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Alginate Properties and Heavy Metal Biosorption by Marine Algae

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ABSTRACT

The physical properties of the alginate component in four different brown seaweeds (*Sargassum fluitans, Ascophyllum nodosum, Fucus vesiculosus*, and *Laminaria japonica*) were characterized using potentiometric titration, ^{13}C-nuclear magnetic resonance (NMR), chemical analysis, and viscosity measurements. The heavy metal binding capacities of the corresponding seaweeds were directly proportional to their respective total carboxyl group content, and related to the electronegativity of the elements investigated (Ca, Zn, Cd, Cu, and Pb). The uronic acid composition or sequence of the alginate component did not affect the metal uptake properties of the biosorbents studied here. However, the alginate leaching owing to its solubilization by Na ions was observed to decrease with increasing intrinsic viscosity of the extracted alginate, related to its molecular weight, and with increasing apparent acidic dissociation constant, related to the alginate density inside the biomass.

Index Entries: Brown seaweed; biosorbent; biorecovery; cation exchanger; carboxyl groups; intrinsic viscosity; extraction; nuclear magnetic resonance.

INTRODUCTION

Biosorption is the ability of biological materials to concentrate organic or inorganic species dissolved in solution. The accumulation of heavy metals by microorganisms or algae has been studied extensively for biomonitoring or

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bioremediation purposes *(1–5)*. The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and functional groups (carboxyl, carbonyl, amino, amido, sufonate, phosphate, and so forth) present on the surface or inside the porous structure of the biological material. Recently, it was demonstrated that carboxyl groups of alginate play a major role in the complexation of heavy metals (Cd and Pb) by dead biomass of the brown seaweed *Sargassum fluitans (6)*. Alginate is a common term for a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged in a nonregular, blockwise fashion along the chain *(7)*. The linear arrangement of the uronic residues can vary widely among algal species. Even in the same species, it can vary depending on the age and part of the plant *(8)*. M-block and G-block conformations may differ considerably, and their proportions are very important for the physical properties and the reactivity of alginate *(9)*. G-blocks are responsible for the "egg box" formation with calcium ions during alginate gelation *(10,11)*, whereas the MG-heteroblock frequency governs the solubility of alginic acid at low pH *(9)*. Fractions of alginate enriched with G-blocks were also found to be more selective for calcium ions, whereas M-blocks had more affinity for cadmium ions *(12,13)*.

The selective affinity for heavy metals and the retention of physical integrity are two major criteria for a biosorbent to be used in water detoxification processes. Despite its high capacity for Cu, Cd, and Zn, *Ascophyllum nodosum* biomass was found to release alginate easily when contacted with metal-bearing solutions *(14)*, making it difficult to assess quantitatively the sorption of the metal. *Ascophyllum nodosum* biosorbent stabilization was attempted by using the acidic formaldehyde crosslinking procedure *(15)*. However, extensive rinsing of the material was necessary to prevent any further leaching of alginate *(16)*, making questionable the real efficiency of the formaldehyde crosslinking. *Sargassum fluitans* biomass was found to be much more stable in heavy metal solutions and presented a similar metal uptake capacity *(17)* to *A. nodosum*. These observations prompted examination of the relationship between the nature of the alginate component in various brown seaweeds and their respective metal biosorption properties. The four algal species selected for this study are known to contain high amounts of alginate in their cell walls. However, the samples described here should not be considered completely representative of each species, since the alginate composition can vary greatly even in the same species.

MATERIALS AND METHODS

Biological Materials

Biomass of *S. fluitans* was harvested from the Gulf of Mexico coast in Naples, Florida and sundried on the beach. *Ascophyllum nodosum* and *Fucus*

vesiculosus were collected in October from the Atlantic Ocean on the coast of Nova Scotia, Canada. *Laminaria japonica* originated from the South Korean sea. After processing in a laboratory blender and sieving to select an appropriate particle size distribution (d = 0.30–0.84 mm), the biomass was washed with 50 vol (v/w) of 0.1*M* hydrochloric acid to convert the acidic groups into their hydrogen forms, and then rinsed extensively with distilled and deionized water to remove the solubilized salts (Na, K, Mg, and Ca). The biomass was dried to constant weight at 60°C. Sodium alginate (lot no. 03916HV, Aldrich Chemical Company, Milwaukee, WI) was converted into alginic acid by two washings in 100 vol of 0.1*M* HCl. After centrifugation (10 min at 9800*g*), excess HCl was removed by two additional washings in distilled deionized water. The final pellet of alginic acid was then freeze-dried and stored at 4°C.

Chemicals

Concentrated hydrochloric acid (36.5–38%), certified 0.1*M* sodium hydroxide, heavy metal nitrates were purchased from Fisher Scientific (Ottawa, ON, Canada). ACS-grade sodium carbonate, lab-grade acetone, and absolute methanol were from Anachemia (Montreal, QC, Canada). Poly(hexamethylene-biguanidinium chloride) [$PHMBH^+Cl^-$] was obtained as a 20% solution from Zeneca Biocides (Wilmington, DE).

Titration of the Biomass

Potentiometric titrations of protonated biomass were conducted as described earlier *(18)*. Typically, 500 mg of algal biosorbent, converted to hydrogen form with 0.1*M* hydrochloric acid and washed with deionized water to a constant conductance, was dispersed in 100 mL of 1 m*M* sodium chloride solution prepared with deionized water. Titration was carried out by a stepwise addition of 0.25 mL of 0.1*M* sodium hydroxide, whereas the suspension was stirred under an atmosphere. Aliquots from the supernatant were regularly sampled (0.5 mL) to measure the amount of alginate released from the biomass into the aqueous phase.

Extraction and Chemical Analysis of Alginate

Alginate was extracted from the dry seaweeds using 2% solution of Na_2CO_3, according to the method of Percival and McDowell *(19)*. The concentration of alginate in crude extracts of algal biomass or in the titration supernatants was determined according to the method of Kennedy and Bradshaw *(20)*, using poly(hexamethylene-biguanidinium chloride) [$PHMBH^+Cl^-$]. The intrinsic viscosities of the alginate samples were measured for the crude extracts by extrapolation at zero concentration according to Haug and Smidsrod *(21)* using a Cannon viscometer #50 (k = 0.004 cS/s). The molecular weight, M_w, of the alginate samples can be related to the intrinsic viscosity [η] using the Mark-Houwin equation:

$$[\eta] = K \times \overline{M}_w \quad (1)$$

K and a are parameters depending only on the alginate composition and the ionic strength of the solution.

Purified alginate samples were analyzed by ^{13}C-nuclear magnetic resonance (NMR) using a Varian XL300 spectrometer. Sodium alginate samples (100 mg) dissolved in 5 mL D_2O were placed in 10-mm id NMR test tubes, ^{13}C-NMR spectra of 75 MHz were recorded from 32,000–35,000 scans with proton decoupling using 0.9 acquisition time, 0.8 relaxation delay, and 60° pulse width at a temperature of 80°C. The monomer composition, and the diad and triad frequencies were determined according to Grasdalen et al. *(22)*. The average length of blocks consisting of more than two contiguous G-units was calculated according to the formula:

$$N_{G>1} = (F_G - F_{MGM}) / F_{MGG} \quad (2)$$

where F_G, F_{MGM}, and F_{MGG} are the frequencies of G-residues and MGM or MGG triads, respectively, in the alginate polymer. The heteroblock frequency is $F_{MGM} + F_{GMG}$.

Metal Uptake Comparisons

In order to compare the metal binding properties of the different biosorbents, 50 mg of each sample were contacted with a 5-m*M* metal solution (initial concentration). This allowed complete saturation of the sorption sites of each biosorbent. The suspensions were agitated at room temperature on an orbital shaker, allowing the sorption reaction to attain equilibrium. The pH was adjusted frequently to 4.5 using 0.1 m*M* NaOH. When not controlled, the final pH of each sample was about 2.9 ± 0.1. The initial and final metal concentrations in the solution were determined by Atomic Absorption Spectrophotometry (Thermal Jarell Ash model Smith-Hieftje II). The biosorbent metal uptake capacity (q) was then calculated from the mass balance between the initial (C_i) and final (C_f) metal concentrations according to the formula:

$$q = [(C_i - C_f) \times V / m] \quad (3)$$

where V is the volume of the solution and m is the mass of biosorbent.

RESULTS

Alginate and Sulfonate Content in the Brown Seaweeds

Figure 1A shows the amount of alginate extracted from native and protonated biomass of the four brown seaweeds as measured with

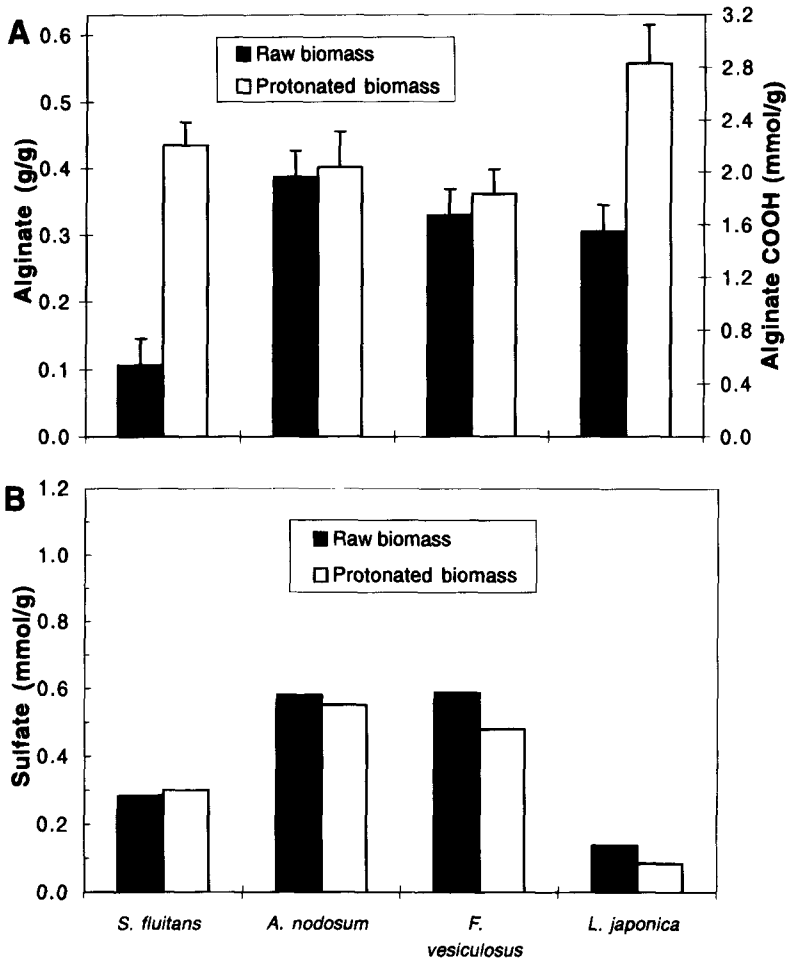

Fig. 1. Concentration of potential ligands for heavy metal cations in brown seaweeds. **(A)** Determination of alginate concentration in crude extracts (0.5 g of sample in 200 mL 2% Na_2CO_3) from native and protonated biomass using the $PHMBH^+Cl^-$ reagent. **(B)** Sulfate determination by ion chromatography after acidic hydrolysis of seaweed samples (4 h, 100°C, in 1 mol/L HCl).

[$PHMBH^+Cl^-$] reagent. The highest values were obtained from protonated algae, with 0.44, 0.40, 0.36, and 0.56 g/g (±10%) for *S. fluitans*, *A. nodosum*, *F. vesiculosus*, and *L. japonica*, respectively. The *Y*-axis indicates the corresponding amount of uronic acid subunits in mmol/g. The yields from extraction are lower with native alga, especially with *S. fluitans* and *L. japonica*. This observation must be related to the fact that the acidic treatment of the seaweeds removes the divalent cations that originally complexed the alginate and compete with the Na ions from Na_2CO_3.

Figure 1B indicates the amount of sulfonate groups determined in the native and protonated seaweed biomass. *Ascophyllum nodosum* and

Table 1
Characteristics of Alginate Samples Determined by NMR and Viscometry

Algal species	Monad Frequency (F_G)	Triad frequency (F_{GG})	Heteroblock frequency ($F_{MGM}+F_{GMG}$)	Mean blocklength $N_{G>1}$	Intrinsic viscosity [η] (g/dL)
F. vesiculosus	0.466	0.27	0.17	5.18	2.5
A. nodosum	0.275	0.136	0.31	3.06	2.8
S. fluitans	0.458	0.283	0.17	4.58	6.3
L. japonica	0.28	0.113	0.30	2.33	15.4

F. vesiculosus contain significantly higher amounts of sulfonate groups, with 0.56 mmol/g. *Laminavia japonica* contains the lowest amount of sulfonate groups, only 0.15 mmol/g. The sulfonate content of the protonated seaweeds is generally lower because of the partial hydrolysis of these groups during the acidic treatment.

Structure and Properties of Extracted Alginates

The compositional characteristics of the alginates extracted from the four seaweeds are given in Table 1 as determined by NMR spectroscopy. The alginates derived from the seaweeds studied here contained <50% of guluronic acid residues and could be classified in two categories: alginates from *S. fluitans* and *F. vesiculosus*, which contain about 46% of G-residues and 27–28% of GGG triads, with only 17% heteroblocks; *A. nodosum* and *L. japonica* alginates, contain only 28% of G-residues and 12% of GGG triads, but more than 30% of heteroblocks. The mean block length of G-homoblocks containing more than one G-residue is more dispersed among the algal samples, with the following increasing order: *L. japonica* < *A. nodosum* < *S. fluitans* < *F. vesiculosus*. Also shown in Table 1 are the intrinsic viscosities of the extracted alginate samples measured as suggested by Haug and Smidsrod *(21)*. The intrinsic viscosity [η] of the alginate samples can be related to their molecular weight using the Mark-Houwink equation:

$$[\eta] = K \times \overline{M}_w^a \tag{4}$$

with $K = 2.0 \cdot 10^{-5}$ and $a = 1.0$.

The values in the last column of Table 1 indicate important mole-wt variations that can exist between alginates obtained from different seaweed samples. The intrinsic viscosity increases in the following order: *F. vesiculosus* < *A. nodosum* < *S. fluitans* < *L. japonica*. The calculated average mol wt ranged from 125,000 ± 15,000 for *F. vesiculosus* to 750,000 ± 50,000 for *L. japonica*.

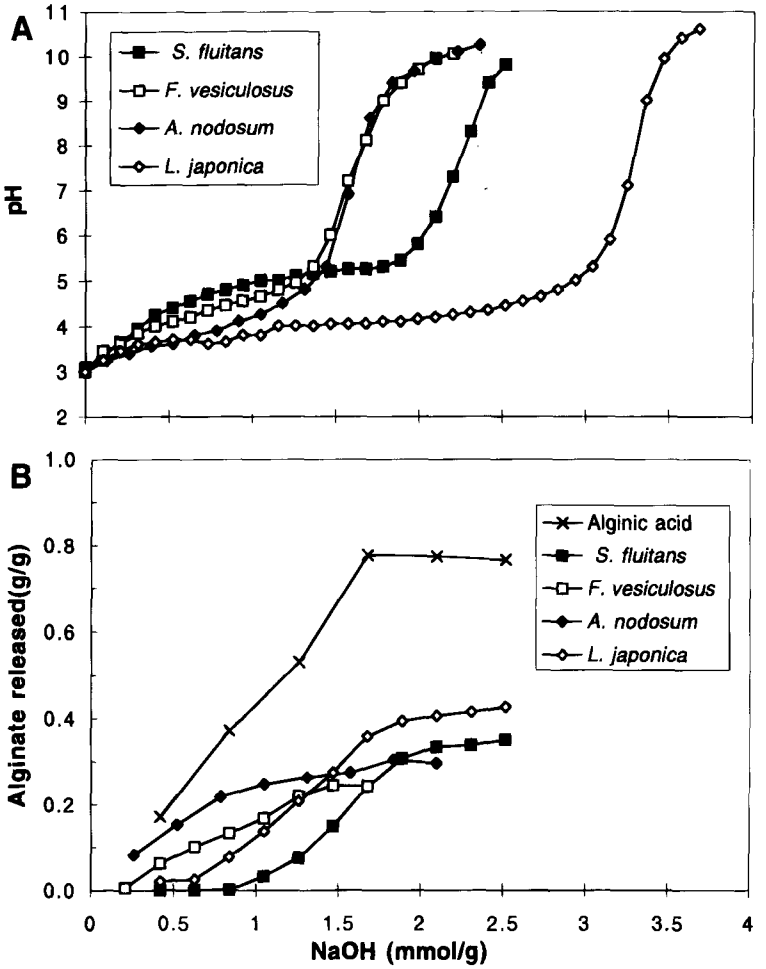

Fig. 2. Titration of seaweed biomass with sodium hydroxide. **(A)** Potentiometric titration (stepwise addition of 0.5 mL NaOH 0.1 mol/L to 100 mL suspensions containing 0.5 g of algal biomass particles). **(B)** Alginate released from seaweed biomass into solution during the NaOH titration (determination by the $PHMBH^+Cl^-$ reagent).

Alginate Leaching During the Titration of the Biosorbents

The potentiometric titration curves of the four algal samples during the addition of sodium hydroxide are shown in Fig. 2A. The first inflection points of the curves, corresponding to the titration of strongly acidic sulfonate groups, could not be determined accurately, but were in the same range as determined by the previous chemical analysis. The total amount of weakly acidic carboxyl groups determined for *S. fluitans* and *L. japonica* (2.0 and 3.2, respectively) were also similar to those determined with [$PHMBH^+Cl^-$] reagent. However, the total titers obtained with *A. nodosum*

and *F. vesiculosus* (1.6) were significantly lower than the sum of uronic acids and sulfonate residues determined chemically. Two reasons could explain this unexpected result. First, *A. nodosum* and *F. vesiculosus* contain larger amounts of sulfated polysaccharides, which could interact with the [$PHMBH^+Cl^-$] reagent, resulting in an overestimation of the total alginate. Second, the alginate from these algal samples could be partially acetylated, making the comparison difficult between the two analytical methods.

The apparent pK_a values of the different alginates can be graphically estimated from the potentiometric curves in Fig. 2A. These values vary from 4.0–5.2 and are very different and higher than the intrinsic values corresponding to carboxyl groups from mannuronic and guluronic acids (3.38 and 3.65, respectively). This discrepancy is explained later in the discussion.

Solubilization of alginate was observed and measured as sodium hydroxide was added to the different algal suspensions (Fig. 2B). It was compared with the solubilization of a standard alginate sample under the same conditions. Whereas the solubilization of the alginate standard was directly proportional to the sodium hydroxide added, the other samples displayed fairly different behavior. Alginates from *A. nodosum* and *F. vesiculosus* readily solubilize, with a short delay for the latter as compared to the standard sample (Aldrich). *Laminaria japonica* alginate was released into the solution only after the addition of 0.5 mmol NaOH/g and was only partially extracted at the end of the titration, when the suspension became very viscous. The alginate from *S. fluitans* was the most resistant to the extraction in the presence of Na. Almost 1.0 mmol NaOH/g was needed before the first release of alginate could be observed. These results are in good agreement with those obtained during the extraction of alginate with sodium carbonate.

Heavy Metal Binding

The metal binding properties of the four algal samples for five different metallic cations (Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}) were investigated at two different pH values (4.5 and 2.8) and the results are summarized in Fig. 3. Figure 3A shows that the maximum metal uptake capacities of the seaweeds are directly related to their acidic titers at pH 4.5. This tendency is reflected for all heavy metals. the presence of higher guluronate concentrations in *S. fluitans* and *F. vesiculosus* alginates does not interfere. The average ratio between the amount of cations bound at pH 4.5 and the total number of acidic groups varies from 0.36–0.50 mol/mol, depending on the metal considered. The selective binding by *L. japonica, S. fluitans*, and *F. vesiculosus* biosorbents of the six metals at pH 4.5 followed the trend Pb > Cu ≥ Cd > Zn ≥ Ca (Fig. 3B), and is related to the electronegativity of the element considered. The same result was observed at pH 2.8.

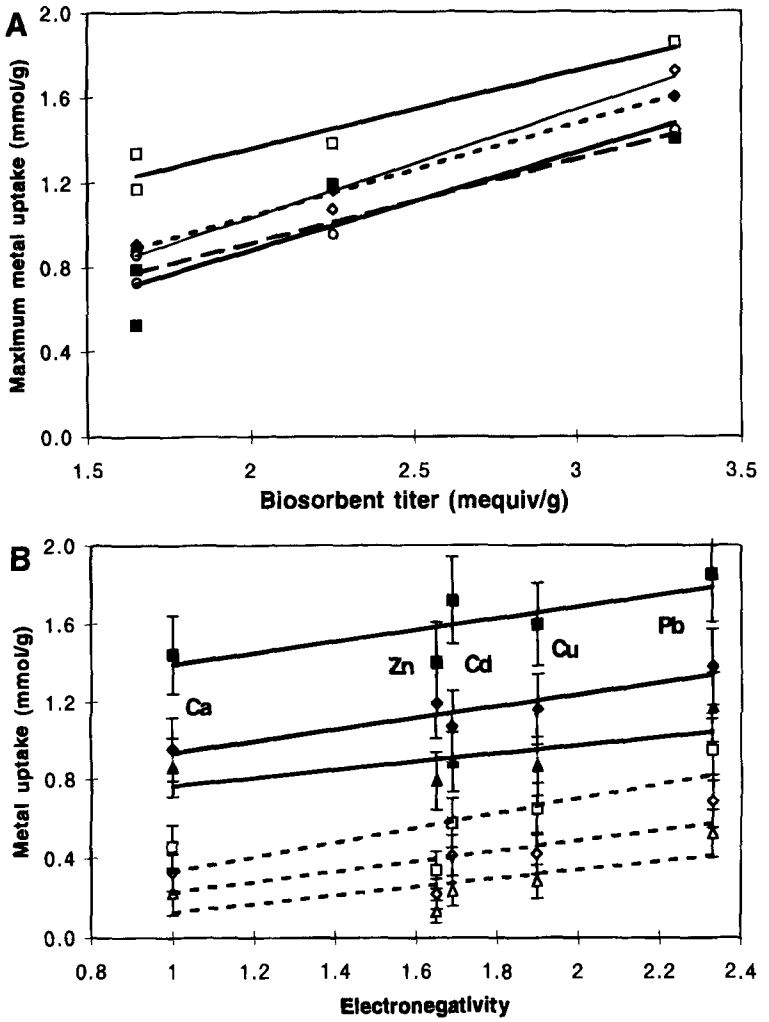

Fig. 3. Heavy metal binding capacities of selected seaweeds. Determined at high soluble metal concentrations leading to the maximum uptake at pH 4.5 and 2.5. **(A)** Relationship between the acidic titer and the maximum Ca, Zn, Cd, Cu, and Pb uptake at pH 4.5. □ Lead; ◆ Copper; ◇ Cadmiun; ■ Zinc; ○ Calcium. **(B)** Relationship between electronegativity and metal uptake at pH 4.5. ▲ *F. vesiculoses*, pH 4.5; △ *F. vesiculoses*, pH 2.8, ■ *L. japonica*, pH 4.5; □ *L. japonica*, pH 2.8; ◆ *S. fluitans*, pH 4.5; △ *S. fluitans*, ph 2.8.

DISCUSSION

In relation to the structure and properties of the biomass alginate component, two different aspects of biosorption were examined: the metal binding capacity and the stability of the biosorbing material. The extractability of alginates from seaweed biomass was observed and quan-

tified in relation to the Na concentration and the solution pH. According to these criteria, the seaweeds examined could be divided into two groups. *Ascophyllum nodosum* and *F. vesiculosus* were observed to release alginate easily at relatively low pH values and in the presence of a low concentration of sodium hydroxide. On the contrary, *L. japonica* and *S. fluitans* alginate required higher amounts of sodium hydroxide to release alginate (0.5 and 1.0 mmol NaOH/g of biosorbent, respectively).

The relative density of alginate inside the seaweed biomass increases in proportion to the increasing values of the apparent degree of acidic dissociation (pK_o), in the following order (Fig 2A): *L. japonica* < *A. nodosum* < *F. vesiculosus* < *S. fluitans*. In addition, the molecular weight of different alginates, as derived from intrinsic viscosity measurements, increased in the order: *F. vesiculosus* < *A. nodosum* < *S. fluitans* < *L. japonica*. Higher solubility of *A. nodosum* alginate in acidic solutions was previously related to a smaller proportion of homopolymeric blocks *(9)*. In the present study, no correlation was found between the M/G ratio or the heteroblock frequency and the extractibility of alginate from the various biomass types examined. The leaching of alginate could thus be qualitatively a function of its molecular weight and its density inside the algal cell wall matrix, reflected by the intrinsic viscosity and the apparent pK_o value of the material.

Electrostatic interactions between adjacent acidic groups in the algal cell walls can modify the acidic properties of these groups *(23)* when compared to the same moieties in solution. An explanation of this variability is also provided by the Gibbs-Donnan interpretation of the counter-ion concentrating domain at the surface of the various alginate components *(24)*. An increase in apparent pK_o values could thus be related to an increasing density of the alginate in the respective algal cell walls. *S. fluitans* biomass, which contains alginate of a relatively high-mol-wt (around 400,000 Dalton) and displays a high pK_o, would tend to retain most of its alginate component. *Fucus vesiculosus* and *A. nodosum* samples, with lower pK_o and molecular weights (150,000 Dalton), were more sensitive to the presence of Na ions, and their alginate leached more easily. Alginate of *L. japonica* had the highest molecular weight, but the lowest pK_o, making its extraction much easier when compared with *S. fluitans*. This tendency was also demonstrated during the alginate extraction experiments from native seaweeds with sodium carbonate. Indeed, the alginate recovery from *F. vesiculosus* and *A. nodosum* was much better than from *S. fluitans* and even *L. japonica*, which makes the latter two seaweeds more suitable for biosorption applications.

The metal binding capacity at high solute concentrations was also analyzed with five metals at two different pH values in order to identify

any potential metal selectivity depending on the algal species and their alginate composition. The binding capacity for each metal was observed to be directly related to the total titer of the respective biomass, with an average binding stoichiometry between 0.36 and 0.50 equivalent metallic cation/equivalent acidic group at pH 4.5, depending on the element. The maximum uptake at pH 4.5 was also observed to increase with increasing electronegativity of the metal, with some minor deviations. A relationship between electronegativity and ion-exchange properties of alginate was observed previously *(25)*. Distinct affinity of pure alginate for Ca ions compared to other alkaline earth elements was attributed to a specific autocooperative binding involving guluronic residues *(10)*. G-rich alginates were also found to display higher affinity for heavy metals than M-rich samples *(13)*. A specific mechanism for Ca binding was recently elucidated by ^{13}C-NMR and SASX studies of alginate during solgel transition *(26,27)*. However, no clear relationship for any metal between the binding capacity and the alginate composition (M/G ratio or heteroblocks frequency) could be established in this work. The stereochemical arrangement of carboxylate groups in the two types of uronic acid residues appears to be less important in the case of alginate immobilized in the biomass than for pure alginate, which has a much higher flexibility. In addition, the frequencies of guluronic acid residues in the seaweed samples studied might be too small to cause any significant metal binding selectivity.

At a low pH value (2.8), the high sulfonate groups content of *F. vesiculosus* and *A. nodosum* was expected to play an increasing role in metal uptake. However, these two biosorbents did not show any increase in their relative binding capacity at low pH value compared to the others.

These results demonstrate the power of simple potentiometric titration in characterizing brown seaweeds as biosorbents for metal removal from aqueous solutions. This technique allows the determination of the maximum cation-exchange capacity, directly related to the total titer of the biomass. It can also yield useful information concerning the stability of the biosorbent by simply monitoring the change in viscosity with increasing additions of sodium hydroxide. The determination of the apparent acidic dissociation constant K_o can also provide a good indication of the density of the alginate in the biosorbent.

This study provides quantitative evidence for the choice of *S. fluitans* as the most suitable algal biosorbent identified so far. The other seaweeds, especially *A. nodosum* and *F. vesiculosus*, would need to be stabilized by crosslinking or alternative techniques to serve as suitable biosorbents. It should be emphasized that high Na concentrations could be damaging to unprocessed brown seaweed-derived biosorbents.

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