REMOVAL OF INORGANIC MERCURY FROM AQUEOUS SOLUTIONS BY BIOMASS OF THE MARINE MACROALGA *CYSTOSEIRA BACCATA*

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Abstract

The ability of *Cystoseira baccata* algal biomass to remove Hg(II) from aqueous solutions is investigated. The mercury biosorption process is studied through batch experiments at 25ºC with regard to the influence of contact time, initial mercury concentration, solution pH, salinity and presence of several divalent cations. The acid-base properties of the alga are also studied, since they are related to the affinity for heavy metals. The studies of the pH effect on the metal uptake evidence a sharp increasing sorption up to a pH value around 7.0, which can be ascribed to changes both in the inorganic Hg(II) speciation and in the dissociation state of the acid algal sites. The sorption isotherms at constant pH show uptake values as high as 178 mg.g^{-1} (at pH 4.5) and 329 mg.g^{-1} (at pH 6.0). The studies of the salinity influence on the Hg(II) sorption capacity of the alga exhibit two opposite effects depending on the electrolyte added; an increase in concentration of nitrate salts ($NaNO_3$, KNO_3) slightly enhances the metal uptake, on the contrary, the addition of NaCl salt leads to a drop in the sorption. The addition of different divalent cations to the mercury solution, namely Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}, reveals that their effect on the uptake process is negligible. Finally, the equilibrium sorption results are compared with predictions obtained from the application of a simple competitive chemical model, which involves a discrete proton binding constant and three additional constants for the binding of the main neutral inorganic Hg(II) complexes, $Hg(Cl)_2$, HgOHCl and $Hg(OH)_2$, to the algal surface sites.

Keywords: *Cystoseira baccata*, mercury, adsorption, marine macroalga.

Introduction

Water pollution by toxic metals in industrial wastewaters has become a major issue throughout the world. In particular, mercury compounds, whose effects on human health and aquatic life are regarded as harmful, must be removed from sewage down to extremely low concentrations. Even though the flux of mercury into the aquatic system has declined in recent years, there is still a lack of effective and cheap resources for such wastewater treatment.

The effluents from chlorine and chlor-alkali manufacturing processes, via electrolysis in mercury cells, represent one of the most important sources of mercury pollution. Other contributions come from pulp, oil refining, plastic and battery manufacturing industries.

Adsorption as a wasterwater treatment process has been found to be an economically feasible alternative for metal removal. Activated carbon is one of the most well-known adsorbents [Bello et al. (1999); Budinova et al. (2003); Gómez-Serrano et al. (1998)] but the high costs of the process has limited its use. A search for a low-cost and easily available adsorbent has led to the investigation of materials of biological origin as potential metal biosorbents. The variety of materials tested includes bark, chitin, lignin, modified wool and seaweeds [Bailey et al. (1999)]. They can be used for the effective removal and recovery of several species from wastewater streams. Among these materials, a few brown marine macroalgae species exhibit higher uptake values than those of activated carbon and natural zeolite, and are comparable to those of some synthetic ion exchange resins.

The algal cell wall plays an important role in metal binding [Crist et al. (1988)], due to its high content in polysaccharides with acid functional groups. In brown algae, the cell wall is mainly comprised of alginates, which usually constitute about 20-40% of the total dry weight, in addition to fucoidans [Percival and McDowell (1967)]. The carboxyl groups of alginates are likely to be the main functionalities involved in metal binding reactions [Schiewer and Wong (2000)] because of their higher abundance with regard to both carboxyl and amine groups of the proteins.

The present work reports a study of the mercury adsorption by non-living biomass of the brown marine macroalga *Cystoseira baccata* originated from the Galician coast (NW, Spain). The biosorption process has been analyzed through batch experiments at 25ºC with regard to the influence of the initial metal concentration, the pH and salinity of the solution, and the presence of different divalent cations. The acid-base properties of the alga have been studied as they are related to its capacity of binding heavy metals. Finally, a complexation model for mercury sorption on *Cystoseira baccata* has been developed in order to explain the adsorption behavior and the different effects studied on the metal uptake.

Materials and methods

The brown alga *Cystoseira baccata* was collected from the coasts of A Coruña (Galicia, NW Spain). The alga was washed twice with running water and once with deionized water. The washed biomass was oven-dried at 60°C for 24 hours, crushed with an analytical mill, sieved (size fraction of 0.5-1 mm) and stored in polyethylene bottles until use. The samples for the potentiometric titrations were acid-treated with diluted HCl following the procedure described elsewhere [Rey-Castro et al. (2003)], in order to transform the biomass into its fully protonated form.

All chemicals used in this work were purchased from Merck. Cellulose nitrate membrane filters were from Whatman and Albet. Throughout this work, all the experiments were conducted at least in duplicate.

Kinetic studies

Kinetic studies of Hg adsorption by the alga *Cystoseira baccata* were accomplished to estimate the time necessary to reach the sorption equilibrium. The experiments were carried out adding 0.25g of dried biomass over 100 ml of a solution that contained 500 $mg \cdot L^{-1}$ of Hg(II) (from $HgCl_2$) and sufficient $NaNO_3$ salt to keep the ionic strength constant at 0.05M. The mercury content of the solution was checked by taking aliquots at certain time intervals. The concentration of dissolved Hg in each aliquot was then analysed by inductively coupled plasma mass spectrometry (ICP-MS).

Potentiometric titrations

For each titration, ca. 0.5 g of protonated *C. baccata* biomass were placed in a thermostated glass cell at a temperature of 25.0±0.1 ºC, and 100 mL of 0.05 M $NaNO_3$ solution were added to keep ionic strength constant. HCl was also added to yield an initial pH value around 2.0. The suspension, magnetically stirred, was allowed to equilibrate and the titration was started solely once the electromotive force measurement was stable. A NaOH solution, prepared with boiled deionized water, was employed as titrant, which was added from a Crison microBu 2031 automatic burette.

Emf measurements were done by a Crison micropH 2000 meter equipped with a GK2401C Radiometer combined glass electrode (saturated Ag/AgCl as reference). After each addition of base, the system was allowed to equilibrate before a stable reading was obtained. A whole titration typically took 6-7 h.

The glass electrode was calibrated in solutions of known proton concentration at a constant ionic strength following the procedure described elsewhere [Brandariz et al. (1998); Fiol et al. (1992)]. All titration experiments were done under a nitrogen stream, intended to remove dissolved O_2 and CO_2.

Adsorption isotherms

The isotherm experiments were carried out at three different values of pH (4.5, 6.0 and 8.0), adjusted by the addition of NaOH or HNO_3 solutions. Different amounts of $HgCl_2$ were dissolved in deionized water to prepare ten mercury solutions in a concentration range from 20 to 2000 $mg\cdot L^{-1}$. A volume of 40 mL of the metal solution was then added to a 100 mL Erlenmeyer flask containing 0.1 g of alga. The mixtures were stirred in a rotary shaker at 175 rpm for 4 hours until equilibrium was reached. Afterwards the suspension was filtered through a 0.45 μm pore size cellulose nitrate membrane filter. The filtrates were analysed for mercury and sodium contents by ICP-MS and for chloride ion (which constitutes a key factor in the inorganic mercury speciation) by capillary electrophoresis.

The amount of mercury sorbed at equilibrium, q_{eq} ($mg\cdot g^{-1}$), which represents the metal uptake, was calculated from the difference in metal concentration in the aqueous phase before and after adsorption, according to the following equation:

$$q_{eq} = \frac{V \cdot (C_i - C_{eq})}{1000 \cdot m_s} \qquad (1)$$

where V is the volume of mercury solution (mL), C_i and C_{eq} are the initial and equilibrium concentration of mercury in solution ($mg \cdot L^{-1}$), respectively, and m_s is the mass of dry alga (g).

Influence on the metal adsorption of pH, salinity and addition of divalent cations

The dependence of *Cystoseira baccata* metal uptake on pH was studied through batch sorption experiments in the pH range from 0.7 to 9.0, with an initial mercury concentration of 500 $mg \cdot L^{-1}$. The pH was adjusted by addition of NaOH and HNO_3 solutions.

The effect of salinity on the adsorption of mercury was tested by addition of different salts (NaCl, $NaNO_3$ and KNO_3) to the solution. The concentration of each salt ranged from 0.001 to 1 M. The initial mercury concentration and pH were 500 mg/L and 6.0, respectively, for all the experiments.

The competition effect of several divalent cations, namely Cd(II), Mg(II), Zn(II), Ca(II), Cu(II) and Pb(II), was tested through batch sorption experiments conducted with 0.1 g of *Cystoseira baccata* in contact with binary mixtures composed of 50 $mg \cdot L^{-1}$ Hg(II) and the competitor metal ion at two different concentrations (500 or 1000 $mg \cdot L^{-1}$) prepared from the respective metal nitrate salt. Throughout the experiments, the pH was kept constant at 4.5 to avoid hydrolysis of the cations, specially Cu(II) and Pb(II).

Results and discussion

Kinetics of the adsorption process: Effect of Contact Time

The batch experiments carried out to study the relationship between contact time and mercury uptake by *Cystoseira baccata* show that the equilibrium time is always reached in less than 100 minutes (see Figure 1 as an example). It can be noticed that the contact time significantly affects the Hg uptake; the metal adsorption increases sharply in the first 50 minutes and tapers off there after, as equilibrium is approached. This relatively rapid mercury uptake indicates that the sorption process occurs mainly on the surface of the adsorbent.

According to these results, it was set a contact time of 4 hours in order to ensure that equilibrium conditions are attained. This equilibrium time is clearly shorter than those usually employed for the adsorption of Hg by other adsorbent materials. Times of 24 hours are proposed for the adsorption by chemically modified chitosan [Jeon and Höll (2003)], pinus pinaster bark [Vázquez et al. (2002)] or ion exchange resins [Chiarle et al. (2000)]. Even longer times, from 80 to 120 hours, are necessary with some carbonaceous materials [Cox et al. (2000)]. However, similar equilibrium times were found for other heavy metal adsorptions by different macroalgae [Cordero et al. (2004); Cruz et al. (2004); Lodeiro et al. (2004a)].

The rapid kinetics has a significant practical importance, as it will facilitate smaller reactor volumes ensuring efficiency and economy.

Acid-base properties of Cystoseira baccata

The total amount of active sites in protonated *C. baccata* biomass was estimated by potentiometric titration with a standard solution of NaOH. The number of acid groups per gram of alga, $[A]_T$ (mmol·g^{-1}), was calculated from the maximum of the first derivative of the titration curves (Figure 2).

Sulfate groups are known to be present in the algal cell wall [Percival and McDowell (1967)]; however, no evidence of their presence was found in the titration curves. The total number of weak acid groups determined in 0.05M $NaNO_3$ was 2.2 ± 0.1 mmol g^{-1}. From titrations in different saline media, it was concluded that ionic strength does not influence the number of acidic groups titrated, but strongly affects their apparent pK values. A physicochemical model based on the Donnan formalism has recently been proposed by Rey-Castro *et al*. [Rey-Castro et al. (2003)], [Rey-Castro et al. (2004b)] in order to account for the effects of pH and ionic strength on the proton binding equilibria of seaweed biomass.

Effect of pH on Hg(II) biosorption

Solution pH values have a significant influence on mercury uptake by *Cystoseira baccata*. Several other researchers have already reported a strong dependency of heavy metal biosorption on pH [Volesky (2003); Wase and Forster (1997)]. As seen from Figure 3 (a), the mercury uptake is small at low pH. Between pH values 2.0 and 7.0 the metal adsorption increases sharply, attaining values that remain almost constant for higher pH values.

As a general rule, the pH influence on metal uptake by seaweeds is closely related to the ionic states of the cell wall functional groups as well as to the metal speciation in solution. In the case of Hg biosorption, the pH dependence is slightly different to that observed for other metals. As an example, Cd(II) also presents an S-shape curve but the maximum uptake is reached at pH 4.5 [Lodeiro et al. (2004b)]. It can well be assumed that cadmium is present in its free ionic form, Cd^{+2}, all along the pH range studied. Therefore, the cadmium biosorption depends on the protonation or deprotonation of the cell wall functional groups, mainly carboxylic groups, whose pK_a values are about 2 to 4 [Rey-Castro et al. (2004a)]. On the contrary, the mercury biosorption process is not only affected by the acid-base properties of the cell wall but also by the metal chemical speciation, which is rather more complex than that of Cd(II), and hence, it may play an important role (see Figure 3 b).

Adsorption isotherms

The metal distribution between the alga and the aqueous solution at equilibrium is of importance in determining the maximum adsorption capacity of the alga for mercury. Figure 4 illustrates the adsorption of mercury on biomass at different pH values. It can be noted that the uptake rises dramatically with increasing pH. The plots of uptake vs. aqueous Hg(II) obtained at pH 4.5 and 6.0 are smooth, continuous curves that eventually reach a saturated value, which suggests monolayer coverage of mercury on the adsorbent surface, a typical behaviour of most metal ions. At pH 8.0, however, together with much greater saturation values, an anomalous S-shaped increasing curve is obtained, which may be explained by a combination of phenomena. On the one hand, similar S-shaped isotherms have already been described in several studies concerning Hg(II) sorption to soils with high contents of organic matter (see Drexel et al. [Drexel et al. (2002)] and references therein), which was attributed to the binding of metal to soluble organic matter released from the sorbent. In fact, there is an important organic leaching from seaweed biomass above pH 6, due mainly to alginate solubilization. On the other hand, the large maximum uptake values determined at pH 8.0 could be assigned to the presence of surface precipitation processes, which may occur at metal concentrations close to the limit of bulk precipitation, leading to an increasing slope of the adsorbed vs. free metal plots (departing from the classical Langmuir isotherm shape) [Schneider et al. (2001)].

From the experiments at constant pH, it can be noticed that an increase in the initial mercury concentration leads to a larger sorption capacity of the biomass at equilibrium, while the percentage of mercury removed from solution shows the opposite trend. Nevertheless, the alga is still able to remove percentages greater than 80% of mercury in solution for relatively high initial mercury concentrations using small amounts of biosorbent.

It can be then concluded that the biomass of the marine alga *Cystoseira baccata* can efficiently remove high concentrations of mercury in solution over a broad range of pH, highlighting its potential for effluent treatment processes. The high sorption capacity of this seaweed is comparable to or even larger than other natural and synthetic materials, such as fungal biomass [Saglam et al. (1999)] (61 $mg \cdot g^{-1}$), the green alga *Ulva lactuca* [Zeroual et al. (2003)] (149 $mg \cdot g^{-1}$), the aquaphyte *Potamogeton natans* [Lacher and Smith (2002)] (180 $mg \cdot g^{-1}$), active carbons from different sources [Budinova et al. (2003); Yardin et al. (2003)] (132-174 $mg \cdot g^{-1}$), the synthetic resin Duolite GT-73 [Chiarle et al. (2000)] (362 $mg \cdot g^{-1}$) or chitosan [Jeon and Höll (2003); Masri et al. (1974); McKay et al. (1989)] (460, 1123 and 815 $mg \cdot g^{-1}$, respectively).

Effect of salinity on mercury uptake

The studies of the salinity influence on the Hg(II) sorption capacity of the alga exhibit two opposite effects depending on the electrolyte added, see Figure 5 (a-b). An increase in concentration of nitrate salts ($NaNO_3$, KNO_3) causes greater mercury uptake, from 5% up to 12-15% as the salt concentration varies from 0.01M to 1M, respectively. On the contrary, a larger presence of NaCl salt leads to a drop in the sorption, which is decreased by more than 80% at salt concentrations around 1 M, whereas low values of salinity have small influence on the metal uptake (declined by 8% at NaCl concentration 0.01M).

It is often stated in literature that light metal ions, such as sodium or potassium, compete with divalent cations for the electrostatic binding to the biomass [Schiewer and Wong (2000)]. Therefore, if a similar mechanism was involved in the present study, the mercury sorption should decrease as the concentration of light metal ions increases.

In view of the results of this work (see Figure 5), it can be concluded that the major effect on the mercury biosorption capacity of the alga is caused by the salt anions, not by the light metal cations. This turns out to be clear from the comparison of the mercury sorption that occurs in two saline solutions containing the same concentration of sodium ion but different type of counterion. As an example, the mercury uptake decreases by 83% in presence of chloride ions whereas it increases by 15% in presence of nitrate, although sodium salt concentration is 1M in both cases. Such behavior can be explained taking into account that the mercury speciation is highly affected by the presence of chloride ions, which induce the formation of $HgCl_3^-$ and $HgCl_4^{-2}$ complexes with low sorption capacity on the algal cell wall. This imposes a limitation in the application of this technology to waters containing high chloride concentrations, such as seawater or wastewaters from brine industries. Nevertheless, the Hg(II) adsorption capacity of algal biomass continues being very high in saline media whose chlorine concentration does not exceed 0.01M.

Effect of the competition of divalent cations on Hg uptake

Figure 6 shows the results of the mercury adsorption experiments carried out in the presence of 500 or 1000 mg·L^{-1} of divalent cation – Cd(II), Mg(II), Zn(II), Ca(II), Cu(II) and Pb(II) –. It can be observed that the uptake remains practically unaffected due to the presence of these divalent metal ions at a much greater concentration than mercury. Only Cu(II) ions seem to decrease slightly the mercury sorption, whereas Pb(II) ions increase it. Such results could be explained in terms of the different chemical speciation of metals at the pH studied, which have a significant effect in the adsorption mechanism. Whereas mercury is largely present as a neutral species, $HgCl_2$, the other metals appear as divalent ions, M^{+2}, which will interact with the algal cell walls mainly through an electrostatic mechanism, thus avoiding any competition with the sorption process of the neutral species of mercury. In fact, different authors have assumed that $Hg(OH)_2$ behaves as a ligand in aqueous solution [Daughney et al. (2002); Sarkar et al. (1999)], and hence a similar behavior may be proposed also for $HgCl_2$. In this way, its interaction with the active groups of the algal wall (represented by A^-) can be illustrated with a reaction of the type:

$$\mathrm{A^- + HgCl_2 = AHgCl_2^-}$$

Such mechanism does not lead to any change in the charge of the algal surface, which, hence, could still bind any other metal cation present in solution. Such specific adsorption of the neutral species $HgCl_2$ is also supported by the small changes found in mercury adsorption when the effect of ionic strength was studied.

Of all the divalent cations studied, only copper appears to compete slightly with mercury for sorption sites. Due to its relatively “soft” character, Cu^{2+} would tend to form covalent bonds more readily than “hard” cations such as Ca^{2+} or Mg^{2+}, which are mainly electrostatically bound [Schiewer and Wong (1999)]. In addition, copper ions start undergoing hydrolisis at pH values as low as 4.5, yielding some amount of neutral species (of higher softness). Since Hg(II) has a strong “soft” character (especially in the form of neutral species like $HgCl_2$), it may therefore be expected that Cu(II) species will compete to some extent with mercury in the complexation process to the algal wall. On the contrary, Pb(II) seems to reinforce the mercury uptake, but authors have not found any consistent explanation.

It is of interest to underline that these studies have been carried out at concentration values of divalent cations significantly higher than those found in natural media. The results obtained make clear the high capacity of *Cystoseira baccata* for the disposal of mercury from polluted natural waters, even in presence of high contents of other divalent ions, which is likely to occur, especially in hard water.

Modelling of Hg adsorption equilibria

The purpose of this section is to explore how well a simple thermodynamically consistent chemical model is able to describe approximately most of the experimental results that have been discussed in previous sections, and to account for the observed influence of the environmental variables (pH, ionic strength, amount of chloride, etc.). Such a model would be useful for the prediction of the sorbent performance under practical conditions.

The basic assumptions of this model are:

(a) The sorption of the inorganic Hg(II) species involves at least the binding to an ionized acid group of the seaweed. Therefore, protons and mercury are assumed to compete with each other for the same acid sites on the algal surface. This is supported by the fact that the observed variation of the Hg(II) biosorption with pH cannot be entirely ascribed to the change in mercury speciation.

(b) Inorganic Hg(II) essentially appears as neutral species, mainly $HgCl_2$, $Hg(OH)Cl$ and $Hg(OH)_2$, which behave as ligands in the sorption process, as discussed above. In fact, calculations of the inorganic Hg(II) speciation using the MINTEQA2 database[Allison et al. (1991)] showed that more than 98% of the total dissolved mercury appears as neutral species between pH 1 and 9, for the range of Hg concentrations of this study.

(c) For the sake of simplicity, a discrete constant for the binding of each mercury species is considered, through the corresponding ideal Langmuir isotherms. It must be admitted that this assumption is quite rough, as shown from potentiometric studies of the acid-base properties [Rey-Castro et al. (2004b); Rey-Castro et al. (2003)]. The algal biomass was proved to behave as a heterogenous proton sorbent with acid sites exhibiting a continuous distribution of affinities for protons, this being a result of different factors (polyelectrolytic effect, intrinsic chemical heterogeneity of the sorbent, etc.). Therefore, a model reflecting a distribution of site affinities would surely be more accurate. However, this option implies the use of a relatively large number of empirical parameters with regard to the amount of available data. On the contrary, the consideration of a discrete binding constant for each neutral Hg(II) species is able to describe roughly the experimental trends observed, with the smallest number of empirical parameters.

Finally, it must be pointed out that the simple model proposed in this study does not take into account other possible mechanisms for the mercury uptake by the biomass postulated in bibliography, such as the sequestering of neutral mercuric species in the lipid environment of the algal cellular membranes [Mason et al. (1995)]. As will be explained below, this mechanism alone would not explain the observed effect of pH on mercury uptake.

Analytical isotherms

Assumptions (a)-(c) lead us to the consideration of the following chemical model, in which each of the neutral mercury species interacts with at least one acid functional group of the algae (A^-):

$$A^- + H^+ \overset{K_H}{=} AH \qquad (2)$$

$$M + n\,A^- \overset{K_i}{=} n\,AM^-_{1/n} \qquad (3)$$

where M = $Hg(Cl)_2$, HgOHCl or $Hg(OH)_2$, K_i (i=1,2,3) represents the binding constant of each species, respectively, and n is the average number of binding sites per metal bound. For instance, the complexation of $Hg(OH)_2$ (assuming a 1:1 stoichiometry) would be represented by:

$$\mathrm{Hg(OH)_2} + \mathrm{A^-} \overset{\mathrm{K_3}}{=} \mathrm{AHg(OH)_2^-} \qquad (4)$$

In this work, only the ideal stoichiometries 1:1 and 1:2 have been considered, for simplicity.

From these equilibria and the mass balance for the ligand, an analytical expression for the sorption isotherm may be easily derived for 1:1 or 1:2 stoichiometries. The 1:1 model leads to

$$\begin{aligned}[\mathrm{A}]_\mathrm{T} &= [\mathrm{AH}] + [\mathrm{A^-}] + [\mathrm{AHg(Cl)_2^-}] + [\mathrm{AHgOHCl^-}] + [\mathrm{AHg(OH)_2^-}] = \\ &= [\mathrm{A^-}](1 + \mathrm{K_H}[\mathrm{H^+}]) + \sum[\mathrm{AM^-}]\end{aligned} \qquad (5)$$

By solving the equilibrium mercury complexation equations (3) the concentration of free ligand can be expressed as

$$[\mathrm{A^-}] = \frac{[\mathrm{AHg(Cl)_2^-}]}{\mathrm{K_1}[\mathrm{Hg(Cl)_2}]} = \frac{[\mathrm{AHgOHCl^-}]}{\mathrm{K_2}[\mathrm{HgOHCl}]} = \frac{[\mathrm{AHg(OH)_2^-}]}{\mathrm{K_3}[\mathrm{Hg(OH)_2}]} \qquad (6)$$

An expression for the overall concentration of bound mercury is readily obtained from eqs.(5) and (6):

$$\sum[\mathrm{AM^-}] = q_{eq} = [\mathrm{A}]_\mathrm{T} \frac{\sum \mathrm{K_i}[\mathrm{M}]}{1 + \mathrm{K_H}[\mathrm{H^+}] + \sum \mathrm{K_i}[\mathrm{M}]} \qquad (7)$$

where q_{eq} and $[A]_T$ must be expressed in the same unities ($mmol \cdot g_{alga}^{-1}$ or $mg_{Hg} \cdot g_{alga}^{-1}$), and the rest of the concentrations are expressed in $mol \cdot L^{-1}$.

Analogously, when a 1:2 stoichiometry, i.e. n=2 in eq. (3), is considered, one gets:

$$q_{eq} = 0.5[A]_T \frac{\sum (K_i[M])^{0.5}}{1 + K_H[H^+] + \sum (K_i[M])^{0.5}} \qquad (8)$$

Eqs. (7) and (8) represent very simple competitive ideal Langmuir isotherms that involve the assumption of the algal binding sites behaving as homogenous ligands, with regard to the binding of protons and mercury. These analytical expressions must be combined with an accurate prediction of the distribution of mercury species in solution.

Inorganic Hg(II) speciation

The chemistry of mercury in aqueous solution is quite complex. Both the distribution of the Hg(II) species and the mercury oxide precipitation are very sensitive to the solution variables, such as pH or chloride concentration. Furthermore, some disagreements about the formation constants of the different inorganic complexes of Hg(II) are still found in bibliography. In this work, the inorganic speciation of Hg(II) was estimated through MINEQL+ [Schecher and McAvoy (1992)] using the thermodynamic database from MINTEQA2 [Allison et al. (1991)]. The activity coefficients were calculated through the Davies equation [Sastre de Vicente (1997)] applied to the bulk ionic strength values estimated either from the acid and/or base additions made for pH adjustment, or from the background salt addition. The experimental measurements of mercury, chloride and sodium in the filtrate solutions were taken as the total concentrations in the MINEQL+ input.

Choice of model parameters and comparison with experimental results

The total number of functional groups, $[A]_T$, was set to 2.2 mmol·g^{-1}, the value determined from the acid-base potentiometric titrations in the absence of Hg species. The fit of the proton binding data to an isotherm model allowed the estimation of an average acid constant K_H, referred to $NaNO_3$ 0.05 M, with a value of $10^{3.6}$. The variation of this constant with ionic strength was estimated by means of an empirical Donnan expression derived elsewhere [Rey-Castro et al. (2004b)] using potentiometric titration data in different electrolytes [Rey-Castro et al. (2003)].

The values of the binding constants for the mercury species were chosen to provide the best simultaneous description of the experiments at pH 4.5 and 6.0, as well as the plots of Hg(II) uptake vs. pH and the influence of salinity. The values of K_1-K_3 were first optimized by least-squares fit for each data set, and then average values (see Table 1) were used to plot the model calculations shown in Figure 3 (a), Figure 4 and Figure 5 (a,b). The isotherm obtained at pH 8.0 was excluded from the model dicussion, since, on the one hand, it does not display the typical Langmuir shape (probably due to the reasons explained above) and, on the other hand, the maximum uptake values obtained exceed the total number of acid groups, which can not be explained by the present complexation model.

It is clear from Figure 4 that the 1:1 model is able to reproduce the shift in the isotherms from pH 4.5 to 6.0, although at low mercury concentrations (and high algal sites to mercury ratio) the uptakes are underestimated. On the contrary, the best fit to the data at pH 4.5 is provided by the 1:2 model, although the latter can not explain the uptake values above 1.1 $mmol\cdot g^{-1}$ obtained at pH 6.0 (1:2 model plot for pH 6.0 not shown).

Figure 3 (b) shows the inorganic speciation of mercury predicted by MINEQL+, together with the estimated fraction of ionized acid sites on the alga, determined from a simple Langmuir equation and a more accurate Langmuir-Freundlich model, both involving an average proton binding constant of $\log K_H = 5$ (corresponding to the estimated average ionic strength of the experiments). It is noticed that (i) $HgCl_2$ is the predominant species below pH 6.0; (ii) the fraction of chloride complex remains almost constant throughout this range, and yet the Hg(II) uptake increases in a remarkable way, thus confirming the postulate that metal speciation is not the only factor in the pH dependence of the Hg(II) uptake; (iii) the observed amount of mercury bound (Figure 3a) varies in parallel with the fraction of dissociated sites (Figure 3b), in agreement with the assumption that these sites are involved in the metal binding; (iv) the hydroxyl mercury complexes are only relevant at the highest pH values, although the Hg(OH)Cl seems to be less important.

The 1:1 and 1:2 model descriptions of the pH dependence are shown in the Figure 3 (a). Note that both models reflect the increasing trend of the experimental data, which is explained as a combination of two factors, namely the growing fraction of dissociated algal sites and the formation of hydroxyl complexes of mercury, with higher binding affinity. It can be observed that both models underestimate the results below pH 4.0, where the fraction of dissociated sites predicted by a discrete acid-base constant is very small. This can be a consequence of neglecting the heterogenous nature of the sorbent with regard to the proton binding. In fact, if the proton binding is represented by the Langmuir-Freundlich isotherm (which assumes a continuous distribution of affinities) then it turns out that a significant fraction of acid sites are already dissociated below pH 4 (see dotted line in Figure 3 b). Therefore, this effect could explain the observed uptakes at low pH. However, the more simple discrete constant description was preferred in this work, with the aim of using the lowest number of empirical parameters.

The discrepancy between the calculated uptakes and the experimental values at low pH may also be attributed to the contribution of a constant (although small) “background” accumulation of Hg due to the solubilization of neutral species in the phospholipid environment of the algal cells. Mason *et al.* [Mason et al. (1995)] showed that neutral inorganic complexes of Hg(II) are fairly hydrophobic compounds, with octanol/water partition coefficients following the trend $HgCl_2$>Hg(OH)Cl>>$Hg(OH)_2$. However, this dependence of the lipid solubility on the inorganic speciation of Hg does not agree with the experimental variation in mercury uptake with pH, which is just the opposite, i.e., the amount of metal sorbed becomes larger as the fraction of the less lipophylic species increases. Therefore, the mechanism of lipid solubilization alone is not enough to explain the experimental results.

Since the model assumes the binding of a neutral species to an acid site of the biomass, it is expected that a change in ionic strength would only influence the inorganic metal speciation in solution and the dissociation state of the algal sites, but not the binding reaction, eq. (3). Therefore, the observed decrease in Hg(II) sorption with the concentration of added NaCl is ascribed to the formation of $HgCl_3^-$ and $HgCl_4^{2-}$ complexes, which are favored by high chloride concentrations. In fact, the uptake values correlate well with the fraction of $Hg(Cl)_2$ (the main neutral species at pH 6.0) estimated through MINEQL+ in the filtrate solutions (Figure 5 c). The model predictions for the uptake vs. added NaCl data are shown in Figure 5 (a). Note that the 1:1 model agrees well with the observed trend, whereas the 1:2 model overestimates the experimental uptakes, although it is still able to reproduce the correct trend.

In contrast with these results, the experiments performed in KNO_3 and $NaNO_3$ (Figure 5 b) show a slight increase in the uptake with background salt concentration. In this case, the background salt anions do not tend to form strong mercury complexes of any kind. Recall that if the sorption mechanism implicated the binding of M^{2+} ions and ionized surface sites, then the electrostatic effect of the supporting electrolyte would cause a sharp reduction in the amount of metal bound with ionic strength. On the other hand, in a model involving complexation of neutral species to ionized acid sites, the factors that may explain the influence of nitrate concentration are the minor changes in mercury speciation (which are already taken into account in MINEQL+ calculations) and the decrease in K_H with ionic strength (incorporated in the model through the Donnan empirical expression). The latter means that an increasing fraction of dissociated sites is formed. These effects seem to account for the trend observed in the experimental data, regardless the stoichiometry considered (Figure 5 b). In addition, the possible contribution of a "salting out" mechanism for the transfer of neutral Hg complexes from saline solution to the algal surface cannot be discarded either. In fact, a similar effect was proposed by Turner *et al*. [Turner et al. (2001)] in order to explain the increase in sediment/water partitioning coefficients with salinity observed for mercury in estuaries.

Conclusions

The results obtained in this study demonstrated that the macroalgae *Cystoseira baccata* could compete with commercial biosorbents for the removal of Hg(II) from wastewaters because of its low cost, among the several reasons studied in this paper summarized below.

The Hg(II) sorption kinetic is relatively fast, reaching equilibrium in 100 minutes. The total number of weak acid groups determined by potentiometric titration was 2.2 ± 0.1 mmol g^{-1}.

C. baccata shows a very high Hg(II) uptake capacity. It is able to remove percentages greater than 80% of mercury in solution for relatively high initial mercury concentrations (120 $mg{\cdot}L^{-1}$ at pH 4.5), using small amounts of biosorbent.

The studies of the salinity influence showed that an increase in concentration of nitrate salts ($NaNO_3$, KNO_3) causes greater mercury uptake, whiles a larger presence of NaCl salt leads to a drop in the sorption, which is decreased by more than 80% at salt concentrations around 1 M, whereas low values of salinity have small influence on the metal uptake. Moreover, the addition of different divalent cations to the mercury solution, namely Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}, reveals that their effect on the uptake process is negligible.

The simple competitive model proposed in the present study is able to reflect the major characteristics of the experimental Hg(II) sorption data, with regard to equilibrium mercury concentration, pH and presence of background salts in solution. However, the stoichiometry of the mercury complexes cannot be clearly assessed. On the one hand, the 1:2 isotherm is able to fit very well the experimental data at low ratios of metal to algal acid sites, on the other hand, the maximum mercury loadings at a relatively high pH can only be explained by a 1:1 relationship. The actual binding mechanism would very probably involve a combination of both mono and bidentate sites, or even some degree of interaction between mercury and non-ionized sites of the algal surface.

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FIGURE CAPTIONS

Figure 1

Sorption of Hg(II) on *C. baccata* vs. time. Biomass concentration: 2.5 g·L^{-1}; initial $HgCl_2$ concentration: 500 mg·L^{-1}; saline medium: 0.05M $NaNO_3$. The final pH at equilibrium was around 4.

Figure 2

Acid-base potentiometric titration of an acid-treated biomass sample of *C. baccata* (5 g·L^{-1}) in 0.05 M $NaNO_3$ at 25ºC. Symbols: pH vs. titrant volume; solid line: first derivative (in arbitrary units).

Figure 3

Influence of pH on Hg(II) uptake. (a): experimental mercury uptake values (symbols) and 1:1 (solid line) or 1:2 (dashed line) model estimates, calculated through eqs. (7) and (8), respectively, using the parameters listed in Table 1 (r^2= 0.92 in both cases). Biomass concentration: 2.5 g·L^{-1}. (b): inorganic speciation of mercury in the aqueous phase at equilibrium with the biomass, calculated by MINEQL+ (solid lines), and fraction of dissociated acid sites on the alga, calculated from Langmuir (dashed line) and Langmuir-Freundlich (dotted line) isotherms.

Figure 4

Hg(II) uptake by *C. baccata* as a function of the aqueous Hg(II) equilibrium concentration at pH 4.5 (circles), 6.0 (triangles) and 8.0 (diamonds). Biomass concentration: 2.5 g·L^{-1}. Each symbol represents the average of two replicate batch sorption experiments performed with the same initial mercury concentration. Lines represent the uptake values calculated through the 1:1 (solid line) and 1:2 (dashed line) complexation models, using the parameter values listed in Table 1.

Figure 5

Sorption of mercury (initial metal concentration: 500 mg·L^{-1}; biomass concentration: 2.5 g·L^{-1}) as a function of the concentration of added (a) NaCl and (b) KNO_3 and $NaNO_3$ at pH 6.0. Lines represent the expected uptake values estimated from the 1:1 (solid line) and 1:2 (dashed line) competitive complexation models using the parameters listed in Table 1. The reference value 100% indicates the mercury sorption in absence of added salt. (c): correlation between the metal uptake of the experiments shown in Figure (a) and the fraction of the total Hg(II) present in aqueous phase as the neutral complex $HgCl_2$. The numbers over each experimental point correspond to the amounts of added NaCl, in mol·L^{-1}. The error bars represent the difference between two replicates of the same experiment.

Figure 6

Effect of the presence of several divalent cations (concentrations shown in the legend) on the Hg(II) uptake by *C. baccata* at pH 4.5. Initial mercury concentration: 50 mg·L^{-1}; biomass concentration: 2.5 g·L^{-1}. The reference value 100% indicates the mercury sorption in absence of competing cations.

FIGURES

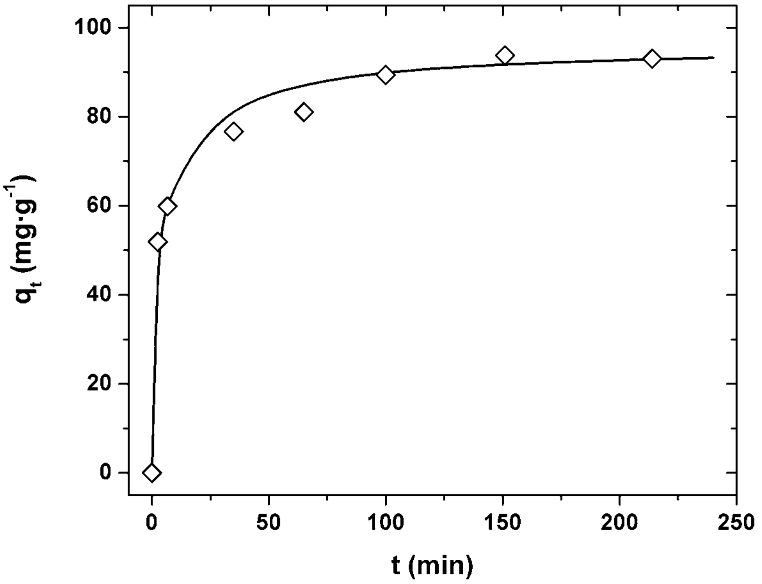

Figure 1

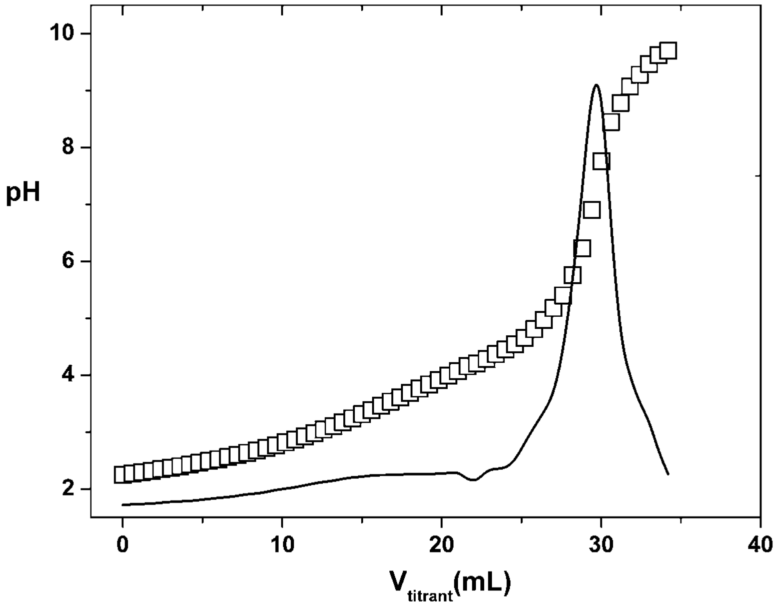

Figure 2

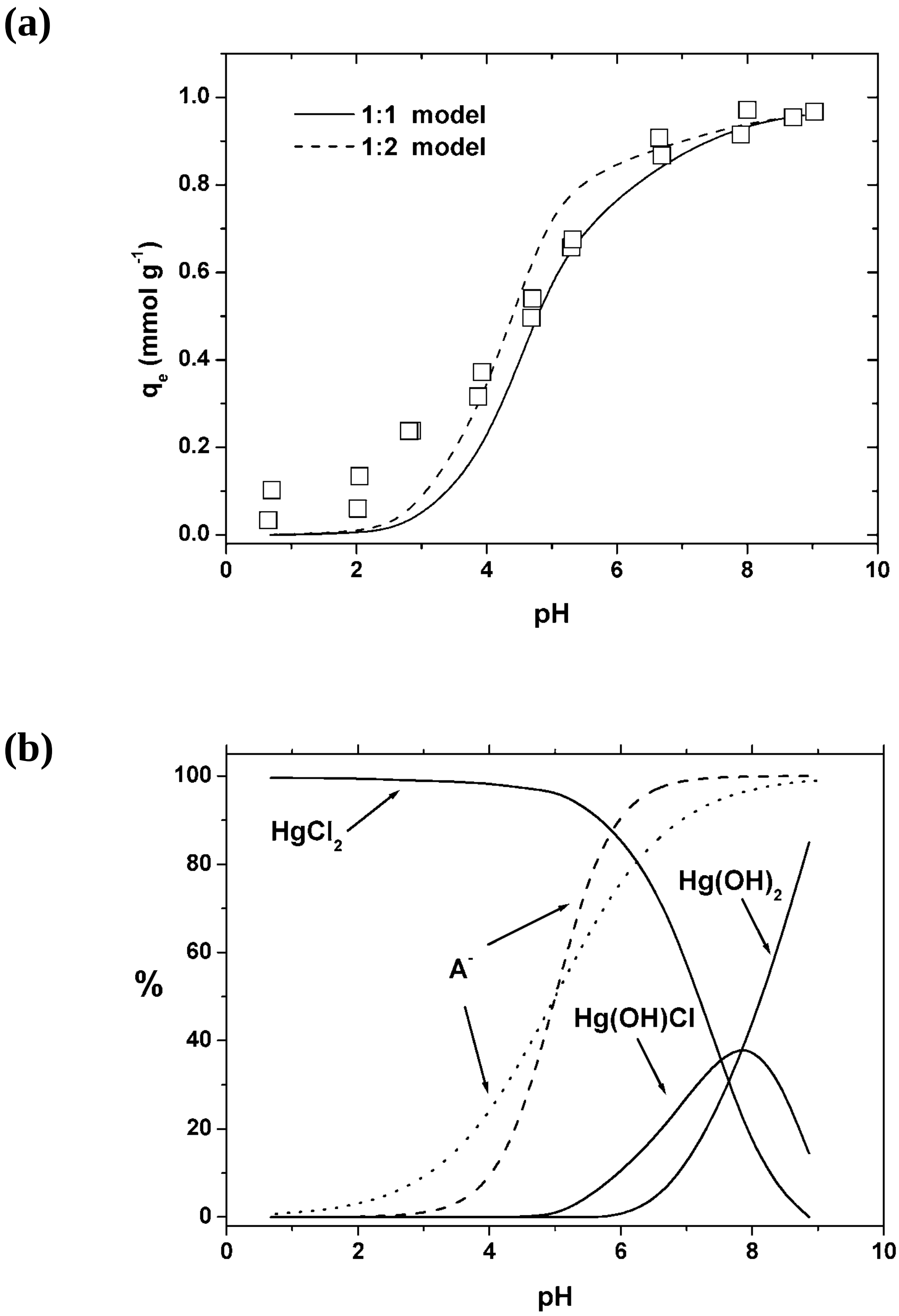
(a)
1:1 model
1:2 model
q_e (mmol g^-1)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
pH
(b)
HgCl2
A^-
Hg(OH)2
Hg(OH)Cl
%
100
80
60
40
20
0
0
2
4
6
8
10
pH

Figure 3

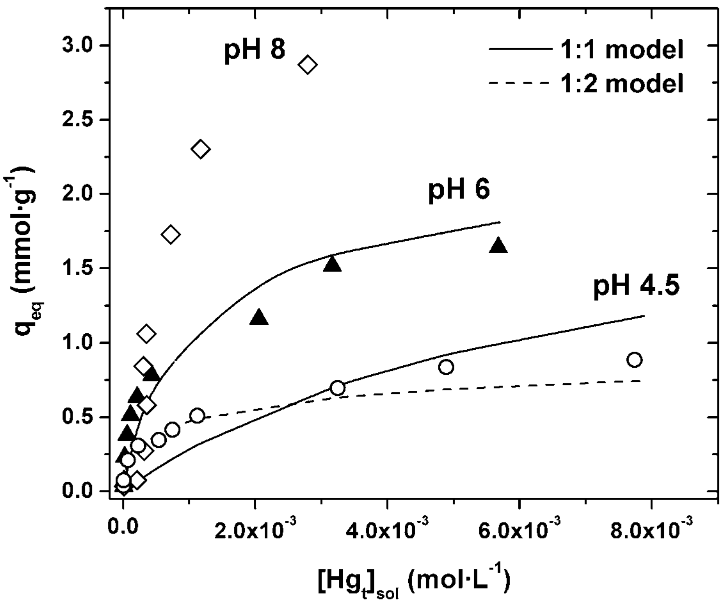
pH 8
1:1 model
1:2 model
pH 6
pH 4.5
q_{eq} (mmol·g^{-1})
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
2.0x10^{-3}
4.0x10^{-3}
6.0x10^{-3}
8.0x10^{-3}
[Hg$_t$]$_{sol}$ (mol·L^{-1})

Figure 4

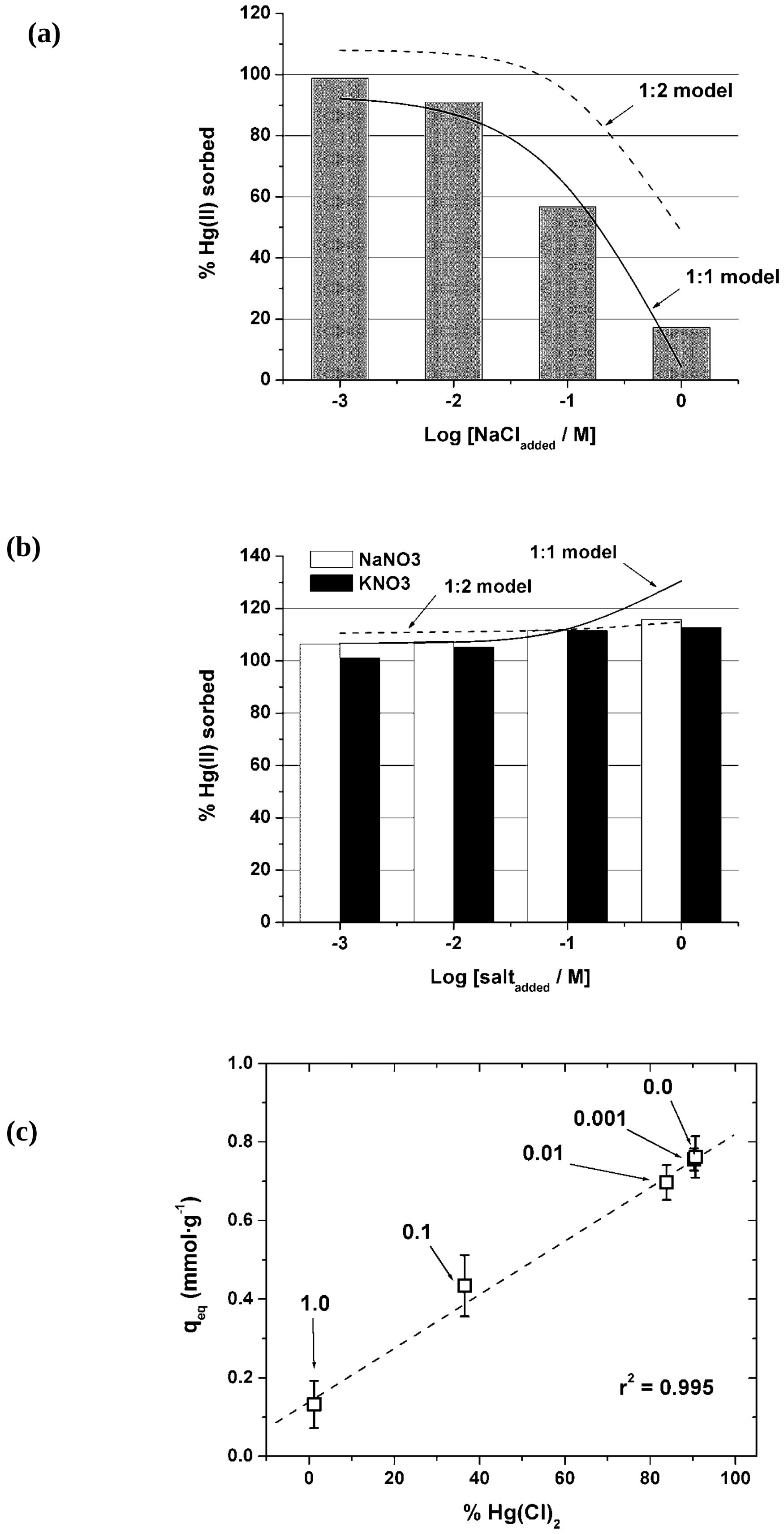
(a)
120
100
80
60
40
20
0
% Hg(II) sorbed
1:2 model
1:1 model
-3
-2
-1
0
Log [NaCl$_{added}$ / M]
(b)
140
120
100
80
60
40
20
0
% Hg(II) sorbed
NaNO3
KNO3
1:1 model
1:2 model
-3
-2
-1
0
Log [salt$_{added}$ / M]
(c)
1.0
0.8
0.6
0.4
0.2
0.0
q_{eq} (mmol·g^{-1})
0.0
0.001
0.01
0.1
1.0
r^2 = 0.995
0
20
40
60
80
100
% Hg(Cl)$_2$

Figure 5

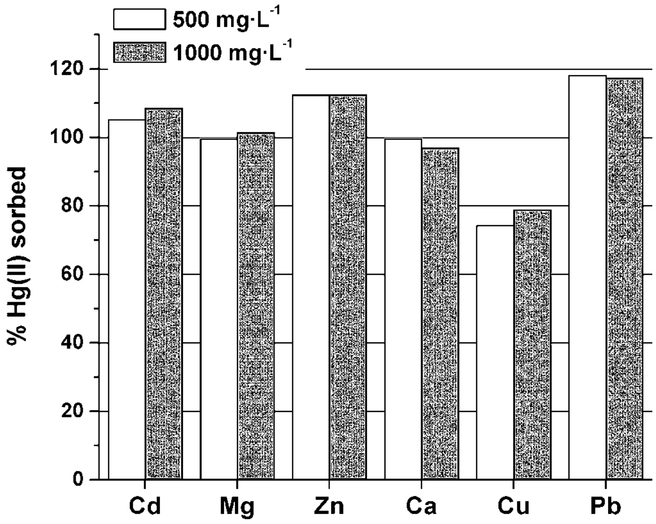
500 mg·L-1
1000 mg·L-1
% Hg(II) sorbed
0
20
40
60
80
100
120
Cd
Mg
Zn
Ca
Cu
Pb

Figure 6

TABLES

Table 1

Parameters of the 1:1 and 1:2 competitive Langmuir models for the binding of neutral Hg(II) species, eqs. (7) and (8). [a] Determined from the equivalence point of the potentiometric titrations; [b] maximum and minimum values in the range 0.004 to 1.0 M ionic strength, estimated through an empirical Donnan expression [Rey-Castro et al. (2004b)] using data from this and previous works [Rey-Castro et al. (2003)]; [c] Average values of the metal binding constants optimized by least squares fit for each set of experiments. These parameters are assumed to remain constant with ionic strength. Errors are shown in brakets.

Model	**Parameters**				
	$[A]_T$ ($mmol \cdot g^{-1}$) [a]	**$\log K_H$** [b]	**$\log K_1$** [c]	**$\log K_2$** [c]	**$\log K_3$** [c]
1:1	2.2 (0.1)	5.0 - 3.2	2.8 (0.1)	4.0 (0.1)	4.0 (0.1)
1:2	2.2 (0.1)	5.0 -3.2	4.0 (0.1)	4.5 (0.5)	5.5 (0.2)

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