Mechanisms of Cu$^{2+}$ biosorption on *Lessonia nigrescens* dead biomass: Functional groups interactions and morphological characterization

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**ABSTRACT**

In this study, the biosorption mechanisms of Cu$^{2+}$ by dead biomass of the brown seaweed *Lessonia nigrescens* were characterized, with special emphasis on the chemical environment provided by functional groups, their interactions with the metal, and the induced morphological changes triggered by the biosorption process. The morphological changes on the seaweed surface were characterized by SEM and AFM techniques, before and after the exposure of the biomass to Cu$^{2+}$ ions. ATR-FTIR, EDS and XPS were used to characterize the chemical atomic environment on the seaweed biomass, to determine the biosorption saturation times of the founded functional groups, and the changes on the biomass surface composition, and main chemical bondings. The use of these techniques revealed that this seaweed biomass interacts with Cu$^{2+}$ ions mainly through carboxyl, sulfonate and hydroxyl groups, by ionic and coordinative bonds by ligand multidentism, and rearrangements of the cell wall stiffness.

1. Introduction

Biosorption is a sorption process that occurs on biological materials, which enables the sequestration of different molecules and ions in solution, being particularly interesting in relation to metal pollutants. Laboratory studies have demonstrated that biosorption is a promising and cost-effective technology for the removal of heavy metals from aqueous solutions [1,2]. Among various biosorbents reported in the literature, marine algal biomass has been identified as a promising one, due to its high metal uptake capacities, low cost and renewability, as well as the ready abundance of biomass sources [3,4]. The cell wall of brown seaweeds, contains three major components: cellulose as structural support, alginate (mannuronic and guluronic acids) complexed with light metals such as Na⁺, K⁺, Mg$^{2+}$ and Ca$^{2+}$; and other sulphated polysaccharides (sulphonates) [3,5]. Alginates and sulphonates have been reported as the predominant reactive molecular components in brown seaweed [6]. The seaweed biomass possesses several chemical groups that can attract and sequester metals: acetamide, amino, amide, sulphhydryl, sulfonate and carboxyl [3,7]. This chemical diversity originates a combination of mechanisms to capture the metals including electrostatic attraction, complexation, ion exchange, covalent binding, Van der Waals attraction, adsorption and microprecipitation [8]. The scarce information about metal biosorption mechanisms of brown seaweed biomass reduces the chances of using this material as a competitive product when compared to traditional well-known metal-removal processes [9]. Understanding how and which chemical active sites present in the biomass sequester metals, can focus the development of chemically modified biosorbents with an improved metal sorption performance [10,11]. Several analytical techniques have been used to study the kinetics of the metal biosorption process, including atomic absorption spectrophotometry (AAS), ion selective electrodes (ISE), UV–vis spectrophotometry and inductively coupled plasma (ICP) [12,13]. However, to give insights into the mechanisms of biosorption, complementary techniques must be used. For instance, potentiometric titration has been used to quantify the amounts of acidic groups and their fractions [14,15] and infrared spectroscopy or Fourier-transform infrared spectroscopy (IR or FTIR) allows the identification of the functional groups present on the biomass [16,17]. Scanning or transmission electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS/TEM-EDS) is used to observe morphological and surface elemental composition changes [18,19]. X-ray photoelectron spectroscopy (XPS) is a useful technique to understand the atomic...
environment, revealing the interactions between the metal and the functional groups present in the biosorbent [12,20,21].

Lessonia nigrescens Bory (Phaeophyceae, Laminariales) is a brown seaweed highly distributed in the South Pacific Chilean coast [22], and represents a source of potential biomaterial for biosorption processes [23]. The goal of this study is to understand the mechanisms underlying the biosorption of Cu^{2+} by dead biomass of the brown seaweed L. nigrescens, using different and complementary techniques. We studied how key functional groups present in the biomaterial interact with the metal in aqueous solution, and the morphological changes and surface reorganization caused by the interaction. This work contributes to the knowledge of the mechanisms underlying the interactions between a complex matrix such as the seaweed biomass and Cu^{2+}, to prospect the potential use of the biomass, for the abatement of the metal from contaminated waters.

2. Materials and methods

2.1. Biomass preparation

Lessonia nigrescens brown seaweed fresh material was collected from the Chilean coast (33°23’S 71°41’O, El Tabo, Fifth Region, Chile). The samples were washed with distilled water to remove salt and sand, and then were dried at 60 °C in an oven (Memmert, Germany) for 72 h. The dry material was chopped into small pieces before grinding with a hammer mill. The dry and ground biomass was sieved to 500–1000 μm particle size. For FTIR and XPS analysis, a finely powder of seaweed biomass was prepared using mortar and pestle, with the addition of liquid nitrogen. For IR and SEM-EDX analysis, the biomass was acid washed [19] using 5 g of ground and dried biomass treated with 100 mL of HCl 0.1 M for 6 h, and then washed 20 times with 100 mL of milli-Q water. Then, the acid treated biomass was dried for 48 h at 60 °C in an oven.

2.2. Cu^{2+} biosorption capacity, optimum pH and equilibrium time

To investigate the effect of pH on the Cu^{2+} biosorption capacity of the biomass, solutions containing 50 mg L^{-1} Cu^{2+} at different initial pH values (2, 3, 4, 5 and 5.5) were prepared using CuCl_{2}-2H_{2}O. The pH was adjusted with 0.1 M NaOH or 0.1 M HCl. For each batch experiment, 50 mg of previously ground and dried biomass were mixed with 50 mL of a Cu^{2+} solution at different pH in 250 mL flasks. The flasks were agitated in an orbital shaker (Cole Parmer, USA) for 4 h at 160 rpm at room temperature (20 ± 1 °C). Then 5 mL of each sample were filtered using a PVDF membrane of 0.45 μm. The filtrate was poured into a propylene tube and 10 mL of an acid solution (7.5% hydrochloric acid and 6% nitric acid) were added to the filtrate. The acid treated samples were analysed by Atomic Absorption Spectrometry (FA-AAS) (Thermo Solar 5, Thermo Scientific, USA). The metal uptake equilibrium q_{e} (mg g^{-1}) was calculated according to Eq. (1):

\[ q_{e} = \frac{V(C_{i} - C_{e})}{ms} \]  

were V is the volume of the copper solution (mL), C_{i} and C_{e} are the initial and equilibrium concentration of copper in solution (mg L^{-1}) and ms is the biomass (g).

To determine the biosorption equilibrium time, at the pH with the maximum performance observed, the same batch procedure described before was used, using a 50 mg L^{-1} Cu^{2+} solution adjusted to the corresponding pH. Samples of 5 mL were taken in triplicate, at 5, 10, 30, 60, 120 and 240 min and were analysed by Atomic Absorption Spectrometry (FA-AAS). To determine the maximum Cu^{2+} biosorption capacity for the biomass, batch experiments were carried out varying the initial concentrations of Cu^{2+}, in a range of 7.5–300 mg L^{-1}. The data were adjusted to the isotherm models of Langmuir and Freundlich [24].

2.3. Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FITR)

To identify functional groups present on the biomass, and their interaction with Cu^{2+}, an ATR-FITR analysis was performed. By this study, the apparent order of saturation of the identified functional groups was determined. The experiments were performed using acid washed biomass, which allowed a better resolution on the identification and analysis of the bands shift [19]. A band shifting in wave number higher than 10 cm^{-1} (both left or right shifts) was associated to a physicochemical interaction between the functional group and the loaded metal [19]. 50 mg of acid-washed biomass were dried for 48 h at 60 °C and then, pellets of KBr at a 5% of biomass were prepared. The pellets were examined with an ATR-FITR (Nicolet Impact 410, Thermo Electronic Corporation, USA) by triplicate. The measurements were taken in a range of 400–4000 cm^{-1} at 32 scans [19]. For the time course analysis, 1 g of acid washed biomass was treated with 100 mL of a solution of 100 mg L^{-1} Cu^{2+} at pH 5 in an orbital shaker at 200 rpm and 20 °C. During the treatment, approximately 50 mg of the biomass were taken at different times (5, 10, 30, 60, 120 y 240 min). The sample preparation for IR analysis was the same used for the functional groups identification, and the band signal assignment was done comparing with the reports in literature.

2.4. Scanning electron microscopy coupled with energy dispersive X-ray spectrometry (SEM-EDS)

This technique was used to examine the morphology and changes on atomic composition on the surface of the biomass (raw biomass), the acid washed biomass, and the acid washed biomass treated with 100 mg L^{-1} Cu^{2+} during 4 h at pH 5, 200 rpm and 20 °C. The dried samples were mounted on a stub and were gold sputtered by 300 s using a Denton Vacuum IV sputter coater (Denton Vacuum Inc, New Jersey, USA). The samples were analysed using a Scanning Electron Microscope SEM (JEOL JSM-6490LV, Jeol Ltd, Tokio, Japan) coupled to a module of Energy Dispersive X-ray Spectrometry (EDS) (INCA Penta FE-Tx3, Oxford Instruments, Abingdon, Oxfordshire, UK). The settings for the equipment were: backscattered electrons mode (BSE), magnification of 5000–20000×, electron beam voltage of 20 kV, work distance of 10.0–10.4 mm, spot size of 4.0–4.8 nm, pressure of 900 mTorr, and temperature of 20 °C. An X-ray spectrum with a microelemental surface composition analysis was obtained for each biomass sample (raw, acid washed and acid washed treated with Cu^{2+}).

2.5. X-ray photoelectron spectrometry (XPS) analysis

A fraction of approximately 1 g of finely ground raw biomass was treated with a solution of 1000 mg L^{-1} of Cu^{2+} at pH 5 for 24 h, 200 rpm of agitation and 20 °C to reach the maximum saturation of the biomass with Cu^{2+}. Then, the treated biomass was dried for 48 h at 60 °C. The control raw biomass and the treated sample were maintained on a vacuum pre-chamber of an XPS spectrometer until a pressure of 10^{-5} Pa was reached in the chamber. Once the system reached the vacuum, the samples were analysed on an XPS-Auger PerkinElmer spectrometer model PHI 1257 which includes an ultra-high vacuum chamber, a hemispheric electron energy analyser and an X-ray source, with X-ray radiation unfiltered from an Al (hv = 1486.6 eV) anode. Changes on the surface composition and differences on chemical bonding’s over atoms on functional groups were tested by fine structure spectra, before and after the treatments with Cu^{2+}.

2.6. Atomic force microscopy (AFM)

Approximately 1 g of raw biomass was treated with a solution at 50 mg L^{-1} of Cu^{2+} at pH 5 for 4 h, 200 rpm and 20 °C. The samples were washed several times with Milli-Q distilled water to remove the
excess of Cu\(^{2+}\) and debris, before they were dried for 48 h at 50 °C. To eliminate any debris on the seaweed surface after the dryness process, the samples were blown with gas nitrogen (99.99% purity) and then stored in sealed airtight containers until required. The seaweed samples were attached to magnetic-sample holders using a thin layer of non-conductive glue, covering an area of approximately 3 × 3 cm\(^2\). Special care was taken to ensure that samples were completely flat during the measurement performed with the AFM equipment (MFP3D-Bio by Asylum Research, Santa Barbara, USA). The probe was a silicon cantilever coated with platinum with a tip radius of 15 nm with a spring constant of 2 N/m and its nominal resonant frequency was 70 kHz (Olympus AC240TM). The measurements were performed on tapping mode to obtain a height and phase retrace profiles with a resolution of 256 × 256 pxl. The software WSxM 5.0 Develop 8.1 was used for the analysis of the images [25].

3. Results and discussion

3.1. Cu\(^{2+}\) biosorption kinetics

The pH is a physicochemical parameter which influences drastically on the biosorption performance. The pH range tested was chosen considering that Cu\(^{2+}\) ions are free species at pH values below 6 [26]. The highest biosorption of Cu\(^{2+}\) by L. nigrescens biomass was achieved at pH 5 (Fig. 1a). The pseudo-second order kinetic model [27] showed that the saturation time of the biomass for Cu\(^{2+}\) was nearly 120 min, without significant variations up to 240 min (Fig. 1B).

To determine the maximum capacity of Cu\(^{2+}\) biosorption by the raw seaweed biomass, Langmuir and Freundlich isotherm models were used [3]. The results showed that Langmuir isotherm modelling had a better adjustment than the Freundlich isotherm model (Table 1), obtaining a maximum capacity of Cu\(^{2+}\) biosorption of 60.4 mg g\(^{-1}\) at pH 5.

The Cu\(^{2+}\) biosorption capacity of L. nigrescens biomass determined in this work was similar to the biosorption of the metal observed with Sargassum sp. and Ascophyllum nodosum biomass (Table 2). Hansen et al. [23] found a Cu\(^{2+}\) biosorption capacity of 54.5 and 58.5 mg g\(^{-1}\), over their blades and their stipes working at pH 3.2, similar to the values obtained in this study at pH 5.0. The biosorption of Cu\(^{2+}\) by L. nigrescens could be improved with chemical modifications on the algal biomass as it has been informed for other biosorbents [28,29].

3.2. Functional groups present in L. nigrescens biomass

The Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) analysis was performed on acid-washed seaweed biomass. The acid treatment eliminates organic components present in the biomaterial such as proteins, lipids, high molecular weight carbohydrate polymers (cellulose, alginic acid and fucoids mainly) and inorganic ions (Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\), Na\(^+\)) [3]. The first distinguishable frequency found on L. nigrescens biomass corresponds to a broad band centred at 3423 cm\(^{-1}\) that is attributed to stretching modes of amino (–NH\(_2\)) and alcohol groups (–OH) [19] (Table 3 and Fig. 2).

According to Svecova et al. [32], in biomass of brown algae, the presence of amine groups is usually confirmed by a shoulder around 3265 cm\(^{-1}\), that is frequently hidden by vibrations of –OH groups. For L. nigrescens biomass, this vibration was found at 3260 cm\(^{-1}\). The vibration at 1315 cm\(^{-1}\), is attributed to amine groups (–CN stretching) on the biomass [18]. The signal at 2923 cm\(^{-1}\) is attributable to C–H asymmetric stretching modes [32]. The asymmetric stretching of carboxylate CO\(_2^-\) and C=O bonds vibration is found at 1625 cm\(^{-1}\). The band at 1384 cm\(^{-1}\) is representative of O–C–O symmetric stretching vibration of the carboxylate group [3,19,34]. The band at 1035 cm\(^{-1}\) can be assigned to the C–O stretching of alcohols groups [7]. The band at 1532 cm\(^{-1}\) can be assigned to amine groups (NH stretching) that usually appear at 1530–1560 cm\(^{-1}\) [20]. The vibration frequency at 1243 cm\(^{-1}\) can be associated to the stretching of sulfonate (–SO\(_3^-\)) that is a functional group present in high sulfonated polysaccharides, such as fucoids [6], that is highly abundant in brown algae cell walls. The presence of sulfonate functional groups was confirmed by the vibrations at 821 and 776 cm\(^{-1}\), that correspond to the stretching of S=O and of S=O, respectively [19]. The results of the FTIR analysis confirm the presence of the functional groups carboxyl, hydroxyl, sulfonate, amine and amide in the algae biomass. These functional groups are mostly present in polymeric compounds such as alginates, cellulose and high sulphated polymers [3].

3.3. Cu\(^{2+}\) saturation time course of functional groups present in L. nigrescens biomass

Using the previously identified band values in the acid-washed biomass, it was possible to observe the bands shifting on the metal loaded biomass during the time course analysis (Table 3 and Fig. 2). After 5 min of Cu\(^{2+}\) exposition, the stretching band of –NH and –OH shifted from 3423 to 3434 cm\(^{-1}\), with a constant increase up to 120 min (3446 cm\(^{-1}\)). The –NH stretching band (1532 cm\(^{-1}\)) did not suffer a considerable shift during the time of the course of the experiment showing that –NH frequencies did not change. Thus, because the amino group associated frequencies did not change, it can be concluded that these functional groups are not saturated with Cu\(^{2+}\) cations, and a minimal interaction occurs between them and the metal. Amide and amine groups are difficult to differentiate by this technique because...
they share common signals; however, the XPS studies give information with respect to both functional groups separately (see XPS section later). The frequency of asymmetrical stretching \( \text{O}==\text{C}==\text{O} \) (1384 cm\(^{-1}\)), presented a shift at 5 min of the biomass Cu\(^{2+}\) treatment (1398 cm\(^{-1}\)), and a constant increase up to 240 min (1416 cm\(^{-1}\)). The other frequency related to carboxyl group behaviour, the symmetrical stretching \( \text{O}==\text{C}==\text{O} \) (1625 cm\(^{-1}\)), was practically constant until the end of the experiment. The shifts on these frequencies are mainly the responsibility of the two oxygen atoms of the COO- moieties. The oxygen can take part on monodentate or bidentate bindings, interacting with cations as has been previously shown on Sargassum family seaweeds alginates [9]. These results suggest that the carbonyl oxygen in the \( \text{COO}^- \) plays a major role during the first 10 min of contact between the biomass and the metal, when most of the Cu\(^{2+}\) ions are sequestered by the biomass, as shown in the kinetic studies (Table 3). The frequency associated to \( \text{SO}_3^- \) stretching changes at 5 min from 1243 to 1224 cm\(^{-1}\). The shift at the frequency of \( \text{SO}_3^- \) (1243 cm\(^{-1}\)) may be the result of the sulfonate groups starting to be saturated [19]. It is observed that on \( \text{L. nigrescens} \) biomass, the amine or amide groups do not play an important role on the biosorption of copper and that the saturation of functional groups is sequential, with carboxyl moieties saturating first, then sulfonate groups and, finally, hydroxyl groups.

### Table 1
Parameters obtained from the biosorption pseudo-second order kinetic model and biosorption isotherm models evaluated.

<table>
<thead>
<tr>
<th>Parameters obtained from the biosorption pseudo-second order kinetic model</th>
<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( q_e ) (mg g(^{-1}))</td>
<td>( k_f ) (g mg(^{-1}) min(^{-1}))</td>
<td>( r^2 )</td>
</tr>
<tr>
<td>28.03</td>
<td>2.98 \times 10^{14}</td>
<td>0.987</td>
</tr>
</tbody>
</table>

### Table 2
Copper biosorption capacities (\( q_m \)) on brown algae biomass.

<table>
<thead>
<tr>
<th>Brown algae biomass</th>
<th>( pH )</th>
<th>( q_m ) (mg g(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascophyllum nodosum</td>
<td>4.0</td>
<td>57.8</td>
<td>[5]</td>
</tr>
<tr>
<td>Durvillaea antarctica</td>
<td>5.0</td>
<td>91.5</td>
<td>[30]</td>
</tr>
<tr>
<td>Fucus serratus</td>
<td>5.5</td>
<td>101.7</td>
<td>[14]</td>
</tr>
<tr>
<td>Fucus spiralis</td>
<td>4.0</td>
<td>69.9</td>
<td>[5]</td>
</tr>
<tr>
<td>Fucus vesiculosus</td>
<td>5.0</td>
<td>105.5</td>
<td>[31]</td>
</tr>
<tr>
<td>( \text{L. nigrescens} ) (blades/stipes)</td>
<td>3.2</td>
<td>54.5/58.5</td>
<td>[23]</td>
</tr>
<tr>
<td>( \text{L. nigrescens} ) (beads)</td>
<td>5.0</td>
<td>58.0</td>
<td>[2]</td>
</tr>
<tr>
<td>Sargassum sp. (immobilized in alginate Cu(^{2+}) beads)</td>
<td>4.5</td>
<td>83.9</td>
<td>[19]</td>
</tr>
</tbody>
</table>

### Table 3
Time course FTIR analysis of \( \text{L. nigrescens} \) biomass Cu\(^{2+}\) biosorption.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Assignment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (untreated)</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>5</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>10</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>30</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>60</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>120</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>240</td>
<td>3434</td>
<td>Bonded –OH, –NH stretching</td>
</tr>
<tr>
<td>320</td>
<td>3256</td>
<td>Peak shoulder, confirm the presence of –NH</td>
</tr>
<tr>
<td>3293</td>
<td>2954</td>
<td>C–H asymmetrical stretching</td>
</tr>
<tr>
<td>1625</td>
<td>1625</td>
<td>Symmetrical stretching ( \text{O}==\text{C}==\text{O} )</td>
</tr>
<tr>
<td>1532</td>
<td>1537</td>
<td>Bending –OH (COOH), asymmetrical stretching of ( \text{O}==\text{C}==\text{O} )</td>
</tr>
<tr>
<td>1384</td>
<td>1354</td>
<td>–CN stretching</td>
</tr>
<tr>
<td>1315</td>
<td>1355</td>
<td>Stretching –SO3</td>
</tr>
<tr>
<td>1243</td>
<td>1225</td>
<td>Stretching C–O (alcohol)</td>
</tr>
<tr>
<td>1035</td>
<td>873</td>
<td>Stretching C–N</td>
</tr>
<tr>
<td>875</td>
<td>873</td>
<td>Stretching C–O (alcohol)</td>
</tr>
<tr>
<td>821</td>
<td>810</td>
<td>Stretching C–O (sulfonate)</td>
</tr>
<tr>
<td>776</td>
<td>777</td>
<td>Stretching S=O (sulfonate)</td>
</tr>
</tbody>
</table>

Fig. 2. Infrared spectra of acid washed \( \text{L. nigrescens} \) biomass after 5, 10, 30, 60, 120 and 240 min of exposure to 100 mg L\(^{-1}\) Cu\(^{2+}\) at pH 5.

3.4. Effect of Cu\(^{2+}\) biosorption on the surface of \( \text{L. nigrescens} \) biomass

The textural properties of the biomass surface were observed by Scanning Electron Microscopy (SEM) analysis. The changes on elemental composition of the surface of acid treated biomass, and after Cu\(^{2+}\) biosorption were observed by Energy-Dispersive X-Ray Spectroscopy (EDS). The images obtained for the seaweed biomass with no acid treatment showed an amorphous and slightly porous surface with some sites that present important porosity (Fig. 3A and B). This biomass shows many free particles of material without a clearly organized pattern, and presents many crystals distributed on the surface (Fig. 3B). The nature of these crystals will be discussed later. In comparison, the surface of the seaweed biomass after the acid treatment acquired a patterned appearance, showing open patterns of around 50 \( \mu \)m diameter surrounded by a sheet structure, which could confer more contact surface to the biomass (Fig. 3A, B, D and E). This pattern was repeated and homogeneous over the surface. Crystals and free particles were not observed in the acid treated biomass (Fig. 3D) probably because the acidic lixiviation removed small material and crystals, homogenizing the surface [35]. After the treatment with Cu\(^{2+}\) at pH 5, the acid washed biomass showed a change in the porous aspect observed without Cu\(^{2+}\) (Fig. 3G and H). Clearly, the presence and
interaction of Cu$^{2+}$ ions induce a change on the biomass surface generating a folding, which is observed as a partial closure of the porosities observed in the biomass without copper treatment.

The EDS analysis of the raw biomass (biomass with no acid treatment) showed a surface atomic abundance of C (73.06%), O (26.27%), Cl (0.29%), K$^+$ (0.21%), Na$^+$ (0.11%), Ca$^{2+}$ (0.03%) and Mg$^{2+}$ (0.03%) (Fig. 3C). The high presence of C and O is due to the biochemical nature of the biomass, with a high content of polysaccharides [3]. Also, the presence of the alkali and alkaline earth metals K$^+$, Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ was expected as common constituents of algae biomass [3]. Based on the observations of the elemental distribution maps (data not shown) the presence of K$^+$, Na$^+$, and Cl$^-$ could be associated to the crystals observed by SEM in the form of KCl and NaCl (Fig. 3B). The high presence of C and O is due to the biochemical nature of the biomass, with a high content of polysaccharides [3]. Also, the presence of the alkali and alkaline earth metals K$^+$, Na$^+$, Ca$^{2+}$ and Mg$^{2+}$ was expected as common constituents of algae biomass [3]. Based on the observations of the elemental distribution maps (data not shown) the presence of K$^+$, Na$^+$, and Cl$^-$ could be associated to the crystals observed by SEM in the form of KCl and NaCl (Fig. 3B). The EDS analysis of the acid washed biomass showed an important change on the surface composition compared with the raw seaweed biomass (Fig. 3H). The elemental abundances were: C (70.93%), O (29.03%), S (0.03%) and Cu$^{2+}$ (0.01%). The alkali and alkaline earth metals observed in the raw biomass were removed with the acid treatment, except for Ca$^{2+}$. The persistence of Ca$^{2+}$ after the acid treatment is explained by the coordination of Ca$^{2+}$ with oxygen atoms provided by carboxyl and hydroxyl groups from alginate fibres, which is a highly stable structure called the 'egg-box' model [3]. In the acid washed samples, the salt crystals and particles present on the surface of the raw biomass were removed and washed away due to the acidic environment and by the mechanical stress of the agitation process. Interestingly, sulphur was detected on the acid washed biomass and not in the raw biomass, indicating that more sulphur containing molecules, like sulphonate groups on fucoidans are exposed after the acid treated samples. Finally, the EDS analysis of the acid washed biomass treated with Cu$^{2+}$ showed elemental abundances of C (70.33%), O (29.63%), S (0.03%) and Cu$^{2+}$ (0.02%) (Fig. 3I). This elemental composition did not suffer important changes compared to the composition observed on acid washed biomass in the absence of Cu$^{2+}$, except for the absence of Ca$^{2+}$ and the detection of Cu$^{2+}$. This observation indicates that Cu$^{2+}$ biosorption by L. nigrescens dead biomass involves the substitution of sites previously occupied by Cu$^{2+}$. To confirm this observation, an XPS analysis was performed.

3.5. X-ray photoelectron spectrometry (XPS) analysis

The atomic concentrations obtained from the spectra analysis of the raw seaweed biomass surface before and after the Cu$^{2+}$ biosorption process is shown in Table 4. After the biosorption of Cu by the raw biomass, there was a 0.4% increase for carbon; 11.9% increase for oxygen (28.07%–31.42%), and a 100% increase of sulphur (from >0.01% to 0.66%). All these elements are present in functional groups of the raw seaweed. Only Nitrogen presents a decrease of 35.9% (2.2%–1.41%) due to the Cu$^{2+}$ biosorption process. The significant increase in sulphur measured by the XPS technique indicates that the biosorption of Cu$^{2+}$ induces changes on the distribution of the molecules present on the biomass surface, causing the exposure of internal layers of the biomass. It is well known that an acid pre-treatment eliminates the leachable fractions of the biomass after the biosorption process by the disruption of hydronium anions, destabilizing the interaction of molecules weakly retained into the biomass [12]. The same effect of surface leaching is usually observed on seaweed biomasses after heavy metal biosorption processes [12]. According to our results, the biosorption of Cu$^{2+}$ by L. nigrescens biomass causes the removal of
surface fractions of exposing internal layers that contain sulfonate groups (–SO₃) or highly sulphated polysaccharides such as fucoidans. With respect to the alkalii and alkaline earth metals, the atomic abundance of Na⁺ and Ca²⁺ on the raw seaweed decreased, contrary to the observed on the EDS analysis. Probably, because of the electron beam penetration, the EDS analysis allows getting information from internal layers of the biomass compared with the XPS analysis. The atomic abundance of Cu after the biosorption process (0.26%) demonstrates that the metal is sequestered on the surface of the biomass.

3.6. Spectral lines C1s and O1s

Table 4 shows the assignments of the components obtained from the deconvolution of the C1s and O1s lines. The four peaks derived from the C1s signal (Table 3 and Fig. S1) are assignable to chemical bonds and functional groups found on large brown seaweed polysaccharides: 284.5 eV peak assigned to C–C/C–H/Cadsorbed, 285.9 eV assigned to C–O (alcoholic groups), 287.2 eV assigned to O–C–O (ether groups) and 288.3 eV assigned to O=C–O (carbonate) groups [20,35,36]. The area of the C–C bonds peak remains practically inalterable after Cu²⁺ biosorption, indicating that no major changes occurs to the carbon heterogeneity of the biomass after the biosorption process. After the treatment of the biomass with Cu²⁺ the signal of C–O decreased from 30.0% to 28.3%, contrary to the increase in the signals of C=C–O (6.0% to 6.3%) and of O=C–O (3.2% to 5.4%). This can be explained by a loss on the electronic densities of the C–O bonds by the donation of electrons when the formation of metal oxides, like CuO, as it has been reported for that carbonyl groups and hydroxyl groups present in alginites and seaweed biomasses [18,37]. With respect to the O1s spectral line (Table 3 and Fig. S1), deconvolution results in three peaks: metal oxides (530 eV), C=O (531.1 eV) and O=C=O (532.4 eV). After the treatment of the biomass with Cu²⁺ the metal oxide peak increased from 21.6% to 27.3%, and the peaks of C=O and O=C–O decreased from 74.9% to 70.8% and 3.47 to 2.05, respectively. These results confirm changes on electron densities on oxygen signals after the Cu²⁺ biosorption. Probably, more oxygen atoms of the carbonyl groups share electrons with Cu²⁺ ions, which results in the increase of metal oxides bonds and a loss of signal due to a change in the angle of carbonyl groups due to the coordination bonding. The decrease in the abundance of the alkali-terrous metals Ca²⁺ and Mg²⁺ observed after the treatment of the biomass with Cu²⁺, correlates with the increment of the area in the oxygen deconvoluted signals for metal oxides. The metal-oxide binding signal is attributable to carboxyl groups but also to hydroxyl groups that can partially donate electrons, or even to groups with no carbon-bonded oxygen, like sulfonate. All together, these results indicate multidentism in metal binding with oxygen-containing functionalities.

3.7. Spectral lines for N1s and S2p

The assignments of the components obtained from the fitting of the N1s and S2p lines are shown in Table 4. Deconvolution of N1s signal of raw seaweed biomass derived in three peaks (Table 4 and Fig. S2), assignable to non-protonated amide (N–C=O at 398.05 eV); amines (NH₂ at 399.38 eV) and protonated amines (NH₃⁺ at 400.58 eV) [18,37]. After the biosorption process, the signal area of amide function increased more than twice (5.32%–12.59%), with a noticeable decrease on amine signal (52.34%–41.54%) and a slight increase on protonated amine deconvoluted signal (42.34%–45.87%). These results indicate that amide groups but not amine groups interact with Cu²⁺ ions on the biosorption process that is correlated with the ATR-FTIR analysis, supporting the idea that amine groups are not involved on Cu²⁺ biosorption. The spectral line for S2p (Table 4 and Fig. S2) showed a doublet S2p1/2 and S2p3/2 signal (167.8 and 169.56 eV, respectively) assignable to sulfonate groups [31,32]. Certainly, this unique signal shows that the chemical environment of Cu²⁺ is mainly composed by oxygen, which is provided by functional groups like carboxyl, sulfonate or hydroxyl, according to the observations on ATR-FTIR kinetic analysis. The decrease of the vibration distance on symmetrical and asymmetrical stretching of COO– of Cu²⁺ loaded biomass, and the XPS changes of O signals on COO– and OH– deconvoluted peaks, confirm that the sorption of Cu²⁺ on the seaweeds biomass is on carboxyl groups as a bidentate binding without
oxidative processes.

The deconvoluted signals of the spectral line of Ca2p (Table 4 and Fig. S3) correspond to a Ca2p_{3/2} and Ca2p_{1/2} doublet (347.43 and 351.10 eV, respectively) and are assignable to calcium oxides [40]. After Cu^{2+} biosorption, Ca^{2+} atoms are displaced and the remaining signal did not show important changes. EDS and XPS analyses showed that Ca^{2+} ions are displaced after Cu^{2+} biosorption but not after the acid treatment, which could be due to an occupation by Cu^{2+} of sites previously occupied by Cu^{2+} [3].

3.9. Atomic force microscopy (AFM) analysis of the seaweed biomass surface

The study of *L. nigrescens* biomass topology by AFM was performed using a lower amount of Ca^{2+} than in the ATR-FTIR, SEM and XPS experiments, to appreciate subtle changes on the surface of the biosorbent. After the Cu^{2+} biosorption, several changes were observed on the surface topology. The two dimension and lineal topological profiles showed a softening effect on the seaweed surface after the Cu^{2+} biosorption process, and some aggregates were observed (Fig. 4A-D). The AFM scan over 100 × 100 nm² areas for the raw biomass (that was not Cu^{2+} treated) showed a rather heterogeneous topology with height and phase profiles sections well correlated (Fig. 4A), and sections with mismatches on the height and phase response (Fig. 4B). This profile explains the presence of different stiffness, in the measured line over the surface observed (Fig. 4A and B, inserts). This agrees with the presence of crystals and free mass particles distributed over the surface. With respect to the AFM scan on Cu^{2+} loaded biomass over 100 × 100 nm² areas, the height profile showed a rather flat surface with some lines on it (Fig. 4C and insert). The phase image (Fig. 4D and insert) showed mainly low phase patches surrounded by high phase areas, indicating a net-like topological pattern.

These patterns were homogeneously distributed over the surface, as it can be seen in the 2D-images for height and phase retrace profiles sections well correlated (Fig. 5A and B) and in the 3D-representations (Fig. 5C and D). According to these results, the raw seaweed biomass presents a rough surface with a practically homogeneous stiffness. When the biomass was treated with Cu^{2+} ions, the surface changed to a different pattern, with patchy soft areas surrounded by stiffed structures by resembling fibres that form a cross-linked net.

Possibly, the patterned stiffness occurs by the reconfiguration of surface molecules when Cu^{2+} interacts with chemical groups on the surface of the biomass.
most exposed layers of the biomass. These interactions might involve carbohydrate polymers like alginates that tend to aggregate in lineal forms. Considering the low amount of Cu$^{2+}$ used for the experiment, it seems that the seaweed biomass surface specifically interacts with the metal reconfiguring the structure of the molecules present in the biomass surface by a multi-ligand interaction.

4. Conclusions

The present study reveals that the maximum biosorption of Cu$^{2+}$ by *L. nigrescens* dead biomass is achieved at pH 5 reaching the saturation at 120 min. The best data adjustment was the Langmuir isotherm model, obtaining a maximum capacity of Cu$^{2+}$ biosorption of 60.4 mg g$^{-1}$. The dead biomass of the brown seaweed *L. nigrescens* captures Cu$^{2+}$ ions in solution by superficial interactions with different functional groups, such as carboxyl, hydroxyl, sulfonate groups and amide groups, but not amine groups. When the metal is in solution, carboxyl groups saturate first, and then sulfonate groups, and then hydroxyl groups. The analyses of the biomass functional groups present in the surface support that carboxyl groups and hydroxyl groups are the most available groups on the surface. Cu$^{2+}$ generates displacement of alkali and alkali-terrous metals, with a high specificity for Ca$^{2+}$ ions. When the biomass is acid washed, Ca$^{2+}$ ions are displaced and these sites are occupied by Cu$^{2+}$ ions. The specific signals found by XPS analysis indicate that Cu$^{2+}$ interacts by coordinative bonding without oxidative reactions, involving a flexion of carboxyl groups’ characteristic of bidentate complexes. The fast saturation times of carboxyl group measured by ATR-FTIR, indicate that Cu$^{2+}$ readily interacts with the oxygen atoms of the carboxyl groups mainly present in alginates. We demonstrate that the physico-chemical interactions of Cu$^{2+}$ with the molecules of *L. nigrescens* dead biomass at a surface level, involves molecular reorganization and generates a general smoothening with some stiffness zones in the form of a fibre web.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [https://doi.org/10.1016/j.jece.2018.03.034](https://doi.org/10.1016/j.jece.2018.03.034).

References

[1] H. Jinsong, C. Paul, A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry and modelling simulation tools,


[16] V.O. Arief, K. Trilestari, J. Sunarso, N. Indraswati, S. Ismadji, Recent progress on


[41] O. Raize, Y. Argaman, S. Yannai, Mechanisms of biosorption of di

[9] E. Fourest, B. Volesky, Contribution of sulfonate groups and alginate to heavy metal


[38] Y.-M. Zheng, T. Liu, J. Jiang, L. Yang, Y. Fan, A.T.S. Wee, J.P. Chen,

[31] Y.N. Mata, M.L. Blázquez, A. Ballester, F. González, J.A. Muñoz, Biosorption of

[35] R.C. Oliveira, C. Jouannin, E. Guibal, O. Garcia, Samarium(III) and praseodymium

[26] K.J. Reddy, L. Wang, S.P. Gloss, Solubility and mobility of copper, zinc and lead in


[4] F. Pourret, B. Volesky, Contribution of sulfonate groups and alginate to heavy metal


[32] L. Wang, S.P. Gloss, Solubility and mobility of copper, zinc and lead in

[15] V. Murphy, Investigation into the Mechanisms of Heavy Metal Binding by Selected


