PERGAMON

Water Research 37 (2003) 4311–4330

WATER RESEARCH

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Review

A review of the biochemistry of heavy metal biosorption by brown algae

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Received 3 April 2002; received in revised form 25 April 2003; accepted 6 May 2003

Abstract

The passive removal of toxic heavy metals such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cr^{3+}, and Hg^{2+} by inexpensive biomaterials, termed biosorption, requires that the substrate displays high metal uptake and selectivity, as well as suitable mechanical properties for applied remediation scenarios. In recent years, many low-cost sorbents have been investigated, but the brown algae have since proven to be the most effective and promising substrates. It is their basic biochemical constitution that is responsible for this enhanced performance among biomaterials. More specifically, it is the properties of cell wall constituents, such as alginate and fucoidan, which are chiefly responsible for heavy metal chelation. In this comprehensive review, the emphasis is on outlining the biochemical properties of the brown algae that set them apart from other algal biosorbents. A detailed description of the macromolecular conformation of the alginate biopolymer is offered in order to explain the heavy metal selectivity displayed by the brown algae. The role of cellular structure, storage polysaccharides, cell wall and extracellular polysaccharides is evaluated in terms of their potential for metal sequestration. Binding mechanisms are discussed, including the key functional groups involved and the ion-exchange process. Quantification of metal–biomass interactions is fundamental to the evaluation of potential implementation strategies, hence sorption isotherms, ion-exchange constants, as well as models used to characterize algal biosorption are reviewed. The sorption behavior (i.e., capacity, affinity) of brown algae with various heavy metals is summarized and their relative performance is evaluated.

Keywords: Alginate; Biosorption; Brown algae; Heavy metals; Remediation; *Sargassum*

Contents

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0043-1354/03/$ - see front matter © 2003 Published by Elsevier Science Ltd.
doi:10.1016/S0043-1354(03)00293-8

1. Introduction

Biosorption is a term that describes the removal of heavy metals by the passive binding to non-living biomass from an aqueous solution. This implies that the removal mechanism is not metabolically controlled. In contrast, the term bioaccumulation describes an active process whereby removal of metals requires the metabolic activity of a living organism. In recent years research on the mechanisms of biosorption has intensified since biomass can be employed to sequester heavy metals from industrial effluents (e.g. from the mining or electroplating industry) or to recover precious metals from processing solutions.

Of the many types of biosorbents (i.e. fungi, bacteria, and yeasts) recently investigated for their ability to sequester heavy metals, brown algal biomass has proven to be highly effective as well as reliable and predictable in the removal of, for example, Pb^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} from aqueous solutions. Some reviews that deal with biosorption by different types of biomass include [1–3]. This review is devoted to biosorption by brown algal biomass and an effort was made to outline their classification. As model predictions of heavy metal biosorption become more sophisticated, there is an underlying need to appreciate the basic cell biology and biochemistry of the brown algae and how these compare to other algae. To this end, the emphasis is placed on outlining the fundamental parameters that are at play in biosorption by the brown algae.

2. Characteristics of the brown algae

2.1. A comparison with other algae

The term algae refers to a large and diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis. It is important to note that algae are distinct from Cyanophyta, class Cyanophyceae, the blue-green algae, which are also oxygenic phototrophs, but are eubacteria (*true* bacteria), and are therefore evolutionarily distinct from algae. Although most algae are microscopic in size and are thus considered to be microorganisms, several forms are macroscopic in morphology. These colonial forms of algae occur as aggregates of cells. In turn, each of these cells share common functions and properties, including the storage products they utilize as well as the structural properties of their cell walls.

The algae are included in the plant kingdom and are distinguished from other chlorophyllous plants on the basis of sexual reproduction. The differences between reproduction in the algae (after Bold and Wynne [4]) and that of plants is as follows: (1) in unicellular algae, the organisms themselves can function as gametes; (2) in

certain multicellular algae, the gametes may be produced in special unicellular containers or gametangia; or (3) in others, the gametangia are multicellular, whereby every gametangial cell is fertile and produces a gamete. These characteristics are absent in vascular plants and form the basis by which the algae are studied and classified.

Several characteristics are used to classify algae, including the nature of the chlorophyll(s), the cell wall chemistry, and flagellation. One common characteristic is that all types of algae contain chlorophyll *a*. However, the presence of phytopigments other than chlorophyll *a* is characteristic of a particular algal division. The nature of the reserve polymer synthesized as a result of photosynthesis is also a key variable used in algal classification. It is important to point out, however, that there have been many classification schemes employed to date and the following discussion is based on the work of Bold and Wynne [4]. Accordingly, their divisions include Cyanophyta, Prochlorophyta, Phaeophyta, Chlorophyta, Charophyta, Euglenophyta, Chrysophyta, Pyrrhophyta, Cryptophyta and Rhodophyta. Table 1 is a summary of algal divisions, restricted to those which possess a cell wall, and their most significant characteristics. When comparing Phaeophyta (brown algae) to other common algal divisions such as the Chlorophyta (green algae), important differences are seen in the storage products they utilize as well as in their cell wall chemistry. In the Phaeophyta (brown algae), laminaran is the main storage product, whereas the Rhodophyta (red algae) is distinguished by the floridean starch it produces and stores. Flagella are absent in the Rhodophyta but they are found in the Chlorophyta and Phaeophyta.

Of greater importance to the biosorption mechanism(s), however, is the presence and chemistry of the cell wall. Biosorption in algae has mainly been attributed to the cell wall properties where both electrostatic attraction and complexation can play a role. Cryptophyta, for example, does not have a cell wall [5]. Pyrrhophyta (dinoflagellates) can be "naked" or protected by cellulosic "thecal" plates [4,5]. The Chrysophyceae of the division Chrysophyta can be either "naked" or have scales, cellulosic walls or a cell envelope [5]. None of these types of algae perform very well as heavy metal biosorbents.

Typical algal cell walls of Phaeophyta, Rhodophyta, and many Chlorophyta are comprised of a fibrillar skeleton and an amorphous embedding matrix. The most common fibrillar skeleton material is cellulose (Fig. 1). It can be replaced by xylan in the Chlorophyta and Rhodophyta in addition to mannan in the Chlorophyta. The Phaeophyta algal embedding matrix is predominately alginic acid or alginate (the salt of alginic acid; Fig. 7) with a smaller amount of sulfated polysaccharide (fucoidan; Fig. 6) whereas the Rhodophyta contains a number of sulfated galactans (e.g. agar, carregeenan, porphyran, etc.). Both the Phaeophyta and

Table 1
Three algal divisions and significant characteristics

Division	Common name	Pigments	Storage product	Cell wall	Flagella
Chlorophyta	Green algae	Chlorophyll *a*,*b*; α-, β- and γ-carotenes and several xanthophylls	Starch (amylose and amylopectin) (oil in some)	Cellulose in many (β-1,4-glucopyroside), hydroxyproline glucosides; xylans and mannans; or wall absent; calcified in some	Present
Phaeophyta	Brown algae	Chlorophyll *a*,*c*; β-carotene and fucoxanthin and several other xanthophylls	Laminaran (β-1,3-glucopyranoside, predominantly); mannitol	Cellulose, alginic acid, and sulfated mucopolysaccharides (fucoidan)	Present
Rhodophyta	Red algae	Chlorophyll *a* (*d* in some Florideophyceae); R- and C-phycocyanin, allophycocyanin; R- and B-phycoerythrin. α- and β-carotene and several xanthophylls	Floridean starch (amylopectin-like)	Cellulose, xylans, several sulfated polysaccharides (galactans) calcification in some; alginate in corallinaceae	Absent

Information in this table is from a similar, more extensive table compiled by Bold and Wynne [4].

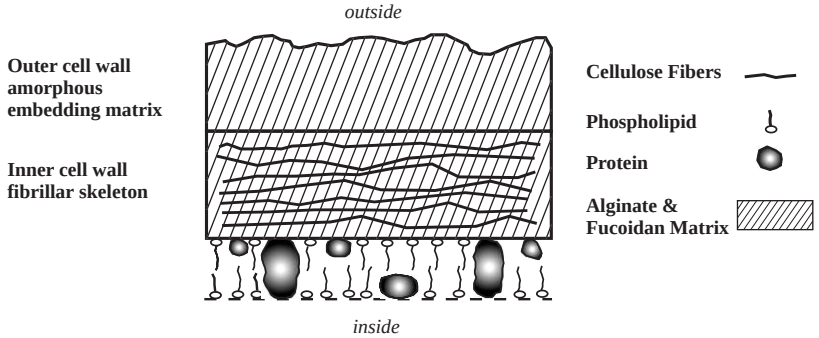

Fig. 1. Cell wall structure in the brown algae. After Schiewer and Volesky [2].

Rhodophyta divisions contain the largest amount of amorphous embedding matrix polysaccharides. This characteristic, combined with their well known ability to bind metals, makes them potentially excellent heavy metal biosorbents.

2.2. The brown algae

The brown algae are an important assemblage of plants that are classified in about 265 genera with more than 1500 species [4]. They derive their characteristic colour from the large amounts of the carotenoid fucoxanthin (which yields a brown colour) contained in their chloroplasts and the presence of various pheophycean tannins. They occur mainly in the marine environment, where they appear as an intertidal component. Some marine forms penetrate into brackish environments, and can be an important part of the salt marsh fauna [5]. Brown algae flourish in temperate to subpolar regions where they exhibit the greatest diversity in species and morphological expression.

The division Phaeophyta is subdivided into *orders*, which subsequently are divided into *families*, and then the familiar *genus* and *specie* are specified. There are 13 orders in the Phaeophyta according to the classification of Bold and Wynne [4]; however, from the point of view of biosorption, only two are of importance, namely the orders Laminariales and Fucales (Fig. 2). Both orders are abundant in nature and include the most structurally complex algae. Laminariales are collectively referred to as ''kelps'' and are harvested for many commercial uses (e.g. water holding property for frozen foods, syrups, and frozen deserts; gelling property for instant puddings and dessert gels, or even explosives; emulsifying properties for polishes; stabilizing properties in ceramics, welding rods and cleaners) [6]. The order Fucales is a large and diversified order, with a great amount of morphological diversity [4]. For example, the family Sargassaceae contained within it, is well known for the algal genus *Sargassum* which is found in the tropical waters of the Sargasso Sea. Fig. 2 outlines some of the genuses and species studied for their heavy metal binding ability. The boxes outline the orders and families of principal interest in biosorption.

3. Cellular structure and biochemistry

3.1. Cellular structure

A typical brown algal cell is depicted in Fig. 3. The chloroplast envelope (Ce) contains the chloroplasts which have three thylakoids (an interconnected set of disc-like sacs) per band. This structure (known as a plastid; the most common of which is the chloroplast) stores food material and contains chlorophyll *a*, c_1, and c_2. In addition to the chloroplast envelope, the chloroplasts are surrounded by the two membranes of the chloroplast endoplasmic reticulum (Cer). The outer membrane which encloses an inner membrane is discontinuous with the nuclear envelope (Ne) in Fucales and Laminaria although it is continuous as seen in Fig. 3 for the brown algal order Ectocarples [5].

Although most of the algal cellular functions are coded for by nuclear DNA, a few organellar proteins are coded from within the chloroplast. These microfibrils of DNA (Fib) occur in the plastids. They can be either linear or of closed circular form and are attached to the thylakoid membranes [7]. As in all eukaryotes, DNA is housed in the nucleolous (Nu) of the nucleus (N). The prenoid (P) is responsible for CO_2 fixation and the formation of storage products. It is enclosed by the prenoid sac (Ps) and extends out from the chloroplast endoplasmic reticulum.

Production and secretion of the polysaccharides take place in the dictyosome (D), otherwise known as the golgi apparatus or golgi dictyosome. The mitochondrian (M) is the site of cellular respiration where ATP is formed. Each mitochondrian is bounded by a double membrane which creates two different compartments within the organelle (''organelle'' or ''little organ'' describes the various internal structures of the cell, each

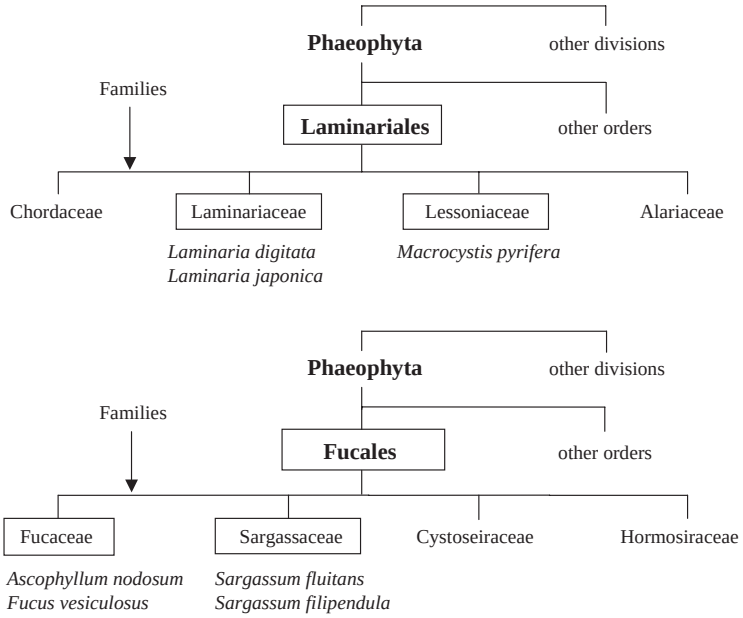

Fig. 2. Classification scheme and important brown algae in the study of biosorption. Boxes enclose orders and families of principal interest in biosorption. Classification is after that of Bold and Wynne [4].

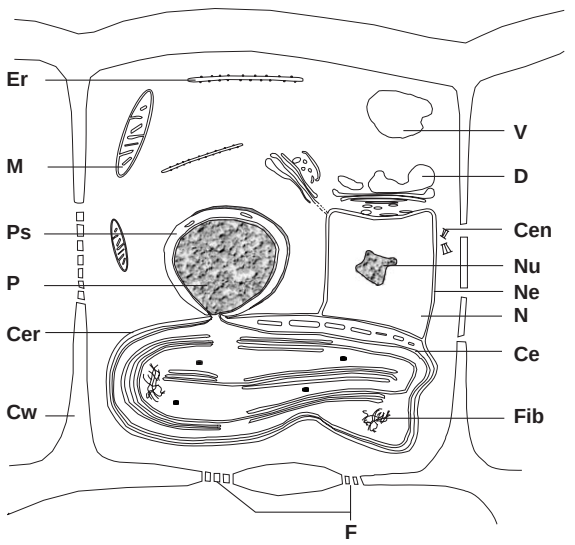

Fig. 3. Schematic diagram of a brown algal cell. (Ce) Chloroplast envelope; (Cer) chloroplast endoplasmic reticulum; (Er) endoplasmic reticulum; (Ne) nuclear envelope; (Fib) DNA fibrils; (Nu) nucleolus; (N) nucleus; (P) prenoid; (Ps) prenoid sac; (D) dictyosome (also known as the golgi apparatus or golgi dictyosome); (M) mitochondrian; (V) vacuole; (F) plasmodesma pit field; (Cw) cell wall; (Cen) centrioles. After Bouck [77].

of which performs specific functions required for the cell's survival). The inner mitochondrial membrane is impermeable to the passage of protons and the resulting proton gradient generated across it couples phosphorylation with oxidation. This combination leads to the production of ATP from ATP synthetase, the former of which can then be used for cellular metabolism [8].

The principal function of the vacuoles (V) is storage and transport of various macromolecules within and to the exterior of the cell [5]. A significant proportion of these organelles contains alginic acid and lie near the exterior of the cell. In addition to their protective function (i.e. from light rays), they are probably "en-route" to the cell wall where they replenish alginic acids. Furthermore, in some species these vacuoles are small colourless vesicles or physodes, and contain reducing phenolic compounds [73,74].

Ultimately, cells are interconnected by plasmodesma pit fields (F). These plasmodesmata or pores are found between most of the cells. The pores are bounded by the plasmalemma, and protoplasm is continuous from one cell to another by these pathways. In contrast to the more primitive Phaeophycea where the plasmodesmata are scattered in the cell wall, the Laminariales and Fucales display pores that are grouped together in primary pit areas [5].

3.2. Storage polysaccharides

Carbon may be stored in monomeric compounds (e.g. mannitol) or in the polymeric state. Storage in the latter state is advantageous since polymers have a smaller effect on osmotic potential than an equivalent amount of carbon in monomeric form [9]. Nevertheless,

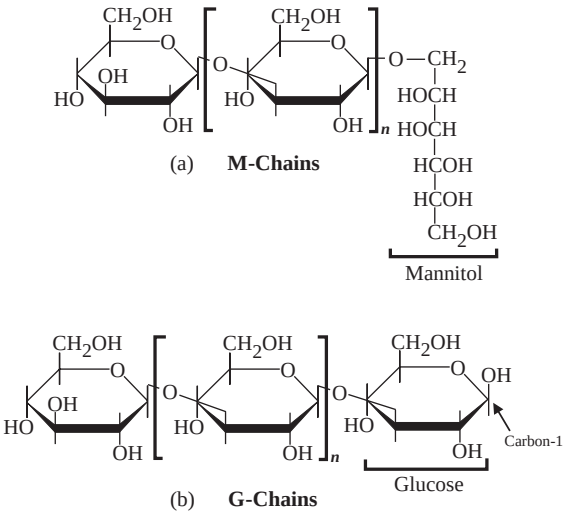

Fig. 4. The structure of laminaran (principally a $\beta(1\rightarrow3)$-linked unbranched glucan) consists of two types of chains. In (a) *mannitol* is attached to the reducing end (M-chains), whereas in (b) *glucose* is attached to the reducing end (G-chains). After Percival and McDowell [12].

mannitol (Fig. 4) occurs in all brown algae [10] and can constitute up to 30% of their dry weight [11]. For example, two *Sargassum* species were reported to contain between 4–12% and 7–9% of mannitol, with the minimum amounts being observed after reproduction [6]. The first accumulation product of photosynthesis, mannitol, has osmoregulatory properties [5,12] and is derived from the six-carbon sugar, D-mannose.

The second major storage product in brown algae is laminaran (Fig. 4). This glucan was first characterized by Schmiedeberg in 1885 [13] and is made up of a mixture of polysaccharides. The glucose in this molecule is in the β form (which means that the hydroxyl group present on C1, the anomeric/chiral carbon, is present above the plane when drawn in a Haworth projection; see Fig. 4). The linkages are primarily $\beta(1\rightarrow3)$ (Fig. 4 illustrates only the $\beta(1\rightarrow3)$ configuration), but a small amount of $\beta(1\rightarrow6)$-links has also been observed [12]. Two types of laminaran chains exist—M, with mannitol attached to the reducing end; and G, with glucose attached to the reducing end [12]. The absolute and relative storage of these compounds is variable but correlated with growth, plant tissue, and reproductive conditions. Variations in the relative abundance of mannitol and laminaran have been shown to exist by Black [14,15] and Jensen and Haug [16].

3.3. Cell wall and extracellular polysaccharides

As mentioned above, the cell wall of algae is composed of at least two different layers. The innermost layer consists of a microfibrillar skeleton that imparts rigidity to the wall (Fig. 1). The outer layer is an amorphous embedding matrix [17,5]. Of these two components the latter is usually viewed as forming a non- or para-crystalline matrix in which the former (as a set of microfibrils) is embedded [18]. There is some evidence that the matrix does not penetrate the fibers, but rather is attached to this layer via hydrogen bonds [18]. The inner, rigid fibrillar layer of brown algae is mainly comprised of the uncharged cellulose polymer (a $\beta(1\rightarrow4)$-linked unbranched glucan; Fig. 5a). Two other fibrillar molecules, xylan (principally a $\beta(1\rightarrow3)$-linked D-xylose) and mannan (a $\beta(1\rightarrow4)$-linked D-mannose) occur in the red and green algae (Fig. 5b and c). Finally, alginate contributes to the strength of the cell wall of brown algae in addition to imparting flexibility [19]. Even if alginate is present within the inner layer, cellulose remains the principal structural component. Fucoidan (discussed below) occurs not only in the matrix but also within the inner cell wall [6,20,12].

Studies of the ultrastructure of cellulose microfibrils by electron microscopy [21] have shown that they are usually flattened, with diameters that can vary between 100 and 200 Å. The microfibrils occur as a network of more or less curved threads. In general, their orientation appears to be random but a transverse style may also be exhibited [21]. When cells have undergone extensive

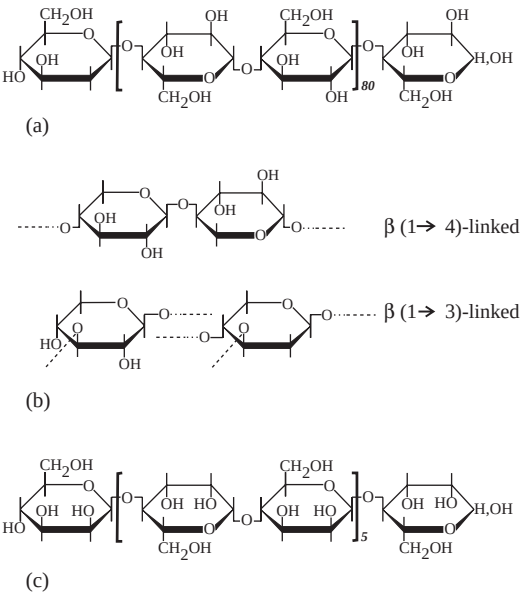

Fig. 5. The fibrillar molecules of algal cell walls. (a) Algal *cellulose*, a $\beta(1\rightarrow4)$-linked unbranched glucan, of the brown algae; (b) structural units present in *xylan* from the red algae, both $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$-linked forms have been isolated; (c) *mannan*, a $\beta(1\rightarrow4)$-linked D-mannose from the red algae. After Percival and McDowell [12].

growth in length, they can display a predominantly longitudinal orientation. The mass fraction of cellulose may be 2–20% of the dry weight according to Kreger [17]. For example, the cellulose content of *Ascophyllum* and *Laminaria* (Fig. 2) were determined to be 7% and 20%, respectively [21].

3.3.1. Extracellular polysaccharides: fucoidan

Fucoidan has been found to occur in several members of the family Laminariaceae (Fig. 2), with dry mass percentages between 5 and 20 [22]. It also occurs abundantly in species of *Fucus* and *Chordaria*, as well as other brown algae. The compound was first isolated by Kylin [23] who prepared and isolated L-fucose phenylhydrazone from the hydrolyzate [12].

Fucoidan is a branched polysaccharide sulfate ester with L-fucose 4-sulfate building blocks as the major component (Fig. 6). They are predominantly α(1→2)-linked [75]. Acid hydrolysis of fucoidan also yields various amounts of D-xylose, D-galactose, and uronic acid [18].

3.3.2. Extracellular polysaccharides: alginic acid

Alginic acid occurs in all brown algae [12]. It may be present in both the cell wall matrix and in the mucilage or intercellular material (Figs. 1,7; [6,18]) and can constitute between 10% and 40% of the dry weight (untreated) of the algae [12]. Its abundance is dependent on the depth at which the algae are grown and it also displays seasonal variations. The latter may reflect changes associated with growth stages [24,17]. The alginic acid content in *Sargassum longifolium* was found to be 17%. For *Sargassum wightii* and *Sargassum tenerium* this value reaches between 30% and 35% [6]. The alginate of *Sargassum fluitans* has been reported [25] to account for 45% of its dry weight once it has been stripped of its sea salts and converted to the protonated form. Davis et al. [26] also reported similar alginate yields for protonated *Sargassum fluitans* and *Sargassum oligocystum* of approximately 45% and 37%, respectively.

Alginic acid or alginate, the salt of alginic acid, 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 non-regular, blockwise order along the chain ([27]; Fig. 7a–c). The residues typically occur as $(–M–)_n$, $(–G–)_n$, and $(–MG–)_n$ sequences or blocks. The carboxylic acid dissociation constants of M and G have been determined as $pK_a = 3.38$ and $pK_a = 3.65$, respectively, with similar pK_a values for the polymers [28], where

$$pK_a = -\log K_a$$

and

$$K_a = \frac{[-COO^-][H^+]}{[-COOH]}.$$

The salts of alginic acid with monovalent ions (alkali metals and ammonium) are soluble, whereas those with divalent or polyvalent metal ions (except Mg^{2+}) and the acid itself are insoluble [12].

M- and G-block sequences (see Fig. 7) display significantly different structures and their proportions in the alginate determine the physical properties and reactivity of the polysaccharide [29]. Polymannuronic acid is a flat ribbon-like chain; its molecular repeat is

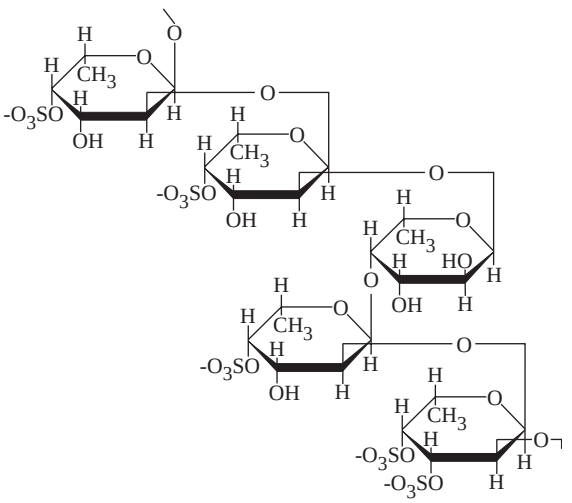

Fig. 6. The structure of fucoidan, a branched polysaccharide sulfate ester with L-fucose building blocks as the major component with predominantly α(1→2)-linkages.

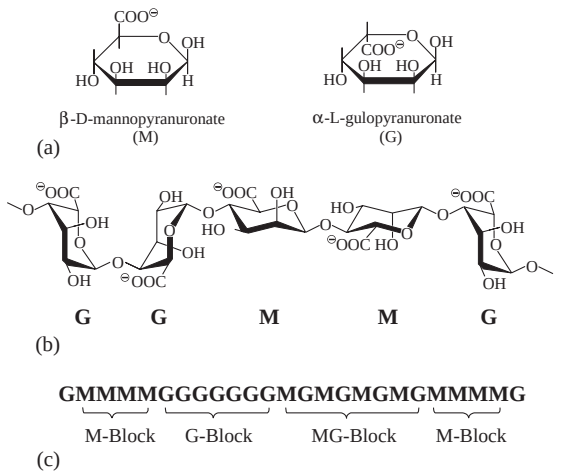

Fig. 7. Alginate structural data: (a) alginate monomers (M vs. G); (b) the alginate polymer; (c) chain sequences of the alginate polymer. After Smidsrød and Draget [19].

10.35 Å, and it contains two diequatorially (1e→4e) linked β-D-mannuronic acid residues in the chair conformation [30]. In contrast, polyguluronic acid contains two diaxially (1a→4a) linked α-L-guluronic acid residues in the chair form which produces a rod-like polymer with a molecular repeat of 8.7 Å [31]. This key difference in molecular conformation between the two homopolymeric blocks is believed to be chiefly responsible for the variable affinity of alginates for heavy metals.

Haug et al. [32] were the first to perform a systematic study of the variability of the uronic acid sequence in alginates from different sources. They determined the mannuronic acid to guluronic acid ratios (M:G) of alginates by using a simple method involving partial hydrolysis with acid, followed by fractional precipitation of the acid-resistant part of the alginate. They found a marked difference among the M:G ratios of alginates from different brown algae.

Grasdalen and others [33,34] pioneered the development of ^{13}C and ^{1}H NMR spectroscopy of slightly depolymerized alginates to characterize their composition. The sequence of monomer residues (M and G) markedly influences the chemical shifts. The relative intensities of protons on the carbon-1 of both guluronic and mannuronic acid can be used to calculate the monomeric composition: the M:G ratio. At 50 MHz, individual ^{13}C resonances for both block residues are resolved into four lines which reflect the presence of either a G or M preceding a particular block. Relative peak intensities from the ^{13}C NMR spectra can then be used to determine the monomeric sequence in terms of a complete set of four diad (e.g. MG or GG) and eight triad (e.g. MGM or GGM) frequencies as well as the M:G ratios of end residues and of the residues adjacent to M-residues at the non-reducing end (no hydroxyl group on carbon-1).

3.3.3. Alginate metal affinity and binding

In the previous sections we have seen that not only is the polymer conformation of the two block residues (M and G) in alginate different, but also that the proportion of these two constituents changes depending on the genus of the algae and from which part of the plant the polysaccharide is extracted. Furthermore, variations in M:G ratio exist from species to species [32]. Variation in the affinity of some divalent metals to alginates with different M:G ratios was demonstrated early on by Haug [28]. He showed that the affinity of alginates for divalent cations such as Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Ca^{2+}, etc. increased with the guluronic acid content. The selectivity coefficients for the ion-exchange reaction between sodium and divalent metals were determined for two alginates [35] and confirmed the higher affinity of guluronic acid rich alginates for divalent metals (Table 2).

Table 2
Selectivity coefficients for two alginates

Metal ions	*L. digitata*[a] M:G = 1.60	*L. hyperborea*[a] M:G = 0.45
Cu^{2+}–Na^{+}	230	340
Ba^{2+}–Na^{+}	21	52
Ca^{2+}–Na^{+}	7.5	20
Co^{2+}–Na^{+}	3.5	4

[a] Ratio of monomer mannuronic acid to guluronic acid residues for a given alginate sample. After Haug and Smidsrød [35].

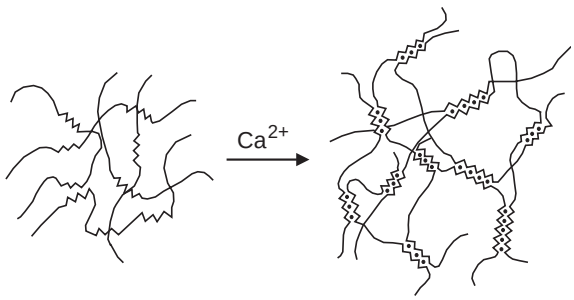

Fig. 8. Schematic representation of the calcium-induced gelation of alginate in accordance with the "egg-box" structure. After Christensen et al. [78].

The higher specificity of polyguluronic acid residues for divalent metals is explained by its "zigzag" structure which can accommodate the Ca^{2+} (and other divalent cations) ion more easily. The alginates are thought to adopt an ordered solution network, through inter-chain dimerization of the polyguluronic sequences in the presence of calcium or other divalent cations of similar size (Fig. 8). The rod-like shape of the poly-L-guluronic sections results in an alignment of two chain sections yielding an array of coordination sites, with cavities suitable for calcium and other divalent cations because they are lined with the carboxylate and other oxygen atoms of G residues. This description is known as the "egg-box" model [36,37]. The regions of dimerization are terminated by chain sequences of polymannuronic acid residues. As a result, several different chains may become interconnected and this promotes gel network formation. The higher the degree of linkage, the greater the resulting viscosity.

4. Mechanisms of biosorption

4.1. Key functional groups

The carboxylic groups are generally the most abundant acidic functional group in the brown algae. They

constitute the highest percentage of titratable sites (typically greater than 70%) in dried brown algal biomass. The adsorption capacity of the algae is directly related to the presence of these sites on the alginate polymer, which itself comprises a significant component (up to 40% of the dry weight, [12]) of the dried seaweed biomass. Furthermore, the majority of metals of interest (i.e. Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, Pb^{2+}) display maximal or near maximal sequestration at pHs near the apparent dissociation constant of carboxylic acids observed in brown algal biomass (pK_0 near 5). The role of carboxylic groups in the adsorption process has been clearly demonstrated by a reduction in cadmium and lead uptake by dried *Sargassum* biomass following partial or complete esterification of the carboxylic sites [25]. Finally, Fourier-transformed infrared (FTIR) spectral analyses have shown that cadmium biosorption to *Sargassum* arises from bridging or bidentate (see below) complex formation with the carboxylate groups of the alginate [25], consistent with the above described ''egg-box'' model of Rees and co-workers [38,39].

The second most abundant acidic functional group in brown algae is the sulfonic acid of fucoidan. Sulfonic acid groups typically play a secondary role, except when metal binding takes place at low pH. Hydroxyl groups are also present in all polysaccharides but they are less abundant and only become negatively charged at $pH > 10$, thereby, also playing a secondary role in metal binding at low pH.

4.2. Ion-exchange

Ion-exchange is an important concept in biosorption, because it explains many of the observations made during heavy metal uptake experiments. Furthermore, it is a natural extension to the premise that alginate plays a key role in biosorption by brown algae, since it has been shown that ion-exchange takes place between metals when binding to alginate [76]. Kuyucak and Volesky [40,41] reported an enhanced release of ions (Ca^{2+}, K^+, Mg^{2+}, Na^+) from the alga *Ascophyllum nodosum* when reacted with a cobalt bearing aqueous solution rather than cobalt-free solution. Furthermore, when the alga was pre-treated with $CaCl_2$ and HCl, a 2:3 stoichiometric relationship was observed between Ca^{2+} release and Co^{2+} uptake. Schiewer and Volesky [2] point out, however, that a ratio closer to one would have been achieved if protons were included in the charge balance. It was concluded that ion-exchange was the dominant mechanism.

Untreated biomass generally contains light metal ions such as K^+, Na^+, Ca^{2+}, and Mg^{2+}. These are originally bound to the acid functional groups of the alga and were acquired from seawater. Treated biomass generally implies one of two chemical alterations. The first is protonation of the biomass with a strong acid such as HCl whereby the proton displaces the light metal ions from the binding sites (i.e. carboxylic, sulfonic, and others). In the second, the biomass is reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by, for example, calcium or potassium. In cases where the non-treated marine alga *Sargassum* was reacted with a (heavy) metal-bearing solution, a pH increase and the release of light metal ions was observed. This also was explained in terms of ion-exchange, whereby the observed release of light metals balanced the uptake of protons and heavy metals. When the heavy metal concentration was increased, little pH increase was observed and this was attributed to the fact that the maximum binding capacity of the biomass had been reached and all exchangeable sites were occupied by the heavy metal [42]. In related metal uptake experiments with *treated* biomass (protonated), the pH of *Sargassum* suspensions decreased. This was observed as either a continual but initially steep drop in pH in a free-drift system, or by the addition of base in a pH-stat system. Again, this was interpreted as ion-exchange between protons and the heavy metal ions at the binding sites [42].

It should be pointed out that the term *ion-exchange* does not explicitly identify the binding mechanism, rather it is used here as an umbrella term to describe the experimental observations. The precise binding mechanism(s) may range from physical (i.e. electrostatic or London–van der Waals forces) to chemical binding (i.e. ionic and covalent). The term *sorption* would refer to binding of a metal cation to a *free* site as opposed to one that was previously occupied by another cation. It is distinct from *adsorption* that, strictly speaking, defines binding in terms of a physical rather than chemical surface phenomenon. In the case of biosorption of heavy metals by brown algal biomass, the mechanisms can be viewed, in principle, as being *extracellular*, or occurring discretely at the cell wall. *Intracellular* sorption would normally imply bioaccumulation by a viable organism.

4.3. Definitions

The following definitions are based on the text by Stumm and Morgan [43]. Any combination of cations with molecules or anions containing free electron pairs (bases) is termed coordination, also known as complex formation. Coordination or complex formation, in turn, may be either electrostatic (i.e. *Coulombic*) or covalent in character. The heavy metal cation that is bound is often designated as the central atom, and is distinguished from the anions or molecules with which it forms a coordination compound, the ligand(s). When the ligand is composed of several atoms, the atom responsible for the basic or nucleophilic nature of the ligand is termed the ligand atom. A base containing more than one ligand atom, a *multidentate* complex, may occupy more

than one coordination position in the complex. Complex formation with multidentate ligands is termed *chelation*; complexes are *chelates*. Furthermore, the *coordination number* refers to the number of ligand atoms surrounding the central atom, where most metal cations engage in coordinations of 2, 4, 6, and 8, with 4 and 6 being the most common. In the case of polymers these values may be lower due to steric effects. A proton complex has a coordination number of one, as opposed to the higher coordination numbers found in metal complexes [43]. Although this terminology is typically employed for aqueous complexation with small ligands, the terms are often applied in the literature when dealing with more complex molecules, thus this outline is intended to serve as a basis for its usage.

The terms *inner-sphere* and *outer-sphere complex* are used to distinguish between binding which is, respectively, largely covalent in character or chiefly electrostatic in nature. In the first case, the interacting ligand is immediately adjacent to the metal cation. In the second case, ions of opposite charge are attracted and approach each other within a critical distance and effectively form what is termed an ion pair. In outer-sphere complexes, the metal ion or the ligand or both generally retain their coordinated water when the complex is formed. In other words, the metal ion and the ligand are most often separated by one or more water molecules [43].

4.4. Complexation

The nature of complexation as it occurs within the brown algae will be addressed by reviewing reported observations in binding of heavy metals by alginate. Haug [28], in his study of metal-ion binding to alginic acid extracted from *Laminaria digitata*, reported that the amount of protons released into solution decreased in the order $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+} > Mg^{2+}$. He explained these results in terms of the relative ability of the binding metal to compete with protons for organic binding sites. The affinity sequence for metal-ion binding to alginate extracted from *L. digitata* followed a similar trend: $Cu^{2+} > Ba^{2+} > Ca^{2+} > Co^{2+}$ [28]. The binding strength of alkaline earth metals to both polymannuronate and polyguluronate was found to decrease in the order $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$ [44]. Haug and Smidsrød interpreted the preferential binding of heavier ions to stereochemical effects, since larger ions might better fit a binding site with two distant functional groups.

The "egg-box" model, in addition to other models with more accurate steric arrangements have been supported by X-ray diffraction [45] and NMR spectroscopic analyses [46]. Accordingly, we can view metal sequestration as the complexation (or coordination) of a central heavy metal to a multidentate ligand, the alginate. Regions of the alginate polymer rich in 'G' residues (G-blocks), which display a higher selectivity for divalent metal ions, provide a multi-dentate environment for complexation whereas in regions rich in mannuronic acid complexation would be predominantly monodentate and therefore weaker. In other words, the higher specificity of guluronic acid supports the hypothesis that the coordination number of the metal bound to guluronic acid residues is larger than with mannuronic acid residues. More specifically, the key appears to be the orientation of the oxygen atoms with respect to $-COO^-$. In guluronic acid the ring oxygen and the axial O-1 form a spatially favorable environment with $-COO^-$, as opposed to the equatorial O-1 which occurs in mannuronic acid residues (see Fig. 7).

Much less work has been carried out on the metal binding ability of fucoidan. The affinity sequence has been reported [47] as $Pb^{2+} > Ba^{2+} > Cd^{2+} > Sr^{2+} > Cu^{2+} > Fe^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > Cr^{3+} > Ni^{2+} > Hg^{2+} > Ca^{2+}$.

5. Biosorption by brown algae

5.1. Quantifying metal–biomass interactions

5.1.1. Sorption isotherms

From a scientist's perspective, the field of biosorption is a challenging one, since it requires the application of first principles of organic chemistry and geochemistry. The main objectives are the elucidation of binding mechanisms, the relative affinity of heavy metals for the biomass, and how both are affected by varying environmental conditions. Ultimately, the goal is the successful implementation of a remediation program.

The first step towards these objectives is to quantify the capacity of a given biomass to sequester heavy metals from an aqueous solution. This is traditionally done by characterizing the equilibrium state after the biomass (i.e. treated or untreated brown alga) has been allowed to react with an aqueous solution of the metal of interest. The reaction is commonly monitored by measuring the amount of metal remaining in solution until it becomes time invariant. The model used to describe the results should be capable of predicting heavy metal binding at both low and high concentrations. Ideally the model should not only be predictive but should rest on our understanding of the mechanism of biosorption.

The Langmuir adsorption isotherm has traditionally been used to quantify and contrast the performance of different biosorbents. However, in order to evaluate the appropriateness of this model we must look at its underlying assumptions.

The Langmuir isotherm was originally developed to describe the gas–solid phase adsorption of activated

carbon. In its formulation, binding to the surface was primarily by physical forces (i.e. electrostatic or London–van der Waals forces) and implicit in its derivation was the assumption that all sites possess equal affinity for the adsorbate. Its use was extended to empirically describe equilibrium relationships between a bulk liquid phase and a solid phase.

One of the simplest representations of the adsorption phenomenon calls for the migration to and the occupation of a surface site, S, on a solid (adsorbent) by an adsorbate, A. This can be represented by an equilibrium reaction:

$$S + A \Leftrightarrow SA, \tag{1}$$

where SA is the adsorbed complex. Surface species concentrations may be expressed as moles per liter of solution, per gram of solid, per unit area of solid surface or per mole of solid. Assuming that all surface sites have the same affinity for the solute A, a mass action law can be written as

$$K_{ads} = \frac{[SA]}{[S][A]} = \exp\left(-\frac{\Delta G^{\circ}_{ads}}{RT}\right). \tag{2}$$

The maximum or total concentration of surface sites, S_T, is given by:

$$[S_T] = [S] + [SA]. \tag{3}$$

From Eqs. (2) and (3) we find

$$[SA] = [S_T]\left(\frac{K_{ads}[A]}{1 + K_{ads}[A]}\right). \tag{4}$$

Defining the surface concentration as

$$\Gamma = \frac{[SA]}{\text{mass adsorbent}} \tag{5}$$

and

$$\Gamma_{max} = \frac{[S_T]}{\text{mass adsorbent}}, \tag{6}$$

we obtain

$$\Gamma = \Gamma_{max}\left(\frac{K_{ads}[A]}{1 + K_{ads}[A]}\right). \tag{7}$$

Eq. (7) is the general form of the Langmuir equation, although other forms do exist. Compliance to the Langmuir isotherm theory [43] requires that (1) adsorption is limited to the formation of a monolayer, or the number of adsorbed species, [SA], does not exceed the total surface sites $[S_T]$; and (2) the energy of adsorption is independent of the $[SA]/[S_T]$, (all surface sites have the same energy or equal affinity for the adsorbate). A typical Langmuir adsorption isotherm is shown in Fig. 9.

At least one of these conditions is implicitly not met in the case of biosorption. We have seen in the previous sections that there is more than one type of functional group contributing to the biosorption process, each of which has a different affinity for a sorbing heavy metal. Furthermore, the one-to-one stoichiometry is also not complied with, since ion-exchange has been shown to be a dominant mechanism, and typically approximately two protons are released upon the binding of one divalent heavy metal ion. Despite this fact, the Langmuir equation is frequently used to fit experimental data (Fig. 10). In this case, the following form of the Langmuir equation (Eq. (7)) is traditionally applied:

$$q = q_{max}\left(\frac{bC_f}{1 + bC_f}\right), \tag{8}$$

where q is the uptake (mmol heavy metal/gram biosorbent) and q_{max} is the maximal uptake (mmol heavy metal/gram biosorbent) of the biosorbent. C_f is the final equilibrium solution concentration of the heavy metal, which is typically determined by atomic

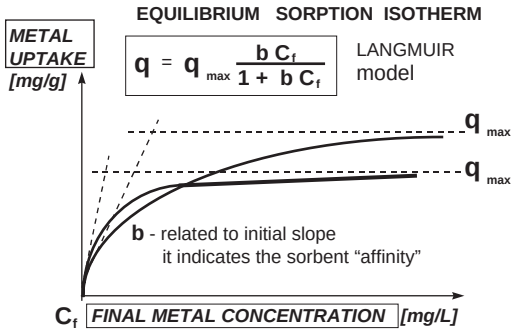

Fig. 9. Biosorption–Langmuir isotherm relationship curves. After Volesky and Schiewer [79].

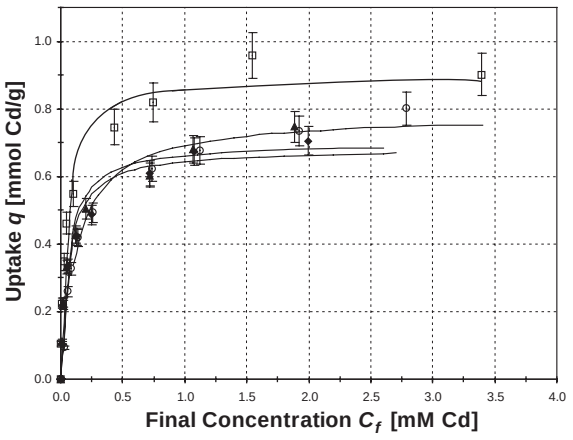

Fig. 10. Cadmium sorption isotherms for raw and unsorted (all size fractions) *Sargassum* species at pH = 4.5: (□) *S. polycystum*, Cebu, Philippines; (○) *Sargassum*, unidentified, Australia; (▲) *S. filipendula*, Brazil; (◆) *S. muticum*, UK. Solid curves are calculated with the Langmuir equation. After Davis et al. [54].

adsorption or inductively coupled plasma spectrometry. In this context, b is not truly the Langmuir adsorption constant but, rather, a simple fitting parameter because, as indicated above, the system does not comply with the assumptions of the model and cannot be related to the Gibbs free energy (Eq. (2)) of a specific reaction. The parameter is nonetheless quite useful as a measure of the biosorption affinity or efficiency of different biomasses. High values of b are reflected by the steep initial slope of a sorption isotherm and indicate a high affinity for the adsorbate. In terms of implementation, biosorbents with the highest possible q_{max} and a high value of b are the most desirable.

The Freundlich isotherm has also been employed to quantify equilibrium biosorption systems. Like the Langmuir isotherm, the extent of adsorption/sorption is determined as a function of the equilibrium concentration of the metal in solution, without reference to pH or other ions in the same aqueous system.

The Freundlich isotherm [48] is originally of an empirical nature, but was later interpreted as sorption to heterogeneous surfaces or surfaces supporting sites of varied affinities. It is assumed that the stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation. Specifically, the Freundlich isotherm is obtained when a log-normal affinity distribution is assumed [49,43]. The Freundlich isotherm is defined by the following expression:

$$q = k[\mathrm{M}]^{1/n}, \tag{9}$$

where k and n are empirically determined constants, with k being related to the maximum binding capacity, and n related to the affinity or binding strength [50,51].

5.1.2. Ion-exchange constants

It is possible to incorporate the concept of ion-exchange into the formulation of the Langmuir equation. The ion-exchange constant for the binding of a metal ion M^+ (for simplicity a monovalent ion) that replaces a proton H^+ at a complexation or coordinating site may be defined as follows, where B^- represents the biomass (Eqs. (1)–(6) use S to designate the surface, which now is replaced by the biomass, B^-).

Langmuir:

$$\mathrm{B}^- + \mathrm{M}^+ \Leftrightarrow \mathrm{BM}, \tag{10a}$$

$${}^{\mathrm{BM}}K^* = \frac{[\mathrm{BM}]}{[\mathrm{B}^-][\mathrm{M}^+]} \quad \text{and} \quad [\mathrm{B}]_t = [\mathrm{B}^-] + [\mathrm{BM}]. \tag{10b}$$

Ion-exchange:

$$\mathrm{BH} + \mathrm{M}^+ \Leftrightarrow \mathrm{BM} + \mathrm{H}^+, \tag{11a}$$

$${}^{\mathrm{BM}}K = \frac{[\mathrm{BM}][\mathrm{H}^+]}{[\mathrm{BH}][\mathrm{M}^+]} \quad \text{and} \quad [\mathrm{B}]_t = [\mathrm{BH}] + [\mathrm{BM}]. \tag{11b}$$

Therefore:

$${}^{\mathrm{BM}}K^* = \frac{{}^{\mathrm{BM}}K}{[\mathrm{H}^+]}. \tag{12}$$

The difference between the models is that the ion-exchange model assumes that all sites to which the metal ions can be bound are initially occupied. This ion-exchange model is a more factual representation of the active biosorption mechanism than the simple Langmuir isotherm. Nevertheless, it does not completely and accurately describe the biosorption phenomenon. For example, the cation-exchange capacity of the biomass increases with increasing pH, whereas the stoichiometry of the reaction varies with increasing metal concentration (i.e. from approximately 1:3 [Me]:[H^+] at low [Me] to approximately 1:1.7 at high [Me] for Cd^{2+}, where low and high are 0.25 and 3.5 mM initial concentrations, respectively). Therefore, one cannot simply model the competitive binding of metals and protons by using a metal-proton-ion-exchange constant. At least one reaction in which a metal cation reacts with a free site should be included such as in Eq. (10a).

5.1.3. Other models

The ion-exchange model is certainly a better representation of the adsorption process since it reflects the fact that most brown algal biomass is either protonated or contains light metal ions such as K^+, Na^+ and Mg^{2+}, which are released upon binding of a heavy metal cation. However, the model cannot account for the influence of important variables such as pH and ionic strength. Generally, as the ionic strength of the solution increases, binding of the heavy metals of interest is reduced. This may reflect the electrostatic component of the complexation reactions or the formation of stable metal complexes in solution, or both. The mechanism can only be resovled by modeling the speciation of the solution and affinity of the individual aqueous complexes.

Following a study of uranium biosorption by the brown algae *Sargassum fluitans*, a model based on the ion-exchange between protons in the biomass and hydrolyzed uranium ion species was developed [80]. At acidic to near circum-neutral pH values, the uranium cation UO_2^{2+} is hydrolyzed in aqueous solution; it exists predominantly as $(UO_2)_2(OH)_2^{2+}$, UO_2OH^+, or $(UO_2)_3(OH)_5^+$, which are related to one another by their hydrolysis constants. The hydrolyzed ion exchange model (HIEM) takes this equilibrium speciation into account, whereby ion-exchange takes place between various hydrolyzed ions and protons at the biomass binding sites. An equilibrium is reached between the various forms of the complexes assumed present in solution. Given the total uranium concentration and pH, the model was capable of predicting uranium and proton binding, as well as the speciation of uranium in

the solution and on the biosorbent (*S. fluitans*). Furthermore, the model was capable of fitting and predicting biosorption isotherms for different pH values as well as the equilibrium uranium desorption concentrations.

Schiewer and Volesky [42,52,53] presented a series of equilibrium biosorption models which incorporate the metal-ion concentration, pH and ionic strength. One of the proposed models utilizes one fitting parameter in which the formation of $BM_{0.5}$ complexes was assumed. They employed a combined Donnan-biosorption-isotherm equation that allowed for direct calculation of cation binding without interactions when swelling proportional to the number of free sites was assumed. The Donnan model considers that the intraparticle concentration of ionic species may be different from the bulk concentration and, therefore, swelling becomes an important parameter since the biomass (*Sargassum fluitans* in these experiments) swells with increasing pH (albeit to a lesser extent in the presence of divalent cations). Using the parameters (number of binding sites, proton binding constant, and specific particle volume) obtained from pH titrations, it was possible to predict the effect of Ca concentration, pH and ionic strength on the binding of Ca for a brown algal biosorbent.

5.1.4. Biosorption of different heavy metals by brown algae

There has been an intense research effort aimed at characterizing the metal binding properties of various forms of biomass. These have included fresh and salt water algae, bacteria, fungi and yeasts. Metal sequestration by this diverse group of biosorbents was summarized by Volesky and Holan [1] and serves as a good comparison to the biosorptive behavior of marine brown algae. Uptake of metals by different brown algae is summarized in Table 3.

The algae most frequently studied were *Ascophyllum nodosum*, *Fucus vesiculosus*, *Laminaria japonica*, and various species of the genus *Sargassum*.

As shown in Table 3, the metal uptake in mmol/g biomass typically does not exceed approximately 2 mmol/g. The values were tabulated from experimental data acquired at sufficiently high initial metal concentrations so that maximal uptake (corresponding to the q_{max} of the Langmuir isotherm) was achieved. The only exception is for Au uptake (2.03 mmol/g) by *Sargassum natans* where the experiments do not appear to have been run at sufficiently high gold concentrations. It can also be seen that the pH at which the maxima were achieved is not the same for all heavy metals, as would be expected on the basis of the varying affinity of the metals for the acidic functional groups (i.e. carboxylic and sulfonic). The pH was held constant in each case by, typically, adding small amounts of base or acid as sorption proceeded. Base was added in most cases since the biomass was generally pre-treated with acid, effectively removing the associated light metal ions. Protonated acid functional groups therefore exchange for the binding metals and release H^+ to the solution. The purpose of maintaining pH is to optimize total metal uptake. The salts of the various heavy metals used for these experiments as well as discussions on the various forms and binding mechanisms of the metals can be found in the corresponding references.

5.2. Alginate conformation: influence on selectivity of the raw biomass

In accordance with the concept that metal binding to brown algal biomass can be approximated by the ion-exchange process, as was indicated earlier in the text, we will now extend the discussion to incorporate the influence of the macromolecular conformation of alginate on the metal selectivity as it pertains to applied biosorption. To this end, recent advances in the characterization of biopolymer conformational variability have allowed us to demonstrate the importance of the biochemical composition of the raw biomass in determining heavy metal selectivity (e.g. metal binding affinity sequence, selectivity of the raw biomass, and the influence of ionic strength on binding in multiple-metal systems). By documenting the variability of alginate conformation that exists naturally among samples of raw biomass, we will be able to correlate parameters such as alginate co-polymer block structure to metal binding behavior in flow-through remediation systems.

5.2.1. Conformational variability of alginates derived from Sargassum spp.

It has been reported [58] that most *Sargassum* alginates have M:G ratios ranging from 0.8 to 1.5, whereas alginates from species such as *A. nodosum* ($\sim$1.2; Order Fucales) and *L. japonica* ($\sim$2.2; Order Laminariales) possess relatively high M:G ratios [6,33,34,36,59,60]. As will be discussed in more detail below and, as indicated in preceding sections of this review, low M:G ratios (i.e. <1.0) are indicative of higher G content and are, therefore, deemed highly advantageous for the implementation of the biosorption process. This reflects the established selectivity for divalent cations of the guluronic block sections, in accordance with the "egg-box" model of Rees and co-workers.

Recent studies on the macromolecular conformation [26,61] of Na-alginates extracted from various species of *Sargassum* reveal a consistent and unusual enrichment in homopolymeric α-L-guluronic acid (G-blocks). Table 4 lists the monomer and diad guluronic acid

Table 3
Uptake of metals by different brown algae

Metal/complex	Brown alga	pH	Metal uptake (mmol/g)	Reference
Au	*Sargassum natans*	2.5	2.03[a]	Volesky and Kuyucak [81]
	Ascophyllum nodosum	2.5	0.12	Kuyucak and Volesky [82]
Cd	*Ascophyllum nodosum*	4.9	1.91	Holan et al. [83]
	Ascophyllum nodosum	3.5	1.18	Holan et al. [83]
	Sargassum natans	3.5	1.17	Holan et al. [83]
	Sargassum vulgare	4.5	0.79	Davis et al. [54]
	Sargassum fluitans	4.5	0.71	Davis et al. [54]
	Sargassum muticum	4.5	0.68	Davis et al. [54]
	Sargassum filipendula	4.5	0.66	Davis et al. [54]
	Fucus vesiculosus	3.5	0.65	Holan et al. [83]
Co	*Ascophyllum nodosum*	4.0[b]	1.70	Kuyukak and Volesky [40,41]
Cu	*Laminaria japonica*	4.5	1.59	Fourest and Volesky [55]
	Fucus vesiculosus	4.5	1.18	Fourest and Volesky [55]
	Sargassum vulgare	4.5	0.93	Davis et al. [54]
	Sargassum filipendula	4.5	0.89	Davis et al. [54]
	Sargassum fluitans	4.5	0.80	Davis et al. [54]
Fe	*Sargassum fluitans*	4.5	0.99	Figueira et al. [56]
Ni	*Sargassum fluitans*	3.5	0.75	Holan and Volesky [84]
	Ascophyllum nodosum	3.5	0.69	Holan and Volesky [84]
	Sargassum natans	3.5	0.41	Holan and Volesky [84]
	Fucus vesiculosus	3.5	0.39	Holan and Volesky [84]
	Sargassum vulgare	3.5	0.09	Holan and Volesky [84]
Pb	*Ascophyllum nodosum*	3.5	1.31	Holan and Volesky [84]
	Sargassum natans	3.5	1.22	Holan and Volesky [84]
	Fucus vesiculosus	3.5	1.11	Holan and Volesky [84]
	Sargassum vulgare	3.5	1.10	Holan and Volesky [84]
UO_2^{2+}	*Sargassum fluitans*	4.0	1.59	Yang and Volesky [57]
Zn	*Laminaria japonica*	4.5	1.40	Fourest and Volesky [55]
	Sargassum fluitans	4.5	1.18	Fourest and Volesky [55]
	Fucus vesiculoses	4.5	0.80	Fourest and Volesky [55]

[a] Metal uptake reported was not maximal.
[b] Initial pH.

frequencies (F_G, F_{GG}) and the M:G ratios measured for several of these extracts. Davis et al. [61] demonstrated that for these Na-alginates, the frequency of diad pairs approximated the frequency of homopolymeric guluronic acid triads (GGG or F_{GGG}). Accordingly, it is possible to use the frequency, F_{GG}, to quantify the degree of guluronic acid homopolymeric block formation (e.g. a low M:G ratio does not explicitly indicate a high G-block frequency). As such, the results presented in Table 4 clearly indicate that these alginates are highly enriched in G-blocks when compared to the vast majority of alginates extracted from other brown algae [32,62,85]. Similar compositions, among other brown algae, are only known to occur in the stipes of *Laminaria hyperborea*, which typically yield a F_{GG} on the order of 0.4. The metal selectivity imparted by these G-block rich alginates may provide additional advantages for the use of these brown algae in implementation scenarios (see below).

Table 4
Compositional data of Na-alginates extracted from species of *Sargassum*

Source of alginate	F_G	F_{GG}	M:G ratio
S. fluitans[a]	0.84	0.81	0.19
S. siliquosum[a]	0.58	0.57	0.72
S. filipendula[b]	0.84	0.76	0.19
S. muticum[b]	0.76	0.59	0.31
S. polycystum[b]	0.82	0.77	0.21

[a] Davis et al. [61].
[b] Davis et al. [26].

5.2.2. Selectivity studies relevant to the biosorption process

In the section on alginate metal affinity and binding, we reviewed some of the earlier work (e.g. [35]) that demonstrated the selective binding of divalent cations by alginates containing significant levels of guluronic acid. These early investigations were followed by studies of mixed-metal pair systems, in which both the extracted alginate and the raw biomass were compared on the basis of their selectivity coefficients. Haug and Smidsrød [87] demonstrated that selectivity coefficients of extracted alginates for the strontium–calcium and strontium–magnesium metal pair systems are closely correlated to coefficients obtained with the raw brown algal tissue. In both cases, there was an enhanced selectivity for strontium over magnesium or calcium as the guluronic acid content of the alginate in the algal tissue increased. The experiments performed by Haug's group were typically carried out at a relatively high ionic strength (on the order of 0.2 M) but the selectivity coefficients determined in their studies still reflect the relative affinity of the alginates and raw biomass at environmentally relevant metal concentrations.

A significant amount of work was published on the binding of Cu^{2+} to alginate by Jang et al. [64–66]. In particular, they demonstrated the usefulness of the selective nature of Na-alginate gels in the field of heavy metal remediation. For example, Jang et al. [66] performed competitive uptake experiments between Cu^{2+} and Co^{2+} by a Na-alginate gel at intermediate (100 ppm or ~1.6 mM) as well as low Cu (18 ppm or 0.28 mM) and high Co^{2+} (300 ppm or 5.1 mM) concentrations which revealed a high selectivity for Cu^{2+} over Co^{2+} (Cu/Co selectivity = 20.9, based on batch adsorption and an extended Langmuir model). In both cases more than 90% of the Cu^{2+} was sequestered preferentially over Co^{2+} from the solution. The Na-alginate (CP Kelco; likely extracted from *M. pyrifera* or *L. hyperborea*) was characterized and found to contain only 31% guluronic acid ($F_G = 0.31$), and the authors acknowledged that an even greater selectivity for Cu^{2+} over Co^{2+} could be expected for Na-alginates containing higher amounts of guluronic acid residues. On the basis of these observations and those of Haug and Smidsrød [87,35], raw brown algal tissues that contain guluronic acid-rich alginates should display a high metal selectivity. This conclusion is supported by the work of Figueira et al. [56] in which they investigated the interference of Fe^{2+} on Cd^{2+} biosorption by raw *Sargassum* biomass. They documented a marked selectivity for Cd^{2+} over Fe^{2+} at concentrations ranging from 0.1 to 10 mM Cd^{2+}. The authors did not specify the guluronic acid composition of the alginates contained within the thallus of their *Sargassum* but the data of Davis et al. [26,61] indicate that the frequency of guluronic acid diads (F_{GG}) of *Sargassum* are likely higher than the Na-alginates employed by Jang et al. [66].

The relationship between alginate conformation in *Sargassum* species and metal selectivity has recently been established [26] for the Ca–Cd–, Ca–Mg– and Cd–Mg–alginate systems. In that study, the selectivity coefficient for the ion-exchange of two cations, A and B, is defined by the following reaction and equilibrium relationship, and was determined according to the method of Haug and Smidsrød [35,63]:

$$\equiv S - A_{(g)} + B_{(aq)} \rightleftharpoons \equiv S - B_{(g)} + A_{(aq)}, \quad (13)$$

$$K^{*B}_{A} = X_B C_A / X_A C_B, \quad (14)$$

where (g) is the gel or alginate phase; (aq) the aqueous phase; S the binding site; X_A and X_B the equivalent fractions of the counterions in the polymer phase, whereby $X_A + X_B = 1$, and C_A and C_B are the concentrations of the same ions in solution. The selectivity coefficients for the Cd–Ca–alginate system, K^{*Ca}_{Cd}, varied from between 0.43 ± 0.10 and 1.32 ± 0.02 for a range in F_{GG} of 0.23–0.81. In contrast, the Mg–Ca– and Mg–Cd–alginate systems yielded maximal values of K^{*Ca}_{Mg} (18.0 ± 1.4) and K^{*Cd}_{Mg} (16.0 ± 0.9) for the alginates extracted from *Sargassum fluitans* ($F_{GG} = 0.81$) and *Sargassum thunbergii* ($F_{GG} = 0.75$). The lack of significant selectivity between Ca^{2+} and Cd^{2+} for a wide range of F_{GG} may reflect the importance of steric placement in the alginate gel network, since the two cations have similar ionic radii (1.00 and 0.95 Å, for a 6-fold coordination, respectively). The high selectivity coefficients observed for the Ca–Mg– and Cd–Mg–alginate systems may, in part, be ascribed to the difference between the ionic radius of Mg^{2+} (0.72 Å, for a 6-fold coordination) and that of either Ca^{2+} or Cd^{2+}. Hence, the size of the cation appears to be an important variable in metal binding to alginates, both due to the rigid nature of the GG-linkages, as well as to the steric arrangement of the electronegative ions surrounding the divalent cation. In contrast to Ca^{2+} and Cd^{2+}, the lower selectivity of magnesium for the G-blocks likely results from its inability to form as tight a coordination environment within the alginate network.

All of the above observations can be related to the original observations of Haug and Smidsrød [35], who determined the amount of various divalent metal cations required to bring about gel-formation and precipitation of alginates extracted from *Laminaria digitata* and the stipes of *Laminaria hyperborea*. The relative amount of each metal ion required for gelation does not correspond exactly with the affinity sequence of the individual metals for the alginate (see *Complexation*) but accurately reflects the relative selectivity of alginate in mixed-metal systems. The discrepancies are attributed to the difference in the absolute quantity of a given metal ion required to obtain a gel. The amount of divalent metal

ions required for precipitation of the alginate increases from right to left and, thus, their relative selectivity increases to the right:

$$Mg > Mn, Fe > Co > Ni > Zn > Ca > Cd > Sr > Cu > Pb > Ba.$$

For example, measured selectivity coefficients in both alginate gels and raw brown algal tissue correspond to the above sequence for the following metal-pair systems: $Sr > Mg$; $Sr > Ca$; $Cu > Co$; $Cd > Fe$; $Cd \sim Ca$; $Ca > Mg$ and $Cd > Mg$. Although these experiments were performed with different alginates and raw algal thalli, a common property of all these substrates was the presence of a minimum frequency of guluronic acid residues (i.e. $F_G > \sim 0.30$). An exact comparison is therefore not possible, but the evidence of selective binding obtained through the variety of experiments outlined in this review (e.g. selectivity experiments, batch metal uptake experiments) clearly lends credibility to the application of the 'egg-box' model in explaining the metal selectivity displayed by brown algal biosorbents.

5.3. Potential for applied remediation

Biosorption can be used to eliminate heavy metals from industrial effluents or to recover precious metals from processing solutions. The fully "loaded" biosorbent may concentrate heavy metals a thousand fold from their concentration in the liquid phase. This loading of the biomass may be reversed in order to "desorb" the metals and several studies have shown [41,67,68] elution of the biomass by acid aqueous solutions to be highly effective. The elution process does not significantly reduce the binding capacity of the biomass and several cycles may be employed. For example, Yang [86] used a *Sargassum fluitans* loaded fix-bed column to study uranium biosorption. He used a 0.1 N HCl solution to elute the bound uranium and recovered 99.5% of the metal. Furthermore, the column was maintained continuously for 1 month over which time five biosorption–desorption cycles were carried out. The biosorption capacity of the substrate decreased by approximately 7% after the first cycle and was about 20% less than the fresh biomass on the fifth cycle. The observed drop in biosorption capacity between cycles was attributed to leaching of alginate. The overall metal concentration factor, defined as the ratio of the elution concentration to the influent concentration for a given biosorption cycle, was determined to be approximately 25.

With a high concentration factor, it should be possible to reduce the volume of waste that is produced by applying an iterative metal sorption–desorption process such that only a small volume of solid waste is generated. According to this scenario, the biosorbent is regenerated and a highly concentrated metal solution is obtained. This concentrate may then be treated by either co-precipitation, flocculation or electro-winning. A toxic sludge would be generated by co-precipitation whereas the solid metal, a more desirable end-product, would be recovered from the concentrate by electro-winning. A simplified schematic representation of the proposed "zero discharge" technology is shown in Fig. 11 where multiple "sorption" and "desorption" cycles are carried out.

The application of biosorption is particularly well suited as a refining technique where wastewater heavy metal concentrations range from 1 to 100 ppm. These levels can be lowered to drinking water standards with the existing biosoption technology. The main advantages of the biosorption process over traditional techniques are the high effluent quality it generates, its terms of operation under a broad range of service conditions and its cost-effectiveness. The bottom line is the inexpensive nature of brown algal biosorbents.

5.4. Relating alginate conformation and metal selectivity to applied biosorption

It is beyond the scope of this work to go into the engineering details of the implementation of flow-through column systems. However, as outlined earlier, it is the natural ion-exchange property of the biomass that lends itself to the task of selective removal of toxic divalent heavy metals. This review is intended to highlight factors that need to be considered in investigations of the relationship between substrate biochemistry and heavy metal binding mechanisms in the biosorption process. In this regard, it is clear that the critical parameters in brown algal biosorption systems are the alginate content and its specific macromolecular conformation in the brown algae. Our research group has focused on developing *Sargassum* biomass biosorption technology for both recovery of strategic metals as well as detoxification of heavy metal bearing industrial wastes. Several investigations (e.g. [69–72]) have specifically focused on the implementation of flow-through column systems with mock-waste feed solutions in the mM range (e.g. [69]). These studies, coupled with equilibrium batch experiments, have consistently demonstrated that selectivity of *Sargassum* biomass is a key feature in the design of such systems. The relative affinity of this raw biomass for various divalent metal cations was determined at environmentally relevant concentrations to be

$$Cu > Ca > Cd > Zn > Fe$$

and is well reflected by the breakthrough curves which evolve following passage of the simulated waste-feed streams through *Sargassum* packed columns. This is

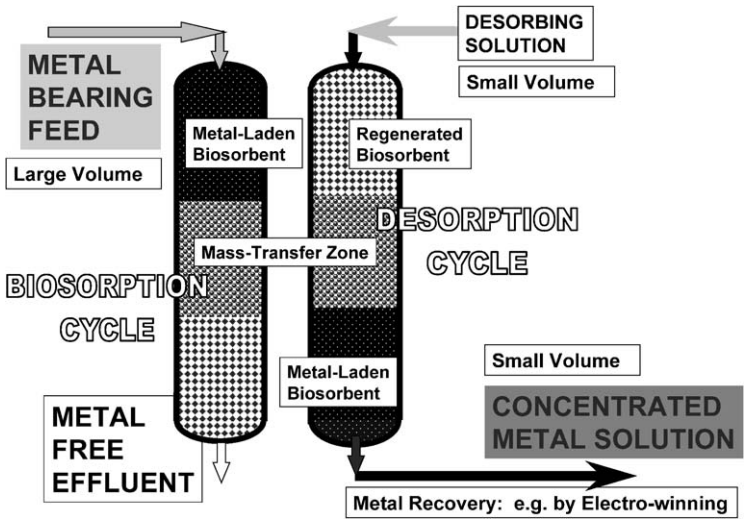

Fig. 11. Schematic diagram of possible biosorption implementation using packed bed columns for biosorption and desorption.

consistent with the divalent metal–alginate gelation sequence of Haug and Smidsrød [35] discussed above. What is therefore important to recognize is that divalent metal ion sequestration by brown algae in the context of remediation should be viewed as being a direct consequence of the formation of network junctions by the cations of homopolymeric guluronic acid blocks. Although the ion-exchange properties of alginates and raw algal biomass have been thoroughly documented in the fields of marine biochemistry, food technology and metal bioremediation, the selective binding behavior observed in flow-through remediation systems can now be specifically attributed to the presence of abundant G-blocks.

6. Conclusions

The brown algae represent an especially efficient and resilient class of biosorbents relative to other biomass types. Fortunately, due to their economic value in many industrial applications, there is much information about their basic structure and biochemical constitution. This in turn makes the interpretation of the sorption behaviors and elucidation of metal binding mechanisms more rigorous. The order Laminariales and Fucales of the brown algae (division Phaeophyta) are the most important groups of algae to the field of biosorption because of the abundance of their cell wall matrix polysaccharides and extracellular polymers. The alginate polysaccharide is mainly responsible for the natural ion-exchange capacity of the brown algae. Its unique macromolecular structure gives rise to selective metal binding whose mechanism is commonly represented by the "egg-box" model.

One of the outstanding tasks in evaluating the role of brown algal polysaccharides in metal binding is the determination of the extent to which the structure of the alginate and its hompolymeric guluronic acid content, or G-block structure determine selectivity among various metals. An identification of the biochemical factors that control this parameter, coupled with a determination of its alginate content, should allow us to predict the sorption behavior of brown algal biomass.

A complete characterization of brown algal substrate biochemistry will be necessary to credibly emphasize the advantages of biosorption over conventional ion-exchange resins and materials. The biochemical concepts used in defining the equilibrium state and heavy metal binding mechanisms are becoming more realistic than ever. Nevertheless, whereas important advances in knowledge have been realized in recent years, the goals stated earlier are paramount toward the implementation of biosorption technology in industrial and environmental remediation.

Acknowledgements

We wish to acknowledge our colleague, Dr. Silke Schiewer whose previous work greatly influenced this text. 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 scholarships. Finally, the authors would like to thank Prof. H. Seki and one anonymous journal

reviewer who provided constructive comments on a previous draft of this manuscript.

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