticum* in contact with binary mixtures (40 mL) of several copper ion concentrations  
159 (the same used in adsorption isotherms) and the competitor metal ion at two different  
160 initial concentrations (0.1 and 5.0 mmol·L<sup>-1</sup>). The solution pH was adjusted to 4.0 by  
161 addition of NaOH solution.

162 *Desorption experiments.* For batch desorption experiments, copper-loaded *S. muticum*  
163 samples (obtained from previous adsorption process with 0.1 g of biomass, at a fixed  
164 pH of 4 and initial copper concentration of 2.5 mmol·L<sup>-1</sup>) were placed in 100 mL  
165 Erlenmeyer flasks and contacted with 10 mL of two different desorbents (HCl and  
166 HNO<sub>3</sub>) at three different concentrations (0.05, 0.1 and 0.5 mol·L<sup>-1</sup>). The mixtures were  
167 agitated on a rotary shaker for 2 hours at room temperature.

168 From these experiments, the optimal eluent concentration was selected (0.05 mol·L<sup>-1</sup>)  
169 and then, the effect of contact time was studied. In this case, the volume of HCl or  
170 HNO<sub>3</sub> solutions was increased to 20 mL, in order to ensure better contact between algae  
171 and solution and to facilitate the samples extraction. Aliquots of 100 µL of solution  
172 were analyzed after 2 and 4 hours of contact.

173

174 **3. Results and discussion**



175 **3.1 Kinetic studies**

176 *3.1.1 Mathematical model*

177 Kinetic experiments are the first necessary stage in every biosorption study. They are  
178 needed to determine the time required for sorption equilibrium to be reached and,  
179 moreover, the resulting data may be used to extract kinetic parameters for the modelling  
180 of column biosorption experiments. Several independent processes, including transport  
181 phenomena and chemical reaction kinetics, which usually act in conjunction, determine  
182 the dynamics of metal biosorption. In the case of porous sorbents, the following steps  
183 may be present (Garcia-Reyes and Rangel-Mendez, 2010; Volesky, 2003): transport of  
184 sorbate within the bulk solution (advection and diffusion), transfer of sorbate from bulk  
185 solution to the sorbent surface through the boundary layer of fluid immediately adjacent  
186 to the particle (external film diffusion), diffusion of sorbate within the particle  
187 (intraparticle diffusion), and chemical reaction of the sorbate with the binding sites of  
188 the biomass.

189 The experimental conditions and setup are very often chosen so that mass transfer  
190 resistance, due to metal transport in the bulk solution, and film diffusion through the  
191 boundary layer of biosorbent are minimized. In particular, an adequate mixing, created  
192 by proper agitation, allows a fast transport of metal in bulk solution, and, at the same  
193 time, it can contribute to suppress the boundary layer surrounding the particles. In these  
194 cases, intraparticle diffusion and/or chemical reaction may be the rate-limiting steps in  
195 the sorption kinetics. However, in biosorption of metal ions from aqueous solution it is  
196 common to assume that the overall rate of uptake is controlled mainly by the diffusivity  
197 of the sorbate within the particle, whereas the chemical reaction of binding with the  
198 sorbent sites is assumed to be relatively faster.

199 In the present work an intraparticle-diffusion linear driving force model (LDF) was  
 200 chosen for the quantitative description of the copper uptake kinetics. Further details  
 201 about this model can be found in bibliography (Tien, 1994; Vilar et al., 2006). In  
 202 summary, this model is based on the following assumptions:

203 -The rate of metal uptake is controlled by the homogeneous diffusion of the sorbed  
 204 species within the biomass particles. The external film diffusion resistance is assumed  
 205 negligible in the actual conditions selected for the experiments (agitation rate: 180 rpm,  
 206 biomass particle size: 0.5-1 mm). Similar conditions were already tested in bibliography  
 207 for *Sargassum* biomass (Yang and Volesky, 1999) and they were proved to ensure the  
 208 exclusion of these kinetic limitations.

209 -The particles are modelled as homogeneous thin plates of thickness  $2L$ . Therefore, the  
 210 uni-dimensional diffusion of the sorbed metal ion along the direction normal to the  
 211 particle surface determines the overall diffusion rate (Volesky, 2003).

212 -The equilibrium concentration of sorbed copper at the particle interface is described by  
 213 the Langmuir isotherm (see section 3.2.1 below).

214 Based on the model assumptions, the mass balance equations for copper in the batch  
 215 reactor are as follows:

$$V \cdot \frac{dC_{Cu,t}}{dt} + m_s \cdot \frac{dq_{Cu,t}}{dt} = 0 \quad (1)$$

$$\frac{dq_{Cu,t}}{dt} = \frac{D_h}{L} \left( \frac{dq_{Cu,t,z}}{dz} \right)_{z=L} \quad (2)$$

216 where  $q_{Cu,t}$  represents the average metal concentration in the particle,  $C_{Cu,t}$  the metal  
 217 concentration in solution at any time  $t$ , and  $D_h$  is the intraparticle homogeneous  
 218 diffusion coefficient of the sorbed species within the algae.

219 From the assumption that the sorbed copper concentration profile,  $q_{Cu,t,z}$ , is parabolic,  
 220 the following expression (known as *linear driving force* approximation) can be derived

221 for the variation of the copper uptake with time (Tien, 1994; Vilar et al., 2006):

$$\frac{dy}{dt} = k \cdot (y_{eq} - y) \quad (3)$$

222 where  $y = \frac{q_{Cu,t}}{Q_{max,Cu}}$ ,  $y_{eq} = \frac{Q_{Cu}}{Q_{max,Cu}}$  and  $k$  is a rate constant;  $Q_{max,Cu}$  is the maximum copper

223 sorption capacity and  $Q_{Cu}$  is the equilibrium sorption capacity of the alga.

224 From the mass balance (Eq. 1) at every instant we get:

$$V \cdot C_{Cu,t} + m_s \cdot q_{Cu,t} = V \cdot C_{Cu,i} \quad (4)$$

225 Reordering this equation and introducing the definition of  $x = \frac{C_{Cu,t}}{C_{Cu,i}}$ , where,  $C_{Cu,i}$  is the

226 initial copper concentration in solution, and  $n_0 = \frac{V \cdot C_{Cu,i}}{m_s \cdot Q_{max,Cu}}$  (the ratio between the

227 initial total amount of copper and the maximum quantity that can be sorbed in a mass,

228  $m_s$ , of sorbent), the following expression is obtained:

$$y = n_0 \cdot (1 - x) \quad (5)$$

229 Substituting this equation in eq. 3 and using Langmuir isotherm, we obtain the

230 following expression:

$$\frac{dy}{dt} = k \cdot \left[ 1 - x - \frac{b \cdot C_{Cu,i} \cdot x}{n_0 \cdot (1 + b \cdot C_{Cu,i} \cdot x)} \right] \quad (6)$$

231 where  $b$  is the Langmuir affinity constant (see Eq. 7). The initial conditions are:  $t=0$ ,

232  $x=1$ ,  $y=0$ . This ordinary differential equation was solved numerically using *ode45*

233 routine from Matlab (MATLAB® v.2008b, The Mathworks Inc.), which is based on the

234 Runge-Kutta algorithm.

235 Experimental kinetic data were fitted to this model by a least-squares minimization

236 procedure using *fminsearch* routine from Matlab. Only one parameter ( $k$ ) was adjusted

237 to fit the four experimental data series simultaneously. The  $Q_{max,Cu}$  ( $0.32 \pm 0.02$  mmolg<sup>-1</sup>)

238 and  $b$  ( $0.9 \pm 0.1 \text{ L}\cdot\text{mmol}^{-1}$ ) parameters were taken from fitting results of equilibrium  
239 isotherm data obtained at pH 3 using Langmuir model ( $r^2= 0.994$ ).

240

### 241 3.1.2 Copper kinetic sorption rate description

242 Experimental data show that the equilibrium is attained within 1 hour, and no further  
243 significant adsorption is noted beyond this period (Figure 1). In addition, it can be  
244 observed that 50% of the total copper uptake occurred within 10 minutes, showing that  
245 the rate of copper uptake is rather fast. It can also be observed that the percentage of  
246 copper removed from solution diminishes as the initial copper concentration increases  
247 (Table 1). The evolution of solution pH was also followed. As it is shown in Figure 2,  
248 the pH was around 3 in all the kinetic experiments, and only a slight increase was  
249 observed with time.

250 Figure 1 shows the copper kinetic data at four different initial metal concentrations and  
251 their adjustment using the linear driving force model, as described above. As it is  
252 denoted, this simple model could describe kinetic data accurately. The calculated  
253 determination coefficients ( $r^2$ ), used as an indication of model goodness, are showed in  
254 Table 1.

255 The rate constant,  $k$ , regressed from the LDF model was  $0.0642 \text{ min}^{-1}$ . The validity of  
256 this model is also demonstrated by the fact that the same rate constant is valid to  
257 describe the experimental points for different initial metal concentrations. The rate  
258 constant can be related with the intraparticle homogeneous diffusion coefficient,  $D_h$ , as:  
259  $k= 3D_h/L^2$ . Since  $L$  (half of the particle thickness) has an estimated average value  
260 between 0.25 and 0.5 mm, then  $D_h$  must be in the range  $0.2\text{-}0.9 \times 10^{-10} \text{ m}^2\cdot\text{s}^{-1}$ . This  
261 parameter is independent of concentration and, as expected, much lower than the  
262 molecular diffusion coefficient for Cu in water ( $7.2 \times 10^{-10} \text{ m}^2\cdot\text{s}^{-1}$ ) (Heyrovsky and Kuta,

263 1966). The fact that *S.muticum* seaweed material is a porous gel-like particle makes  $D_h$   
 264 for Cu to be about ten times lower than the corresponding diffusion coefficient in pure  
 265 water or aqueous salt solutions.

266

### 267 3.2 Equilibrium studies.

#### 268 3.2.1 Mathematical models

269 The development of a technology based on biosorption implies the use of adequate  
 270 models for the metal ion binding to biomaterials. These models can be employed to  
 271 analyse equilibrium data and to compare quantitatively different biosorbents under  
 272 several conditions. Ideally, they would constitute a useful tool to predict the metal  
 273 biosorption, to deduce the binding mechanism and to determine the influence on  
 274 biosorption of variables such as pH, ionic strength or the presence of competing species  
 275 (Lodeiro et al., 2006).

276 The most common isotherms used in sorption studies, Langmuir-Freundlich (Eq. 7) and  
 277 Langmuir (Eq. 7 with  $n'=1$ ), are able to accurately reproduce equilibrium experimental  
 278 data if environmental parameters, such as pH, are controlled carefully during  
 279 experiments (Carro et al., 2009; Lodeiro et al., 2004).

$$Q_{Cu} = \frac{Q_{\max,Cu} (b C_{Cu})^{1/n'}}{1 + (b C_{Cu})^{1/n'}} \quad (7)$$

280 where  $b$  represents the affinity for the sorbate, which can be used to compare the  
 281 adsorption performance,  $n'$  is an empirical parameter that varies with the degree of  
 282 heterogeneity, and  $C_{Cu}$  is the copper concentration in solution at equilibrium.

283 In order to account for stoichiometry and pH effects, a modified competitive Langmuir  
 284 sorption model, Eq. 8, was proposed by Schiewer *et al.* (Schiewer and Wong, 1999).

285 The metal binding at equilibrium is described as a function of pH and free metal ion  
 286 concentration in solution.

$$Q_{Cu} = n Q_{\max,H} \frac{(K_{Cu} C_{Cu})^n}{1 + K_H C_H + (K_{Cu} C_{Cu})^n} \quad (8)$$

287 where  $K_{Cu}$  and  $K_H$  are the equilibrium constants for the binding of copper and protons,  
 288 respectively;  $C_H$  is the proton concentration in solution, the parameter  $n$  defines the  
 289 stoichiometry ratio, 1:1 ( $n=1$ ) or 1:2 ( $n=0.5$ ), and  $Q_{\max,H}$  is the maximum binding  
 290 capacity for protons, which has been calculated from the equivalence point of the acid-  
 291 base titrations in absence of heavy metal.

292 However, another aspects that characterize algal biomass, such as chemical  
 293 heterogeneity, polyelectrolytic effects and conformational changes (Buffle, 1988), are  
 294 not considered by these equations. As a consequence, new models with additional  
 295 parameters, that reflect the complexity of the system, would be required.

296 The NICA model, developed by Kinniburgh (Kinniburgh et al., 1999) for the  
 297 description of metal adsorption in heterogeneous materials, addresses to binding site  
 298 heterogeneity, interactions between ionic species and reaction stoichiometry. It is a  
 299 semi-empirical, competitive, non-ideal and thermodynamically consistent model, whose  
 300 application is fairly simple. This model, which is able to describe different types of  
 301 experiments (acid-base titrations, influence of pH on biosorption, metal sorption and  
 302 competition with other metals in solution), constitutes a powerful tool to describe  
 303 biosorption processes with both great accuracy and relatively small number of  
 304 parameters (see below) (Herrero et al., 2006; Lamelas et al., 2005; Pagnanelli et al.,  
 305 2005). However, despite the obtained encouraging results, the knowledge of the  
 306 geometric parameters that determine the electrostatic description of the system would be  
 307 required in order to derive the intrinsic binding parameters (i.e., independent of the bulk

308 ionic strength) (Li and Englezos, 2005; Rey-Castro et al., 2004; Rey-Castro et al.,  
309 2003).

310 The basic NICA equation for the overall binding of species  $i$  in the competitive situation  
311 is:

$$\theta_i = \frac{(\tilde{K}_i c_i)^{n_i}}{\sum_i (\tilde{K}_i c_i)^{n_i}} \times \frac{\left[ \sum_i (\tilde{K}_i c_i)^{n_i} \right]^p}{1 + \left[ \sum_i (\tilde{K}_i c_i)^{n_i} \right]^p} \quad (9)$$

312 where  $\theta_i$  is the coverage fraction of the species  $i$ ,  $\tilde{K}_i$  is the median value of the affinity  
313 distribution for species  $i$ ,  $p$  is the width of the distribution (usually interpreted as a  
314 generic or intrinsic heterogeneity seen by all ions),  $n_i$  is an ion-specific non-ideal term  
315 and  $c_i$  is the local concentration of species  $i$  at the binding site.

316 The following normalization condition is used to calculate the amount of species  $i$   
317 bound,  $Q_i$ :

$$Q_i = \theta_i \left( \frac{n_i}{n_H} \right) Q_{\max, H} \quad (10)$$

318 The ratio  $n_i/n_H$  has been interpreted by Kinniburgh *et al.* (Kinniburgh et al., 1999) in  
319 terms of stoichiometry and cooperativity. When this ratio is less than one, then the  
320 maximum binding of species  $i$  is lower than the total amount of sites (defined as the  
321 amount of titratable protons), which would be a consequence of certain degree of  
322 multidentism. On the other hand, a value of  $n_i/n_H$  greater than one would reflect some  
323 degree of cooperativity. Finally, if  $n_i/n_H=1$ , it can be demonstrated that the maximum  
324 proton/metal exchange ratio is one.

325 If only the proton binding is considered (i.e., absence of competing ions), Equations 9  
326 and 10 simplify to the Langmuir-Freundlich (LF) isotherm:

$$Q_H = Q_{\max,H} \frac{(\tilde{K}_H c_H)^{m_H}}{1 + (\tilde{K}_H c_H)^{m_H}} \quad (11)$$

327 where this time the heterogeneity parameter  $m_H$  describes the combined effect of  $n_H$  and  
 328  $p$  ( $m_H = n_H \cdot p$ ). In the case of a homogeneous system (where all the binding sites behave  
 329 as independent, chemically equivalent sites)  $n_i$  and  $p$  are 1, and then the mono or  
 330 multicomponent Langmuir isotherms are obtained.

331 The development of a physico-chemical model to describe metal ion binding to seaweed  
 332 biomass needs a previous description of proton binding as a function of pH in 1:1  
 333 electrolytes. Rey-Castro et al. (Rey-Castro et al., 2003) reported proton binding data  
 334 from potentiometric titrations of biomass from different seaweed species (*Sargassum*,  
 335 *Cystoseira* and *Saccorhiza sp.*). Moreover, in a previous work, Lodeiro *et al.* (Lodeiro  
 336 et al., 2005) obtained the maximum amount of acid functional groups of the algae  
 337 *Sargassum muticum* ( $2.61 \text{ mmol}\cdot\text{g}^{-1}$ ), through acid-base titrations of protonated biomass  
 338 samples. The fit of the proton binding data to an isotherm model allowed the estimation  
 339 of an average acid constant  $K_H$ , referred to  $\text{NaNO}_3$   $0.05 \text{ mol}\cdot\text{L}^{-1}$ , with a value of  $10^{3.8}$ ,  
 340 and the heterogeneity parameter, 0.54, calculated from least-squares fit of the LF  
 341 isotherm. These results were used in this work for the interpretation of both proton and  
 342 metal binding in terms of competitive adsorption isotherms.

343

### 344 3.2.2 Copper uptake and the effect of pH

345 The copper uptake capacity of the biomass was tested using the empirical Langmuir-  
 346 Freundlich (Eq. 7) and Langmuir isotherms (Eq. 7 with  $n'=1$ ). As it is showed in Figure  
 347 3 (pH 4), both models describe with great accuracy the experimental points and  
 348 identical fitted parameters are obtained ( $Q_{\max,\text{Cu}} = 1.12 \pm 0.02 \text{ mmol}\cdot\text{g}^{-1}$  and  $\text{Log } b =$   
 349  $1.4 \pm 0.3$ ), since the heterogeneity parameter ( $n'$ ) has a value about the unity, which turns



350 Langmuir-Freundlich equation into Langmuir one. However, one must be aware that the  
351 validity of these models or their underlying assumptions are not proved.

352 The maximum uptake capacity obtained for copper is around  $1.1 \text{ mmol}\cdot\text{g}^{-1}$ , which  
353 corresponds to  $71 \text{ mg}\cdot\text{g}^{-1}$  (equivalent to 7% of the total dry weight of the alga). This  
354 value is comparable with maximum capacities obtained with other marine algae, such as  
355 *Fucus serratus* ( $1.60 \text{ mmol}\cdot\text{g}^{-1}$ ) (Ahmady-Asbchin et al., 2008), *Sargassum filipendula*  
356 ( $1.30 \text{ mmol}\cdot\text{g}^{-1}$ ) (Luna et al., 2007) or *Posidonea oceanica* ( $1.35 \text{ mmol}\cdot\text{g}^{-1}$ ) (Izquierdo et  
357 al., 2010). Moreover, there are a great variety of useful materials proposed for copper  
358 removal from solution with different maximum metal uptake capacities, such as: spent-  
359 grain ( $0.165 \text{ mmol}\cdot\text{g}^{-1}$ ) (Lu and Gibb, 2008), activated poplar sawdust ( $0.085\text{-}0.21$   
360  $\text{mmol}\cdot\text{g}^{-1}$ ) (Acar and Eren, 2006), *Trametes versicolor* ( $0.63 \text{ mmol}\cdot\text{g}^{-1}$ ) (Sahan et al.,  
361 2010), *Mansonina* wood sawdust ( $0.67 \text{ mmol}\cdot\text{g}^{-1}$ ) (Ofomaja et al., 2010) or chitosan ( $1.59$   
362  $\text{mmol}\cdot\text{g}^{-1}$ ) (Paulino et al., 2008).

363 The effect of pH on Cu(II) ion adsorption capacity of *S. muticum* was studied at  $2.22$   
364  $\text{mmol}\cdot\text{L}^{-1}$  of initial Cu(II) concentration. Heavy metal sorption studies have shown that  
365 pH is one of the most important parameters affecting the process (Schiewer and  
366 Volesky, 2000). Both algae structure and copper speciation are affected by solution pH.  
367 The structure of the alga can be damaged by extremely acidic pH, due to the acid ability  
368 to dissolve certain groups of polysaccharides found on the surface of the biomass. The  
369 pH can also change the state of the active binding sites of the algae, mainly the carboxyl  
370 groups of alginates. Moreover, the MINEQL+ speciation programme shows that  
371 insoluble CuO appears at pH values greater than 5.5, decreasing free Cu(II) ion  
372 concentration.

373 As seen from Figure 4, the copper uptake capacity is almost negligible at pH values less  
374 than 2.5, the metal adsorption increases sharply between pH values 2.5 and 4.0, whereas

375 a plateau is reached around pH 4.5. To explain this behaviour, it is important to consider  
376 both the metal speciation in solution and the ionic state of cell wall functional groups,  
377 mainly carboxyl groups, at various pH values (Davis et al., 2003). Since Cu(II) is  
378 present in its free ionic form ( $\text{Cu}^{2+}$ ) at pH values lower than 5, copper biosorption  
379 depends on the protonation or deprotonation state of the cell wall polymer functional  
380 groups, which have a  $pK$  value of 3.8 (Lodeiro et al., 2005).

381 At pH values less than 2.0, these functional groups are clearly associated with hydrogen  
382 ions, restricting the approach of Cu(II) cations; so low uptake capacities could be  
383 explained by the metal ion binding to strongly acidic groups that do not become  
384 protonated at these pH, like sulfonic groups from fucoidans, that are known to be  
385 present in *Sargassum* biomass (Davis et al., 2003). As the pH increases, more  
386 carboxylate groups would be exposed and the available negative charges would lead to  
387 a rise in the binding of Cu(II) ions. Above pH 4.5, the increase in the Cu(II) sorption is  
388 almost negligible and the uptake reaches a plateau. Similar behaviour has been reported  
389 for Cd(II) ion biosorption by protonated *Sargassum* (Lodeiro et al., 2004; Lodeiro et al.,  
390 2005).

391 The single component (proton) model was then extended to the general multi-  
392 component case (interpretation of competitive ion binding). The conditional proton  
393 binding parameters obtained in Eq. 11 ( $Q_{\max,H}$ ,  $\log \tilde{K}_H$  and  $m_H$ ) were assumed to apply  
394 also in the presence of copper. The values of  $Q_{\max,H}$ , and  $m_H$  were taken as fixed in all  
395 subsequent calculations using the NICA model.

396 The binding parameters for the copper ion were chosen to provide the best simultaneous  
397 description of the isotherm at constant pH (4) and the data of copper adsorption vs. pH.  
398 The values of  $\log \tilde{K}_{Cu}$ ,  $n_{Cu}$  and  $p$ , in the NICA isotherm, or  $\log K_{Cu}$ , in the Langmuir  
399 competitive models, were first optimized by least squares fit for each data set, and then

400 average values (see Table 2) were used to fit the experimental data according to the  
401 different models, as shown in Figures 5 and 6. In the NICA model, the separation of  $n_H$   
402 and  $p$  was made using the constraint  $m_H = n_H \cdot p$ .

403 The fits to NICA and Langmuir competitive models of the copper binding data at  
404 different pH values are shown in Figure 4. It can be observed that the NICA equation is  
405 able to reproduce experimental data satisfactory. On the other hand, Langmuir  
406 competitive 1:2 model describes with accuracy the “S-shaped” curve in Figure 4, but it  
407 does not reproduce the plateau reached at pH values higher than 4, whereas the  
408 Langmuir competitive 1:1 model is not able to reproduce the pH effect.

409 Experimental data from isotherm at pH 4 were also fitted using NICA and Langmuir  
410 competitive models (Figure 5). The obtained results demonstrate, like in the study for  
411 the adsorption of copper as a function of pH, that only NICA model can explain  
412 experimental data properly, employing the same constants attained through proton  
413 binding studies.

414

### 415 3.2.3 Cadmium competition on copper uptake

416 The values listed in Table 2, together with the obtained in a previous article for  
417 cadmium adsorption ( $\log \tilde{K}_{Cd} = 3.1$  and  $n_{Cd} = 1.8$ ) (Lodeiro et al., 2005) were also used to  
418 predict the competition between copper and cadmium ions for the biosorbent binding  
419 sites. The comparison between experimental results from copper isotherms (at two  
420 different initial cadmium concentration) and NICA model prediction is shown in Figure  
421 6. Note that there is good agreement between model and experimental data, using the  
422 same model parameters, estimated from the batch sorption experiments and acid-base  
423 titrations in the absence of metal.

424 One must be aware that the use of the initial concentration of the competitor metal  
425 (cadmium) does not reflect the sorption equilibrium; however, for modelling purposes  
426 only final equilibrium concentrations were considered. In general terms, it can be  
427 observed that as copper initial concentration is incremented, the effect of cadmium  
428 competition decreases. So that, little difference between the two isotherms is observed  
429 at the highest initial copper concentration. Moreover, the copper isotherm is practically  
430 identical to the respective one in absence of the competitive cation at any copper  
431 concentration for the lowest initial cadmium concentration (compare experimental  
432 points in Figure 5 and filled circle data in Figure 6). This result demonstrates a greater  
433 affinity of the active binding sites in the alga for copper ions than for cadmium ions.  
434 This fact is also supported by the median values of the affinity distribution for cadmium  
435 and copper determined with the NICA model,  $10^{3.1}$  (Lodeiro et al., 2005) and  $10^{4.3}$   
436 (Table 2), respectively. This average Cu binding affinity reported lies between the  
437 values of 5.0 and 3.6 for the formation constant of Cu-alginate complexes (assuming  
438 langmuirian complexation) reported by De Stefano et al. (De Stefano et al., 2010) at  
439 infinite dilution and 0.1 M ionic strength, respectively. It is also probe that the main  
440 responsible for the metal sorption in brown algae is alginate.

441

### 442 **3.3 Desorption studies**

443 In general, the application of biosorption as a useful alternative in wastewater treatment  
444 implies the sorbent regeneration, in order to recover the bounded metal and to reduce  
445 process costs.

446 Desorption studies require a great amount of experimental work to determine both the  
447 ideal desorbent and its best conditions of use. In batch studies, it must be taken into  
448 account that the metal, once desorbed, remains in solution and it continues in contact

449 with the biosorbent, so a new adsorption equilibrium could be established, affecting  
450 desorption process.

451 An important parameter that requires special consideration is the solid/liquid ratio  
452 (biosorbent mass/solution volume). Ideally, this relation should be as high as possible,  
453 which implies low desorbent volume and/or a great amount of biomass.

454 Two acids,  $\text{HNO}_3$  and  $\text{HCl}$ , were both tested in batch studies and evaluated according to  
455 their effectiveness to release copper from the metal-laden biomass, estimating the  
456 percentage of metal desorbed (Table 3). The acid elution efficiency was based on the  
457 competition between protons and the heavy metal ions bound to active sites, which will  
458 be released if eluant concentration is high enough and there is not steric impediment.  
459 Both acids were found to be very powerful metal-desorbing agents (Lodeiro et al., 2006;  
460 Vilar et al., 2007), with the added advantage that both the release of metal and the  
461 regeneration of the alga can be achieved just in one step.

462 First of all, three acid concentrations ( $0.05$ ,  $0.1$  and  $0.5 \text{ mol}\cdot\text{L}^{-1}$ ) were tested at fixed  
463 both contact time (2 hours) and solution volume (10 mL). As it is shown in Table 3, the  
464 percentage of desorbed copper was similar for the different concentrations of both acids,  
465 although not as high as it was desirable (around 75% of copper removal). So that, the  
466 lowest acid concentration was selected and the solution volume was increased to 20 mL  
467 in order to improve the mix, making that the solution was not so dense. In this way, the  
468 percentage of desorbed copper was raised up to 87%. As the contact time was  
469 augmented to 4 hours, , percentages very close to the complete copper desorption were  
470 achieved (Table 3).

471 To summarize, it can be said that the effectiveness of  $\text{HNO}_3$  and  $\text{HCl}$  acids is very  
472 similar. Their optimal conditions of use are an acid concentration of  $0.05 \text{ mol}\cdot\text{L}^{-1}$ , a  
473 solid/liquid ratio of 5 g/L and a biomass-desorbent contact time of 4 hours.

474

475 **4. Conclusions**

476 Kinetic experiments showed that equilibrium was attained within 1 hour. An  
477 intraparticle-diffusion linear driving force model (LDF) was able to correctly explain  
478 these dynamic experiments.

479 A simple Langmuir or Langmuir-Freundlich isotherm can be used to accurately describe  
480 equilibrium experiments. However, only the application of a model that takes into  
481 account the complexity of macromolecular systems, e.g. NICA model, allows a good  
482 description of all equilibrium experiments tested (isotherm, pH influence and  
483 competition between copper and cadmium) employing the same constants attained  
484 through proton binding studies.

485 Batch studies proved the high efficiency of HNO<sub>3</sub> and HCl acids as copper desorbing  
486 agents.

487

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496 **FIGURE CAPTIONS**497 **Figure 1**

498 Sorption of copper as a function of contact time at different initial metal concentrations,  
499 for aqueous suspensions of the protonated *S. muticum* in  $0.05 \text{ mol}\cdot\text{L}^{-1} \text{ NaNO}_3$   
500 (temperature of  $25.0 \pm 0.1 \text{ }^\circ\text{C}$ , alga dose  $2.5 \text{ g}\cdot\text{L}^{-1}$ ). The symbols correspond to the  
501 experimental points at natural pH (see Figure 2) and different copper initial  
502 concentrations:  $0.25 \text{ mmol}\cdot\text{L}^{-1}$  (squares),  $0.50 \text{ mmol}\cdot\text{L}^{-1}$  (up triangles),  $1.00 \text{ mmol}\cdot\text{L}^{-1}$   
503 (circles) and  $3.00 \text{ mmol}\cdot\text{L}^{-1}$  (down triangles). Lines represent modelled results  
504 calculated using Equation 6.

505 **Figure 2**

506 Evolution of solution pH values with time at different initial metal concentrations:  $0.25$   
507  $\text{mmol}\cdot\text{L}^{-1}$  (dash line),  $0.50 \text{ mmol}\cdot\text{L}^{-1}$  (solid line),  $1.00 \text{ mmol}\cdot\text{L}^{-1}$  (dot line) and  $3.00$   
508  $\text{mmol}\cdot\text{L}^{-1}$  (dash-dot line), for aqueous suspensions of the protonated *S. muticum* in  $0.05$   
509  $\text{mol}\cdot\text{L}^{-1} \text{ NaNO}_3$  (temperature of  $25.0 \pm 0.1 \text{ }^\circ\text{C}$ , alga dose  $2.5 \text{ g}\cdot\text{L}^{-1}$ ).

510 **Figure 3**

511 Copper biosorption isotherms for suspensions of protonated *S. muticum* (alga dose:  $2.5$   
512  $\text{g}\cdot\text{L}^{-1}$ ) at  $\text{pH } 4.0 \pm 0.1$  (open triangles) at  $25 \text{ }^\circ\text{C}$ . Lines represent the fits to the Langmuir  
513 model, Eq. 7 with  $n'=1$ , (dashed line) and Langmuir-Freundlich (solid line) model, Eq.  
514 7.

515 **Figure 4**

516 Effect of pH on copper adsorption by  $2.5 \text{ g}\cdot\text{L}^{-1}$  of protonated *S. muticum* at  $25^\circ\text{C}$ , with  
517 initial copper concentrations of  $2.22 \text{ mmol}\cdot\text{L}^{-1}$  (open squares). Lines represent the fit of  
518 the data to different equations: NICA isotherm, Eqns. 9 and 10 (solid line), competitive  
519 Langmuir isotherm assuming 1:1 stoichiometry (dotted line) and assuming 1:2  
520 stoichiometry (dashed line), Eq. 8.

521 **Figure 5**

522 Copper binding by *S. muticum* at pH= 4.0 ± 0.1. Symbols represent experimental points  
523 (the same showed in Figure 3), solid line is the fitted NICA isotherm, Eqns. 9 and 10,  
524 dotted line is the competitive Langmuir isotherm assuming 1:1 stoichiometry and  
525 dashed line assuming 1:2 stoichiometry, Eq. 8.

526 **Figure 6**

527 Effect of cadmium ions competition on copper elimination by *S. muticum* at pH= 4.0 ±  
528 0.1 at 25°C. Symbols represent experimental points at two different cadmium initial  
529 concentrations: 0.1 mmol·L<sup>-1</sup> (filled circles) and 5 mmol·L<sup>-1</sup> (open circles). Solid lines  
530 represent the fitted NICA isotherm.

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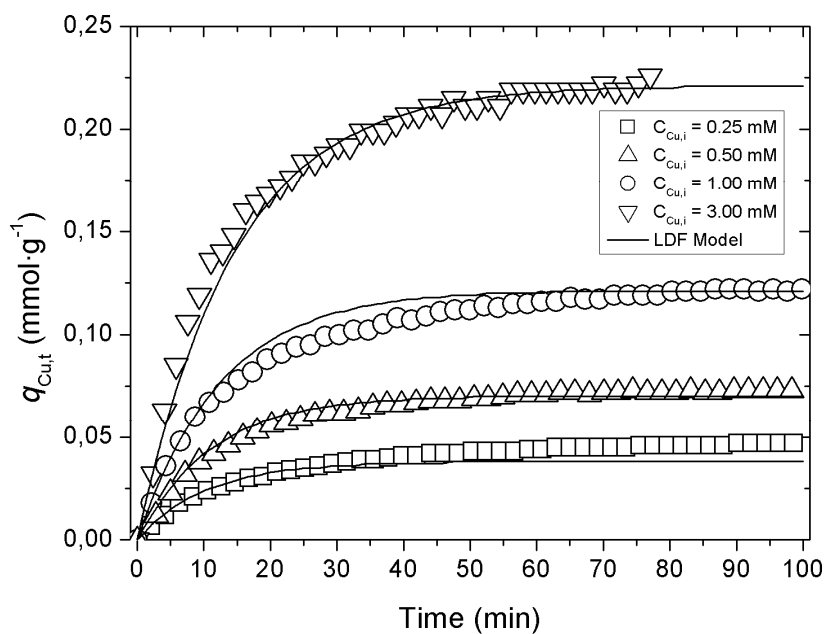


Figure 1

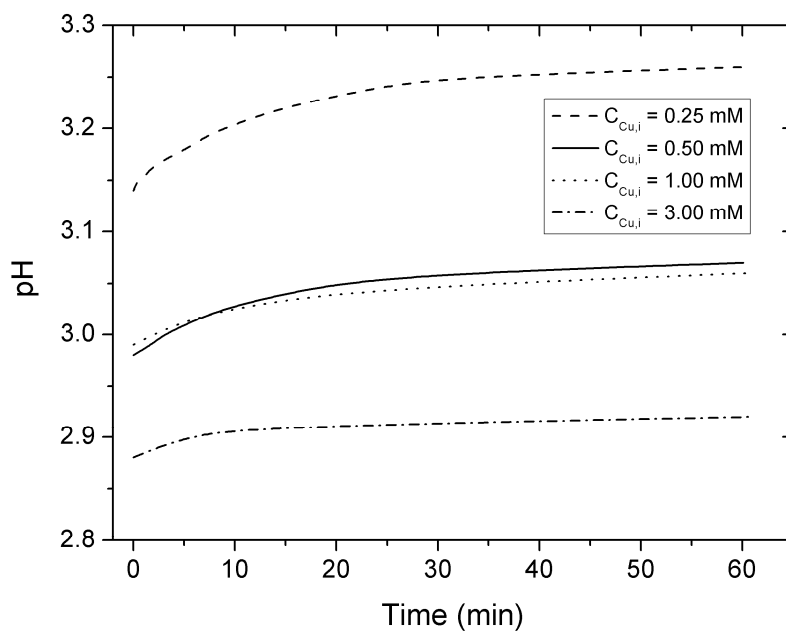


Figure 2

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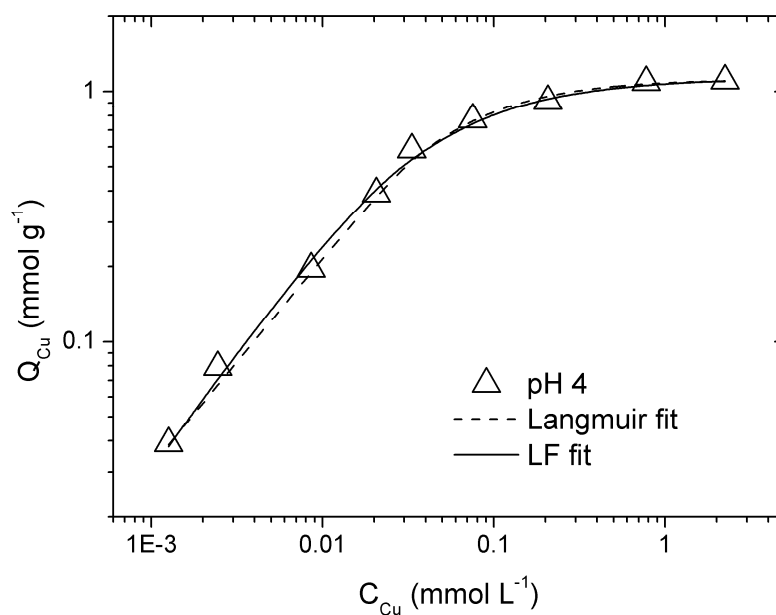


Figure 3

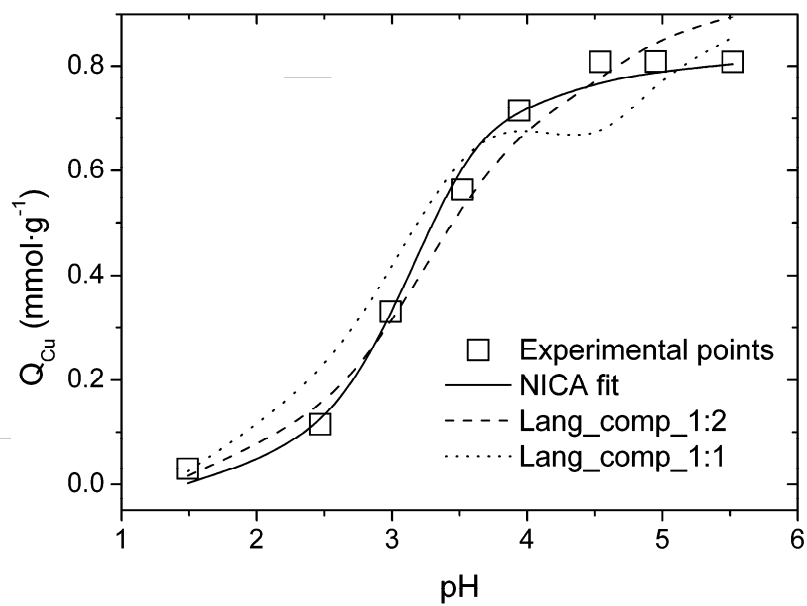


Figure 4

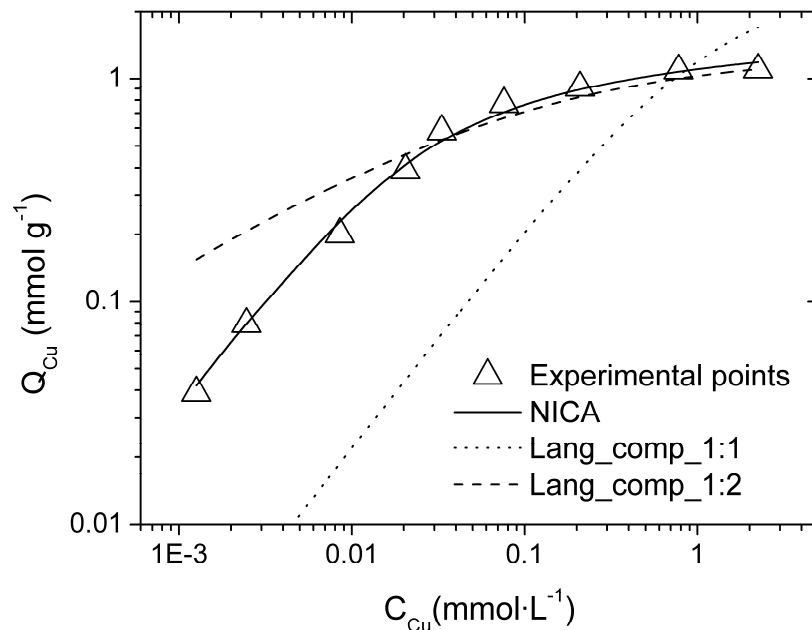


Figure 5

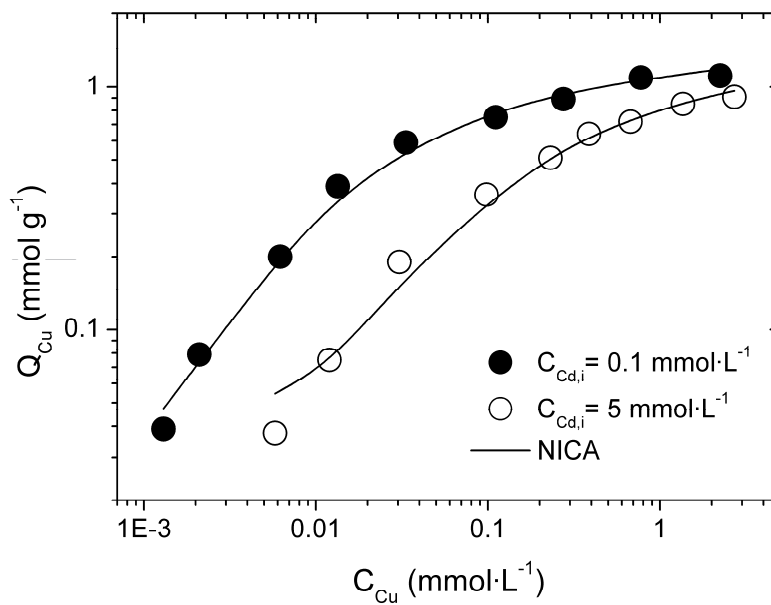


Figure 6

621 **TABLES**

622 **Table 1.** Kinetic rate constant for copper uptake by protonated *Sargassum muticum* at  
 623 several initial metal concentrations (T=298 K, pH= 3), obtained by fitting experimental  
 624 data to Equation 6 (LDF model). The percentage of Cu removed from solution at the  
 625 end of the kinetic process is also included.

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$C_i$ (mmol·L <sup>-1</sup> )	%Cu removed	$k$ (min <sup>-1</sup> )	$r^2$
0.25	43	0.0642	0.91
0.50	35	0.0642	0.98
1.00	30	0.0642	0.96
3.00	18	0.0642	0.992

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641 **Table 2.** Optimal parameters estimated for copper binding by the acid-treated biomass.

<i>Copper binding parameters</i>			
	$\log \tilde{K}_{Cu} / \log K_{Cu}$	$n_{Cu} / n$	Heterogeneity parameter, $p$
<b>NICA fit</b>	$4.3 \pm 0.1$	$1.05 \pm 0.05$	$0.31 \pm 0.02$
<b>Lang. fit</b>	$3.5 \pm 0.1$	1	
<b>Lang. fit</b>	$5.0 \pm 0.5$	0.5	

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660 **Table 3.** Percentage of copper released from protonated *S. muticum* biomass (previously  
 661 metal-loaded), employing HNO<sub>3</sub> and HCl acids as desorbent agents at different  
 662 concentrations, solution volumes and contact times.

Concentration (mol·L <sup>-1</sup> )	Contact time (h)	Volume (mL)	%Cu removed
<b>HCl</b>			
0.05	2	10	78
0.1	2	10	73
0.5	2	10	76
0.05	2	20	86
0.05	4	20	94
<b>HNO<sub>3</sub></b>			
0.05	2	10	69
0.1	2	10	75
0.5	2	10	80
0.05	2	20	87
0.05	4	20	95

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