Cadmium Biosorption by S. fluitans: Treatment, Resilience and Uptake Relative to Other Sargassum spp. and Brown Algae

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Species of the brown algae Sargassum have been targeted for use in the implementation of strategies to remediate toxic heavy metal contamination in effluents and drinking waters. This work focuses on some of the intrinsic physico-chemical properties of the algal material and aspects of the sorption mechanism, in particular: their maximal metal uptake, the influence of particle size and their resilience to leaching during equilibrium batch experiments. In addition to S. fluitans, the database on cadmium uptake capacities by Sargassum is extended to include S. thunbergii and S. oligocystum, and these are compared to those of two common brown algae. Results of our experiments demonstrate that cadmium sorption is independent of the range of particle sizes investigated (<2 and 3–6 mm), thereby indicating that sorption is not a function of the specific surface area of the biomass exposed to the solution. Dissolved organic carbon (DOC) analyses reveal that leaching to the cadmium solutions during the metal sorption reaction is independent of the biomass preparations used to obtain the two size fractions but decreases with increasing final cadmium concentration.

Key words: alginate, biosorption, cadmium, Sargassum fluitans, Sargassum thunbergii, Sargassum oligocystum, leaching

Introduction

The contamination of aquatic environments by toxic heavy metals due to the increased activities of metal mining and metal processing industries has become a major source of concern over the last several decades. The removal of these contaminants from industrial effluents is, therefore, a priority both in current environmental research and legislation. Consequently, there is a strong demand for economic remediation technologies, particularly in developing countries. Bioremediation is a viable alternative, especially if the sorbent material can be reused and the heavy metals recovered. The use of natural biomass as a substrate for metal-ion chelation has been termed biosorption and refers to a passive, or rather, nonmetabolically mediated process. Numerous articles have been published in which the metal sorption properties of a variety of biosorbents, including fungi, yeast, bacteria and algae (e.g., Chen et al. 1990; Ho et al. 1995; Kapoor and Viraraghavan 1995; Volesky 1990) have been characterized.

The brown algae Sargassum was shown to possess the required mechanical properties (Fourest and Volesky 1997), chemical affinity (Davis et al. 2003a,b) and sorption capacity (Fourest and Volesky 1997; Davis et al. 2000) to bind metals such as Pb, Cu, Hg and Cd in an effective, reversible and cost-effective manner. Previous studies of Sargassum have focussed on the implementation of a fully operational bioremediation system that employs a fixed-bed column design (Kratochvil et al. 1995, 1997; Kratochvil and Volesky 1998, 2000), modelling of electrostatic effects (Schiewer and Volesky 1995, 1997a,b), and elucidation of the primary binding mechanism (Fourest and Volesky 1996). Amongst the constituents of Sargassum algae, alginate was identified as the most important with respect to metal binding (Fourest and Volesky 1996). Alginate is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged in a nonregular, block-wise order along the chain. The relative abundance of the M and G residues and their macromolecular conformation determine the physical properties and the affinity of the alginate for divalent metals (Haug et al. 1967). A detailed description of the cellular structure and biochemistry of brown algae can be found in Davis et al. (2003a).

Cadmium was chosen for binding studies to Sargassum species because it is highly toxic and is included in the so-called “Red-List” of priority pollutants and in List I of the EEC Dangerous Substances Directive (1976). Furthermore, binding of this target element by Sargassum seaweed was described previously (e.g., Fourest and Volesky 1996, 1997; Davis et al. 2000) and it was used in modelling and implementation studies rel-
evant to *Sargassum* biosorption (Figueira et al. 2002a), but these previous studies were not designed to highlight the influence of size fractionation and metal uptake on biomass degradation.

The objectives of this work were: (i) to investigate the influence of particle size on the total divalent metal uptake and resilience (i.e., leaching of dissolved components) of *S. fluitans* during the equilibrium sorption process, and (ii) to compare the divalent metal (i.e., cadmium) uptake capacity of three *Sargassum* species with respect to other brown algal species. Accordingly, this study was designed to characterize some of the intrinsic properties of the biomass and further elucidate the metal binding mechanism. It does not focus on the engineering and feasibility of remedial processes to drinking or effluent waters, including competitive binding; these issues are addressed elsewhere (e.g., Kratochvil and Volesky 1998, 2000; Figueira et al. 2002a). Conventional, equilibrium batch experiments were performed to determine the cadmium uptake capacity of *S. fluitans*, *S. thunbergii* and *S. oligocystum*. Results of these experiments are pivotal to the interpretation of other laboratory studies (e.g., Davis et al. 2003b,c) carried out with brown algae under similar conditions.

**Materials and Methods**

**Samples**

*Sargassum fluitans* originates from the Sargasso Sea of the northwest Atlantic Ocean and is one of the few known pelagic species of *Sargassum* algae. It is carried by winds and tides to the shores of Cuba where it accumulates in copious quantities along the beaches. The biomaterial used in this work was collected fresh at several locations along Guanabo Beach, 30 km east of Havana. *Sargassum oligocystum* was collected from the reef flat, 1 to 4 m below tide level, on the fringing reef at Goold Island (18°10.9'S, 146°10.2'E) on the inshore, central Great Barrier Reef, Australia. This benthic species is found as part of mixed species assemblages that dominate the reef flat of many inshore reefs on the Great Barrier Reef (McCook 1996, 1997). *Sargassum thunbergii* was collected at Songjong Beach, Pusan Bay, Korea. *Macrocystis pyrifera* and *Laminaria digitata* were sampled in Nova Scotia, Canada. The latter three species are also benthic species. Each sample was treated with a 0.2 N HNO₃ solution at a 1:50 (w/v) ratio in order to remove light ions bound to the biomass. The biomass was stirred in solution for 3 h, followed by successive rinses with deionized water (3 times, 45 min each) or until the pH of the solution reached 4.5. The resulting biomass was dried in an oven overnight at 60°C.

Two different particle size fractions were prepared from *S. fluitans*. For the first (<2 mm), the dried biomass was placed in a high-speed blender and minced for at least 2 min until the minimum particle size was reached. For the second, the dried *S. fluitans* was cut with a sharp knife into 3- to 6-mm particles. The other four seaweed samples were prepared by the first method, using a high-speed blender with a resulting biomass particle size of <2 mm. Compared to the uniformly sized, knife-cut samples, the lower size fraction (i.e., <2 mm) includes a much wider range of particle sizes and a very fine powder is produced by the vigorous blending/mincing. A different batch of *S. fluitans* was used for the maximal metal binding experiments than for the leaching experiments. This resulted in slightly different cadmium uptake values for the two *S. fluitans* samples, as will be discussed below.

**Metal Ion Binding and Organic Carbon Leaching Experiments**

Cadmium metal-bearing solutions were prepared by dissolving Cd(NO₃)₂·H₂O salt (ACP Chemicals) into deionized distilled water and adjusting the pH to 4.5 with a dilute LiOH solution. The chemical equilibrium program MINEQL⁺ (Schecher 1991) was used to calculate the speciation of cadmium in the aqueous solutions as a function of total salt concentration and solution pH. For all experiments, the cadmium was determined to exist almost solely in the free divalent form (i.e., Cd²⁺). Batch equilibrium sorption experiments were performed in 125-mL Erlenmeyer flasks containing 50 mL of a cadmium solution of known initial concentration to which 100 mg of size-fractionated, dried biomass particles were added. The initial solution cadmium concentrations used to carry out the isotherm experiments were 0.25 to 3.5 mM Cd whereas the concentrations ranged from 2.58 to 5.92 mM Cd for the maximal metal uptake experiments (also isothermal). The flasks were then covered with Parafilm in order to minimize evaporation. The suspensions were mildly agitated on a rotary shaker (New Brunswick Scientific) at either 180 or 300 rpm and maintained at room temperature (22°C) throughout the equilibration period (i.e., up to 26 h). During this period, pH was monitored at fixed intervals, ranging from 5 min to 1.5 h, using a Thermo Orion combination pH electrode (Model 91-07) calibrated against three NIST-traceable buffers (i.e., pH = 4.01, 7.00 and 10.00) and the solution pH was maintained at pH = 4.50 ± 0.05 by adding incremental amounts of a 0.1 N LiOH solution. Volume additions were recorded in order to correct the solution volume and, hence, final cadmium concentration. Metal-free and biosorbent-free blank solutions were used as controls. Organic carbon leaching experiments, performed in the same manner as the metal binding experiments, were also carried out in distilled water, in which the pH was maintained at 4.5. Cadmium uptake increases with increasing pH (e.g., Davis et al. 2000) but its solubility is limited beyond a pH of 6.8.
whereupon the formation of hydrolysis products and precipitation can occur. Furthermore, leaching of seaweed constituents is significant at a high pH (e.g., Smidsrød 1970; Fourest and Volesky 1997). On the other hand, the pH must be sufficiently higher than the acid dissociation constant, $K_a$, of the poly-uronic acids ($pK_a \approx 3.5$) that make up the alginate polysaccharide to ensure that significant binding occurs.

After 24 to 26 h of equilibration (determined by a separate set of time-series experiments), the samples were left to stand unstirred for several minutes in order to let the solid particles settle to the bottom of the Erlenmeyer flasks. Separate aliquots of the supernatant were then taken for both cadmium and dissolved organic carbon (DOC) analyses. Samples destined for dissolved cadmium analysis were syringe-filtered through a 0.45-µm nitrocellulose Millipore membrane. Samples destined for DOC analysis were vacuum-filtered through a 0.3-µm nominal pore size, glass fibre filter (EPM-2000, Whatman). The cadmium concentration in the filtered supernatant (both initial and final) and control solutions were determined by atomic absorption spectrometry (AAS, Thermo Jarrel Ash, model Smith-Hiefje II; 0.1 ppm detection limit, reproducibility >96%). An external calibration curve was obtained with solutions prepared from dilution of a 1000-ppm standard stock solution (Fisher Scientific). The DOC was measured using a total organic carbon (TOC) analyzer (Dohrman DC-80) following a UV-persulfate oxidation (Bauer et al. 1991). Samples were acidified prior to analysis and the TOC analyzer was calibrated using 200 and 400 mg/L solutions of K-phtalate prepared from a 1000-mg/L standard solution.

**Results and Discussion**

**The Biosorption Process**

The ion-exchange reaction (Myklestad 1968) which conventionally describes the biomass sorption process is:

$$2BH + M^{2+} \rightleftharpoons B_2M + 2H^+ \quad (1)$$

where B is the binding site and $M^{2+}$ represents the binding ion. Accordingly, protons are released into solution causing the solution pH to drop. In order to maintain the pH at a constant value, LiOH is added during the course of the experiment. Characterization of metal binding capacity typically requires measurements at 6 to 9 initial metal concentrations over a broad range (e.g., 0.25 to 3.5 mM) of concentrations. If triplicate experiments are performed for each metal concentration, including the controls, at least 20 batch experiments will be run in parallel. In the absence of an automated system, simultaneous monitoring of the solution pH of each experiment is not possible and, hence, the amount of time required to reach metal-biomass equilibrium for each experiment is prolonged by delays introduced by the pH adjustments (also see Yun and Volesky 2003). Consequently, whereas ion-exchange equilibrium between the solutions and the biomass may be achieved within a few hours or less at a fixed pH, experiments were carried out over a 24- to 26-h period.

**Leaching of Organic Carbon in the Absence of Cadmium Biosorption**

Leaching of organic carbon from the biomass in pure water was monitored for 24 h as the pH was maintained at 4.5 by incremental additions of a dilute LiOH solution. The experiment was carried out only with the smaller size fraction (i.e., <2 mm) under the premise that it would be more susceptible to leaching.

Results of the experiment, shown in Fig. 1, reveal a rapid rise of the DOC concentration in the solutions within the initial 4 h. The DOC concentration reaches a near maximal value of approximately 56 mg of DOC/g of *S. fluitans* at this time and very little material is leached to solution thereafter. An independent set of experiments was performed at a faster stirring rate (300 rpm, data not shown) and the same trend and amount of leaching were observed. Leaching of alginate from brown algal biomass has previously been reported (Fourest and Volesky 1997; alginate specifically assayed according to the method of Kennedy and Bradshaw 1987) during titration of various algal species by NaOH using the batch experimental approach or with pre-treated [i.e., KOH or Ca(OH)$_2$; DOC measured] biomass (Figueira et al. 2000b).

**Biomass Particle Size and Cadmium Sorption Behaviour**

Parallel, triplicate Cd sorption experiments were performed with two different size fractions (<2 and 3–6 mm) and the organic carbon leached during the equilibration period was measured at the end of each experi-

![Fig. 1. DOC leaching as a function of time by *S. fluitans* of the less than 2-mm particle size fraction. Solution held at constant pH = 4.5.](https://iwaponline.com/wqrj/article-pdf/39/3/183/230335/wqrjc0390183.pdf)
ment (duplicate analyses). Biosorption metal uptake \((q)\) was calculated from the sorption system mass balance:

\[
q = V(C_i - C_f)/S
\]

where \(V\) is the solution volume, \(S\) is the amount of solids (biomass), and \(C_i\) and \(C_f\) are the initial and final metal concentrations, respectively.

The experimental data were fit to the Langmuir sorption model isotherm:

\[
q = (q_{max} C_f)/(b^{-1} + C_f)
\]

The two parameters, \(q_{max}\) and \(b\), conventionally reflect the nature of the sorbent material (i.e., sorption capacity and affinity, respectively) and can be used to compare its biosorption performance. The data depicted in Fig. 2 were fit to the Langmuir equation using the KaleidaGraph software with an internal algorithm based on a least squares approach. The curve drawn in Fig. 2 is the model fit to the data. The maximum experimental error (to a 95.4% level of confidence) was calculated to be less than 4% on the basis of the triplicate experiments/measurements, and is depicted by error bars (Fig. 2). There was little observable difference in cadmium uptake for the two different \(S.\ fluitans\) particle size fractions over the entire range of final cadmium concentrations. Accordingly, the combined sets of data were fit to the Langmuir equation and yield values of 0.77 and 11.2 for \(q_{max}\) and \(b\), respectively.

These results clearly demonstrate that sorption by this biomaterial is not surface controlled (i.e., determined by the exposed surface area of the solid), since a higher uptake would have been expected for the smaller size fraction with the greater specific surface area. The absence of a particle-size effect on cadmium uptake capacity is consistent with the notion that sorption takes place throughout the biomass particle (Andresen et al. 1977) and reflects the fact that the algal thallus is composed of a network of individual brown algal cells. Each of these plant cells possesses a cell wall primarily composed of macromolecules such as cellulose, fucoidan and alginate (Bold and Wynne 1985). It is the alginate present in both the cell wall matrix and in the mucilage or intercellular material (Mackie and Preston 1974; Chapman 1980) that is responsible for most of the metal binding at pH 4.5 (Fourest and Volesky 1996; Davis et al. In press). This assumption was implicit in the equilibrium models elaborated by Scheiwer and Volesky (1995, 1997a,b) that account for variations in metal uptake as a function of the metal-ion concentration, pH and ionic strength. In their conceptual models, the individual biomass particles were treated as a gel matrix, with the metal ions free to permeate throughout the gel and bind to the macromolecules.

**Cadmium Sorption and Leaching of Organic Carbon**

The amount of organic carbon lost from the biomass to the solutions during cadmium uptake in equilibrium batch experiments decreased with increasing \(C_f\), and a minimum value of approximately 55.0 mg of DOC/g biomass was measured at a \(C_f\) of approximately 1.80 mM Cd (Fig. 3). The decrease in organic carbon loss was not linear over the range of cadmium concentrations studied but is a mirror image of the cadmium sorption isotherm. It would appear that the amount of organic carbon leached during the sorption experiments is related to the quantity of cadmium bound to the biomass. Figueira et al. (2000a) measured the leaching of organic carbon from biomass during metal biosorption in a fixed-bed column packed with \(S.\ fluitans\). They found that leaching, expressed in terms of measured total organic carbon in the eluted solution, decreased when high concentrations of Zn (7.5 mM) were passed through the column relative to either distilled water or low concentrations of Zn (0.075 mM). These observations were attributed to cross-linking of the alginate matrix by the divalent zinc cations.

**Fig. 2.** Cadmium biosorption isotherm for \(S.\ fluitans\). Solid line depicts the modelled Langmuir isotherm for the two size fractions. (●) <2 mm; (▲▲) 3 to 6 mm; Langmuir isotherm parameters: \(b = 11.2\), \(q_{max} = 0.774\).

**Fig. 3.** DOC leaching as a function of equilibrium cadmium concentration \((C_f)\) for cadmium biosorption isotherms for \(S.\ fluitans\) for two size fractions. (●) <2 mm; (▲▲) 3 to 6 mm.
The binding of divalent cadmium ions to the biomass results in a greater drop in pH relative to the control system in deionized water. Consequently, more LiOH was added to the cadmium bearing solutions in order to maintain pH fixed during the course of the equilibration. The addition of Li⁺ to these solutions likely promotes the dissolution of the alginate and leaching of organic material to the solutions but cross-linking of the alginate by the divalent cadmium ion limits its solubility. Therefore, despite cross-linking at the higher cadmium concentrations, a minimal amount of organic material (i.e., 55.0 mg of DOC/g of biomass) is leached into solution due to the LiOH additions, a value corresponding to the plateau reached in the control experiment (i.e., 60 ± 2 mg of DOC/g of biomass). A recent study performed by Yun and Volesky (2003) on the influence of Li⁺ on cadmium biosorption by Sargassum polycystum reveals that lithium also binds to the carboxyl sites of the biomass and may interfere with the uptake of protons and cadmium. Based on their model and estimated dissociation constants, less than 3% of the carboxyl sites would be occupied by Li⁺ at pH 4.5 and over the range of [Li⁺]:[Cd²⁺] ratios used in our experiments.

No significant differences were observed in the amounts of organic carbon leached from the two different size fractions over the entire range of final cadmium concentrations. In that respect, no additional degradation of the biomass could be attributed to the vigorous blending/mincing it was subjected to during the preparation of the smaller size fraction (<2 mm). Finally, since equilibrium metal uptake is independent of size fraction, the standard technique of mincing/blending of the biomass is deemed suitable for laboratory-based studies.

Maximal Cadmium-Uptake Experiments

In an effort to extend the database on biosorption performance by species of Sargassum seaweed, the maximal cadmium uptake capacity of two species, S. thunbergii and S. oligocystum, for which no data are available in the literature, were determined at pH 4.5 and relatively high levels of equilibrium cadmium concentration. Their cadmium uptake capacities and that of two other brown algae, Macrocystis pyrifera and Laminaria digitata, were compared to that of the S. fluitans samples measured in this study. The maximal cadmium uptake by the algae was characterized by performing four sorption experiments (each in triplicate) for each algae, at different and increasingly high concentrations of cadmium. All solution concentrations were sufficiently high (Davis et al. 2000) to saturate the sorption sites and be well within the isotherm plateau or qₘₐₓ region. Results of all 12 experiments (9 for M. pyrifera) were considered to reflect maximal cadmium binding and, hence, were averaged in order to obtain a mean value of cadmium uptake for each species. This experimentally determined quantity is herein designated as max q, in order to distinguish it from the Langmuir model fitting parameter qₘₐₓ. The 12 replicates yielded a maximum uncertainty of 3% at the 95.4% level of confidence. The results are shown in Fig. 4 and the max q values for each species are summarized in Table 1.

The max q for S. thunbergii (0.69 ± 0.02) falls within the range of values previously obtained for Sargassum species. Davis et al. (2000) reported a range in modelled qₘₐₓ values of 0.66 for S. filipendula (b = 5.20) to 0.90 for S. polycystum (therein termed Sargassum sp. 1; b = 21.5). The max q values for S. fluitans and S. oligocystum reported here, therefore, reflect a larger cadmium uptake capacity relative to the species studied in that work. The qₘₐₓ value reported previously for S. fluitans (0.71, b = 5.19) by Davis et al. (2000) closely matches the modelled value depicted in Fig. 2, but is lower than the max q value of 0.95 ± 0.02 obtained in the maximal cadmium binding experiments. Although the S. fluitans used in this and our previous study (Davis et al. 2000) was sampled from the same locality, at the same time, and treated the same way, the DOC leaching and cadmium isotherm experiments (Fig. 1 to 3) were carried out using a distinct subsample (Fig. 4). The differential behaviour of the two subsamples most likely reflects a natural compositional variability within the biomass (Davis et al. 2003b,c).

It is well documented that the alginic content of brown algae varies according to the depth at which the algae are grown as well as the harvest season, reflecting various growth stages (Black 1948; Kreger 1962; Grasdalen 1983). Furthermore, S. fluitans is one of the only two known pelagic Sargassum species and it lives free-floating in the Sargasso Sea. It is, therefore, likely that the samples, carried by the winds and tides to the shores of Cuba where they were collected, display a greater variability in alginate composition than benthic species that can be harvested in situ.

![Fig. 4. Maximal metal uptake experiments for cadmium biosorption by five brown seaweeds. Cadmium uptake as a function of equilibrium cadmium concentration (Cᵦ). (■) L. digitata, (●) M. pyrifera, (△) S. oligocystum, (○) S. fluitans, (▲) S. thunbergii.](https://iwaponline.com/wqrj/article-pdf/39/3/183/230335/wqrjc0390183.pdf)
The two maximal cadmium uptakes determined for *S. fluitans* \( (q_{\text{max}} = 0.77 \text{ and } q = 0.95) \) are lower than the one determined for *S. oligocystum* \( (q = 1.07 \pm 0.03) \) and all three *Sargassum* spp. investigated in this study display lower cadmium uptakes than the two Laminariales, *M. pyrifera* and *L. digitata*. The latter two species are well known for their higher alginate content (Chapman 1980), yet *Sargassum* spp. have been identified (Fourest and Volesky 1997) as the brown algae most suitable for implementation in the biosorption remediation process because of their greater resilience to degradation when compared to other brown algae such as *Ascophyllum, Fucus* and *Laminaria*. For example, Fourest and Volesky (1997) reported that *S. fluitans* was most resistant to leaching of alginate by Na\(^+\) as more than 1.0 mmol NaOH/g was required before soluble alginate was detected from a suspension of *S. fluitans* whereas 0.5 mmol NaOH/g was sufficient to dissolve the alginate from *L. japonica*.

With the addition of these new measurements of the maximal cadmium uptake by *S. thunbergii* \( (0.69 \pm 0.02 \text{ mmol Cd/g })\), *S. oligocystum* \( (1.07 \pm 0.03 \text{ mmol Cd/g })\), and *S. fluitans* \( (q_{\text{max}} = 0.77 \text{ versus } q = 0.95 \text{ mmol Cd/g })\), it now appears that *Sargassum* species display considerable variability in their capacity to sorb divalent metals. Nevertheless, a composite of these and results from Davis et al. (2000) for 8 different species from a global sampling show that maximal cadmium uptake by *Sargassum* spp. ranges from 0.68 mmol Cd/g \( (S. muticum)\) to 1.07 mmol Cd/g \( (S. oligocystum)\) with five of these species within the range of 0.70 to 0.90 mmol Cd/g. Therefore, despite the variability, a characteristic binding range may be assigned to *Sargassum* spp., especially when comparing their performance to other genuses of the brown algae.

**Conclusions**

The database on maximal cadmium uptake capacity by *Sargassum* under batch equilibrium conditions at pH 4.5 was extended to include *S. thunbergii* and *S. oligocystum*. Our results indicate that a characteristic binding range, on the order of 0.7 to 0.9 mmol Cd/g biomass, can be assigned to *Sargassum* spp. Experiments performed on two different particle size fractions of *S. fluitans* biomass indicate that cadmium uptake capacity is not a function of the specific surface area exposed to the solution.

Our work demonstrates that the long equilibrium times required (24 to 26 h) for multiple samples to be brought to equilibrium under our experimental protocol does not adversely affect the degradation of the brown algal particles of *Sargassum fluitans* in terms of leaching \( \text{(i.e., DOC loss)} \) to the solution. It also reveals that increasing concentrations of cadmium in equilibrium batch experiments reduces DOC loss to the solution. This observation is believed to result from the cross-linking by cadmium of the alginate present in both the cell wall matrix and in the mucilage or intercellular matrix.

Despite a lower maximal cadmium uptake capacity than other brown algal, *S. fluitans* remains a most suitable candidate for implementation of biosorption remediation strategies because it has a stronger resilience to leaching and other studies (Smidsrod 1970; Davis et al. 2003b) have shown that it displays a greater selectivity for divalent metal ions. Ultimately, it is a combination of properties including uptake capacity, alginate content, toxic metal selectivity and resilience that will determine the choice of the most suitable biosorbet for remediation applications.

**Acknowledgements**

This work was made possible by a Natural Sciences and Engineering Research Council of Canada (NSERC) Seed Grant to A.M. Additional financial support was provided by individual NSERC research grants to A.M. and B.V. as well as funding from NSERC and the Fonds pour la Formation des Chercheurs et l'Aide à la Recherche du Québec (FCAR) to T.D. in the form of a post-graduate scholarship. Courtesy samples of *Sargassum oligocystum* were obtained from G. Diaz-Pulido and L. McCook (Australian Institute of Marine Science and CRC Reef Research) and *Sargassum thunbergii* from Y.S. Yun (Postech University, S. Korea). The hospitality of Lissy Wong-Hernandez at BIOMAT, University of Havana, Cuba, and technical assistance by the staff of the McGill Geochemical Laboratories for AAS analyses and Ed Siliauskas for DOC determinations were greatly appreciated. Finally, the authors would like to acknowledge the journal’s reviewers for their critical comments.

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