PHYSICOCHEMICAL STUDIES OF CADMIUM (II) BIOSORPTION BY THE INVASIVE ALGA IN EUROPE *Sargassum muticum.*

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ABSTRACT

In recent years, there has been a significant increase in the studies concerning brown seaweed as biosorbents for metal removal owing to their high binding ability and low cost. This work reports the results of a study regarding the cadmium binding equilibria of dead biomass from the seaweed *Sargassum muticum*; this alga is a pest fouling organism that competes with the local fucalean species and may also interfere with the "sea industry"; therefore, it would constitute an ideal material to be used as biosorbent.

Seven different treatments were tested in order to obtain a stable biomass that could be suitable for industrial use under a broad range of operational conditions. The treatments employed were protonation, chemical crosslinking with formaldehyde, KOH, $Ca(OH)_2$ and $CaCl_2$ or physical treatments with acetone and methanol.

The equilibrium adsorption isotherms of Langmuir, Freundlich and Langmuir-Freundlich, were obtained for the quantitative description of the cadmium uptake. The effect of pH on biosorption equilibrium was studied at values ranging from 1 to 6, demonstrating the importance of this parameter for an accurate evaluation of the biosorption process. Maximum biosorption was found for pH higher than 4.5. The

maximum biosorption uptake for the raw biomass was 65 mg·g^{-1}, while for formaldehyde crosslinking biomass the uptake increases to 99 mg·g^{-1} and for protonated biomass to 95mg·g^{-1}.

Potentiometric titrations were carried out to estimate the total number of weak acid groups and to obtain their apparent pK value, 3.85, using the Katchalsky model.

Kinetic studies varying cadmium concentration, algal dose and ionic strength were carried out. Over 95% of the maximum cadmium uptake was achieved within 45 minutes, so the process can be considered relatively fast. A pseudo-second order model, for the kinetics of cadmium biosorption, showed to be able to reproduce experimental data points with accuracy.

INTRODUCTION

Sargassum muticum is an invasive alga in Europe. It was first found in Bembridge, Isle of Wight, in 1971. Its native habitats are Japanese and Chinese waters, where it is much smaller than in the European coast. This alga is an alien species which interferes with recreational use of waterways, blocking propellers and intakes; it is also a fouling organism in oyster beds and a nuisance to commercial fishermen (Critchley et al. 1990).

On the other hand, environmental pollution from industrial wastewaters, that contain heavy metal ions, is an important issue in many parts of the world; several technologies have been developed for treating wastewater streams in recent years. Biosorption, which uses the ability of biological materials to accumulate heavy metals,

is one of those technologies that have gained more importance; an increasing amount of work is being focused on it.

Many types of biomass have been reported to have high uptake capacities for heavy metals, including cadmium (Bailey et al. 1999; Wase and Forster 1997), which is included in the "red list" of pollutants by all environment departments and may be regarded as highly toxic. Among these materials, some species of brown marine macroalgae exhibit much higher uptake values than other types of biomass, higher than activated carbon and natural zeolite, and comparable to those of synthetic ion exchange resins. This is the case of the different species of *Sargassum* genus, which have been studied by several authors (Cruz et al. 2004; De França et al. 2002; Vegliò et al. 2003), especially Volesky and coworkers (Davis et al. 2003a; Volesky 1990; Volesky et al. 2003).

The algae cell wall plays an important role in metal binding (Crist et al. 1988), due to its high content in polysaccharides with acid functional groups. The main substances of this type in brown algae are alginates, which usually constitute about 20-40% of the total dry weight (Percival and McDowell 1967), and fucoidans. Alginic acid is a linear polymer of 1,4-linked β-D-mannuronic and α-L-guluronic acids. The monomer sequence and its relation with proton dissociation and metal binding equilibria has been studied by Haug and co-workers (Haug 1961; Haug et al. 1966; Haug et al. 1974). The term "fucoidans" covers a group of partially sulphonated heteropolysaccharides that often contain uronic acids (Lobban and Harrison 1994). In brown algae, the carboxyl groups of alginates are more abundant than either carboxyl or amine groups of the proteins. Therefore, they are likely to be the main functionalities involved in metal binding reactions (Schiewer and Wong 2000).

The purpose of this study was to evaluate the physical chemistry of cadmium biosorption: isotherms, kinetics and acid-base properties of the invasive brown seaweed *Sargassum muticum*; moreover, several chemical modifications of the native alga were tested for their possible use as adsorbent. As stated above, this species is a problem and no general commercial use has been described for it jet, despite its possible value to the alginate industry.

Also, the eradication of this species in European waters has been attempted unsuccessfully; furthermore, no systematic study of the metal biosorption on this alga has been carried out and only a brief mention to the *Sargassum muticum* can be found in bibliography (Davis et al. 2003a; Davis et al. 2000; Rey-Castro et al. 2003).

MATERIALS AND METHODS

Biomass and chemical modifications

Samples of the brown marine alga *Sargassum muticum* were collected from the coast of A Coruña (Galicia, NW Spain) in July 2002 (before the Prestige sinking). The alga was washed with tap and distilled water to eliminate impurities, oven dried at 60 ºC overnight, crushed with an analytical mill, sieved (size fraction of 0.5-1 mm) and stored in polyethylene bottles until use.

In this study *Sargassum muticum* biomass with several pretreatments was examined: alga protonation (Figueira et al. 2000b) was carried out by soaking it in 0.2 M HNO_3 with regular shaking in a rotary shaker (175 rpm) for 3 h, at a biomass concentration of 10 g L^{-1}. Afterwards the material was rinsed thoroughly with deionized water until pH 4.5 was attained and then dried in an oven at 60 ºC. The conversion of this protonated biomass to Ca-, K- derivated (Figueira et al. 2000a), was carried out adding stepwise a 20 mM solution of $Ca(OH)_2$ or KOH, respectively, from an automatic

burette to a solution with biomass avoiding pH from exceeding a value of 6, in order to prevent alga leaching, until the pH value remained constant at 5. For Ca- pretreatment another method was tested with 0.2 M $CaCl_2$ solution for 24 hours under slow stirring (Matheickal et al. 1999).

Washings in methyl alcohol and acetone were carried out with a 100 mL of 50% (v/v) solutions respectively, added to 1 g of biomass and agitated for 24 h (Bai and Abraham 2002).

The formaldehyde crosslinking procedure was essentially the same as reported by Leusch et al. (Leusch et al. 1995): 2.5 g of raw biomass were soaked for 1 hour with 50 mL of a mixture (1:2, v/v) of formaldehyde (36%) and HCl (0.1 M); after that, the alga was filtered and washed with deionized water and a 0.2 M Na_2CO_3 solution.

Following filtration, biomass was dried in an oven at 60 ℃ overnight for all the pretreatment. Biomass weight loss was determined during and after each treatment. Moreover, the spent supernatant solution was analysed for the total organic carbon (TOC). All the experiments were done at least by duplicate.

Equilibrium studies

A volume of 40 mL of eight cadmium(II) solutions of several concentrations (10, 20, 50, 100, 150, 200, 250 and 350 mgL^{-1}), prepared by dissolving $Cd(NO_3)_2 \cdot 4H_2O$ (Merck pro analysis) in distilled water, was placed in a 100 mL Erlenmeyer flask containing 0.1 g of alga. The mixtures were stirred in a rotary shaker at 175 rpm for 3-4 hours until equilibrium was reached; solutions of NaOH and HNO_3 (Merck pro analysis) were used for pH adjustment. After that, the algal biomass was filtered through a 0.45 μm pore size cellulose nitrate membrane filter and the filtrate was analysed for the remaining cadmium ion concentration by differential pulse anodic stripping voltammetry (DPASV) using a 757 VA Computrace (Metrohm) with a

conventional system of three electrodes: hanging mercury drop electrode as working electrode, Pt auxiliary electrode and 3 M Ag/AgCl as reference electrode.

Equation (I) was used to obtain q_{eq} (mg·g^{-1}), the cadmium ion sorbed at equilibrium that represents the metal uptake.

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

where V is the volume of cadmium solution (mL), C_i and C_{eq} are the initial and equilibrium concentration of cadmium in solution (mgL^{-1}), respectively, and m_s is the mass of alga (g).

Influence of pH on metal biosorption

The dependence of *Sargassum* metal uptake on pH was studied for 10 and 250 mgL^{-1} cadmium concentrations in the pH range from 1 to 6. The pH adjustments were carried out using NaOH and HNO$_3$ solutions.

Potentiometric titrations of the biomass samples

For each titration, ca. 0.2 g of protonated *Sargassum* biomass was placed in a thermostated glass cell at a temperature of 25.0±0.1 ºC, and 40 mL of 0.05M NaNO$_3$ solution were added to keep ionic strength constant. A certain amount of HCl was also added to yield an initial pH value ca. 2. The stirred suspension was allowed to equilibrate until the electromotive force (emf) measure was stable before the titrations started. The titrating solutions were added from a Crison microBu 2031 automatic burette. Electromotive force measurements were done with a Crison micropH 2000 meter equipped with a Radiometer GK2401C combination glass electrode (sat. Ag/AgCl as reference). After each addition of titrant (NaOH, prepared with boiled deionized water) the system was allowed to equilibrate until 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 describe elsewhere (Brandariz et al. 1998; Fiol et al. 1992). The electrodes were calibrated in the acid pH range, slopes were within 4% of the theoretical Nernst value, coefficients of determination (r^2) for the Nernst type equation were always greater than 0.9999.

A nitrogen stream was used to remove dissolved O_2 and CO_2 during titration experiments.

Kinetic studies

A cadmium ion selective electrode (CdISE, Orion) with a Ag/AgCl reference electrode (Orion), previously calibrated in cadmium concentration, was employed to follow the reaction kinetics. Comparative measurements were also made using DPASV technique. Experiments were carried out in a glass cell furnished with a thermostated jacket at a temperature of 25.0±0.1 °C maintaining pH=4.5±0.1; a nitrogen stream was used to remove dissolved O_2 and CO_2. Three groups of experiments were run:

Effect of initial metal concentration: A sample of 0.25 g of alga was added to 100 mL volume of several cadmium solutions (10, 50, 100 and 250 mgL^{-1}) with ionic strength adjusted to 0.05M with $NaNO_3$.

Effect of alga dose: Several alga weights (0.12, 0.25, 0.40, 0.50, 0.60 g) were added to a 100 mL volume of 100 mgL^{-1} cadmium solution with ionic strength adjusted to 0.05M with $NaNO_3$.

Effect of ionic strength: Four concentrations of $NaNO_3$ (0.05, 0.1, 0.2, and 0.5 M) were used for ionic strength adjustment of a 100 mgL^{-1} cadmium solution (0.25 g algal mass).

RESULTS AND DISCUSSION

Equilibrium studies

Adsorption isotherms

The adsorption of a substance from one phase to the surface of another in a specific system, leads to a thermodynamically defined distribution of that substance between the phases as the system reaches equilibrium. This distribution can be expressed in terms of adsorption isotherms (Kinniburgh 1986), whereby the metal species sequestered by the sorbent (alga) through a number of several mechanisms, is in equilibrium with its residue left free in the solution.

In order to developed an operational procedure, different absorbents could be compared quantitatively under several conditions by using an equation to analyse equilibrium data. Ideally, the models used for quantitative description of biosorption should be capable of predicting metal biosorption, reflecting the mechanisms of this sorbate uptake. However, the adsorption isotherms most commonly used in biosorption, the Langmuir and Freundlich models, were developed under many assumptions that are well known not met in the case of biosorption; for instance, they do not take into account the fact that metal ion biosorption is mainly an ion exchange phenomenon (Crist et al. 1992).

We selected the simple Langmuir, Freundlich and Langmuir-Freundlich isotherm equations (Table I) to fit the experimental data points. The main reason for the extended used of these isotherms is that they incorporate constants easily interpretable. These constants reflect the feature of the sorbent and can be used to compare the biosorption performance: as showed in Table I, q_{max}, represents the maximum biosorption and b is an affinity parameter. A high value of parameter b indicates a steep desirable beginning of the isotherm, reflecting the high affinity of the biosorbent for the

sorbate; *n* is an empirical parameter, which varies with the degree of heterogeneity and K_f relates to biosorption capacity. Moreover, these models are capable of reflecting satisfactorily the experimental data if environmental parameters, such as pH, are controlled carefully during experiments.

Figure I shows Langmuir fitted data, corresponding to the cadmium biosorption by raw *Sargassum muticum* biomass at three different pH values (3, 4.5 and 6). The adjustable parameters obtained (q_{max}, *b, n* and K_f) are listed in Table II, showing that Langmuir model exhibits lower errors and better fits than Freundlich model; in addition, Langmuir-Freundlich isotherm can describe experimental data with similar accuracy, although three parameters are necessary to fit data, so the errors increase (data fit not shown).

To obtain biomass stable and suitable for industrial use, it may be necessary to reinforce the algae. Reinforcement can be achieved by chemical crosslinking (e.g., with formaldehyde) or by simple physical treatment (Volesky 1990).

Figure II shows biosorption isotherms at pH=4.5±0.1 for all the treatments carried out with *Sargassum muticum*; the experimental data points were fitted to the Langmuir isotherm equation and the corresponding adjustable parameters are shown in Table III; in the case of $CaCl_2$ a slight reduction in the biosorption capacity (per total mass) as result of the addition of inert materials or blocking or consumption of binding sites can be observed. However, most of the treatments used enhance the alga cadmium uptake and the affinity of the biosorption due probably to a better accessibility of the binding sites for the metals or an increase in the quantity of these sites.

In order to control biomass stability, Table III shows the percentages of algal weight loss in water during 3-4 h of stirring (W_2) and the percentages of weight loss due to treatments as well (W_1), based in the final dry weight compared to the initial weight.

Total organic carbon leached from the biomass was measured in the spent supernatant solution after biosorption equilibrium was attained with 350 mgL^{-1} of cadmium solution (the maximum cadmium concentration used in the experiments).

The results from Table III demonstrate that the major biomass leaching and weight loss occur with the raw biomass. As showed, metal ions can stabilize biosorbents, in many cases, by crosslinking of the active sites. In all the procedures, biomass weight loss and TOC values decrease, indicating stabilization of the biomass attained through the modification process.

The raw biomass is stabilized with the majority cations present in seawater, mainly Na, K, Ca and Mg. As a result of the acid treatment, these ions binded to active sites are substituted by protons, obtaining one of the greatest weight losses(Crist et al. 1992). Evidences of this fact are supported by comparing the potentiometric titration of protonated (Figure V) and raw biomass (data not shown); in the latter, no evidence of protons is found. The acid wash releases significant quantities of organic carbon, although lower than raw biomass. The acid treatment is one of the most aggressive for the stability of the cell structure, by releasing a substantial portion of soluble biomass material, smaller organic molecules and ions. Similar percent weight losses were found with methanol and acetone washes, in this case probably due to the extraction of proteins and lipid fractions of the biomass surface (Bai and Abraham 2002). Formaldehyde crosslinking has a previous acid treatment included in the weight loss value; even so, the high value obtained account for the weight loss for acid wash, in contrast to KOH and Ca(OH)$_2$ treatments, with lower leaching values.

On the other hand, no significant differences were found between the two calcium treatments, both giving very low values for biomass losses and organic leaching; the effectiveness of these treatments in stabilizing the biomass is due to the

crosslinking reaction taking place in the alginate molecules, which results in a cooperative association of long regions of polymer chains, leading to an "egg-carton" structure (Lobban and Harrison 1994).

As showed in Table III, there is a small biomass weight loss after the treatment, so treated alga can still leach some material during the biosorption process; nevertheless, these biomass losses do not affect significantly to the uptake processes.

As a result of this study, different useful choices are possible in order to modify the raw biomass. Both methods, protonation and crosslinking with formaldehyde, lead to higher uptakes that increase nearly 50 % the uptake of the raw biomass. But both of them are also associated with the highest values of TOC in solution. This fact reveals an important leaching of biomass during the modification of the alga. On the other hand, uptakes of calcium-treated biomass remain practically the same as raw biomass. The effect of calcium ions is to stabilize the biomass by means of the formation of an "egg-box structure", which is reflected by the low TOC leached to solution and the lowest values of the percent loss of mass during treatment and during the biosorption process. This behaviour can suppose a great advantage when the algal biomass has to be employed in columns for several adsorption/desorption cycles.

The maximum cadmium(II) uptake values found in this work for modified or raw biomass (65-99 mg·g^{-1}) are similar to values found in the literature for other algae of the *Sargassum* genus (70-120 mg·g^{-1}) or other marine algae (Cruz et al. 2004; Davis et al. 2003b). Usually, biosorption capacities of marine algae are higher than the exhibited by many of the adsorbents commonly used in practical metal recovery applications. As an example, reported values of 43 mg·g^{-1} for yeast (Chang et al. 1997), 19-31 mg·g^{-1} for fungus (Holan and Volesky 1995), 14 mg·g^{-1} for chitin (Benguella and Benaissa 2002) or 8 mg·g^{-1} for active carbon (Leyva-Ramos et al. 1997) can be found in

literature. More extensive compilations of the adsorption of metals by different biosorbent materials can be found in the literature.(Bailey et al. 1999; Volesky 1990; Wase and Forster 1997)

Effect of pH on cadmium uptake

The biosorption capacity of the algae strongly depends on the initial solution pH, so characterization of the pH effect on biosorption equilibrium is necessary for an accurate evaluation of biosorption process (Wase and Forster 1997).

The speciation diagram calculated with the program MINEQL+ (Schecher and McAvoy 1992) (Figure III) confirms that cadmium (II) ion is the relevant species in the range of pH studied; at pH values greater than 8, insoluble cadmium hydroxides are formed with total cadmium concentration less than 450 mgL^{-1}, so precipitation may occur; on the other hand, an extremely acid pH can damage the structure of the algae. Moreover, the pH can change the state of the active binding sites of the algae, mainly carboxyl groups of alginates.

Figure IV shows the dependence of cadmium uptake with the solution pH at two different initial metal concentrations. From the s-shape of the curve, it is seen that there is a slight increase in the pH range between 1.0 and 2.0, a fast increase in cadmium uptake with increasing pH from 2.0 to 4.0, while around pH 4.5, the cadmium biosorption capacity levelled off at a maximum value reaching a plateau. However, at pH less than 2, the cadmium uptake capacity is almost negligible, but not zero; it could be explained by metal ion binding to strong acidic groups that do not become protonated at this pH (Schiewer and Volesky 1995). Equilibrium experiments were carried out at pH 4.5, where the maximum uptake capacity was achieved, avoiding cadmium precipitation.

The increase in cadmium biosorption from pH 2.0 to pH 4.0 can be explained by the change in the ionic state of the carboxyl functional groups, which present a pK_a value between 2-4 (Haug and Smidsrod 1970; Rey-Castro et al. 2004a) (this work, see below). This implies that the metal biosorption depends on the protonation or deprotonation of the cell wall polymer functional groups. At pH values lower than pK_a, carboxylate groups are closely associated with the hydrogen ions, restricting access to sites to cadmium ions as a result of repulsive forces, and resulting in a low cadmium uptake. This effect is stronger at lower pH. At pH values higher than pK_a, more carboxylate groups carry negative charge and the positively charged cadmium ions will be bound, reducing the electrostatic repulsion and increasing the cadmium uptake. These experimental observations can be explained by an exchange between the metal ions and protons initially present in the biomass and the metal in solution (Crist et al. 1999).

Acid-base properties

In order to estimate the total amount of active sites in protonated *Sargassum* biomass, a simple potentiometric titration was carried out with a NaOH solution. The number of carboxyl groups per gram of alga, $[COOH]_{total}$ (mmol g^{-1}), was calculated by estimation of the position of inflection points (V_{eq}) in the resulting titration curves (Figure V), using the following equation:

$$[COOH]_{total} = \frac{V_{eq} \cdot [NaOH]}{m_s} \qquad (II)$$

Sulfate groups are known to be present in the *Sargassum* alga (Percival and McDowell 1967), however, no evidence of their presence was found in the titration curves. The total number of weak acid groups found was 2.61 mmol g^{-1}, determined in 0.05M NaNO$_3$. As expected, ionic strength does not influence in the number of acidic

groups titrated, but strongly affects their apparent pK values. A physicochemical model that accounts for the effects of pH and ionic strength on the proton binding equilibria of algae was recently proposed by Rey-Castro et al.(Rey-Castro et al. 2003)

The proton binding active zone of the algal biomass is supposed to be constituted of a polyelectrolyte that forms a charged, three-dimensional structure. Moreover, surface charge models also reproduced with similar accuracy experimental equilibrium data (Rey-Castro et al. 2004b).

Katchalsky found that the titration curve of a polyacid could be empirically described by two constants, pK and n (Katchalsky et al. 1954), according to the equation:

$$pH = pK - n \cdot \log \frac{1-\alpha}{\alpha} \qquad \text{(III)}$$

where α represents the degree of dissociation, defined in Equation (IV), and n is an empirical parameter that accounts for the chemically heterogeneous nature of the algal biomass, and whose value is greater than one,

$$\alpha = \frac{\left[COO^-\right]}{(C_0 \cdot V_0)/(V_0 + V_{NaOH})} \qquad \text{(IV)}$$

where C_0 is the initial concentration of the carboxylic acid, V_0 is the initial volume and V_{NaOH} is the volume of NaOH added during titration.

From the slope and intercept of straight line plots of pH against $log((1-\alpha)/\alpha)$, $pK_{\alpha=0.5}$ and n values were experimentally determined. Figure VI shows these values and the fit of the experimental data points to Equation (III) with the coefficient of determination.

The obtained $pK_{\alpha=0.5}$ value is 3.85 which agrees well with the values corresponding to carboxyl groups from mannuronic and guluronic acids (3.38 and 3.65)

of alginate (Haug 1961) or to lately calculated pK values for alginic acid (Rey-Castro et al. 2004a); so these groups are likely to be responsible for cadmium biosorption.

Kinetic studies

Equilibrium relationships comprise different conditions for biosorption processes attributed to the necessary time for a system to achieve thermodynamic stability. Whereas the extent of biosorption is dependent only on the initial and final equilibrium states, the rate of biosorption is dependent on the way leading from the initial to the final state. In porous media, these include three steps (Weber Jr. et al. 1991): film diffusion of the sorbate (metal) from the bulk solution to the surface of the biosorbent particle, followed by diffusion through the particle and chemical binding reaction of the sorbate.

External film diffusion resistance can be eliminated if shaking rate is enough to assure that all the cell wall binding sites are really available for metal uptake; we selected a shaking rate of 175 rpm, which is enough to neglect the effect of film diffusion (Cruz et al. 2004). The rate at which biosorption takes place has great importance when designing biosorption systems; kinetic models are important for investigating the biosorption mechanism and rate controlling steps, such as pore diffusion and chemical reaction process. Several kinetics models have been used to fit experimental data, so it is important to establish the time dependence of such systems under various process conditions (Aharoni and Sparks 1991).

Biosorption of metals by biomass cell surfaces is generally considered a fast process; the decrease of cadmium concentration in solution with time showed that the rate of cadmium uptake is rather fast; in general, the system reached over 50% of the maximum cadmium uptake by the biomass within 5 minutes of contact and it was observed that over 95% of the maximum cadmium uptake was removed from solution

within 45 minutes; on the other hand, it was observed that there were not significant differences in the time necessary to achieve the 50 % of maximum metal uptake when experiment conditions were changed (see Table IV).

In an attempt to present an equation representing kinetic biosorption of cadmium onto alga, several mechanisms were proposed: Elovich, pseudo-first order, diffusion (Aharoni and Sparks 1991) and pseudo-second order (Ho 2003); the Elovich and intraparticle diffusion models fit well until the first 5 or 10 minutes of experimental data respectively (data fit not shown), although best data fits, in the whole data range, were found with a pseudo-second order process. In this model, the rate limiting step is a biosorption mechanism involving chemisorption, where metal removal from solution is due to purely physicochemical interactions between biomass and metal solution (Aksu 2001). However, the fact that experimental data may be fitted by a given rate expression is not sufficient evidence that the molecularity of the reaction is that implied by the rate expression.

The kinetic rate equation proposed by Ho (Ho and McKay 2000; Ho et al. 1996), Equation (V), can be considered a pseudo-second order chemical biosorption process with respect to the alga biosorption sites, due to the fact that cadmium displace divalent ions, among others, from these sites.

$$\frac{dq_t}{dt} = k \cdot (q_{eq} - q_t)^2 \tag{V}$$

where k (g mg^{-1}min^{-1}) is the pseudo-second order constant of biosorption, q_t (mg g^{-1}) is the metal uptake at time t and q_{eq} (mg g^{-1}) is the cadmium ion sorbed at equilibrium, which is given by Equation (I).

Integrating and separating variables in Equation (V) for the boundary conditions: $q_t = 0$ at $t = 0$ and q_t at time t, the following equation is obtained:

$$q_t = \frac{q_{eq}^2 \cdot k \cdot t}{1 + q_{eq} \cdot k \cdot t} \tag{VI}$$

This equation can be rearranged to obtain a linear form law for a pseudo-second order process:

$$\frac{t}{q_t} = \frac{1}{k \cdot q_{eq}^2} + \frac{1}{q_{eq}} \cdot t \tag{VII}$$

The equilibrium biosorption capacity and the pseudo-second order rate constant were experimentally determined from slope and intercept of straight line plots of t/q_t against t. The pseudo-second order kinetic model has been applied successfully to metal biosorption by several biomasses like microalga (Aksu 2001), yeast (Vasudevan et al. 2003), peat (Ho et al. 2000), macroalga (Cruz et al. 2004) or tree fern (Ho 2003).

A cadmium ion selective electrode (CdISE) was employed to obtain the cadmium concentration in the kinetic studies; moreover, additional measurements were made with DPASV technique in order to compared both methods; better results were found with CdISE, since this technique allows to obtain easier and faster a great number of experimental points.

Effect of initial metal concentration

Experimental data points obtained for the biosorption of cadmium by *Sargassum muticum* at several cadmium concentrations are shown in Figure VII. Good fits between predicted curves, with a pseudo-second order model, and experimental data points were found (fits not shown; see coefficients of determination in Table V), supporting the idea that chemisorption is the rate-limiting step and that the mechanism follows a pseudo-second order process model.

Table V shows the percentage of cadmium removed from solution and the constants obtained from Equation (VII), q_{eq} and k. All the fits show very good coefficients of determination. The equilibrium biosorption capacity, q_{eq}, increases with

an increase in the initial cadmium concentration, however, the percentage of removed metal decreased. The values of q_{eq} obtained from the fitting to the pseudo second order model are very similar to the experimental values obtained from the equilibrium biosorption isotherm. This fact constitutes an additional confirmation of the pseudo second order model. On the other hand, the rate constant, k, were found to increase with the reduction in cadmium concentration.

The corresponding linear plots of the values of q_{eq}, and k against C_i (initial cadmium concentration) were regressed to obtain empirical expressions for these values in terms of C_i. The best fits were found with exponential expressions (fits not shown). Equation parameters are the following:

$$q_{eq} = (0.66 \pm 0.12) \cdot C_i^{(0.82 \pm 0.04)} \qquad r^2 = 0.998$$
$$k = (0.56 \pm 0.07) \cdot C_i^{-(0.58 \pm 0.04)} \qquad r^2 = 0.993$$

(VIII)

Substituting the values of q_{eq} and k from the equations above in Equation (VI), the rate law for a pseudo-second order reaction and the relationship between q_t, C_i and t can be represented as:

$$q_t = \frac{0.25 \cdot C_i^{1.06} t}{1 + 0.37 \cdot C_i^{0.24} t}$$

(IX)

This equation can be used to derive the amount of cadmium removed at any given ion concentration and reaction time, as showed in Figure VII (solid lines), where very good accuracy with experimental data can be observed .

Effect of alga dose

The results of the effect of *Sargassum muticum* dose on experiments carried out are shown in Figure VIII. The rate constant and the equilibrium biosorption capacity were calculated from the intercept and slope of the straight line plots of t/q_t against t in

Equation (VII), and are shown in Table V with the corresponding coefficients of determination.

The q_{eq} values increased with a decrease in alga mass but, as expected, there was an increase in the percentage of cadmium removed from solution with an increase in alga mass, due to the raise in the amount of activated biomass sites (see Table V).

The data showed a good compliance with the pseudo-second order model and the coefficients of determination of the linear plots were very good. The corresponding linear plots of the values of q_{eq} and k against m_s were regressed to obtain expressions for these values in terms of the m_s parameters as follows:

$$
\begin{aligned}
&q_{eq} = (10.9 \pm 0.2) m_s^{-(0.67 \pm 0.01)} && r^2 = 0.999 \\
&k = (0.037 \pm 0.004) m_s^{(0.81 \pm 0.14)} && r^2 = 0.963
\end{aligned}
\tag{X}
$$

Substituting the values of q_{eq} and k from the equations above in Equation (VI), the rate law for a pseudo-second order reaction and the relationship between q_t, m_s and t can be represented as:

$$
q_t = \frac{4.4 \cdot m_s^{-0.53} t}{1 + 0.4 \cdot m_s^{0.14} t}
\tag{XI}
$$

This equation can be used to derive the amount of cadmium sorbed at any given alga dose and the reaction time, as showed in Figure VIII (solid lines). Very good compliance with experimental data can be observed, likewise in the study of the effect of initial cadmium concentration.

Effect of ionic strength

Results obtained from the biosorption of cadmium by *Sargassum muticum* at several ionic strengths are shown in Figure IX. The rate constant and the equilibrium biosorption capacity were calculated from the intercept and slope of the straight line plots of t/q_t against t in Equation (VII), and are listed in Table V with the corresponding

coefficients of determination. As expected, a decrease in cadmium biosorption was observed with an increase in ionic strength, due to the strong competitiveness of electrolyte ions for the biomass adsorption sites (Schiewer and Wong 2000).

In order to explain the influence of electrolyte concentration on cadmium biosorption, we fitted the parameter q_{eq} to an empirical expression like Debye-Hückel equation, which accounts for the effect of ionic strength (Sastre de Vicente 1997), :

$$\log(q_{eq}) = A + B \cdot I^{1/2} \tag{XII}$$

parameter k seems to remain practically constant in the ionic strength range studied. The corresponding equations are the following:

$$q_{eq} = 10^{\left[(1.58\pm0.03)-(0.44\pm0.07)\cdot I^{1/2}\right]} \qquad r^2 = 0.95 \tag{XIII}$$

$$k = 0.013 \pm 0.003$$

Substituting the values of q_{eq} and k from the equations above in Equation (VI), the rate law for a pseudo-second order reaction and the relationship between q_t, I and t can be represented as:

$$q_t = \frac{0.013 \cdot 10^{\left(3.16-0.88\cdot I^{1/2}\right)} \cdot t}{1 + 0.013 \cdot 10^{\left(1.58-0.44\cdot I^{1/2}\right)} \cdot t} \tag{XIV}$$

This equation can be used to derive the amount of cadmium sorbed at any given ionic strength, as showed in Figure IX (solid lines); the results obtained with this equation were not as good as with Equation (IX) and (XI) due probably to the simplicity of the model accounting for the ionic strength effects (Sastre de Vicente 1997).

CONCLUSIONS

The invasive alga in Europe, *Sargassum muticum*, can be considered as a promising biosorbent material. It could compete with commercial biosorbents because of its low cost, among several reasons studied in this paper summarizes below.

High metal uptake values were found under many operational conditions, such as: pH, cadmium concentration, alga mass or ionic strength. Moreover, several pretreatments were proposed in order to increase the metal removal and affinity constant: the protonated and formaldehyde crosslinked biomass showed around 50% of increase in the q_{max} value and more than 175% in b value. These parameters were determined using the Langmuir isotherm model. The maximum metal uptake for the raw biomass was 65 mg·g^{-1}, for the protonated biomass 95 mg·g^{-1} and for the formaldehyde-biomass 99 mg·g^{-1}.

The stability of the alga, attained through the modification process, was also improved by the decrease in biomass weight loss (between 60-99.67%) and TOC values (between 31-96%) for all the pretreatments studied.

The solution pH is an important parameter, which affects biosorption of cadmium by the alga. The fact that at pH value lower than 2 the cadmium uptake capacity is almost negligible, is interesting in order to regenerate the biomass in desorption cycles. Maximum uptakes were found for pH higher than 4.5.

On the other hand, cadmium biosorption kinetic is relatively fast, with 95% of the maximum metal uptake remove from solution within 45 minutes. A pseudo-second order empirical model can describe this process, supporting the idea that chemisorption is the rate-limiting step. Simple empirical equations were obtained to derive reaction time and the amount of cadmium removed at any given ion concentration, alga mass or ionic strength.

These results obtained in the study, demonstrated the suitability of the alga *Sargassum muticum* for industrial use as biosorbent, although more experiments have to be carried out for its practical applicability.

REFERENCES

Aharoni C, Sparks DL. 1991. Kinetics of Soil Chemical Reactions-A theoretical treatment. In: Sparks DL, Suárez DL, editors. Rates of soil chemical processes. Madison (WI): Soil Science Society of America. p 1-18.

Aksu Z. 2001. Equilibrium and kinetic modeling of cadmium (II) biosorption by C. vulgaris in a batch system: effect of temperature. Separation and Purification Technology 21:285-294.

Bai RS, Abraham TE. 2002. Studies on enhancement of Cr(VI) biosorption by chemically modified biomass of Rhizopus nigricans. Water Research 36:1224-1236.

Bailey SE, Olin TJ, Bricka RM, Adrian DD. 1999. A review of potentially low-cost sorbents for heavy metals. Water Research 33(11):2469-2479.

Benguella B, Benaissa H. 2002. Cadmium removal from aqueous solutions by chitin: kinetic and equilibrium studies. Water Research 36:2463-2474.

Brandariz I, Vilariño T, Alonso P, Herrero R, Fiol S, Sastre de Vicente ME. 1998. Effect of ionic strength on the formal potential of the glass electrode in various saline media. Talanta 46:1469-1477.

Chang JS, Law R, Chang CC. 1997. Biosorption of lead, copper and cadmium by biomass of Pseudomonas Aeruginosa PU21. Water Research 31(7):1651-1658.

Crist RH, Martin JR, Crist DR. 1999. Interaction of metal ions with acid sites of biosorbents peat moss and Vaucheria and model substances alginic and humic acids. Environmental Science & Technology 33(13):2252-2256.

Crist RH, Oberholser K, McGarrity J, Crist DR, Johnson JK, Brittsan JM. 1992. Interaction of metals and protons with algae. 3. Marine algae, with emphasis on lead and aluminum. Environmental Science & Technology 26(3):496-502.

Crist RH, Oberholser K, Schwartz D, Marzoff J, Ryder D, Crist DR. 1988. Interactions of metals and protons with algae. Environmental Science & Technology 22(7):755-760.

Critchley AT, Farnham WF, Yoshida T, Norton TA. 1990. A bibliography of the invasive alga Sargassum muticum (Yendo) Fensholt (Fucales; Sargassaceae). Botanica Marina 33:551-562.

Cruz CCV, Costa ACA, Henriques CA, Luna AS. 2004. Kinetic modeling and equilibrium studies during cadmium biosorption by dead Sargassum sp biomass. Bioresource Technology 91:249-257.

Davis TA, Llanes F, Volesky B, Mucci A. 2003a. Metal selectivity of Sargassum spp. and their alginates in relation to their α-L-guluronic acid content and conformation. Environmental Science & Technology 37(2):261-267.

Davis TA, Volesky B, Mucci A. 2003b. A review of the biochemistry of heavy metal biosorption by brown algae. Water Research 37:4311-4330.

Davis TA, Volesky B, Vieira RHSF. 2000. Sargassum seaweed as biosorbent for heavy metals. Water Research 34(17):4270-4278.

De França FP, Tavares APM, Da Costa ACA. 2002. Calcium interference with continuous biosorption of zinc by Sargassum sp. (Phaeophyceae) in tubular laboratory reactors. Bioresource Technology 83:159-163.

Figueira MM, Volesky B, Azarian K, Ciminelli VST. 2000a. Biosorption column performance with a metal mixture. Environmental Science & Technology 34(20):4320-4326.

Figueira MM, Volesky B, Ciminelli VST, Roddick FA. 2000b. Biosorption of metals in brown seaweed biomass. Water Research 34(1):196-204.

Fiol S, Arce F, Armesto XL, Penedo F, Sastre de Vicente ME. 1992. Analysis of systematic errors in calibrating glass electrodes with H^+ as a concentration probe. Fresenius Journal of Analytical Chemistry 343:469-472.

Haug A. 1961. The affinity of some divalent metals to different types of alginates. Acta Chemica Scandinavica 15(8):1794-1795.

Haug A, Larsen B, Smidsrod O. 1966. A study of the constitution of alginic acid by partial acid hydrolysis. Acta Chemica Scandinavica 20(1):183-190.

Haug A, Larsen B, Smidsrod O. 1974. Uronic acid sequence in alginate from different sources. Carbohydrate Research 32:217-225.

Haug A, Smidsrod O. 1970. Selectivity of some anionic polymers for divalent metal ions. Acta Chemica Scandinavica 24(3):843-854.

Ho YS. 2003. Removal of copper ions from aqueous solution by tree fern. Water Research 37:2323-2330.

Ho YS, McKay G. 2000. The kinetics of sorption of divalent metal ions onto sphagnum moss peat. Water Research 34(3):735-342.

Ho YS, McKay G, Wase DAJ, Forster CF. 2000. Study of the sorption of divalent metal ions on to peat. Adsorption Science & Technology 18(7):639-650.

Ho YS, Wase DAJ, Forster CF. 1996. Kinetic studies of competitive heavy metal adsorption by sphagnum moss peat. Environmental Technology 17:71-77.

Holan ZR, Volesky B. 1995. Accumulation of cadmium, lead and nickel by fungal and wood biosorbents. Applied Biochemistry and Biotechnology 53(2):133-146.

Katchalsky A, Shavit N, Eisenberg H. 1954. Dissociation of weak polymeric acids and bases. Journal of Polymer Science 13:69-84.

Kinniburgh DG. 1986. General purpose adsorption isotherms. Environmental Science & Technology 20(9):895-904.

Leusch A, Holan ZR, Volesky B. 1995. Biosorption of heavy metals (Cd, Cu, Ni, Pb, Zn) by chemically-reinforced biomass of marine algae. Journal of Chemical Technology and Biotechnology 62:279-288.

Leyva-Ramos R, Rangel-Mendez JR, Mendoza-Barron J, Fuentes-Rubio L, Guerrero-Coronado RM. 1997. Adsorption of cadmium(II) from aqueous solution onto activated carbon. Water Science and Technology 35(7):205-211.

Lobban CS, Harrison PJ. 1994. Seaweed ecology and physiology. Cambridge [England] ; New York, NY, USA: Cambridge University Press. ix, 366 p.

Matheickal JT, Yu Q, Woodburn GM. 1999. Biosorption of cadmium (II) from aqueous solutions by pre-treated biomass of marine alga Durvillaea potatorum. Water Research 33(2):335-342.

Percival E, McDowell RH. 1967. Chemistry and enzymology of marine algal polysaccharides. London New York: Academic Press. xii, 219 p.

Rey-Castro C, Herrero R, Sastre de Vicente ME. 2004a. Gibbs-Donnan and specific ion interaction theory descriptions of the effect of ionic strength on proton dissociation of alginic acid. Journal of Electroanalytical Chemistry 564:223-230.

Rey-Castro C, Herrero R, Sastre de Vicente ME. 2004b. Surface charge and permeable gel descriptions of the ionic strength influence on proton binding to seaweed biomass. Chemical Speciation and Bioavailability 16(1-2):61-69.

Rey-Castro C, Lodeiro P, Herrero R, Sastre de Vicente ME. 2003. Acid-base properties of brown seaweed biomass considered as a Donnan Gel. A model reflecting electrostatic effects and chemical heterogeneity. Environmental Science & Technology 37(22):5159-5167.

Sastre de Vicente ME. 1997. Ionic strength effects on acid-base equilibria. A review. Current Topics in Solution Chemistry 2:157-181.

Schecher WD, McAvoy DC. 1992. MINEQL+: A software environment for chemical equilibrium modeling. Computers, Environment and Urban Systems 16(1).

Schiewer S, Volesky B. 1995. Modeling of the proton-metal ion exchange in biosorption. Environmental Science & Technology 29(12):3049-3058.

Schiewer S, Wong MH. 2000. Ionic strength effects in biosorption of metals by marine algae. Chemosphere 41:271-282.

Vasudevan P, Padmavathy V, Dhingra SC. 2003. Kinetics of biosorption of cadmium on Baker´s yeast. Bioresource Technology 89:281-287.

Vegliò F, Esposito A, Reverberi AP. 2003. Standardisation of heavy metal biosorption tests: equilibrium and modelling study. Process Biochemistry 38:953-961.

Volesky B. 1990. Biosorption of heavy metals. Boca Raton, Flo.: CRC Press. 396 p.

Volesky B, Weber J, Park JM. 2003. Continuous-flow metal biosorption in a regenerable Sargassum column. Water Research 37:297-306.

Wase J, Forster CF. 1997. Biosorbents for metal ions. London: Taylor & Francis. x, 238 p.

Weber Jr. WJ, McGinley PM, Katz LE. 1991. Sorption phenomena in subsurface systems: concepts, models and effects on contaminant fate and transport. Water Research 25(5):499-528.

Tables

Table I. Adsorption isotherm equations

Isotherm	Equation	Number of adjustable parameters
Langmuir	$q_{eq} = \dfrac{q_{max} \cdot b \cdot C_{eq}}{1 + b \cdot C_{eq}}$	2
Freundlich	$q_{eq} = K_f \cdot C^{1/n}$	2
Langmuir-Freundlich	$q_{eq} = \dfrac{q_{max} \cdot (b \cdot C_{eq})^{1/n}}{1 + (b \cdot C_{eq})^{1/n}}$	3

Table II. Adsorption parameters of Langmuir, Freundlich and Langmuir-Freundlich isotherm equations at different pH values, for 0.1g of raw *Sargassum* biomass without electrolyte addition.

Isotherm	Langmuir		
	q_{max} (mg g^{-1})	b (mg^{-1}L)	r^2
pH 3	154 ± 40	0.0025 ± 0.0009	0.9868
pH 4.5	69 ± 2	0.065 ± 0.003	0.9954
pH 6	74 ± 2	0.044 ± 0.003	0.9970
Isotherm	Langmuir-Freundlich		
	q_{max} (mg g^{-1})	b (mg^{-1}L)	n	r^2
pH 3	96 ± 35	0.006 ± 0.003	0.83 ± 0.21	0.9886
pH 4.5	75 ± 3	0.052 ± 0.006	1.19 ± 0.07	0.9984
pH 6	70 ± 2	0.051 ± 0.006	0.87 ± 0.04	0.9988
Isotherm	Freundlich		
	K_f	n	r^2
pH 3	0.8 ± 0.3	1.25 ± 0.11	0.9807
pH 4.5	12 ± 2	2.94 ± 0.35	0.9489
pH 6	10 ± 3	2.63 ± 0.42	0.9185

Table III. Adsorption parameters obtained using the Langmuir isotherm equation, total organic carbon measurements (TOC), percentage of weight loss of algal biomass due to treatments (W_1) and percentage of weight loss of algal biomass in water measured after treatment (W_2), of the different pretreatments for *Sargassum* biomass.

Treatment	q_{max} (mg g^{-1})	b (mg^{-1}L)	TOC (mgL^{-1})	W_1	W_2
Formaldehyde	99 ± 2	0.16 ± 0.01	24.8 ± 0.3	39	9
H$^+$	95 ± 2	0.16 ± 0.01	83.9 ± 0.6	37	12
Acetone	88 ± 4	0.08 ± 0.01	5.2 ± 0.2	31	6
Methanol	86 ± 3	0.12 ± 0.02	7.4 ± 0.2	30	4
KOH	86 ± 2	0.40 ± 0.05	13.4 ± 0.2	19	8
Raw biomass	65 ± 2	0.06 ± 0.01	122 ± 1		30
Ca(OH)$_2$	61 ± 3	0.06 ± 0.01	8.8 ± 0.1	6	0.1
CaCl$_2$	57 ± 2	0.07 ± 0.01	11.1 ± 0.3	6	0.2

Table IV. Necessary time for removing 50 and 95 % of the maximum cadmium uptake at different experimental conditions: * 0.25 g alga, I=0.05M NaNO$_3$; **C_i=100 mgL^{-1}, I=0.05M NaNO$_3$; ***0.25 g alga, C_i =100 mgL^{-1}.

	t (min)			t (min)			t (min)	
*C_i (mgL^{-1})	50 %	95 %	**m_s /g	50 %	95 %	***I / M	50 %	95 %
10	2.5	9	0.12	4	45	0.05	3	30
50	1.5	15	0.25	4	30	0.1	2.5	25
100	1.5	16	0.40	3	25	0.2	3	16
250	1.5	25	0.50	3	18	0.5	3	15
			0.60	2.5	14			

Table V. Percentage of cadmium removed and parameters obtained from the pseudo-second order rate reaction for the effect of initial cadmium concentration, alga mass and ionic strength on metal uptake kinetics. Experimental conditions were: * 0.25 g alga, I=0.05M NaNO$_3$; **C_i=100 mgL^{-1}, I=0.05M NaNO$_3$; ***0.25 g alga, C_i =100 mgL^{-1}.

*C_i / mgL^{-1}	Cd removed %	r^2	q_{eq} (mg g^{-1})	k (g mg^{-1}min^{-1})
10	81 ± 5	0.9992	3.28 ± 0.02	0.150 ± 0.010
50	82 ± 3	0.9999	15.73 ± 0.04	0.065 ± 0.004
100	75 ± 3	0.9999	30.14 ± 0.03	0.034 ± 0.001
250	63 ± 3	0.9999	61.00 ± 0.08	0.020 ± 0.001
**m_s /g				
0.12	54 ± 4	0.9999	44.96 ± 0.09	0.007 ± 0.001
0.25	69 ± 3	0.9997	27.35 ± 0.13	0.014 ± 0.001
0.40	83 ± 2	0.9998	20.73 ± 0.08	0.016 ± 0.001
0.50	87 ± 2	0.9999	17.32 ± 0.05	0.030 ± 0.001
0.60	88 ± 2	0.9994	14.92 ± 0.10	0.026 ± 0.002
***I / M				
0.05	75 ± 3	0.9995	30.97 ± 0.17	0.013 ± 0.001
0.1	69 ± 3	0.9998	28.33 ± 0.11	0.012 ± 0.001
0.2	55 ± 2	0.997	22.53 ± 0.28	0.018 ± 0.002
0.5	48 ± 2	0.9990	19.21 ± 0.04	0.012 ± 0.001

Figure captions

Figure I: Nonlinearised isotherms for cadmium biosorption by *Sargassum muticum* at different pH values and biomass concentration of 2.5 gL^{-1}. The lines correspond to Langmuir isotherm fits.

Figure II: Nonlinearised isotherms for cadmium biosorption at pH=4.5±0.1 by 2.5 gL^{-1} of *Sargassum muticum* with several pretreatments. The lines correspond to Langmuir isotherm fits.

Figure III: Speciation diagram for cadmium(II) species present in solution at different pH and cadmium concentrations.

Figure IV: Effect of pH on cadmium biosorption by 2.5 gL^{-1} of *Sargassum muticum* with initial cadmium concentration of 10 mgL^{-1} (open squares) and 250 mgL^{-1} (solid squares).

Figure V: Titration curve (pH vs. NaOH volume) for 0.2 g of *Sargassum muticum* with ionic strength adjusted to 0.05M with $NaNO_3$. Squares represent experimental data points and solid line is the derivative of the experimental curve.

Figure VI: Katchalsky model (straight line) for 0.2 g of *Sargassum muticum* with ionic strength adjusted to 0.05M with $NaNO_3$. Squares represent experimental data points.

Figure VII: Kinetics for cadmium uptake by 2.5 gL^{-1} of *Sargassum muticum* dose with ionic strength adjusted to 0.05M with $NaNO_3$ at different initial cadmium concentrations. The lines correspond to the modelled results using the pseudo-second order equation including the dependence of q_t and k on C_i according to Equation (IX).

Figure VIII: Kinetics for cadmium uptake by *Sargassum muticum* at different alga mass with 100 mgL^{-1} cadmium solution and ionic strength adjusted to 0.05M with

NaNO$_3$. The lines correspond to the modelled results using the pseudo-second order equation including the dependence of q_t and k on m$_s$ according to Equation (XI).

Figure IX: Kinetics for cadmium uptake by 2.5 gL^{-1} of *Sargassum muticum* dose at several ionic strengths with 100 mgL^{-1} of cadmium solution. The lines correspond to the modelled results using the pseudo-second order equation including the dependence of q_t and k on I according to Equation (XIV).
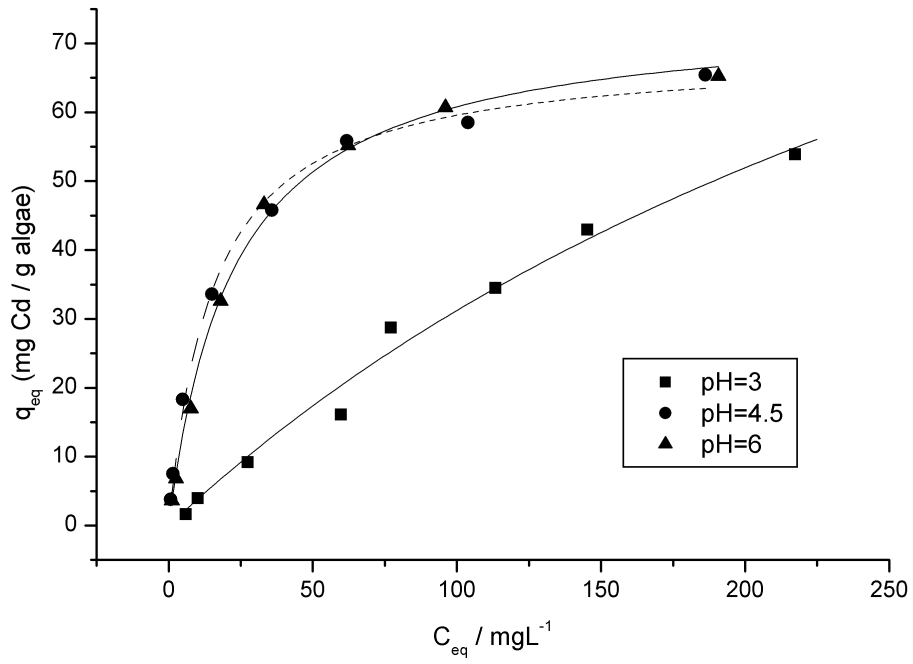
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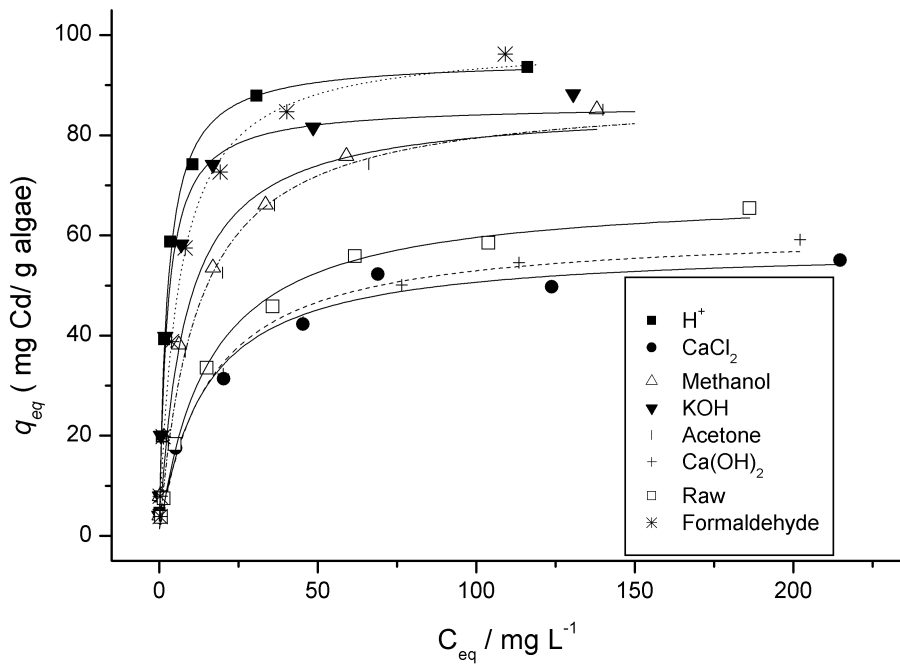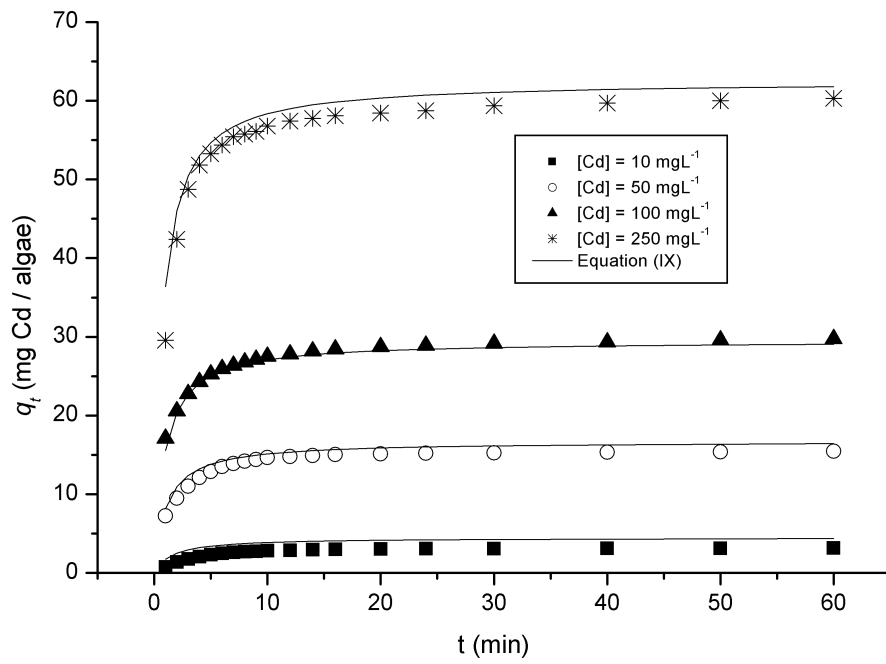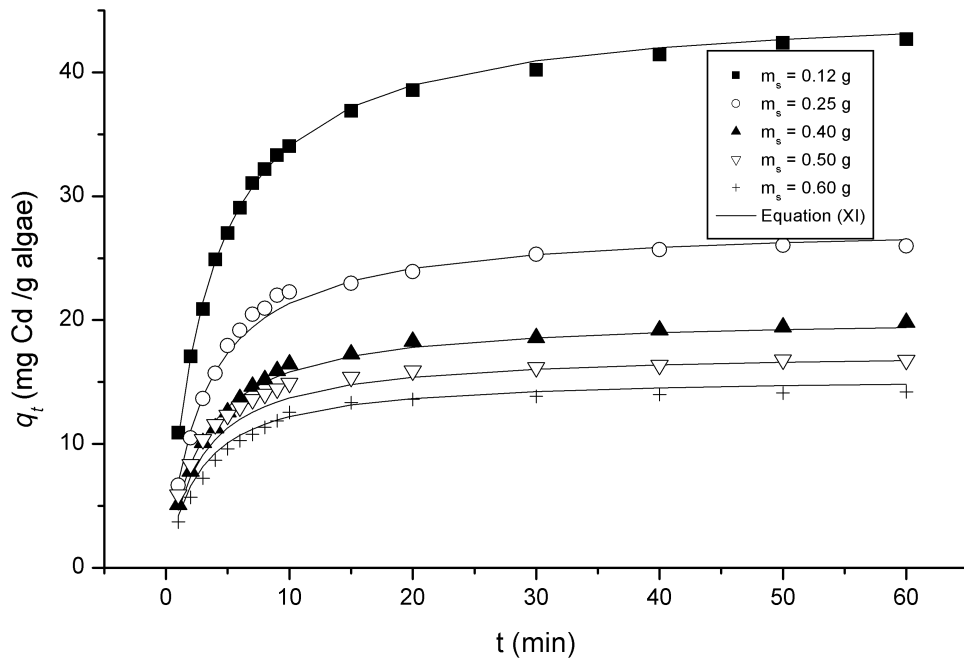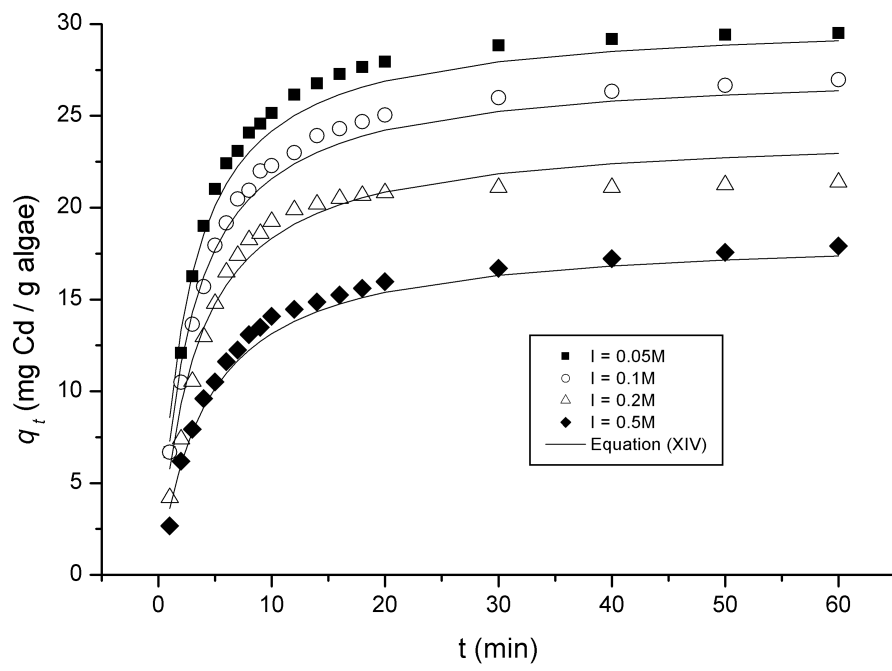

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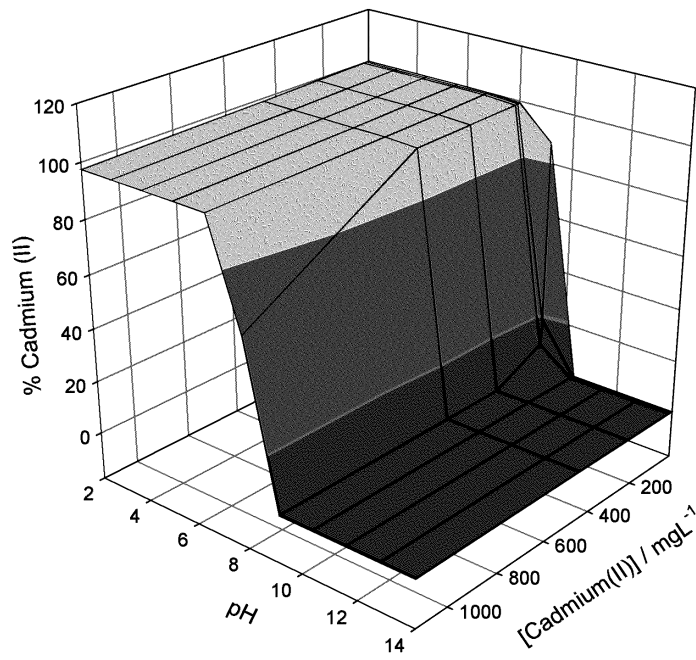
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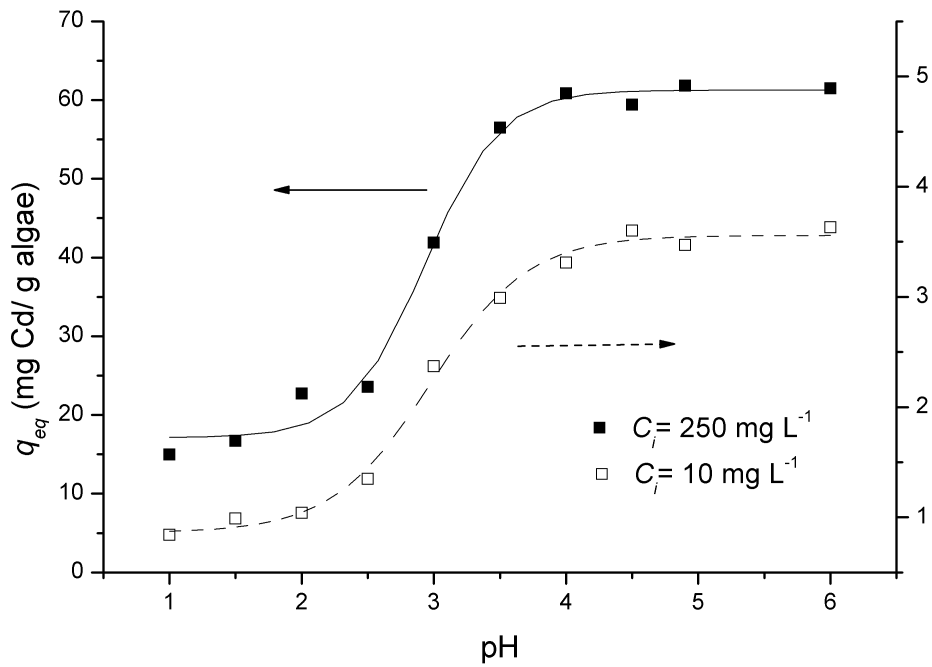
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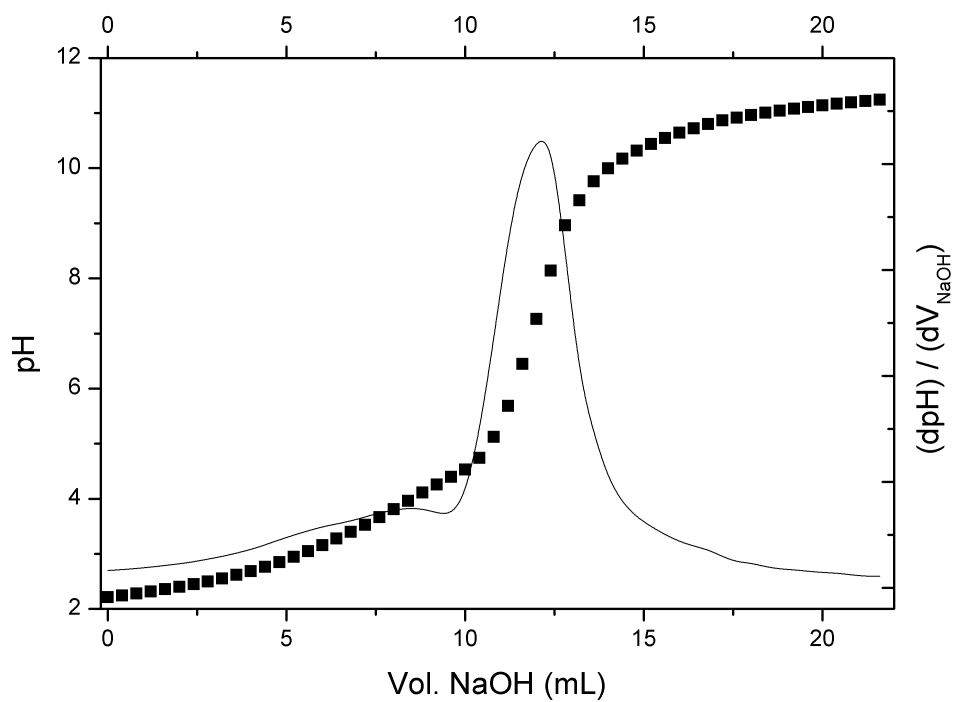
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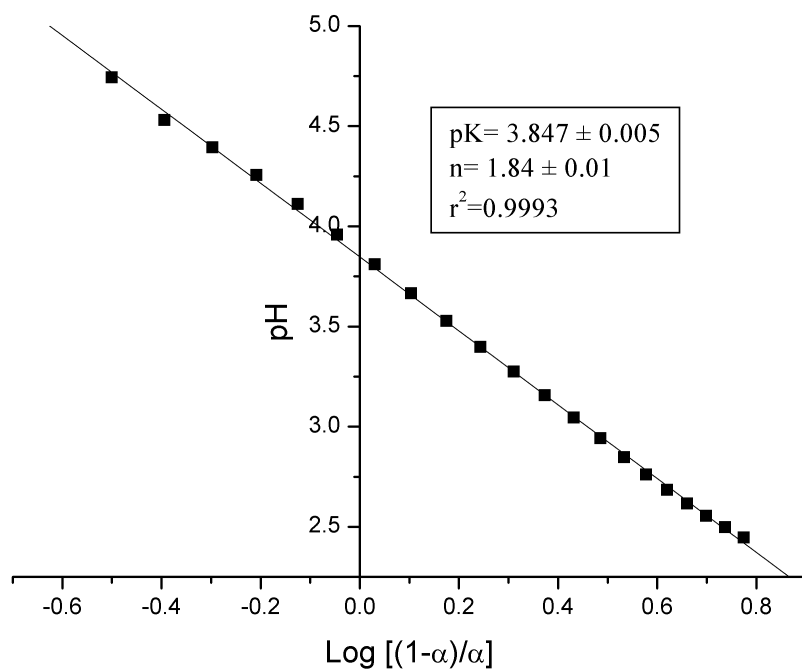
Figure VI

Figure VII

Figure VIII

Figure IX