Phycologia Volume 53 (1), 100–108 Published 3 February 2014

Biochemical characterization of *Macrocystis pyrifera* and *Undaria pinnatifida* (Phaeophyceae) in relation to their potentiality as biosorbents

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Abstract: *Undaria pinnatifida* and *Macrocystis pyrifera* from Patagonia were characterized in terms of the alginate, protein, total carbohydrate and organic matter content, cation exchange capacity and acid-base properties to assess the suitability of both species as potential biosorbents of metals. Environmental scanning electron microscopy equipped with an energy dispersive X-ray microanalysis system, Fourier transform infrared spectroscopy (FT-IR) and Brunauer, Emmett and Teller surface area analysis were also applied for characterization. The results showed that *U. pinnatifida* had a higher percentage of organic matter, alginate and protein and a larger specific surface area than *M. pyrifera*, although the latter exhibited a higher ionic exchange capacity. The groups with a weak acid behaviour represented 84% and 97% of the total acid groups in *M. pyrifera* and *U. pinnatifida*, respectively. FT-IR analysis confirmed the presence of different chemical groups such as sulphonate in both biomaterial *M. pyrifera* and *U. pinnatifida*. Besides, Zn(II) adsorption capacity was different among different parts of the thallus of each species. The difference founded in the physicochemical properties of both algae could affect their biosorption capacities.

Key Words: Biochemical characterization, Biosorption, *Macrocystis pyrifera*, *Undaria pinnatifida*, Zinc

INTRODUCTION

Biosorption and bioaccumulation are biological methods suitable for metal removal from wastewater. Bioaccumulation is an intracellular metal accumulation that involves metal binding to intracellular compounds, intracellular precipitation, methylation and other mechanisms. Bioaccumulation depends on cellular metabolism and can be inhibited by metabolic inhibitors such as low temperature and lack of energy sources (light). Biosorption describes the removal of metal from an aqueous solution by passive binding to nonliving biological materials. It is a quick process independent of the presence of specific nutrients or cell growth conditions. This implies that the biosorption removal mechanism is not metabolically controlled (Davis *et al.* 2003). From a terminological point of view, the term bioaccumulation is usually used for metal uptake by living cells and the term biosorption for passive sequestering by dead cells (Volesky 1990).

Biosorption is considered promising for wastewater treatment because it is a cheaper and faster process than conventional wastewater technology (Norton *et al.* 2004; Orhan *et al.* 2006). Moreover, the possibility of employing dead biomass avoids living cell toxicity problems and biosorption allows the regeneration and reuse the biological materials for several adsorption–desorption cycles. Although many biological materials bind metals, only those with sufficiently high metal binding capacity and selectivity can be considered useful in full-scale biosorption processes (Volesky 2003). Among these materials, marine macroalgae are one of the most studied groups (e.g. Areco & dos Santos Afonso 2010; Luna *et al.* 2010; Ghasemi *et al.* 2011; Ibrahim 2011; Montazer-Rahmati *et al.* 2011; Bulgariu & Bulgariu 2012). Natural algal populations and seaweeds cast ashore could be used for biosorption purposes, making the process economically viable.

Both chemical structure and composition affect the ability of the biological material for metals removal. Structure refers to rigidity, porosity, flexibility, swelling effects (expansion and contraction by addition of water during biosorption process). Chemical composition reflects different chemical groups present on cell wall that act as active sites for binding metal. Hence, knowledge about the physicochemical properties of the biomaterial allows one to potentially modify the chemical and physicochemical aspects to induce or enhance its adsorption capacity.

Biosorption in algae has mainly been attributed to the cell wall properties where both electrostatic attraction and complexation can play an important role. In a comparison of the Phaeophyta to other algal groups, important differences in the storage products as well as in their cell chemistry can be found. The Phaeophyta have an embedding matrix that is predominately composed by alginic acid or alginate with a smaller amount of sulphated polysaccharide (fucoidan); whereas, the Rhodophyta contain sulphated galactans. Large amounts of amorphous embedding matrix polysaccharides, combined with their well-known ability to bind metals, makes both algal groups potentially excellent metal biosorbents (Davis *et al.* 2003).

Two species of Phaeophyta, *Macrocystis pyrifera* (Linnaeus) C. Agardh and *Undaria pinnatifida* (Harvey) Seringar, were chosen for this study. *Macrocystis pyrifera* is a native species broadly distributed in Argentina from Chubut to

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DOI: 10.2216/12-106.1

Tierra del Fuego. Large quantities of this and others seaweeds are washed up and deposited on the beaches of Patagonia, Argentina. This algal biomass interferes with recreational uses of the beach, and therefore must be periodically collected and disposed (Eyras *et al*. 1998). Sometimes this biomass is used as raw material for cattle feed additives as well as producing fertilizers and alginic acid (Boraso & Zaixo 2012). On the other hand, the invasive species *U. pinnatifida* today occupies more than 1000 km of the Patagonic coastline. *Undaria* annually develops the macroscopic sporophytic phase during winter that dies in summer, which strongly impacts tourism uses of the shores and submarine reefs. In addition, recent studies have shown negative impacts on native macroalgal assemblages and reef fish (Dellatorre *et al*. 2012).

The aim of this study was to evaluate the possibility of using *Macrocystis pyrifera* and *Undaria pinnatifida* as biosorbents through the analysis of alginate, organic matter, total carbohydrate and protein content, cation exchange capacity, acid-base properties, functional groups, structural characterization and adsorption capacity.

MATERIAL AND METHODS

Materials were collected in Bahía Camarones (Chubut Province; 44° 47′ 24″ S, 65° 43′ 12″ W) during November and December in 2006 and 2009. Blades, stipes and floats were separated from *Macrocystis pyrifera*, and blades and holdfast were selected from *Undaria pinnatifida*. Pieces were ground using a food processor and sieved with a metal drum sieve with different meshes. To remove the excess of salts, particles less than 1.8 mm were chosen and washed several times with distilled water until the electrical conductivity of washing water was equal or less than 1 mS cm^{-1}. Particles were then filtered with technical paper and dried at 50°C for 48 h and stored for the chemical and physical analyses.

Dry biological material (2.00 g) was weighed and placed in porcelain evaporating dishes and burned for 24 h at 405°C. The percentage of organic matter was calculated by subtracting the ash weight from the algal dry weight. Total carbohydrate was measured following the method proposed by Andrade *et al*. (2004), with some modifications. Dried material (4.0 g) was weighed and placed in 250 ml flask with distilled water and stirred for 24 h. Then it was centrifuged at 4°C, 10,000 × *g* for 20 min. This first step yielded a polysaccharide supernatant (fraction A). Another sample of 4.0 g of dried material was weighed and treated with 5% w/v KOH and stirred for 24 h at room temperature. After that, algal material was separated from the supernatant by centrifugation (4°C, 10,000 × *g* for 20 min). This second step yielded a polysaccharide supernatant (fraction B). Finally fractions A and B were mixed to obtain only one extract, which was employed for total carbohydrate determination. Two milliliters of extract were mixed with 0.5 ml 3% w/v aqueous phenol in glass tubes, and 5.0 ml of concentrated H_2SO_4 was immediately added and mixed. The tubes were cooled in an ice bath for 30 min, and optical density was measured at 490 nm using a Beckman DU 640 spectrophotometer (Beckman Coulter Inc., Brea, California USA). The standard curve was prepared with 2.0 ml of a glucose solution containing 6 to 60 μg, and the blank was prepared with 2.0 ml of distilled water.

The total protein extraction procedure was based on Barbarino & Lourenço (2005) with some modifications: 50 mg of freeze-dried (–70°C for 24 h) algal sample was manually ground with a pestle and mortar for 5 min to break down the tissues. Four milliliters of distilled water were added and the sample was kept at 4°C for 12 h. After that, it was centrifuged at 4°C, 10,000 × *g* for 20 min, and the supernatant was collected and kept at 4°C. With stirring, the pellet was re-extracted with 1.0 ml of 0.1 mol l^{-1} NaOH for 1 h. Then, it was centrifuged at 4°C, 10,000 g for 20 min. The supernatant was collected and mixed with the first supernatant. Cold 25% v/v of trichloroacetic acid (TCA) was added in a proportion of 2.5:1 (TCA:supernatant). Tubes containing TCA and protein were kept in an ice bath for 30 min and then centrifuged for 20 min at 4°C (10,000 × *g*). The supernatant was discarded, and the pellet was washed with cold 10% TCA and centrifuged again. The pellet that formed after the second centrifugation was suspended in 5% TCA at room temperature, in a proportion of 5:1 (5% TCA:precipitate) and centrifuged at 21°C (10,000 × *g*) for 20 min. The supernatant was discarded, and the pellet was kept in the tubes for protein quantification. If protein analysis was not performed immediately, the pellet was stored at –20°C until analysis. Precipitated proteins were quantified by the Lowry method (Barbarino & Lourenço, 2005); for this purpose, the precipitate proteins were solubilised in 0.25 ml of 1.0 mol l^{-1} NaOH for 5 min at 100°C. Next, 2.5 ml of reactive C (50 ml 2% w/v of Na_2CO_3 + 1 ml 0.5% w/v of $CuSO_4 \cdot 5H_2O$ in 1% w/v of potassium and sodium tartrate, prepared at the moment) was added; the solution was maintained at room temperature for 10 min. Finally, 0.5 ml of diluted Folin reactive (1 volume of commercial Folin reagent + 1 volume of distilled water) was added and the solution was kept for 20 min at 37°C. Blank and standard samples (0.25 ml bovine serum albumin with a concentration of 200 mg l^{-1}) were prepared. The absorbance of the samples was read at 750 nm against a blank with a Beckman DU 640 spectrophotometer.

Extraction and quantification of alginate

The pretreatment and extraction procedures were based on Arvizu-Higuera *et al*. (1995), with several modifications: 10 g of dried, ground and sieved algal samples were placed in vessels with 200 ml of 0.5% w/v formaldehyde and agitated at 600 rpm for 30 min. The solutions were drained off, and the algae were washed with distilled water. Then, the algae were put in contact with 150 ml of distilled water (pH 4.0 adjusted with 12 mol l^{-1} HCl) and agitated as recommended for 15 min. The aqueous solutions were drained off and the algal samples were washed several times with distilled water. The extraction was carried out mixing the washed algae with 250 ml distilled water, adjusting the pH to 10.0 using 10% w/v Na_2CO_3. The mixtures were kept for 2 h at 70°C–75°C with continuous agitation. The algal residues were separated from the viscous liquid by filtration with gauze and cotton. Next, 0.5% v/v HCl was added to the filtered solutions up to the alginate-complete precipitation. The alginic acid fibres were placed in ethanol–water (1:1) to eliminate excess acid and other residues. The

Table 1. Physicochemical characteristics of *Macrocystis pyrifera* and *Undaria pinnatifida*. $t_{\text{theoretical}(4,0.025)} = 2.77$.

Species	Organic matter %	Total carbohydrate %	Proteins %	Alginate %	CEC, meq (100 g)$^{-1}$	Specific area, BET (m^2 g^{-1})
M. pyrifera	84.4 ± 0.3	22.3 ± 0.07	0.21 ± 0.01	10 ± 0.03	80 ± 4	0.2
U. pinnatifida	88.7 ± 0.3	23.0 ± 0.05	1.31 ± 0.04	30 ± 0.05	69 ± 5	0.3
t_{exp}	17.91	1.43	47.81	606.33	3.03	

precipitate was separated by vacuum filtration and mixed with distilled water. Next, 10% w/v Na_2CO_3 was slowly added to achieve the complete dissolution of alginic acid. Then the alginate was precipitated by drop-wise addition of 10% w/v $CaCl_2$ solution. The precipitates were recovered by filtration and treated with 5% w/v NaClO for 30 min under constant agitation for whitening. Then they were washed three times with ethanol:water (1:1). To transform the calcium alginate in alginic acid, the fibres were mixed with distilled water and 12 mol l^{-1} HCl, which was slowly added until the pH of the mixture was 2.0. A second washing step was performed while keeping the pH at a lower value (1.8) during a 15-min wash. This process was repeated twice. Finally, the fibres were washed with small amounts of water, filtered and dried at 30°C–40°C up to constant weight. The alginate yields were calculated as a weight percentage (%w/w), based on the initial dry weight of the algae.

Cation exchange capacity

The ion exchange capacity of the biomass to hold cations (i.e. the number of negatively charged sites per gram of biomass) was quantitatively expressed in a measured property known as the cation exchange capacity (CEC). The CEC was measured by cation displacement as described by Hawari & Mulligan (2006): 20 ml of 1 mol l^{-1} potassium acetate was added to 1.0 g of the algal biomass in a 50-ml centrifuge tube. Samples were shaken for 30 min at 75 rpm and then were centrifuged for 10 min at 650 × *g*. The clear supernatant was discarded. This process was done twice. Distilled water (20 ml) was added to the algal material of each sample, and the samples were shaken for 30 min at 75 rpm. Then they were centrifuged for 10 min at 650 × *g*. The clear supernatant was discarded. This process was performed twice. Ammonium acetate (25 ml, 1 mol l^{-1}) was added to the material. The samples were shaken for 30 min at 75 rpm and then centrifuged for 10 min at 650 × *g*. The clear supernatant was collected in a clean 50-ml centrifuge tube. This process was repeated twice by pouring the clear supernatant into the same centrifuge tube. The concentration of potassium was measured by atomic absorption spectrophotometry (Shimadzu AA6650, Shimadzu Corporation, Kyoto, Japan). The value for the concentration of potassium was equal to the CEC.

Acid–base characterization

The algal material was analysed after treating 2.0 g with $CaCl_2$ (0.2 mol l^{-1}). Then it was placed in a flask containing 100 ml of HCl (0.10 mol l^{-1}) and kept under agitation at 160 rpm, room temperature for 1 h. It was then filtered, washed three times with distilled water and dried at 50°C for 24 h. The amount of calcium released from the algae after the acid contact was measured by atomic absorption spectrophotometry. The protonated material (0.1 g) was dispersed in flasks with 100 ml of 1.0 mmol l^{-1} NaCl solution. Titration was carried out by addition of successive aliquots standardized NaOH to the flask while the suspension was stirred under nitrogen atmosphere. After each addition, the system was allowed to equilibrate until stable pH. The pH measurements were recorded using a pH meter (ORION 3 STAR pH Benchtop, Thermo Electron Corporation, Singapore). Potentiometric titrations were performed in triplicate.

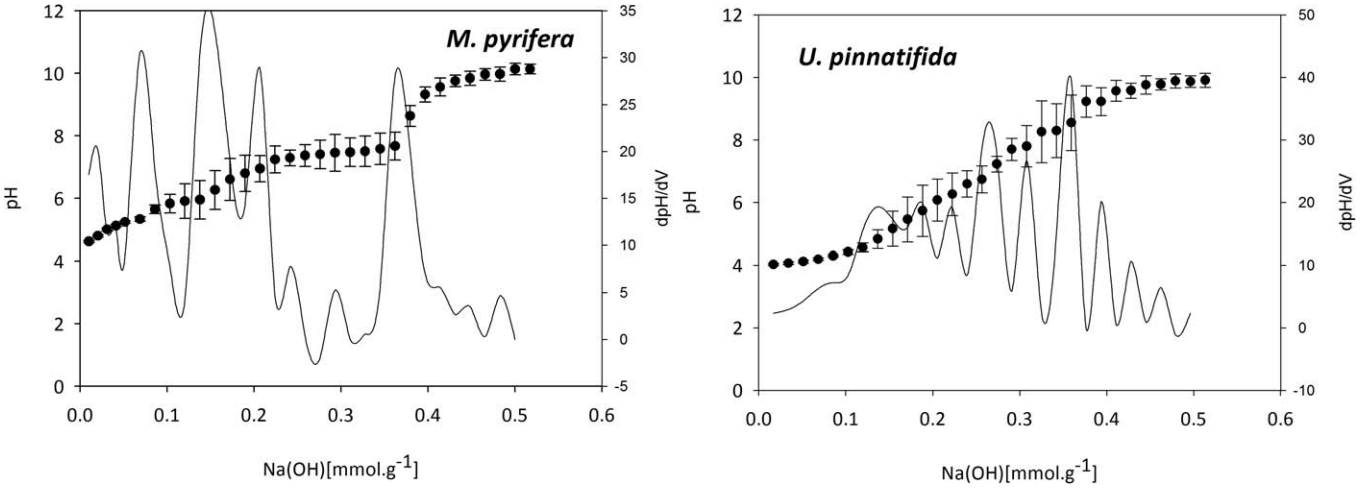

Fig. 1. Potentiometric titration curves (dots) and first derivative curves (continuous line) of average pH during titration of calcium-treated algae: *Macrocystis pyrifera* (left) and *Undaria pinnatifida* (right).

Table 2. Acid-base properties of calcium-treated algae.

Biosorbent	Total amount of acid groups (mmol g^{-1})	Strong acid group (mmol g^{-1})	Weak acid group (mmol g^{-1})	Ca^{2+}exchange with total acid groups (mmol g^{-1})
M. pyrifera	0.62	0.02	0.60	0.26
U. pinnatifida	0.46	0.069	0.39	0.198

Metal adsorption capacity by different thallus parts

Blades, stipes, floats and holdfast of both species were washed separately several times with distilled water (until the electrical conductivity reached values less or equal than 1 mS cm^{-1}), filtered and dried at 50°C for 48 h. To evaluate the adsorption properties of each thallus part, adsorption experiments were performed with Zn(II) as the adsorbate. Individual solutions of Zn(II) at 50 mg l^{-1} were prepared by dilution of stock solutions [1000 mg Zn (II) l^{-1}]. The pH was set at 4.0 by adding H_2SO_4, and 0.4 g of material was weighed and placed in Erlenmeyer flasks containing 100 ml of the Zn(II) solution. Flasks were kept at 20°C in a rotary shaker at 160 rpm. Samples were collected at different time intervals and filtered through 0.45-μm membranes. Zn(II) remaining in solution was measured by atomic absorption spectrophotometry (Shimadzu AA6650) according to the analytical method 7950 EPA (atomic absorption, direct aspiration) under the following operating conditions: lamp with Zn hollow cathode, wavelength 213.9 nm, acetylene fuel, oxidizing air, correction background, lamp current 8 mA and slot 0.5 nm.

The amount of metal adsorbed was calculated by the mass balance equation, according to:

$$q = \frac{V(C_i - C_f)}{m} \quad (1)$$

Where q was the adsorbate uptake (mmol g^{-1}); C_i and C_f were the initial and final adsorbate concentrations in solution (mmol l^{-1}), respectively; V was the solution volume (litres); and m was the mass biosorbent (g, dry weight basis).

ESEM-EDX and FT-IR

Macrocystis pyrifera and *Undaria pinnatifida* were ground and sieved; the particle size fractions collected by the 10–16 mesh (i.e. 1.2–2.0 mm) were retained and washed several times with distilled water (until the electrical conductivity in the washing water was less than 1 mS/cm) and dried at 50°C for 48 h. Next, the biological material was examined using Fourier transform infrared spectroscopy (FT-IR) and environmental scanning electron microscopy (ESEM; Model Quanta 200 FEG, FEI, Hillsboro, Oregon USA), equipped with the OXFORD Inca 350 Energy Dispersive X-ray microanalysis (EDX) system (Oxford Instruments, Abingdon, Oxfordshire, UK).

FT-IR analysis was performed on a Nicolet 740 FT-IR spectrometer (Thermo Electron Corporation, Madison, Wisconsin USA). Infrared spectra were recorded in the region of 400–2000 cm^{-1} at a resolution of 2 cm^{-1} using 32 scans and the OMNIC software version 4.1 for numerical treatment of spectra. Analysis was performed on KBr discs: dry biomass was carefully ground then mixed with KBr (final concentration of sorbent in the powder close to 0.1% in weight), dried under vacuum before being conditioned in the form of thin discs (about 1.5 mm) under mechanic press (Svecova *et al.* 2006). Overlapped and hidden peaks were analysed by spectra differentiation (second derivative of the spectrum; De Aragao & Messaddeq 2008).

Surface area by BET analysis

The algal material treated as described above was used for determining the porosity by the Brunauer, Emmett and Teller (BET) surface area analysis method. A gas adsorption analyser (Coulter SA 3100; Beckman Coulter Inc., Brea, California USA) was employed with 1.0 g of material and krypton gas at 24°C. This analysis was done in triplicate. The method is based on the gas adsorption technique performed by the addition of a known volume of gas (the adsorbate, krypton in our case), to a solid material in a sample vessel at cryogenic temperature. At cryogenic temperatures, weak molecular attractive forces will cause the atoms of Kr to adsorb onto the solid material. The Kr is added in a series of controlled doses, the pressure in the sample vessel is measured

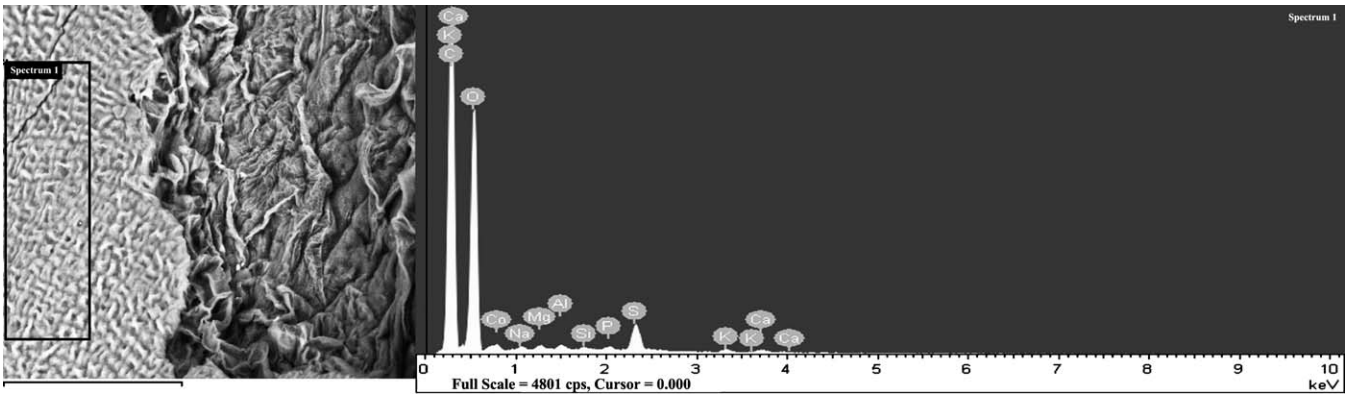

Fig. 2. ESEM-EDX analysis of *Macrocystis pyrifera* particle: external surface. Scale bar = 60 μm.

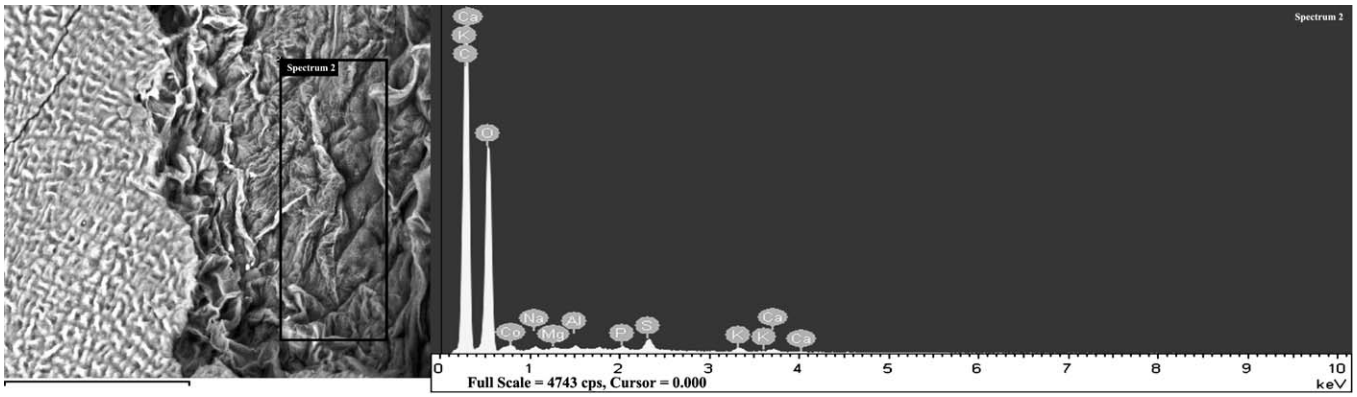

Fig. 3. ESEM-EDX analysis of *Macrocystis pyrifera* particle: inner surface. Scale bar = 60 μm.

after each dosing. There is a direct relationship between the pressure and the volume of gas in the vessel. By measuring the reduced pressure due to adsorption, the ideal gas law can then be used to determine the volume of gas adsorbed by the sample. The resulting relationship of volume of gas adsorbed vs relative pressure at constant temperature is known as an adsorption isotherm. From the analysis and the cross-sectional area of the adsorbate gas molecule, the surface area and pore size distribution of the sample can be derived.

RESULTS

Table 1 shows percentage of organic matter, alginate, total carbohydrate, protein and specific superficial area. Although *Undaria pinnatifida* exhibited higher values in all the parameters, the statistical test (unpaired two-sample *t*-test) confirmed significance differences with $\alpha = 0.05$ in all case except total carbohydrate, which was not statistically different.

The potentiometric titrations of the calcium treated biomaterial (after protonation) are shown in Fig. 1. The total acid groups and the strong acid groups were identified from the inflexion points of the derivative plot. The first inflexion point and the last one corresponded to the strong acid groups and the total amount of acid groups, respectively (Oliveira *et al*. 2011). The weak acid groups were obtained as result of the subtraction between the total amount of acid groups and the strong acid groups. The weak acid group represented 84% and 97% of the total acid groups for *Macrocystis pyrifera* and *Undaria pinnatifida*, respectively (Table 2).

During protonation (before titration), Ca^{2+} ions, bound to the cell wall surface as a consequence of calcium treatment, were exchanged with protons. The calcium amount released to the solution was measured to determine the number of active sites occupied originally by calcium. The amount of Ca^{2+} exchanged with protons was 0.13 mmol g^{-1} and 0.099 mmol g^{-1} for *M. pyrifera* and *U. pinnatifida*, respectively. As calcium ions have two charges, 0.26 mmol g^{-1} (*M. pyrifera*) and 0.198 mmol g^{-1} (*U. pinnatifida*) of protons were needed to replace calcium present on the cell walls (Table 2), which means that 41.9% (*M. pyrifera*) and 43.0% (*U. pinnatifida*) of the total acid groups were occupied by calcium when the material was treated with $CaCl_2$ (0.2 mol l^{-1}) under the experimental conditions.

The EDX spectra revealed the presence of different elements such as Ca, K, Na, Mg, Al, S, P and Si at the external surface of the cell wall of both species, as well as in the inner surface in case of *M. pyrifera* (Figs 2–4). The inner surface was exposed due to the grinding process. Some of the elements present at the cell wall participated in ionic exchange process during adsorption or remained in the

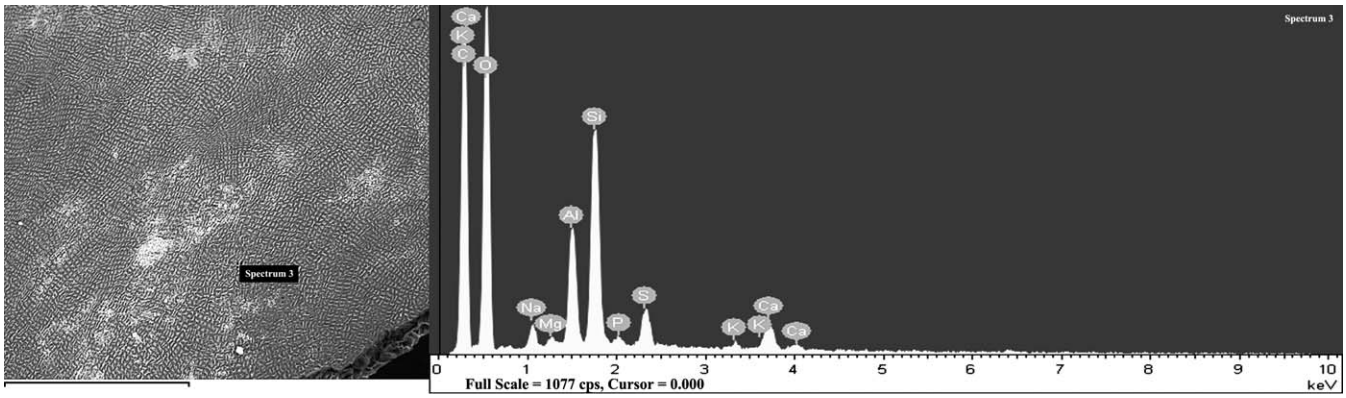

Fig. 4. ESEM-EDX analysis of *Undaria pinnatifida* external surface. Scale bar = 200 μm.

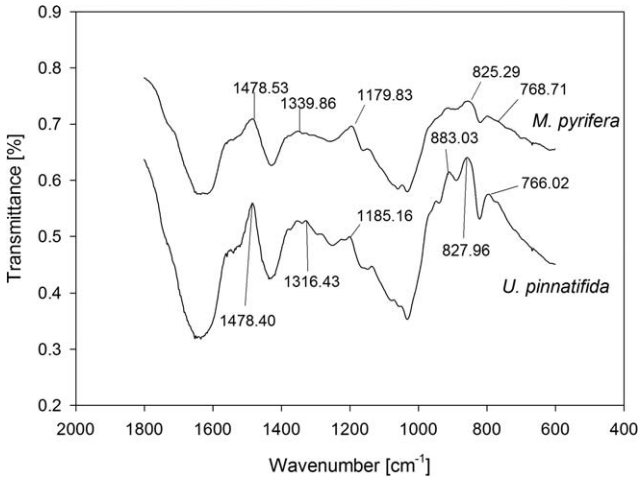

Fig. 5. *Macrocystis pyrifera* and *Undaria pinnatifida* material characterized by FT-IR. Peaks corresponding to functional groups on algal surface (Table 3).

active sites on the algal material blocking them for metal targets during the biosorption process.

The FT-IR spectrum analysis determined the main chemical groups present in both biomaterials and that was reflected in their complex nature (Fig. 5). The spectrum of *U. pinnatifida* showed more bands than the spectrum of *M. pyrifera*, and therefore *U. pinnatifida* should have more diversity of active sites for biosorption (Fig. 5).

The peak determined in both biomaterials was C-O amino stretching. The *M. pyrifera* spectrum revealed the presence of the following functional groups: -C-O-C vibration of polysaccharides and $–SO_3$ asymmetric bands. On the other hand, the *U. pinnatifida* spectrum presented the following chemicals groups: N–H amine stretching, C–N stretching, vibration of amine groups, P–O–C links of the organic phosphated groups and P–O vibration of the ($C–PO_3^{-2}$) moiety, S=O stretching and S–O stretching peaks (Table 3).

The blades, stipes and floats exhibited different adsorption capacity as can be seen in Fig. 6. The adsorption capacity of *M. pyrifera* blades was 100% higher than for stipes and floats. On the other hand, blades of *U. pinnatifida* presented only 12% more adsorption capacity than the holdfast.

DISCUSSION

Although the literature on the use of brown algae as a biosorbent is abundant (Luna *et al.* 2010; Montazer-Rahmatia *et al.* 2011; Oliveira *et al.* 2011), there is a lack of information about their physicochemical characteristics and their implication in biosorption capacity. *Macrocystis pyrifera* and *Undaria pinnatifida* analysed in this study showed higher organic matter percentage but a lower total carbohydrate content (26.4 mg of carbohydrate/100 mg of organic matter for *M. pyrifera* and 25.9 for *U. pinnatifida*) in comparison with other brown algae such as *Cytoseira trinodis* (Forsskål) C. Agardh, *C. myrica* (Gmelin) C. Agardh, *Sargassum dentifolium* (Turner) C. Agardh, *S. asperifolium* (Turner) C. Agardh ex Kützing and *S. latifolium* C. Agardh (Table 4).

Protein content reported for different brown algae varied from 4% to 9% (Barbarino & Lourenço 2005). Seasonal variability in protein content was reported, being higher in spring than in winter (Sagredo *et al.* 2007). The values found here are lower than those of the species mentioned in Table 4. This could be due to the fact that the material analysed in this work was collected from banks deposited on the shores.

Generally, alginic acid can represent between 10% and 40% of the brown algae dry weight (Volesky 2003), but the levels of alginate also depend on different environmental factors such as the season of the year, life cycle, collection site, etc. (Stengel *et al.* 2011). The alginate yield was determined by a procedure proposed in the present work that allowed obtaining an alginate salt with fewer colours and lower viscosity. The alginate yield percentage values determined here were within the reported range for brown algae (10% and 40%; Volesky 2003). These values could be compared with the alginate yields for different brown algae reported in the literature (Table 4). The alginate yield expressed in percentage obtained for *M. pyrifera* (from Patagonia) was lower than those found for *Sargassum* sp., *Chnoospora* sp., *Laminaria digitata* (Hudson) J.V.F. Lamouroux, *Spatoglossum* sp., *Zanaria* sp., *M. pyrifera* (from New Zealand) and *U. pinnatifida* (Table 4). On the other hand, *U. pinnatifida* had a higher alginate yield than *Spatoglossum* sp. and *M. pyrifera* (present work; Hernández-Carmona *et al.* 1999). However, *U. pinnatifida* had

Table 3. Wave number (cm^{-1}) of the dominant peaks in the FT-IR spectra of *Macrocystis pyrifera* and *Undaria pinnatifida* materials.

Functional groups	*M. pyrifera* wave number (cm^{-1})	*U. pinnatifida* wave number (cm^{-1})	References
C-O amino stretching	1478.40	1478.53	Pavasant *et al.* 2006
C-H amine stretching	-	1316.43-	Pavasant *et al.* 2006
C–N stretching vibration of amine groups, P–O–C links of the organic phosphated groups and P–O vibration of the ($C–PO_3^{-2}$) moiety	-	1185.16	Pan *et al.* 2006
C-O-C and O-H Vibration of polysaccharides	1179.83	-	Pan *et al.* 2006
$–SO_3$ asymmetric bands	1339.86	-	Murphy *et al.* 2008
	-	883.03	Ahmady-Asbchin *et al.* 2008
S=O stretching	825.29	827.9	
S-O stretching	768.71	766.02	Ahmady-Asbchin *et al.* 2008

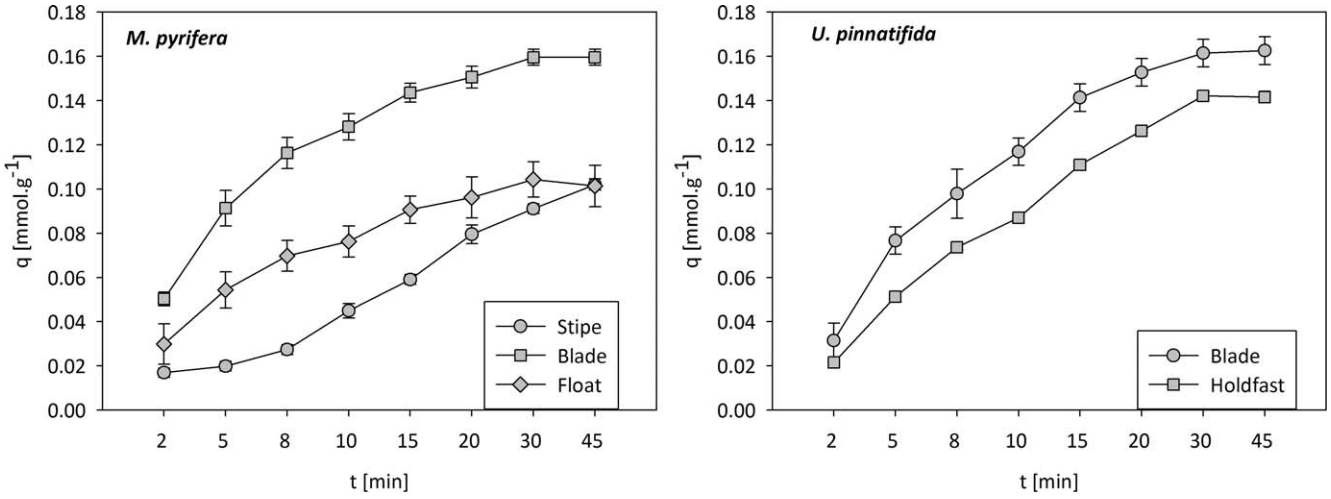

Fig. 6. Zn(II) adsorption kinetic employing separately blades, stipes and floats of *Macrocystis pyrifera* (left) and blades and holdfasts of *Undaria pinnatifida* (right).

almost the same alginate percentage as the following algae species: *Sargassum* sp. and *Zanaria* sp., but lower than *Laminaria digitata, Chnoospora* sp. and *M. pyrifera* (from New Zealand) (Table 4).

The potentiometric titration allows qualitative and quantitative determination of the nature and number of active sites present at the algal cell wall. The results of the calcium-treated algal potentiometric tritation reveals that the acid weak groups (carboxylate groups from alginate) were the main component of the cell wall of *M. pyrifera* and *U. pinnatifida*. Accordingly, Oliveira *et al.* (2011) reported than more than 90% of the total acid groups were represented by carboxylate groups in *Sargassum* sp. protonated treated algae. Also carboxylic groups are the main chemical groups present in *Pelvetia canaliculata* (Linnaeus) Decaisne & Thuret (protonated form) (Vilar *et al.* 2012). The strong groups determined were 0.02 and 0.069 mmol g^{-1} on calcium-treated *M. pyrifera* and *U. pinnatifida*, respectively. These groups have been identified as sulphonate groups of fucoidan that represent 5% and 20% of the dry weight of brown algae (Chapman 1980). These groups were also detected in *Sargassum* sp. in a concentration of 0.15 mmol g^{-1} (Oliveira *et al.* 2011). Considering the amount of calcium released during titration, less than one half of the total acid groups would be involved in metal binding. A similar relation was found for *Sargassum* sp. (Oliveira *et al.* 2011).

The chemical heterogeneity of the cell wall, related to the presence of different functional groups, can be characterised

Table 4. Biochemical characterization of selected brown algae.

	Organic matter %	Total carbohydrate (mg/100 mg organic matter)	Protein %	Alginate %	Reference
Cytoseira trinoide	35.50	74.93			Larsen *et al.* 2003
C. myrica	17.63	72.39			Larsen *et al.* 2003
Sargassum dentifolium	26.34	57.87			Larsen *et al.* 2003
S. asperifolium	42.69	32.16			Larsen *et al.* 2003
S. latifolium	44.57	42.26			Larsen *et al.* 2003
S. vulgare			6.9		Barbarino & Lourenço, 2005
Sargassum sp.				30.5	Adriamanantoanina & Rinaudo, 2010
Chnoospora sp.				50.8	Adriamanantoanina & Rinaudo, 2010
Chnoospora minima			7.8		Barbarino & Lourenço, 2005
Padina gymnospora			8.7		Barbarino & Lourenço, 2005
Dictyota menstrualis			4.0		Barbarino & Lourenço, 2005
Durvillea antartica			5.81 (spring) 8.97 (winter)		Sagredo *et al.* 2007
Laminaria digitata				38.7	Vauchel *et al.* 2009
Spatoglossum sp.				17.4	Adriamanantoanina & Rinaudo, 2010
Zanaria sp.				30	Adriamanantoanina & Rinaudo, 2010
Macrocystis pyrifera				19.4 (70°C) 21.9 (90°C)	Hernández-Carmona *et al.* 1999
M. pyrifera				38 (New Zealand)	Panikkar & Brasch, 1996
M. pyrifera	84.4	26.4	0.21	10	This study
Undaria pinnatifida	88.7	25.9	1.31	30	This study

by FT-IR and ESEM-EDX analysis. The FT-IR methodology employed in the present work to make the pellet with KBr in agate mortar destroyed the cell and released the cytoplasmatic content; however, it was possible to determine by FT-IR some functional groups that form part of the main chemical components of the algal cell wall (Graham & Wilcox 2000). The FT-IR spectra confirm the presence of polysaccharides and amino and sulphonate groups in both biomaterials; however, phosphate groups were detected only in *U. pinnatifida*. ESEM-EDX revels the presence of different light metals (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) adsorbed onto the cell wall.

These light metals, bound to sulphonate groups of fucans or carboxylate from alginic acid can act as cation exchangers (where light cations are substituted by metal) or block the active sites (Romera *et al*. 2006, Plaza Cazón *et al*. 2012). The CEC for calcium-treated *M. pyrifera* and *U. pinnatifida* was lower than the CEC determined for anaerobic granules of microbial aggregates (111 meq/100 g in Hawari & Mulligan 2006).

The adsorption capacity of each component (holdfast, floats, stipes and blades) of *M. pyrifera* and *U. pinnatifida* thalli was different. This could be attributed to the natural variability of biochemical composition of each thallus part (Stengel *et al*. 2011). The chemical and physical characteristics of the biological materials are controlled by several external factors such as location, growth conditions and seasonality even within species (Stengel *et al*. 2011). The coexistence of different algal groups or even species within a group in a common habitat does not necessarily imply common metabolic pathways, biochemical composition or life strategies. It is generally reasonable to assume that the biochemical composition of closely related algal species are more similar to each other than they are to unrelated species, but the biochemical composition even within a single species or indeed individual organism can be highly variable. The seaweeds exhibit enormous plasticity, with regard to chemical composition in response to single or combinations of multiple environmental factors (Stengel *et al*. 2011); all of that must be taken into account when natural algal populations or banks of algal depositions (our case) are selected to remove metal from wastewater or to develop high-quality commercial products from sustainable (natural or cultivated) algal resources.

Undaria pinnatifida had higher percentage of organic matter, alginate and protein as well as a larger specific surface area when compared with *M. pyrifera*, although the latter had a higher ionic exchange capacity. However the proportion of acid weak groups (carboxylic groups) present on the cell walls was very similar for both algae. The FT-IR spectra indicated the presence of phosphate groups only on *U. pinnatifida* cell wall. EDX spectra revealed the presence of light metals on the raw algae suggesting that ion exchange could be the main mechanism in biosorption process. It was proved that each thallus part had a different Zn(II) adsorption capacity. Blades exhibited the highest adsorption capacity. On the basis of the difference on biochemical characteristics and adsorption capacity between the two algal species, *U. pinnatifida* should be a better biosorbent than *M. pyrifera*, but this affirmation must be proved through specific biosorption assays.

ACKNOWLEDGEMENTS

We thank to Dr Cecilia Eyras (CENPAT, Argentina) for providing the analysed biomaterial.

REFERENCES

Ahmady-Asbchin S., Andrès Y., Gèrente C. & Le Cloirec P. 2008. Biosorption of Cu(II) from aqueous solution by *Fucus serratus*: surface characterization and sorption mechanisms. *Bioresource Technology* 99: 6150–6155.

Andrade L.R., Salgado L.T., Farina M., Pereira M.S., Mouraño P.A.S. & Amado Filho G. M. 2004. Ultrastructure of acidic polysaccharides from the cell walls of brown algae. *Journal of Structural Biology* 145: 216–225.

Andriamanantoanina A. & Rinaudo M. 2010. Characterization of the alginates from five Madagascan brown algae. *Carbohydrate Polymers* 82: 555–560.

Areco M.M. & dos Santos Afonso M. 2010. Copper, zinc, cadmium and lead biosorption by *Gymnogongrus torulosus*. Thermodynamics and kinetics studies. *Colloids and Surfaces B: Biointerfaces* 81:620–628.

Arvizu-Higuera D.L., Hernández Carmona G. & Rodríguez Montensinos E. 1995. Sistemas de Carga y de Flujo Continuo durante la etapa de preextracción ácida en el proceso de extracción de alginatos. *Ciencias Marinas* 21: 25–37.

Barbarino E. & Lourenço S.O. 2005. An evaluation of methods for extraction and quantification of protein from marine macro and micro algae. *Journal of Applied Phycology* 17: 447–460.

Boraso A. & Zaixo J.M. 2012. Algas marinas bentónicas. In *Atlas de Sensibilidad Ambiental de la Costa y el Mar Argentino* (Ed. by D. Boltovskoy). http://seaweedafrica.org/pdf/562DF4FA1149b1C475TSK191032E/48288.pdf; searched on 18 October 2012.

Bulgariu D. & Bulgariu L. 2012. Equilibrium and kinetics studies of heavy metal ions biosorption on green algae waste biomass. *Bioresource Technology* 103: 489–493.

Chapman V.J. 1980. *Seaweeds and their uses*. Chapman and Hall, London, UK. 287 pp.

Davis T.A., Volesky B. & Mucci A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research* 37: 4311–4330.

De Aragao B.J.G. & Messaddeq Y. 2008. Peak separation by derivative spectroscopy applied to FT-IR analysis of hydrolyzed silica. *Journal of the Brazilian Chemical Society* 19: 1582–1594.

Dellatorre F.G., Amoroso R. & Barón P.J. 2012. *El alga exotica Undaria pinnatifida en Argentina: biología, distribución y potenciales impactos*. Editorial Académica Española. 60 pp.

Eyras M.C., Rostagno C.M. & Defossé G.E. 1998. Biological evaluation of seaweed composting. *Compost Science & Utilization* 6(4): 74–81.

Ghasemi M., Keshtkar A.R., Dabbagh R. & Safdari S.J. 2011. Biosorption of uranium (VI) from aqueous solutions by Ca-pretreated *Cystoseira indica* alga: breakthrough curves studies and modeling. *Journal of Hazardous Materials* 189: 141–149.

Graham L.E. & Wilcox L.W. 2000. *Algae*. Prentice Hall International (UK) Limited, London. 603 pp.

Hawari A.H. & Mulligan C.N. 2006. Biosorption of lead(II), cadmium(II), copper(II) and nickel(II) by anaerobic granular biomass. *Bioresource Technology* 97: 692–700.

Hernández-Carmona G., McHugh D.J. & López-Gutiérrez F. 1999. Pilot plant scale extraction of alginates from *Macrocystis pyrifera* 2. Studies on extraction conditions and methods of separating the alkaline-insoluble residue. *Journal of Applied Phycology* 11: 493–502.

Ibrahim W.M. 2011. Biosorption of heavy metal ions from aqueous solution by red macroalgae. *Journal of Hazardous Materials* 192: 1827–1835.

Larsen B., Salem D.M.S.A., Sallam M.A.E., Mishrikey M.M. & Beltagy A.I. 2003. Characterization of alginates from algae harvested at the Egyptian Red Sea Coast. *Carbohydrate Research* 338: 2325–2336.

Luna A.S., Costa A.L.H., da Costa A.C.A. & Henriques C.A. 2010. Competitive biosorption of cadmium (II) and zinc(II) ions from binary systems by *Sargassum filipendula*. *Bioresource Technology* 101: 5104–5111.

Montazer-Rahmati M.M., Rabbania R., Abdolalia A. & Keshtkarb A.R. 2011. Kinetics and equilibrium studies on biosorption of cadmium, lead, and nickel ions from aqueous solutions by intact and chemically modified brown algae. *Journal of Hazardous Materials* 185: 401–407.

Murphy V., Hughes H. & McLoughlin P. 2008. Comparative study of chromium biosorption by green and brown seaweed biomass. *Chemosphere* 70: 1128–1134.

Norton L., Baskaran K. & McKenzie T. 2004. Biosorption of zinc from aqueous solutions using biosolids. *Advances in Environmental Research* 8: 629–35.

Oliveira R.C., Jouannin C., Guibal E. & Garcia O. Jr. 2011. Samarium(III) and praseodymium(III) biosorption on *Sargassum* sp.: batch study. *Process Biochemistry* 46: 736–744.

Orhan Y., Hrenovic J. & Büyükgüngör H. 2006. Biosorption of heavy metals from wastewater by biosolids. *Engineering in Life Sciences* 6: 399–402.

Pan J., Ge X., Liu R. & Tang H. 2006. Characteristic features of *Bacillus cereus* cell surfaces with biosorption of Pb(II) ions by AFM and FT-IR. *Colloids and Surfaces B: Biointerfaces* 52: 89–95.

Panikkar R. & Brasch D.J. 1996. Composition and block structure of alginates from New Zealand brown seaweeds. *Carbohydrate Research* 293: 119–132.

Pavasant P., Apiratikul R., Sungkhum S., Suthiparinyanont P., Wattanachira S., & Marhaba T.F. 2006. Biosorption of Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} using dried marine green macroalga *Caulerpa lentillifera*. *Bioresource Technology* 97: 2321–2329.

Plaza Cazón J., Benitez L., Donati E. & Viera M. 2012. Biosorption of chromium (III) by two brown algae *Macrocystis pyrifera* and *Undaria pinnatifida*: equilibrium and kinetic study. *Engineering in Life Sciences* 12: 95–103.

Romera E., González F., Ballester A., Blázquez M.L. & Muñoz J.A. 2006. Biosorption with algae: a statistical review. *Critical Reviews in Biotechnology* 26: 223–235.

Sagredo M.C., Hernández H.S., Herrera C.C. & Franco C.O. 2007. Proteínas totales en tres especies de algas marinas de Lebu, VIII Región Chile. Facultad de Ciencias, Universidad Católica de la Santísima Concepación. *Ciencia Ahora* 20: 46–51.

Stengel D.B., Connan S. & Popper Z.A. 2011. Research review paper. Algal chemodiversity and bioactivity: source of natural variability and implications for commercial application. *Biotechnology Advances* 29: 483–501.

Svecova L., Spanelova M., Kubal M. & Guibal E. 2006. Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies. *Separation and Purification Technology* 52: 142–153.

Vauchel P., Leroux K., Kass R., Arhaliass A., Baron R. & Legrand J. 2009. Kinetic modelling of alginate alkaline extraction from *Laminaria digitata*. *Bioresource Technology* 100: 1291–1296.

Vilar V.J.P., Valle J.A.B., Bhatnagar A., Santos J.C., Guelli U. de Souza S.M.A., U. de Souza A.A., Botelho C.M.S. & Boaventura R.A.R. 2012. Insights into trivalent chromium biosorption onto protonated brown algae *Pelvetia canaliculata*: distribution of chromium ionic species on the binding sites. *Chemical Engineering Journal* 200–202: 140–148.

Volesky B. 1990. *Biosorption of heavy metals*. CRC Press, Boca Raton, FL. 408 pp.

Volesky B. 2003. *Sorption and biosorption*. B. V. Sorbex, Inc. Montreal- St. Lambert, Quebec, Canada. 320 pp.

Received 1 November 2012; accepted 7 November 2013
Associate Editor: John Beardall