

## Characterization of the Residue from Double Alginate Extraction from *Sargassum filipendula* Seaweed

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Residue of alginate extraction from *Sargassum filipendula* brown seaweed shows high potential to be applied as bioadsorbent for the removal of heavy metals from contaminated wastewater, since it contains some constituents of the raw algae. Consequently the waste of the extraction process could be also employed as bioadsorbent, valorising the residue from alginate extraction and making the metal sorption more economically attractive. In front of this, the alginate extraction was carried out twice to verify if after the double extraction the residue presents the same constituents and the same potential to remove toxic metals. Furthermore, the double extraction was used in order to verify if the alginate extraction yield increases and if it preserves the characteristics of the residue as bioadsorbent. The yield (w/w) of alginate and residue in the first extraction was  $24 \pm 5.00\%$  and  $52 \pm 2.51\%$ , respectively. With the second extraction, the yield obtained represented  $11 \pm 1.88\%$  of alginate and  $76 \pm 3.84\%$  of residue. The high amount of residue encourages its use as biosorbent adding value to a waste of the process. The SEM-EDX results indicate that the materials' surface is mainly composed by Si, Na, Mg, Al, S, K, Ca and Fe, being that the presence and reduction of some elements concentration is due the diatoms shells. The spectra obtained from FTIR of first and second residues were similar, indicating that the main functional groups involved in adsorption phenomenon are not totally removed. It suggests that even after extracting more alginate it is possible to use the residue as bioadsorbent.

### 1. Introduction

Industrial wastewater are the major sources of toxic metals, representing serious environmental problems, and can be extremely damaging for humans if are ingested beyond the permitted concentrations, causing serious health disorders (Barakat, 2011). The adsorption, that has become one of the alternatives to remove heavy metals from wastewater, is a mass transfer process in that some constituents presents in a fluid phase are transferred to an adsorbent's surface (McCabe et al., 1993). The different kinds of adsorbents that can be used, encourage the employing of adsorption in order to eliminate and/or reduce this problematic by an efficient and economical way, since they are renewable, abundant and inexpensive materials. Examples of biological materials used as bioadsorbents are pre-treated bentonite clay (Cantuaria et al., 2016), olive stones (Martín-Lara et al., 2014), neem leaves (Ang et al., 2013), aquatic macrophytes (Lima et al., 2013), *Sargassum* seaweed (Kleinübing et al., 2013), and alginate extraction products from *Sargassum seaweed* (Bertagnolli et al., 2014c). In addition, bioadsorption shows some advantages in comparison with other conventional treatment processes, like precipitation and ion exchange, because results in smaller volume of sludge to dispose and presents a high efficiency in low concentrations. It is believed that bioadsorption occurs by the interaction of the adsorbate with functional groups of organic compounds such as proteins and carbohydrates presents in the solid surface. Therefore, identification of the involved functional groups is of great importance (Jianlong, 2002).

Among the biomass which may be used, algae, like brown seaweeds, have been studied a lot because are extremely efficient, given that shows high ability to remove various toxic metals from contaminated

wastewater. The presence and chemistry of algae cellular wall is the major characteristic of them and responsible of the biosorption process (Volesky, 2003). *Sargassum* seaweeds, very common in Brazilian coast, mainly in the southeast and northeast, contains alginic acid or alginate as major component of its cellular wall, constituting between 10 and 40 % of the dry weight of raw algae, varying according to the depth at which the algae are grown and the seasons (Davis et al., 2003). Alginate is a common name given to a linear polysaccharides composed by the 1,4-linked  $\beta$ -D-mannuronic (M) and  $\alpha$ -L-guluronic (G) acid residues arranged in a non-regular and block-wise order along the chain, given that the alginate physical properties depend of M- and G-block sequences and of their proportion (Davis et al., 2000). The alginate extraction process is based in the conversion of insoluble form presented in algae cellular wall to the soluble form, normally sodium alginate (Larsen et al., 2003). The residue from extraction is generally employed in the production of animal feed, but, as even after extraction, it contains some constituents of the raw algae and presents potential for the removal of heavy metals by adsorption from contaminated effluents. Therefore, the waste of the extraction process could be also employed as bioadsorbent, valorising the residue from alginate extraction and leading to an economically more attractive metal adsorption (Davis et al., 2003). There are numerous chemical groups present in *Sargassum* seaweed like carboxyls, phosphate, sulfate, amino and hydroxyl and they are responsible for the physical and chemical interactions between biosorbent and toxic metals, and they are supposed to contribute to metal sorption by residue from biopolymer extraction (Bertagnolli et al., 2014b).

In front of this, the alginate extraction was carried out twice to verify if after the double extraction the residue presented the same constituents and the same potential to remove toxic metals from industrial wastewater, and determine if the double extraction should be used to increase the alginate yield and preserve the characteristics of the bioadsorbents.

## 2. Materials and Methods

The brown seaweed *Sargassum filipendula* was collected in the São Paulo coast, at Cigarra beach, São Sebastião, during the summer (February, 24th, 2015), supposing that would be found higher quantity of guluronic acid in alginate composition (Bertagnolli et al., 2014a). The biomass was washed, dried at 60 °C for 24 h, milled and sieved into fractions smaller than 1 mm.

### 2.1 Alginate extraction

Firstly, 15 g of dry algae with mean diameter of 0.737 mm were put in contact with 500 mL of formaldehyde (0.4 % w/w) for 30 min, washed with deionized water and added in to 500 mL of hydrochloric acid solution (0.1 M) for 2 h. These wash treatments are necessary to remove phenolic compounds and clarify the biomass. The extraction was conducted with 350 mL of sodium carbonate solution (2% w/w) for 5 h at 60 °C. The viscous mixture was vacuum filtered to separate the solid residue and the alginate solution. After, the solid residue was exhaustively washed with deionized water and dried at 60 °C. The alginate was precipitated with ethanol (1:1 v/v) and dried too. For the second extraction, the residue was kept in contact with a proportional volume of sodium carbonate solution (2 % w/w) for 5 h at 60 °C, and the alginate precipitation was again accomplished. The yield of alginate and residue was calculated according to following Eq.(1) and Eq.(2):

$$\%yield (alginate) = \left( \frac{final\ dry\ matter\ of\ alginate}{seaweed\ dry\ matter\ before\ extraction} \right) \times 100 \quad (1)$$

$$\%yield (residue) = \left( \frac{final\ dry\ matter\ of\ residue}{seaweed\ dry\ matter\ before\ extraction} \right) \times 100 \quad (2)$$

### 2.2 Bioadsorption characterization

Scanning Electronic Microscopy (Leo 440i) coupled with Energy Dispersive X-ray (Leo 6070, Oxford) was applied to investigate the samples' morphology. Functional groups and chemical bonds were determined through Fourier Transform Infrared Spectroscopy (Micro Probe, IlluminatIR), from 4,000 to 650  $cm^{-1}$ , and resolution 4.

## 3. Results and discussion

The extraction was carried out in two cycles, with  $24 \pm 5.00$  % and  $11 \pm 1.88$  % from first and second extractions respectively, in agreement with the range of alginate present in brown seaweed, between 10 and 40 % (Davis et al., 2003). Bertagnolli et al. (2014b) found  $17.2 \pm 0.3$  % of alginate in *S. filipendula* from Brazil. Seaweed residues resulting from extractions correspond to  $52 \pm 2.51$  % and  $76 \pm 3.84$  % of the initial seaweed matter used on the first and second extractions, respectively. The high percentage recovered encourages its use as bioadsorbent, given that adds value to a residue of the process. Bertagnolli et al.,

(2014b) recovered about 39 % of residue. The percentage of lost matter (about 23 %) is due to different treatment and rinsing steps (formaldehyde, HCL and deionized water).

### 3.1 Bioadsorbent characterization

*Sargassum filipendula* seaweed and residues from alginate extraction were analyzed by SEM-EDX. The results of SEM are showed in Figure 1.

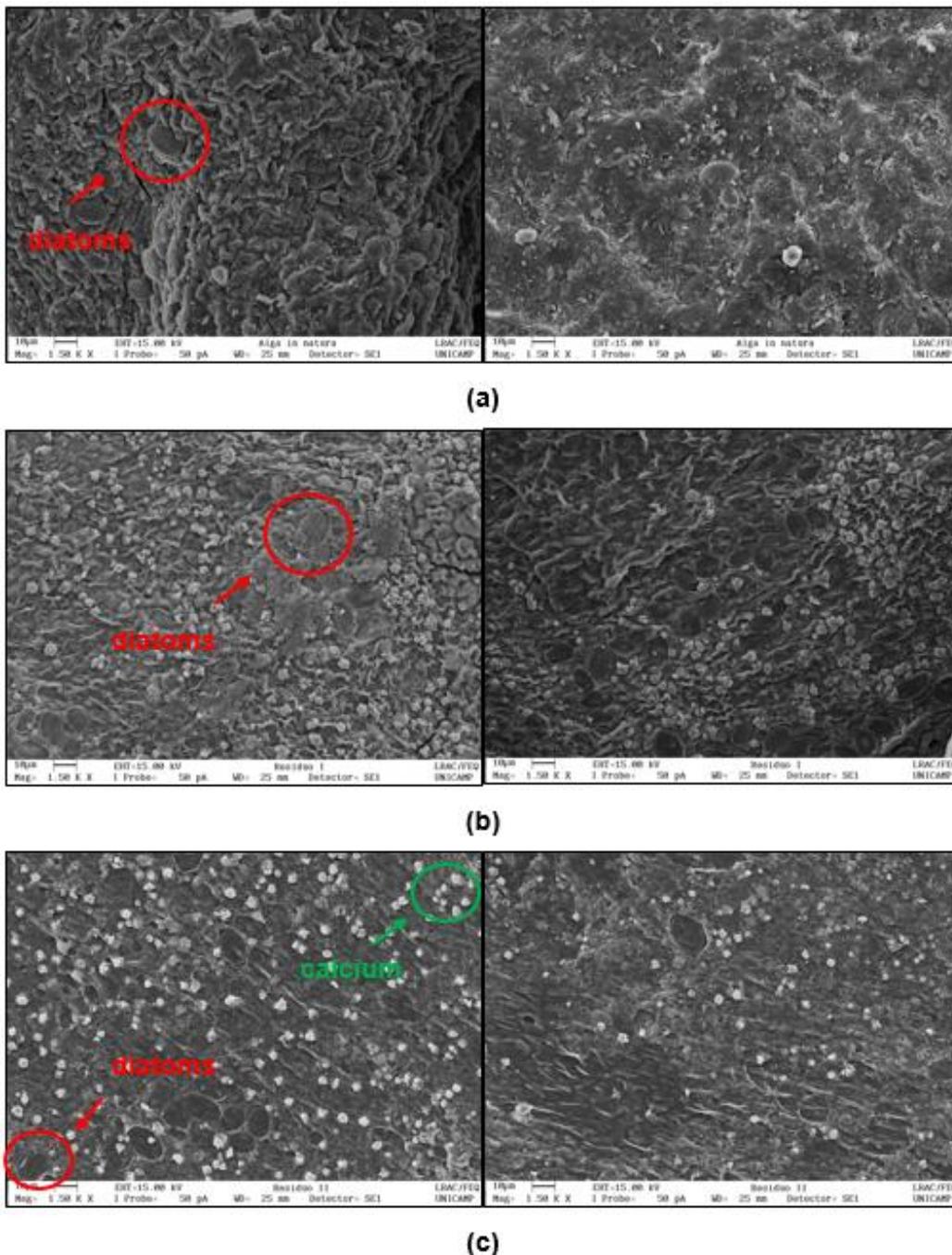


Figure 1: SEM analysis of seaweed (a) and alginate extraction residues: (b) after first extraction and (c) after second extraction

The images show irregular and porous structure with ruptures after the alginate extraction, since it is the major component of the algae's cellular wall. Fucoidan and cellulose are presents too. The EDX analyses were carried out in different five points of the biosorbent surface, giving a representative average of each cation. Table 1 shows the chemical composition of the samples.

Table 1: Materials' chemical composition (%)

Components	Na	Mg	Al	Si	S	K	Ca	Fe
Algae	3.08	10.41	9.20	29.77	11.25	8.02	21.66	6.62
Residue I <sup>a</sup>	30.37	5.12	4.44	7.65	9.31	1.15	39.05	2.93
Residue II <sup>b</sup>	25.51	4.89	3.15	5.99	7.66	-	50.08	2.72

<sup>a</sup>Residue from first extraction;

<sup>b</sup>Residue from second extraction.

Chemical analyses showed that Ca, Mg, Na and Fe are the most common elements in materials' surface. The Si, Na, Mg, Al, S, K, Ca and Fe presence can be related of the presence of diatom shells that was observed in surfaces of algae and residues, even after wash treatments and extractions of alginate (Tesson et al., 2009). So, the decrease of these elements concentration can be associated with diatoms remotion. Sodium concentration was increased from 3.08 % in algae to 25.51 % in residue II, and it can be related with the sodium carbonate used in the extraction stage. Calcium deposited in forms of CaCO<sub>3</sub>, CaO or Ca(OH)<sub>2</sub> is the major metal on the materials' surface after extraction, with percentages varying from about 20 % to 50 % in *S. filipendula* and residues, respectively (Bermúdez et al., 2012).

The FTIR spectra that allow the identification of functional groups of *S. filipendula* and residues are shown in Figure 2.

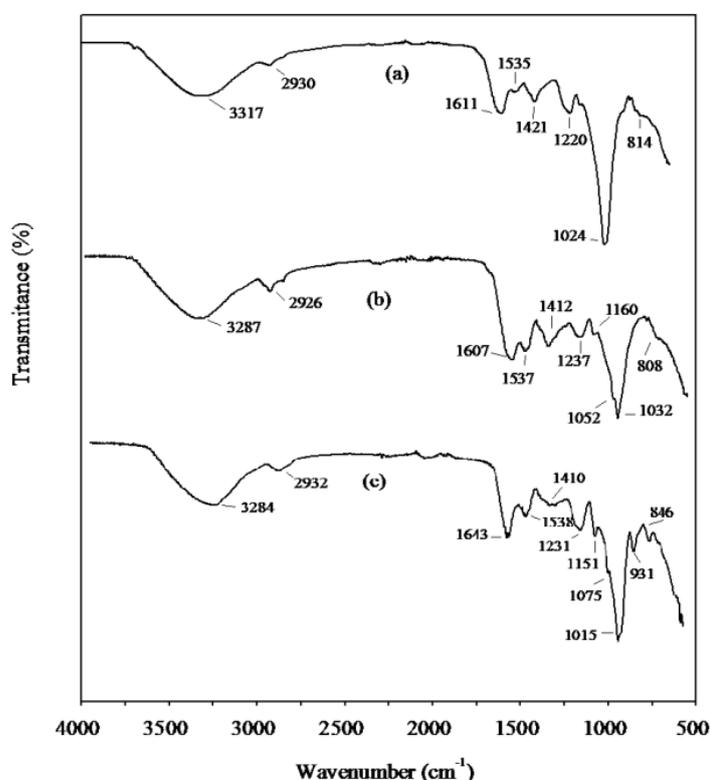


Figure 2: FTIR spectra of *Sargassum filipendula* (a), residue from first extraction (b) and residue from second extraction

In Figure 2, it is possible to note the change in intensity and a shift of the broad band at 3,317 cm<sup>-1</sup> in the algae to 3,287 - 3,284 cm<sup>-1</sup> in the residue after first and second alginate extraction. It may be caused by the alcoholic groups (Chen and Yang, 2005) present in the extracted alginate and the interaction with Na<sup>+</sup> used in extraction (Bertagnolli et al., 2014b). The peaks at 2,930 cm<sup>-1</sup> and 1,611 cm<sup>-1</sup>, corresponding to -CH bond and carboxylic groups (Sheng et al., 2004) respectively, did not show significant changes after extractions, except for the second residue. In this case, the peak at 1,611 cm<sup>-1</sup> shifted to 1,643 cm<sup>-1</sup>. The band at 1,421 cm<sup>-1</sup> in the algae shifted to 1,412 cm<sup>-1</sup> and 1,410 cm<sup>-1</sup> after first and second extraction respectively, probably due to a higher content of calcium in residues (Daemi and Barikani, 2012). The peak at 1,535 cm<sup>-1</sup> is related to amino groups of proteins (Kleinübing et al., 2013). The presence of S=O and C-S-O groups of fucoidan in the raw

algae results in the peaks at  $1,220\text{ cm}^{-1}$  and  $814\text{ cm}^{-1}$  (Fourest and Volesky, 1996). In the first residue, these peaks shifted to  $1,237\text{ cm}^{-1}$  and  $808\text{ cm}^{-1}$ , and in the second residue, to  $1,231\text{ cm}^{-1}$  and  $846\text{ cm}^{-1}$ . The strong peak at  $1,024\text{ cm}^{-1}$  in the raw algae corresponds to guluronic units of alginate (Sinha et al., 2010), and the small shifts to  $1,032\text{ cm}^{-1}$  and  $1,015\text{ cm}^{-1}$  after the two extractions may be related to the alginate extraction. The spectra of residues indicated the presence of functional groups involved in adsorption, such as carboxylic, alcoholic, sulphur and amino groups (Bertagnolli et al., 2014b). It suggests that the residue from alginate extraction may still present a good adsorption capacity.

#### 4. Conclusions

In general, the SEM-EDX results indicate that the materials' surface are composed mainly by Si, Na, Mg, Al, S, K, Ca and Fe, being that the presence and reduction of some elements concentration is due the diatoms shells. The spectra obtained from FTIR of first and second residues were similar, indicating that the main functional groups involved in adsorption phenomenon are not totally removed. It suggests that even after extracting more alginate it is possible to use the residue as bioadsorbent. It would be economically interesting due to the alginate value and the feasibility of the residue reuse. However, whereas the extractions generate similar residues for heavy metals adsorption, it becomes unnecessary a second extraction, due the quantity of reagents and the time spent.

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