Contribution of Sulfonate Groups and Alginate to Heavy Metal Biosorption by the Dry Biomass of *Sargassum fluitans*

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The mechanism of heavy metal complexation by the dry biomass of the brown seaweed *Sargassum fluitans* was investigated at the molecular level using different techniques. Simultaneous potentiometric and conductimetric titrations gave some information concerning the amount of strong and weak acidic functional groups in the biomass (0.25 ± 0.05 mequiv/g and 2.00 ± 0.05 mequiv/g, respectively). Those results were confirmed by the chemical identification of sulfonate groups (0.27 mequiv/g ± 0.03) and alginate (45% of the dry weight) corresponding to 2.25 mmol of carboxyl groups/g of biomass. Modification of these functional groups by methanolic hydrochloride or propylene oxide demonstrated the predominant role of alginate in the uptake of cadmium and lead. However, sulfonate groups can also contribute, to a lower extent, to heavy metal binding, particularly at low pH. Eventually, FTIR spectrophotometry on protonated or cadmium-loaded alginate and *S. fluitans* biomass physically demonstrated that cadmium binding arises by bridging or bidentate complex formation with the carboxyl groups of the alginate.

Introduction

Biomass from various natural or industrial origins can be used as complexing materials to recover toxic or strategic elements from industrial wastewaters (*1*). The accumulation of heavy metals by biomass of bacteria, actinomycetes, fungi, and algae has been described (*2*, *3*). The major mechanisms responsible for it include ionic interactions and complex formation between metal cations and ligands contained in the structure of the biomaterials (*4*−*6*). However, very little is known about the actual tissue structure and composition of different organisms, which also vary widely depending on the growth conditions for industrial or laboratory grown biomass, and the location or the season for natural harvested biomass (*7*). The unknown features of most biosorbents reduce their chance to be used as competitive products compared to well-known synthetic ion exchangers (*1*), even if their costs are expected to be significantly lower. The knowledge of the chemical structure of biosorbents is essential for modeling and predicting their metal binding performance in water purification systems. The overall effectiveness of biosorbent metal removal would also depend on the concentration range, the solution pH, the reaction kinetics, sorption equipment design, and the composition of the actual effluent handled. The identification of the binding sites in efficient biosorbents would be also helpful in the selection process for new biomass types as well as in attempts to improve their complexing properties by biological, chemical, or engineering processes.

Among the huge diversity of biomass available, algae have already proved to be the most promising for heavy metal recovery. The brown seaweed of the genus *Sargassum* was previously selected for its capacity to bind selectively gold at low pH (*8*−*10*) and also cadmium, copper, nickel, lead, and zinc cations up to 20% of the biosorbent dry weight (*11*). The metal binding performance of this alga has been well described, but the mechanisms responsible are still poorly understood. The aim of this study was to investigate qualitatively and quantitatively the contribution of two potential ligands known to be present in the thallus of brown seaweeds: carboxyl and sulfonate groups. Different strategies were employed in order to achieve this objective: potentiometric and conductometric titration, chemical analysis and modification of the biomass in relation with the sorption capacity for cadmium and lead, and infrared spectrophotometry. The two specific metals were selected because of their highly hazardous potential in the environment and their relatively different behavior in the chelation process.

Methods and Materials

Biological Materials. Biomass of *Sargassum fluitans* was harvested from the Gulf of Mexico coast in Naples, FL, and sundried on the beach. After being processed in a laboratory blender and sieved to select an appropriate particle size distribution (d = 0.50−0.84 mm), the biomass was washed with 50 vol (v/w) of 0.1 mol/L hydrochloric acid and then rinsed extensively with distilled and deionized water to remove the salts (sodium, potassium, magnesium, and calcium) and to convert the acidic groups into their hydrogen forms. Drying was performed at 60 °C until constant weight. Sodium alginate (Lot 03916HV), purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI), was converted into alginic acid by two washings in 100 vol of 0.1 mol/L HCl. After centrifugation (10 min at 9800*g*), the excess HCl was removed by two additional washings in distilled deionized water. The last pellet of alginic acid was then freeze-dried and stored at 4 °C.

Chemicals. Concentrated hydrochloric acid (36.5−38%), certified 0.1 mol/L sodium hydroxide, cadmium sulfate, lead nitrate, potassium bromide, and reagent-grade propylene oxide were purshased from Fisher Scientific (Ottawa, ON). ACS-grade sodium carbonate, lab-grade acetone, and absolute methanol were from Anachemia (Montreal, QC). Poly(hexamethylene−biguanidinium chloride) [$PHMBH^+Cl^-$] was obtained as a 20% solution from Zeneca Biocides (Wilmington, DE). Folin−Ciocalteau reagent was from Sigma Chemical Company (St. Louis, MO).

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Determination of Strong and Weak Acidic Groups. Potentiometric and conductometric titrations of native and modified *S. fluitans* biomass and alginic acid were performed together according to the methodology described by Katz et al. (*12*). Typically, 200 mg of raw or modified algal biosorbent, converted to hydrogen form with 0.1 mol/L hydrochloric acid and washed with deionized water to a constant conductance, was dispersed in 100 mL of 1 mmol/L sodium chloride solution prepared with deionized water. Titration was carried out by stepwise addition of 0.25 mL of 0.1 mol/L sodium hydroxide while the suspension was stirred under a nitrogen atmosphere.

Chemical Analysis of Alginate, Sulfonates, and Polyphenols. The concentration of alginate in solutions or in crude extracts of algal biomass was determined according to the method of Kennedy and Bradshaw (*13*), using poly(hexamethylene−biguanidinium chloride) [$PHMBH^+Cl^-$]. Alginate was extracted from the dry alga using 2% solution of Na_2CO_3, according to the method of Percival and McDowell (*7*). The results usually obtained with this technique have been described to be in good agreement with other recognized methods and are quite unaffected by the numerous substances present in crude alginate liquors.

Sulfonate groups were determined as the amount of sulfates released after acidic hydrolysis of algal biomass samples. Typically, 50 mg of native or modified dry algal particles were heated for 4 h at 100 °C in 10 mL of 2 mol/L HCl under reflux. The liquid phase was then filtered and diluted before determination of sulfate using a Dionex DX 100 ion chromatograph equiped with a IonPac AS4A-SC column. All measurements were repeated twice.

Polyphenol extraction was carried out with an acetone/water mixture (1:1) as described by Mole and Waterman (*14*). The amount of extracted polyphenol was then measured using the Folin−Ciocalteau assay.

Chemical Modification of Alginate and Algal Biomass. Partial esterification of *S. fluitans* biomass and alginic acid was performed according to the method described by Jansen and Jang (*15*). One gram of protonated dry algal particles (500−840 μm) or lyophylized alginic acid was suspended in 50 mL of absolute methanol to which 1% (v/v) of concentrated HCl (36%) was added. After one to four cycles of 48 h continuous agitation, with replacement of the methanolic HCl between cycles, the solid was sedimented by centrifugation, washed with 0.1 mol/L HCl, rinsed with distilled deionized water, and dried at 60 °C (algal sample) or freeze-dried (alginic acid).

Carboxyl-free samples of alginic acid and algal biosorbent were prepared in aqueous propylene oxide solution according to the following procedure (*16*). Biomass or alginate, previously converted to hydrogen ion form with 0.1 mol/L hydrochloric acid, was allowed to stand in 37% (w/w) propylene oxide solution (20 mL/g) at room temperature. The esterification period lasted from 4 h to 3 days. In the latter case of more extended periods, the solution was replaced once by fresh propylene oxide solution. To stop the reaction, the excess propylene oxide was removed by washing the sample with deionized water. The treated biomass was then dried at 60 °C.

Metal Uptake Comparisons. A new methodology, referred to as shared batch equilibrium, was developed to compare the metal binding properties of the native and modified biosorbents under the same equilibrium conditions. For that purpose, 50 mg of each sample was confined in a Spectra/Por no. 1 dialysis bag (MWCO = 6−8000) and suspended in 2 mL of distilled deionized water. Alternatively, cellulose tissue bags were used. All the bags were submerged in 1 L of a bufferred metal-containing solution ($CdSO_4$ or $PbNO_3$) and exposed to it overnight at room temperature on an orbital shaker, allowing the sorption reaction to attain equilibrium. Each sample was then transferred into individual 100-mL flasks containing 50 mL of 0.5 mol/L nitric acid solution and contacted for 6 h under agitation in order to completely remove all the metal originally sorbed by the biomass. The concentration of the metal released in the solution was determined by atomic absorption spectrophotometry (Thermal Jarell Ash Model Smith-Hieftje II). In order to ascertain that the metal was completely removed from the biosorbent, a second acidic wash was performed and analyzed for metal traces. The cellulose acetate membrane forming the dialysis bags was preconditioned in the metal solution. The metal uptake was calculated from the concentration of the metal released into the desorbing solution. All these experiments were performed at high metal concentrations (2.0 mM), resulting in metal uptakes close to the maximum, at pH 2.5 and pH 4.5 in 20 mM sodium chloracetate and sodium acetate buffers, respectively.

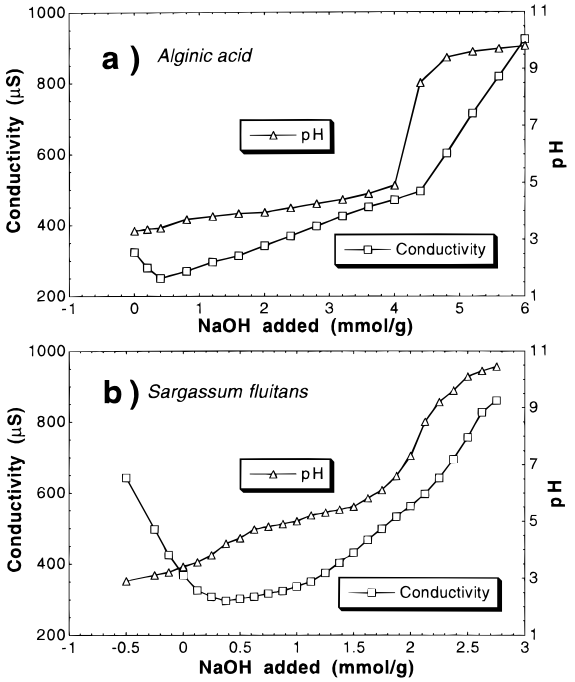

FIGURE 1. Potentiometric and conductometric titrations of alginic acid (a) and protonated biomass of *S. fluitans* (b). Titrations were performed by stepwise addition of 0.25 mL of 0.1 mol/L NaOH while the suspension (200 mg/100 mL) was stirred under a nitrogen atmosphere.

Fourier Transformed Infrared Spectra Analysis. Infrared spectra of protonated or cadmium loaded algal biomass or alginate were recorded on a Michelson 100 FTIR spectrophotometer. Samples of 100-mg KBr disks containing 1% of finely ground powder (<20 μm) of each sample were prepared less than 24 h before recording.

Results

Titration of the Native *Sargassum* Biomass and Alginic Acid. Figure 1 shows the potentiometric and conductometric titration curves of alginic acid (a) and protonated *Sargassum* (b) resulting from the addition of NaOH. The

TABLE 1

Ionic Characterization of Raw and Modified Biomass of *S. fluitans*

S. fluitans treatment	sulfonates[a] (mmol/g)	acidic titre: strong (mequiv/g)	acidic titre: weak (mequiv/g)	alginate[b] (mmol/g)	weight loss (%)	cadmium uptake (mmol/g): pH 4.5	cadmium uptake (mmol/g): pH 2.5	lead uptake (mmol/g): pH 4.5	lead uptake (mmol/g): pH 2.5
no treatment	0.275	0.25	1.95	2.25		0.914	0.335	1.06	0.80
methanol control	0.177	0.20	2.00		15	0.76		0.96	0.66
acetone/H2O 1:1	0.173	0.20	2.05		27	0.95		1.17	0.87
methanol/HCl									
1 × 48 h	0.125	0.11	1.43		14	0.50	0.16		
2 × 48 h	0.05	<0.1	1.35		17	0.46	0.14		
3 × 48 h	0.015		1.22		20	0.34	0.11	0.36	0.12
4 × 48 h	<0.01		1.02		18	0.23	0.06		
propylene oxide									
4 h	0.187	0.18	1.24	2.05	4	0.38	0.09	0.55	0.41
48 h	0.176		0.55	1.55	12	0.063	0.01	0.20	0.13

[a] Determined by ion chromatography after acidic hydrolysis. [b] Determined using the [PHMBHCl] reagent (uronic acid subunits).

NaOH addition in the initial 'negative' part of the diagrams in Figure 1 accounts really for the titration of the known amount of HCl initially added to the samples (Materials and Methods). The conductometric titration curve of alginic acid begins with a short decreasing portion, characterizing the repression of the dissociation of the weak acidic groups. With *Sargassum*, the decreasing portion is slightly longer, because of the presence of additionnal free protons from strongly acidic groups, probably sulfonates. In order to distinguish between the strong acids and the beginning of titration of weak acids, a known amount of strong acid (hydrochloric) was added to the suspension prior to titration. The intersection between the linearly decreasing portion and the linear slowly increasing branch, corresponding to the titration of weak acidic groups, yielded a quantitative estimation of equivalents of strong acidic groups in the biomass (0.25 ± 0.05 mmol/g). This intersection also corresponds to the first equivalent point of the pontentiometric titration curve. When all the strong acidic groups were neutralized, the weaker acidic groups began to dissociate and contributed to the measured conductance. In this region, the change attributed to the proton concentration change was low because of the low level of dissociation of the weak acidic groups. However, the dissolution of sodium alginate contributed significantly to the change in the solution conductivity. With *Sargassum*, the solubilization of alginate, determined by using the PHMB reagent, occurred only during the last portion of the weak acidic group neutralization, while it appeared earlier with pure alginic acid (not shown). When all the weak acids were neutralized, the conductivity increased in the proportion to the excess of sodium hydroxide added. This break point corresponds to the second equivalence point of the potentiometric titration curve and gives the amount of weak acidic equivalent groups in the alginic acid (4.4 mmol/g) and the dry algal biomass (2.25−0.25 = 2.0 mmol/g).

Strong and Weak Acids Determined by Titration in Modified Biomass. In order to determine the extent of chemical modification, the same titration experiments as above were performed on algal samples after the treatment with methanolic HCl, propylene oxide, methanol, or acetone/water (1:1). As shown in Table 1, the amount of NaOH necessary to neutralize weakly acidic groups decreased after successive cycles of methanolic HCl treatment. However, four consecutive treatments did not esterify more than 50% of the initial acidic functions. Propylene oxide esterified the weak acidic groups to a much higher degree. Thirty percent of the acidic groups were blocked after only 4 h of treatment, and up to 80% esterification could be achieved after 48 h. Treatment with methanol alone or acetone/water (1:1) did not show any significant modification of the amount of weakly or strongly acidic functions. The presence of residual strong acidic groups in the materials examined after methanolic HCl treatment was below the sensitivity of the titrating method used.

Chemical Analysis of Sulfonate Groups and Alginate in Native and Modified Biomass. The amounts of sulfonate groups and alginate determined by chemical analysis in protonated or modified algal biomass are also presented in Table 1. The concentration of alginic acid determined by the [$PHMBH^+Cl^-$] technique from crude carbonate extracts of the native protonated *Sargassum* (0.4 g/g) corresponds to 2.25 mmol of carboxyl groups/g of dry biomass. The same measurement performed on propylene oxide treated biomass gave 2.05 and 1.55 mmol/g after 4 and 48 h of treatment, respectively. This reduction of alginate concentration can be attributed to a lower complexation of esterified alginate by the $PHMBH^+$ reagent or to the partial solubilization of alginate during the propylene oxide treatment. The sulfates measured by acidic hydrolysis of the untreated algal biomass represent 0.275 mmol of sulfonate groups/g. These results correlate very well with the amount obtained by titration. The methanolic HCl treatment of the algal biomass resulted in a significant decrease of the sulfonates concentration. After three successive treatments, there were no sulfonates detectable. All other treatment resulted only in about 35% decrease of the sulfonate groups content. These results suggest that the sulfates present in the materials examined fall into weakly bound and strongly bound categories.

Metal Complexation by Untreated and Treated Biomaterials. The ion exchange capacity of native or modified alginic acid and *S. fluitans* biomass was determined after contacting them with concentrations of cadmium and lead, resulting in close to maximum saturation levels of the respective metal uptakes. The corresponding maximum metal uptakes (in mmol/g) are shown in Table 1. The maximum biosorption sorption capacities for Cd and Pb at pH 4.5 with native *S. fluitans* biomass came close (0.91 and 1.06, respectively) and correspond approximately to one-half of the number of equivalent acidic groups as

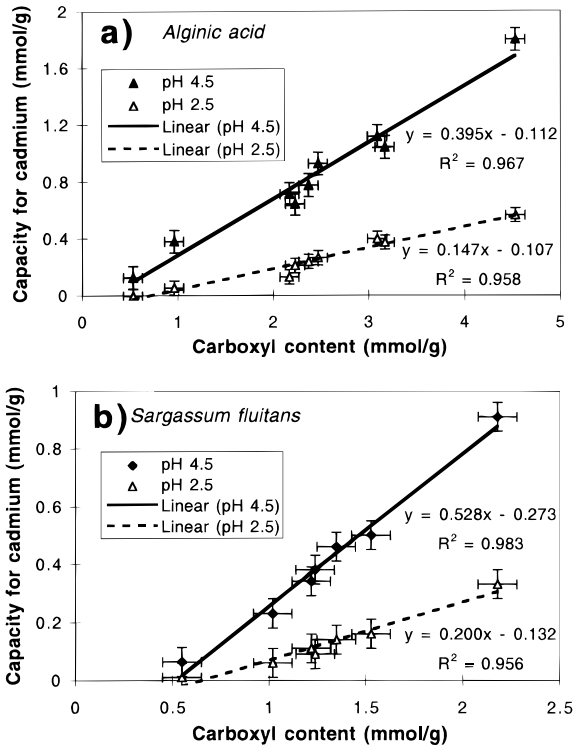

FIGURE 2. Correlation between the carboxyl groups content and the cadmium capacity of alginic acid (a) and *S. fluitans* (b). Carboxyl contents were measured by titration. Error bars correspond to the sensitivity of the measurements.

determined by titration. The metal uptake capacities are lower at pH 2.5, particularly for cadmium. Methanolic HCl-treated samples of *S. fluitans* showed a continuous decrease of their cadmium binding capacity with less than 0.23 mmol/g residual capacity at pH 4.5 for the longest time of exposure for the modification. The treatment with methanol alone performed as a control resulted in only 15% decrease of the uptake for each metal at both pH values. On the contrary, extraction with acetone/water (1:1) intended to partially solubilize tannin-like polyphenolic compounds, resulting in a 5−10% enhancement of heavy metal uptakes. However, complete extraction of these compounds is very difficult and could never be achieved.

The inhibition of the biosorbent metal uptake after propylene oxide esterification of the biomass was very drastic, with a more than 50% decrease after only 4 h of treatment and, respectively, 80% and 95% decrease of Pb and Cd binding after 48 h. The correlations between the heavy metal uptake and the acidic titre of alginic acid or *S. fluitans* biomass are presented in Figure 2, panels a and b. Each plot can be linearized with a regression coefficient above 0.95. The slopes correspond to the metal to acidic groups ratios in the complexes, at the specified pH and residual metal concentration in the solution. At pH 4.5, this ratio was significantly lower with alginic acid (0.39) compared to *Sargassum* (0.52). The same relative difference was observed at pH 2.5 between the two biological materials. No significant deviation could be detected between samples treated with propylene oxide or methanolic HCl. Despite a good linear correlation between cadmium uptake and acidic titre, it should be noticed that a minimum acidic titre of 0.5 mequiv/g is necessary to achieve a significant cadmium uptake.

Infrared Spectroscopy of Alginate and *S. fluitans* Biomass. Infrared spectra of protonated or cadmium-loaded alginate and *Sargassum* biomass samples are shown in Figure 3, panels a and b, respectively. The spectra of protonated biomaterials typically display absorbance peaks at 1738 cm^{-1} corresponding to the stretching band of the free carbonyl double bond from the carboxyl functional group. It can be observed that the two spectra have a high similarity, which supports the above finding that the algal biomass contains a high concentration of alginate (45%). After the contact with a high-concentration cadmium solution, the two biological materials exhibited spectra with a clear shift of the carbonyl stretching band from 1738 to 1630 cm^{-1}. This shift is typical of the complexation of the carbonyl group by dative coordination (*17*), with cadmium in this case. With alginic acid, a good correlation was obtained between the cadmium loading and the area of the chelated C═O bound stretching peak (not shown). Another shift can be observed from 1238 to 1415 cm^{-1}, corresponding to the complexation of the oxygen from the carboxyl C—O bond (*17*). The frequencies corresponding to the C═O and C—O bond stretching in protonated, cadmium-complexed, and sodium-ionized materials are given in Table 2 as well as the differences between C═O and C—O band frequencies. It can be observed that the distance between the two peaks decreased in the order protonated > sodium-ionized > cadmium-complexed. This reflects the increase of the relative symmetry of the carboxyl functional groups (*17*). Absorbance peaks around 850 and

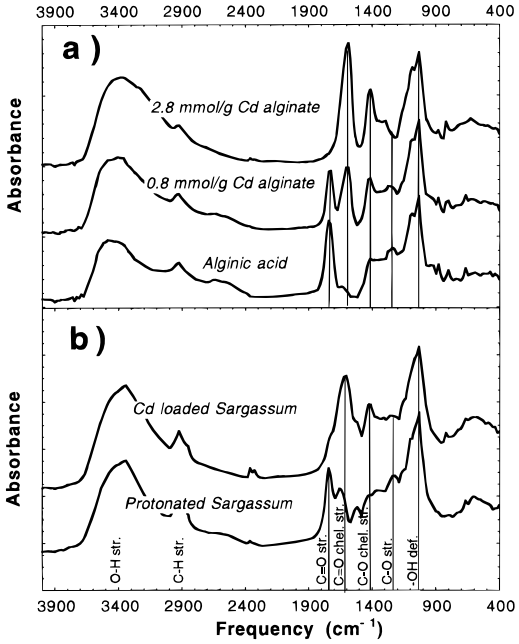

FIGURE 3. Fourier transformed infrared spectra from protonated or cadmium loaded alginate (a) and *S. fluitans* (b). 100 mg KBr disks for FTIR contained 1% of finely powdered materials. str. = stretching, chel. str. = chelate stretching, def. = deformation.

TABLE 2

Carboxyl Stretching Frequencies for Different Ionic Forms of Alginate and *S. fluitans*

materials	ν (C═O)	ν (C—O)	Δ (C═O−C—O)
alginic acid	1739.50	1237.69	501.81
sodium alginate	1620.17	1416.80	203.37
cadmium alginate	1602.31	1419.20	183.11
protonated *Sargassum*	1738.00	1238.48	499.52
sodium *Sargassum*	1630.09	1426.24	203.85
cadmium *Sargassum*	1618.07	1422.36	195.71

1240 cm^{-1}, corresponding to S═O and *C*—*S*—O bonds, respectively, are not clearly and specifically detectable on *Sargassum* spectra, confirming the low amount of sulfonate groups in the biomass.

Discussion

The thallus of Phaeophycae is known to contain extracellular polyuronic acid (alginate) as well as sulfated glucurono-xylo-fucane (fucoidan) embedded in a fibrous cellulose skeleton (*18*, *19*). Alginate extraction was shown to dramatically reduce the cobalt uptake by *Ascophyllum nodosum* (*20*), whereas gold uptake by *Sargassum* was more affected by extraction with cellulase (*10*). The carboxyl groups from polyuronic acid were also proved to be partially responsible for the chelation of copper and aluminium by algae (*21*) and of zinc by filamentous fungi (*22*), but their contribution to the overall ion-exchange capacity can vary widely from one species to another.

Because of an insufficient difference in the dissociation constants of the acidic groups contained in different types of algal biomasses, the classical potentiometric titration curve does not show distinct steps marking the end of the titration of strong or weak acids. This approach cannot accurately determine the separate contribution of the different types of acids to the overall acidity. Katz et al. (*12*) proposed a method to determine sulfonate and carboxyl groups in sulfite wood pulp with a single conductometric titration. The interpretation of the results obtained with the same technique in the present work was tedious because of the low amount of strongly acidic groups present in the biomass material studied. There also was a disturbing increase of the test solution conductivity due to alginate solubilization. However, these results, coupled with additional chemical analysis, clearly demonstrated the predominance of carboxyl groups in alginic acid among the total acidic moieties contained in *S. fluitans* biomass. The apparent dissociation constants pK_o determined from the experimental titration curves of alginic acid and *S. fluitans* biomass ($pK_o = 4.0$ and 5.2, respectively) were above the intrinsic pK_a values of manuronnic and glucuronic acids (3.38 and 3.65) (*18*). This is explained by the electrostatic interactions between adjacent acidic groups in the biomaterials (*23*). *S. fluitans* biomass has a more rigid structure that is responsible for a higher density of carboxyl groups than in free alginate.

In this work, as in others (*21*, *22*), selective esterification was the chosen strategy to investigate the contribution of these carboxyl functional groups in the overall metal binding properties of the algal biomass. The method described by Jansen and Jang (*15*), using HCl in absolute methanol, was initialy employed as a way of esterification of carboxyl groups from galacturonic acid and polyuronides. However, this method also causes desulfonation reactions, as described by Kantor and Schubert (*24*). Unfortunately, the two reactions proceed at the same low rate, making it impossible to distinguish independently their effect on metal complexation. Esterification using propylene oxide in water was found to be faster and more selective. Using the two techniques, two different samples could be obtained with the same content of residual carboxyls (1.4 mmol/g) but different sulfonates (0.19 and 0.015 mmol/g, respectively). These two samples had similar binding capacities for cadmium (0.38 and 0.335 mmol/g at pH 4.5; 0.09 and 0.114 mmol/g at pH 2.5), but lead was more efficiently bound by the more sulfonated sample, especially at a low pH (0.55 and 0.36 mmol/g at pH 4.5; 0.41 and 0.12 mmol/g at pH 2.5). Since lead is known to have higher affinity than cadmium for hard ligands containing sulfur or nitrogen atoms (*25*), it is more sensitive to the low concentration of sulfonate groups. These results indicate that sulfonate contribution to heavy metal binding is small but could be significant at low pH. In addition, recent work has shown that the presence of alginate and sulfonate groups are both necessary to model and correctly predict the ion-exchange properties of *Sargassum* biosorbent at different pH values (*26*).

The correlation between metal binding capacity and the amount of acidic groups in the algal biomass showed that the stoichiometry of metallic complex formation varied with pH and approached 0.5 Cd^{2+} per acidic group at pH 4.5 and high metal concentrations in solution. Since two carboxyl groups are necessary to chelate one metal cation, the average distance between ligands is a crucial parameter, and the high degree of ionization and the important flexibility of the alginate polymer facilitate the formation of metallic complexes (*27*, *28*). At lower pH, the ratio between bound cations and carboxyl groups available is even lower because of a partial protonation of the binding sites. Below 0.5 mequiv of acidic group/g of biosorbent or alginate, the carboxyl group density in the polymer is too low to enable the formation of binding sites.

Brown algae are also known to contain significant amounts of reducing compounds in physodes vesicles, identified as condensed or hydrolyzable polyphenols (*29*) and characterized as phloroglucinol polymers (*30*). According to previous observations (*31*), these polyphenolic compounds were suspected to contribute to the metal binding capacity of the algal biomass. However, the extraction with acetone/water did not reduce the uptake of cadmium or lead. On the contrary, a slight increase was observed, indicating a partial purification of the sorbing components by elimination of nonsorbing polyphenols.

FTIR analysis was already used in the past to investigate the mechanism of gold and cobalt biosorption by the biomass of *S. natans* and *A. nodosum* (*20*, *9*). No significant conclusions could be drawn at that time. However, the samples used, referred to as "free biosorbent", were actually loaded with the alkali and alkaline earth ions (Na^+, K^+, Mg^{2+}, and Ca^{2+}) initially present in seawater. For this reason, no obvious variation could be detected between cadmium-free and cadmium-loaded biosorbent. In the present work, the protonated algal biomass was chosen as a free biosorbent control, and the spectra of pure alginic acid and cadmium alginate were measured in parallel. This approach allowed the identification of two absorbance peak shifts, characteristic of coordination compounds between carboxyl groups and heavy metals. The distance between these two peaks (Δ) is related to the relative symmetry of the carboxyl group and reflects the nature of the coordination compound (*17*). Unidentate complexes and protonated compounds exhibit the highest Δ, while this value is significantly lower with chelating (bidentate) complexes. Ionic compounds and bridging complexes display intermediary Δ. The data obtained with pure alginate (Table 2) showed that the cadmium complexation tends to increase the symmetry of the carboxyl groups as compared to sodium ionization. These results physically demonstrate that cadmium binding arises from bridging or bidentate complex formation with the carboxyl groups of the alginate and are consistent with the "egg-box" model described by Rees and

co-workers (*32*, *33*). With *Sargassum* biomass, the differences between C═O and C─O stretching frequencies with cadmium or sodium were very similar, and the C═O vibration peaks were larger than with alginate. This observation suggests a larger diversity of coordination mode, due to the particular environment of the alginate polymer in the algal biomass. In addition, it should be noticed that the peak corresponding to the complexation of carbonyl still appeared in the spectrum obtained with protonated biomass, even after extensive acidic washing. This suggest that these ligands might form internal complexes with other electrophylic groups of the algal biomass.

These results elucidate the molecular basis of heavy metal biosorption by *S. fluitans* by providing evidence for the pH-dependent ion exchange by complexation. The alginate part of the biomass structure is responsible for most of the metal sequestering. Its gelatinous nature imposes limitations on the application of the microporous diffusion concept to metal ions mass transfer. In fact, algal biosorbents behave to a certain extent like polyelectrolytes separated from the external solution by a semipermeable membrane. Their physical properties, such as swelling and solubilization, are thus expected to modify the ionic state of alginate, all depending on the pH of the solution.

Literature Cited

(1) Brierley, C. L. *Geomicrobiol. J.* **1990**, *8*, 201−223.
(2) Gadd, G. M. In *Biotechnology*; Rehm, H.-J., Ed.; VCH Verlagsgesellschaft: Weinheim, Germany, 1988; Vol. 6b: Special Microbial Processes, pp 401−433.
(3) Volesky, B., Ed. *Biosorption of Heavy Metals*; CRC Press, Inc.: Boca Raton, FL, 1990.
(4) Treen-Sears, M. E.; Volesky, B.; Neufeld, R. J. *Biotechnol. Bioeng.* **1984**, *26*, 123−129.
(5) Fourest, E.; Roux, J.-C. *Appl. Microbiol. Biotechnol.* **1992**, *37*, 399−403.
(6) Crist, R. H.; Martin, J. R.; Carr, D.; Watson, J. R.; Clarke, H. J.; Crist, D. R. *Environ. Sci. Technol.* **1994**, *28*, 1859−1866.
(7) Percival, E., McDowell, R. H., Eds. *Chemistry and enzymology of marine algal polysaccharides*; Academic Press: London, U.K., 1967; pp 137−143.
(8) Kuyucak, N.; Volesky, B. *Biorecovery* **1989**, *1*, 189−204.
(9) Kuyucak, N.; Volesky, B. *Biorecovery* **1989**, *1*, 205−218.
(10) Kuyucak, N.; Volesky, B. *Biorecovery* **1989**, *1*, 219−235.
(11) Leusch, A.; Holan, Z. R.; Volesky, B. *J. Chem. Technol. Biotechnol.* **1995**, *62*, 279−288.
(12) Katz, S.; Bearson, R. T.; Scallan, A. M. *Sven. Papperstidn.* **1984**, *6*, 48−53.
(13) Kennedy, J. F.; Bradshaw, I. J. *Carbohydr. Polym.* **1987**, *7*, 35−50.
(14) Mole, S.; Waterman, P. G. *Oecologia* **1987**, *72*, 137−147.
(15) Jansen, E. F.; Jang, R. *J. Am. Chem. Soc.* **1946**, *68*, 1475−1477.
(16) Sjostrom, E.; Ernstrom, B. *Sven. Papperstidn.* **1966**, *69*, 55−59.
(17) Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*; John Wiley & Sons: New York, 1986; p 231.
(18) Percival, E., McDowell, R. H., Eds. *Chemistry and enzymology of marine algal polysaccharides*; Academic Press: London, U.K., 1967; pp 99−124.
(19) Kreger, D. R. In *Physiology and biochemistry of algae*; Lewin, R. A., Ed.; Academic Press: New York, 1962; pp 315−335.
(20) Kuyucak, N.; Volesky, B. *Biotechnol. Bioeng.* **1989**, *33*, 823−831.
(21) Gardea-Torresday, J.; Becker-Hapak, M. K.; Hosea, J. M.; Darnall, D. W. *Environ. Sci. Technol.* **1990**, *24*, 1372−1378.
(22) Fourest, E.; Serre, A.; Roux, J.-C. *Toxicol. Environ. Chem.*, in press.
(23) Helfferich, F. *Ion Exchange*; McGraw-Hill: New York, 1962; pp 299−319.
(24) Kantor, T. G.; Schubert, M. *J. Am. Chem. Soc.* **1957**, *79*, 152−153.
(25) Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533−3539.
(26) Schiewer, S.; Volesky, B. *Environ. Sci. Technol.* **1995**, *29*, 3049−3058.
(27) Haug, A.; Smidsord, O. *Acta Chem. Scand.* **1965**, *19*, 341−351.
(28) Haug, A.; Myklestad, S.; Larsen, B.; Smidsrod, O. *Acta Chem. Scand.* **1967**, *21*, 768−773.
(29) Haug, A.; Larsen, B. *Acta Chem. Scand.* **1958**, *12*, 650−657.
(30) Ragan, M. A.; Craigie, J. S. *Can. J. Biochem.* **1976**, *54*, 66−73.
(31) Slabbert, N. In *Plant Polyphenols* ; Hemingway, R. H., Lako, P. E., Eds.; Plenum Press: New York, 1992; pp 421−436.
(32) (a) Rees, D. A.; Morris, E. R.; Thom, D.; Madden, J. K. In *Molecular Biology: An International Series of Monographs and Textbooks*; Horecker, B., Kaplan, N. O., Marmur, J., Sheraga, H. A., Eds.; Academic Press: New York, 1982. (b) *The Polysaccharides, Vol. I.*; Aspinal, G. O., Ed.; Academic Press: New York, pp 195−290.
(33) Thom, D.; Grant, G. T.; Morris, E. R.; Rees, D. A. *Carbohydr. Res.* **1982**, *100*, 29−42.

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