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COMPARATIVE STUDY OF ALGINATE EXTRACTION METHODS FROM RED MARINE ALGAE *CALLITHAMNION CORYMBOSUM* SP.

BY

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Abstract. In recent years studies related to the metal ions biosorption on biological materials have increased significantly. Unfortunately, the relatively low biosorptive performances of most of natural biomasses have determined the finding of simple and cheap methods for the separation of various compounds from biomass composition, which are more efficient in the biosorption processes. Alginate is an active compound found in marine algae, and which is a great interest in environmental engineering. In this study are adapted and compared two methods for alginate extraction using red algae biomass (*Callithamnion corymbosum* sp.) as raw material. The main advantages and disadvantages of each extraction procedure are highlighted, in order to provide the best way to extract the alginate from marine algae biomass. Also, the biosoptive performances of the extracted alginate was tested in the biosorption process of Cu(II) ions from aqueous solution.

Keywords: Alginate extraction; red marine algae; biosorption; Cu(II) ions; aqueous solution.

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1. Introduction

The presence of heavy metal ions in aqueous ecosystems is an environmental problem worldwide. Due to their toxicity, persistence and accumulation tendency, high concentrations of heavy metals are found in the environment, becoming an important factor in degradation of ecosystem quality (Hackbarth *et al.,* 2015). The main source of environment pollution with heavy metal ions is industrial activities. The development of the industrial activities has determined the increasing of heavy metals discharge into environment, with negative consequences on soils, plants, rivers, groundwater (Volesky, 2001; Febrianto *et al*., 2009). Therefore it is necessary to find appropriate methods that should be environmentally friendly and should remove heavy metal ions in a more efficient and cheaper way (De Oliveira *et al*., 2013).

The biosorption process is considered a favorable alternative for removing off heavy metal ions from aqueous media. The main advantages of this method are: high efficiency even at low heavy metals concentrations, recyclingof biosorbent material, low energy consumption and ease of operation (Kratochvil and Volesky, 1998; Febrianto *et al*., 2009; Wang and Chen, 2009). Various natural materials or agricultural and industrial waste of biologic origin, general called biosorbent, have demonstrate to have a high efficiency in retention of heavy metal ions from aqueous media, in various experimental conditions (Montazer-Rahmati *et al*., 2011; Nguyen *et al*., 2013).

Marine algae are an example of biosorbent material, which have the ability to retain significant amounts of heavy metal ions from aqueous solutions, by biosorption (Ibrahim, 2011; Gupta *et al*., 2015). The biosorption process is mainly determined by the interactions between heavy metal ions from aqueous solution and functional groups from marine algae biomass surface (such as carboxyl, sulfonic, amine, hydroxyl, etc.) (Vilar *et al*., 2008). When such functional groups from marine algae biomass have high affinity for the heavy metal ions from aqueous solution and the cost of biosorbent preparation is low, the biosorption process can be successfully used to treat wastewater containing such contaminants.

Marine algae are found in significant quantities on the coastal areas in many regions of the world, including in Romania. In most of cases, the marine algae are considered a waste, and are a problem for both local authorities and tourists. Therefore, their use as a biosorbent makes it easier to solve this issue. Unfortunately, the studies from literature (Donmez *et al*., 1999; Romera *et al*., 2007; Bulgariu and Bulgariu, 2014) have shown that the biosorptive performances of most marine algae are quite low, and that multiple biosorptionsteps are required to efficiently remove various heavy metal ions from aqueous media.

A solution for increasing the efficiency of marine algae biomass in biosorption processes could be the extraction of the active compounds from their structure (such as alginate) and the use of these compounds as biosorbent materials.

Alginate extracted from marine algae is an organic biopolymer, which contains numerous free carboxyl and hydroxyl groups (Kumar and Sahoo, 2017; Latufa *et al*., 2017) that can interact readily with the heavy metal ions from aqueous media, compared to the functional groups from marine algae biomass. In addition, if the selected extraction procedure is simple, the alginate extracted from marine algae meets the two requirements of an inexpensive biosorbent.

In this study, two extraction methods been adapted starting from existing information in literature (Calumpong *et al*., 1999; Wang *et al*., 2018), and used for the extraction of alginate from the red marine alga *Cllithamnion corymbosum*. These two methods suppose the extraction of alginate in acid and basic media, and involve only few elementary steps. The main advantages and disadvantages of each extraction method are highlighted, in order to provide the best way to extract the alginate from red marine algae biomass. In addition, the biosoptive performances of the obtained alginate was tested in the biosorption process of Cu(II) ions from aqueous solution.

2. Experimental

2.1. Preparation of Marine Algae Biomass

The red marine algae (*Callithamnion corymbosum*) were collected from the Black Sea coast, in August 2016. The collected biomass was washed several times with distilled water to remove impurities and dried in air at 70°C, for 8 h. After drying, the biomass was crushed and sieved to a particle size of 1.0 – 1.5 mm and stored in desiccators for further use.

2.2. Chemical Reagents

All the chemical reagents used in this study were of analytical grade and were used without further purifications. Solutions of formaldehyde (2%), NaOH (1 M), Na_2CO_3 (2%) and $CaCl_2$ (1 M) have been prepared by dissolving an adequate quantity of solid reagent in distilled water. Solution of HCl (1M) was obtained from concentrated hydrochloric acid solution (37%). The stock solution of Cu(II) ions (635.22 $mg \cdot L^{-1}$) was prepared form copper sulphate salt. The stock solution was then used to obtain the working solutions (12.71 – 50.82 mg $Cu(II) \cdot L^{-1}$) by dilution with distilled water.

2.2. Methods Used for Alginate Extraction

Two methods have been adapted and used for alginate extraction, namely:

i) *in acid media* (adapted after Calumpong *et al*., 1999): 3 g red marine algae biomass have been treated with 30 mL of (2% [w/w])formaldehyde solution for 1 h, for to remove of fenolic compounds from algae composition, filtrated and washed with distilled water. The obtained material has been then treated with 200 mL 2N HCl solution, and left in stand-by for 24 h, for the dissolution of alginic acid from marine algae composition, and then filtrated. The solution, containing alginic acid, was treated with 2% [w/w] Na_2CO_3 solution and heated to 100°C for 3 h, in order to obtain the sodium alginate. The hot solution was then poured, drop by drop, into a cold solution of 1M Na_2CO_3, and vigorously stirred for 2 h. The obtained precipitate of sodium alginate was filtered, washed with distilled water and dried in air.

ii) *in basic media* (adapted after Wang *et al*., 2018) 5 g red marine algae biomass have been treated with 100 mL of 1M NaOH solution for 4 h for the dissolution of sodium alginate from marine algae composition. The obtained liquid phase have been heated for 24 h at 50°C and then cooled to 10°C, using an ice bath. The could solution was treated with 100 mL of 1M $CaCl_2$ solution and vigorously stirred for 2 h, in order to precipitate the alginate salt. The obtained precipitate of calcium alginate was filtered, washed with distilled water and dried in air.

Before the storage for further uses, the alginate obtained from each method was weighed with an analytical balance (RAGWAG XA 60/120, precision ± 10^{-4} g), and alginate extracted was calculated using Eq. (1),

$$\%\ \text{alginate extracted} = \frac{m_{alginate}}{m^{i}_{algae}} \cdot 100 \qquad (1)$$

where: % alginate extracted is the percent of alginate separated by each method, %; $m_{alginate}$ is the mass of alginate weighed after drying, [g] and m^{i}_{algae} is the initial mass of marine algae, [g].

2.3. Biosorption Tests

The biosorption tests were performed in batch systems, mixing a constant amount of alginate extracted by these two methods and raw marine algae biomass (0.1 g) with volume of 25 mL of known Cu(II) ions concentration, in 150 mL Erlenmeyer flasks, with intermittent stirring. After 24 h, the samples were filtered, and Cu(II) ions concentration was determined spectrophotometricaly (VIS Spectrophotometer YA1407020 model) with rubeanic acid (λ = 390 nm; 1 cm glass cells, against blank solution), using a prepared calibration graph (Dean, 1995).

The biosorptive performances of alginate extracted by these two methods to remove Cu(II) ions from aqueous solution was evalueted using biosorption capacity (q, [mg·g^{-1}]) and removal percent (R, [%]) calculated with the following equations:

$$q = \frac{(c_0 - c)\cdot(V/1000)}{m} \quad (2)$$

$$R = \frac{c_0 - c}{c_0}\cdot 100 \quad (3)$$

where: c_0 is the initial Cu(II) ions concentration in solution, [mg·L^{-1}], c is the equilibrium concentration of Cu(II) ions in the solution, [mg·L^{-1}], V is volume of solution, [mL], and m is the mass of alginate and marine algae, respectively, [g].

3. Results and Discussions

3.1. Evaluation of the Efficiency of Alginate Extraction Methods

Generally, the extraction methods of alginate from marine algae biomass are based on the solubilization of alginate by hydrolysis in acid or basic media, followed by its precipitation in solution with high salinity (Rostami *et al.*, 2017). Starting from existing information, in this study the extraction of alginate was performed in both acid and basic media, using original experimental procedures, adapted according to those existing in literature (Calumpong *et al*., 1999; Wang *et al*., 2018).

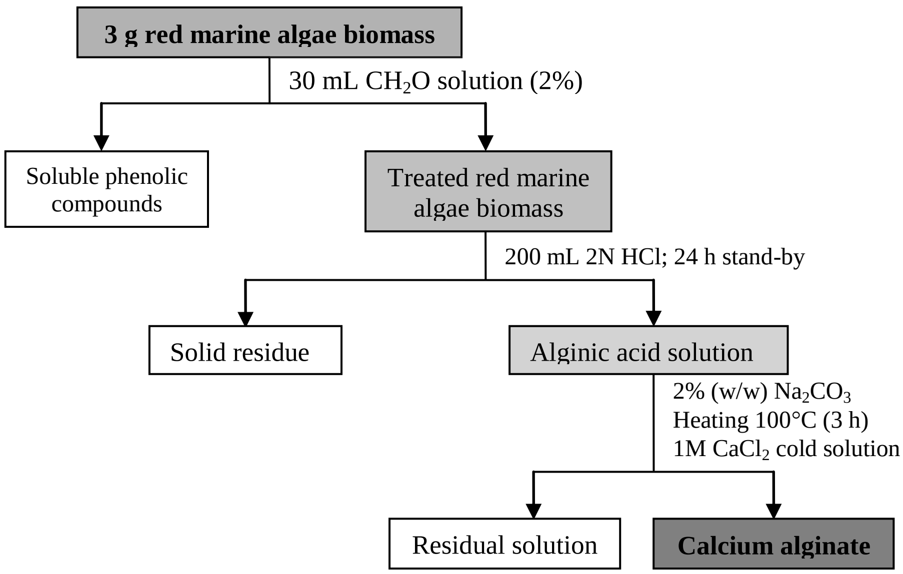


Fig. 1 − Experimental procedure used for extraction of alginate from red marine algae biomass, in acid media.

In Figs. 1 and 2 are illustrated the experimental procedures used in this study for the extraction of alginate from red marine algae biomass *Callithamnion corymbosum* sp.

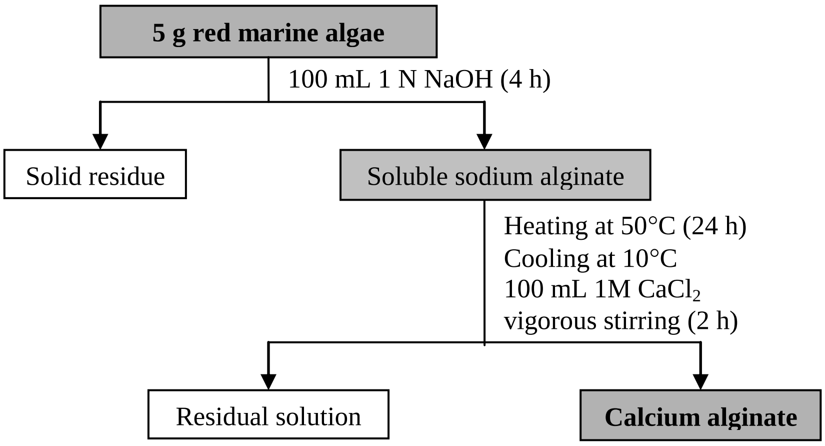


Fig. 2 − Experimental procedure used for extraction of alginate from red marine algae biomass, in basic media.

Besides that these two methods used for the extraction of alginate require different chemical reagents, other advantages and disadvantages are summarized in Table 1.

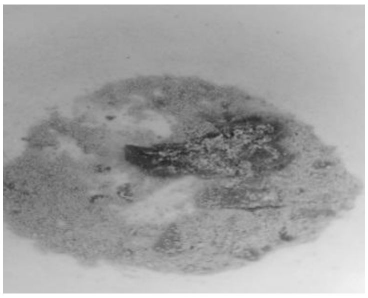
Table 1
Advantages and Disadvantages of the Two Methods Used for Alginate Extraction

	Acid media	Basic media
Advantages	- short work time (6 h) - low energy consumption (due to short heating and cooling time)	- obtained alginate is low soluble in aqueous media - low number of elementary steps
Disadvantages	- obtained alginate is quite soluble in aqueous media - higher number of elementary steps	- long work time (30 h) - high energy consumption (due to long heating and cooling time)

Table 2
Extraction Efficiency of Alginate by the Two Studied Methods

Extraction method	m algae, g	m alginate, g	% alginate extracted
Acid media	3.0032	0.1652	5.50
Basic media	5.0978	0.3245	6.36

On the other hand, the quantitative evaluation shows that there are significant differences between these two extraction methods. Thus, the quantity of alginate extracted in basic media is higher than in acid medium (Table 2), and his morphology is more granular (Fig. 3).


a

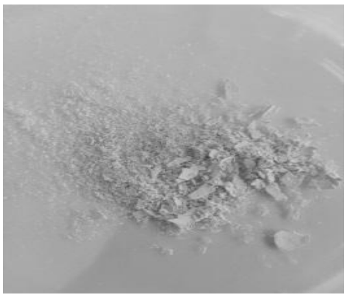

b

Fig. 3 – Morphological aspect of alginate extracted in acid media (*a*) and basic media (*b*).

The obtained experimental results showed that the extraction of alginate in basic media is more efficient and allows for obtaining a solid material with more granular morphology, which is more suitable for subsequent use as a biosorbent. Therefore, this method was selected to obtain the alginate from red marine algae biomass (*Callithamnion corymbosum* sp.).

3.2. Evaluation of the Biosorptive Performances of Extracted Alginate

In order to test if the alginate extracted from red marine algae biomass in basic media is suitable for use as a biosorbent, a known amount of alginate (0.1 g) was mixed with 25 mL of Cu(II) ions solution, with different initial concentration, at room temperature (26 ± 1.0°C). All the biosorption studies were performed in comparison with the red marine algae biomass, which is the raw material, and the experimental results are illustrated in Fig. 4.

During the biosorption process it was observed that the amount of solid alginate does not vary significantly, which means that by extracting the alginate in the basic medium, the obtained solid material does not dissolve in aqueous solutions and can be used as biosorbent material.

It can be observed from Fig. 4*a* that both in case of red marine algae biomass and alginate the values of biosorption capacity highly depends on the initial Cu(II) ions concentration. The highest values of this parameter is obtained at initial Cu(II) ions concentration of 50.82 $mg \cdot L^{-1}$, indicating that both red marine algae biomass and alginate can retain Cu(II) ions from aqueous solution by a biosorption process. Unfortunately, by comparing the values of biosorption capacity obtained for red marine algae biomass and alginate (Fig. 4*a*), no significant differences can be observed. This casts doubt on the necessity of alginate extraction from marine algae, since both materials have approximately the same affinity for Cu(II) ions from aqueous solution.

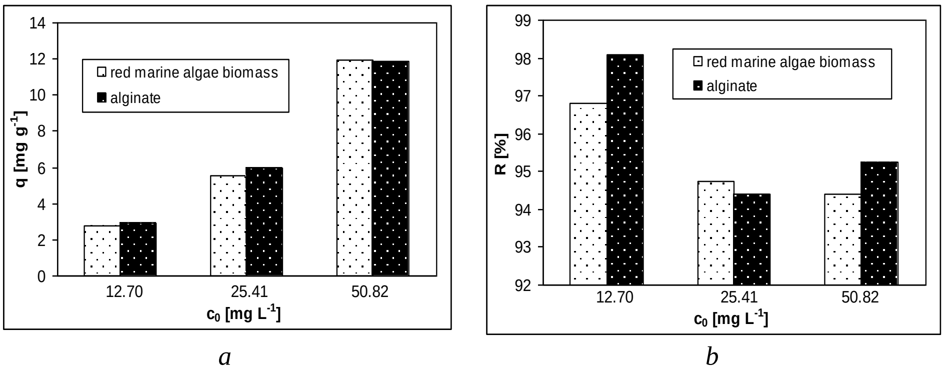


Fig. 4 – Variation of biosorption capacity (*a*) and removal percent (*b*) as a function of initial Cu(II) concentration in studied biosorption processes.

However, by comparing the values of removal percents (Fig. 4*b*), it can be seen that the values obtained for the alginate are higher than for the red marine algae biomass. This gives us the hope that by suitable selection of the experimental conditions (initial solution pH, alginate dosage, contact time, etc.), the biosorptive performances of alginate for Cu(II) ions can be improved. All these aspects will be discussed in a further study.

4. Conclusions

In this study, two extraction methods have been adapted and used for the extraction of alginate from the red marine alga *Callithamnion corymbosum*. These two methods suppose the extraction of alginate in acid and basic media, and involve only few elementary steps. Several advantages and disadvantages of each extraction method are highlighted, in order to provide the best way to extract the alginate from red marine algae biomass. The obtained experimental results showed that the extraction of alginate in basic media is more efficient (6.36%) and allows for obtaining a solid material with more granular morphology, which is more suitable for subsequent use as a biosorbent. Therefore, this method was selected to obtain the alginate from red marine algae biomass (*Callithamnion corymbosum* sp.). In order to test the biosorptive performances of the extracted alginate, a known amount of alginate (0.1 g) was mixed with 25 mL of Cu(II) ions solution, with different initial concentration. The experiments were carried out compared to the red marine algae biomass, which is the raw material. The obtained values of the biosorption capacity have indicated that no significant differences can be observed between alginate and red marine algae biomass. However, the values of removal percents obtained in case of alginate are higher than for the red marine algae biomass. Therefore, by an appropriate selection of experimental conditions, the alginate may become an effective biosorbent to remove heavy metal ions from the aqueous solution.

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STUDIU COMPARATIV AL METODELOR DE EXTRACŢIE ALE ALGINATULUI DIN BIOMASA DE ALGE MARINE ROŞII *CALLITHAMNION CORYMBOSUM* SP.

(Rezumat)

În ultimii ani, studiile legate de biosorbția ionilor metalici pe materiale biologice au crescut semnificativ. Din păcate, performanțele biosorptive relativ scăzute ale majorității biomaselor naturale au determinat găsirea unor metode simple și ieftine pentru separarea diferiților compuși din compoziția de biomasă, care sunt mai eficienți în procesele de biosorbție. Alginatul este un compus activ care se găsește în algele marine și care prezintă un interes deosebit în ingineria mediului. În acest studiu au fost adaptate și comparate două metode de extracție a alginatului folosind biomasă de alge marine roșii (*Callithamnion corymbosum* sp.) ca materie primă. Principalele avantaje și dezavantaje ale fiecărei metode de extracție au fost evidențiate, pentru a găsi cea mai bună modalitate experimentală de a extrage alginatul din biomasa algelor marine. De asemenea, performanțele biosorptive ale alginatului extras au fost testate în procesul de biosorbție a ionilor Cu(II) din soluție apoasă.