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EVALUATION OF THE BIOSORPTION PERFORMANCE OF MARINE GREEN ALGAE BIOMASS (ULVA LACTUCA SP.) IN THE REMOVAL OF INORGANIC POLLUTANTS

ΒY

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Abstract. Contamination of water sources with inorganic pollutants (cations or anions) is still a serious environmental problem, for which solutions are still being sought. Therefore, finding a biosorbent that can retain both cations and anions represent a solution to this problem. In addition, if such a biosorbent has a low preparation cost, it use in decontamination processes is recommended. Based on these considerations, in this study, marine green algae biomass (Ulva lactuca sp.) was used as a biosorbent for the removal of Cu(II) (inorganic cation) and HPO₄²⁻ (inorganic anion) from aqueous media. The experiments were conducted in batch systems, as a function of initial concentration of each inorganic ion, at constant pH (6.53), biosorbent dosage (4.0 g·L⁻¹), contact time (1440 min) and temperature (20 \pm 2°C). The maximum biosorption capacities obtained from Langmuir model under these experimental conditions, were 11.44 mg·g⁻¹ for Cu(II) ions and 7.45 mg·g⁻¹ for HPO₄²⁻ ions, respectively. FTIR spectrometry, SEM microscopy and potentiometric and conductometric titration were used for the characterization of the algae biomass. The structural particularities highlighted by these methods, show that this type of biomass has functional groups on its

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surface that can interact both with cations and anions from aqueous media. Therefore, its use as a biosorbent in decontamination processes is a viable solution.

Keywords: marine green algae biomass, *Ulva lactuca sp.*, Cu(II) ions, HPO_4^{2-} ions, biosorption.

1. Introduction

Industrial activities are the most recognized sources of environmental pollution (Cui *et al.*, 2014). Regardless of their nature (mining, electroplating, fertilizers manufacturing, paints production, etc.), (Fu and Wang, 2011; Volesky, 2015), the discharge into the environment of improperly treated industrial effluents, leads to the pollution of water sources (surface or even underground) and soils with various chemical compounds (inorganic or organic). Such chemical compounds (heavy metal salts, or simple or complex organic compounds) are persistent, and most of the time they accumulate in the environment, with serious consequences on the quality of ecosystems and human health (WHO, 2019). Therefore, finding technologically and economically efficient methods that can be used for the treatment of industrial effluents is still an open research direction.

Many studies in the literature have shown that compared to traditions methods of treating industrial effluents (chemical precipitation, coagulation, flotation, membrane filtration, etc.), the retention of pollutants on cheap solid materials by biosorption is a viable alternative, which can be adapted for use on an industrial scale (Kurniawan et al., 2006; Cui et al., 2014; Joseph et al., 2019; Ezugbe and Rathilal, 2020). This is because biosorption is considered a simple and environmentally friendly process that does not generate toxic sludge, requires a small number of simple operations (such as stirring, filtering, etc.), and can be used in a wide range experimental conditions (pH, initial concentration of pollutant, temperature, etc.) (Chojnacka, 2010; Fu and Wang, 2011; Priya et al., 2022). But all these advantages of biosorption processes are relevant for practical applications only if the solid material used as biosorbent is easy to obtain, available in large quantities and cheap (De Gisi et al., 2016; Crini and Lichtfouse, 2019; Saravanan et al., 2021). Therefore, natural and waste biomasses (results from agriculture or industry) have been testes in the literature for the removal of contaminants from aqueous effluents, to find possibilities for the use of these materials, in accordance with the principles of the circular economy (Yrjälä et al., 2022).

Natural materials are one of the most important sources of low-cost biosorbents, especially if these materials have little or no economic value, and generate additional costs related to their disposal (Duque-Acevedo *et al.*, 2020; Ghosh *et al.*, 2022). Thus, materials such as marine algae, aquatic plants, peat, clay, zeolites, etc., (Negm *et al.*, 2018; Rangabhashiyam and Balasubramanian, 2019; Joseph *et al.*, 2019) have already proven their effectiveness in pollutant removal processes. Special attention was paid to the use of marine algae biomass as

biosorbents in such processes. High abundance (in coastal regions), low cost of preparation, high biosorption capacity for various chemical species, good flexibility, lack of other practical uses, etc., (Farooq *et al.*, 2010; Rangabhashiyam and Balasubramanian, 2019) are just a few characteristics of these materials that support their use as ecological biosorbents. These are the main reasons why *Ulva lactuca sp.* marine green algae biomass was selected as biosorbent in this study.

The marine green algae *Ulva lactuca sp.* is the most abundant species of marine algae on the Romanian coast of the Black Sea (www.info-delta.ro). Every year, tons of such algae end up on beaches, where they cause inconvenience to seasonal tourist activities. Since these marine algae have no practical use (in agriculture of industry), they are collected daily from the beach and discharged in urban landfills. Therefore, the possibility of using marine green algae biomass (*Ulva lactuca sp.*) to retain contaminants in aqueous media would allow finding a practical application and the valorisation of this material.

In this study, marine green algae biomass (*Ulva lactuca sp.*) was used for the removal of Cu(II) ions and HPO₄²⁻ ions from aqueous solution. These two ionic species were selected because they represent models for biosorption studies. The biosorption capacity of algae biomass was determined at different initial concentrations of these ions, and the obtained results were analyzed using the Langmuir isotherm model. Detailed analysis of algae biomass revealed the most important structural characteristics of this material, which were used to explain its efficiency in the biosorption processes.

2. Experimental

2.1. Materials

Marine green algae (*Ulva lactuca sp.*) were collected from Costinești, Romania (in 2019). Algae samples were washed several times, first with tap water (to remove sand and solid impurities), then with distilled water (to remove dissolved salts from algae leaves). Clean algae were air-dried at room temperature $(25 \pm 2^{\circ}C)$ for 5 days, ground and sieved, and stored in hermetically sealed plastic box (to maintain constant humidity).

Aqueous stock solutions of Cu(II) ions and HPO₄²⁻ ions (with concentration of 10^{-2} mol·L⁻¹) were prepared from copper sulphate and potassium phosphate (both reagents were of analytical grade). The working solutions were obtained by diluting an exact measured volume of stock solution with distilled water.

2.2. Biosorption experiments

Biosorption experiments were performed in 100 mL conical flasks, by mixing 0.1 g of algae biomass with 25 mL of aqueous solution with different initial concentrations of Cu(II) ($26.36 - 96.02 \text{ mg}\cdot\text{L}^{-1}$), and HPO₄²⁻ ions ($23.79 - 1000 \text{ mg}\cdot\text{L}^{-1}$)

95.16 mg·L⁻¹), respectively. The initial solution pH, contact time and temperature were kept constant (pH = 6.53, 24 h and 20 \pm 2°C). At the end of contact time period, the two phases were separated by filtration (quantitative filter paper), and the concentration of Cu(II) and HPO₄²⁻ ions in the filtrate was analysed spectrophotometrically. The biosorption capacity of algae biomass (q, [mg·g⁻¹]) and the removal percent (R, [%]), were calculated, for each case, using the equations:

$$q = \frac{(C_0 - C) * (V/1000)}{m} \tag{1}$$

$$R = \frac{c_0 - c}{c_0} * 100 \tag{2}$$

where: c_0 and c are the initial and equilibrium Cu(II) ions and, respectively HPO₄²⁻ ions concentrations [mg·L⁻¹], V is the volume of solution [mL], and m is the mass of algae biomass used in each experiment [g].

2.3. Analytical methods

The concentration of Cu(II) ions and HPO_4^{2-} ions in the solutions obtained after filtration was determined spectrophotometrically (Digital Spectrophotometer S 104 D, 1 cm glass cells). The analytical characteristics of the spectrophotometric methods are summarized in Table 1.

Analytical characteristics of the spectrophotometric methods used in this study		
Parameter	Cu(II)	HPO ₄ ²⁻
Colour reagent	Rubeanic acid	Ammonium molybdate
λ_{max} [nm]	390	650
Buffer solution	Citrate buffer	Ascorbic acid/H ₂ SO ₄
Linear range [mg·L ⁻¹]	0.5 - 2.6	1.66 - 6.65

 Table 1

 An abstical observations of the spectrophotometric methods used in this study.

The functional groups on the surface of the algae biomass were identified by FTIR spectrometry (Bio-Rad Spectrometer, KBr pellet method, spectral domain = 400 - 4000 cm⁻¹, resolution of 4 cm⁻¹). The surface morphology was analyzed by SEM microscopy (SEM Hitach S3000N microscope), and the SEM images were recorded at different magnitude orders.

Potentiometric titration (TitroLine 3000 Titration system, equipped with a combined glass electrode) and conductometric titration (Mettler Toledo FiveEasy F30 Conductometer) were used for the determination of the amount of acid superficial functional groups of algae biomass. Thus 0.14 g algae biomass was treated with 25 mL of NaOH (0.1 N) solution, and the excess of NaOH was potentiometric and conductometric titrated with HNO₃ solution (0.1N). The volume of NaOH solution used for the neutralization of acid functional groups of

algae biomass, obtained by difference, was used in the calculations based on the law of equivalents.

3. Results and discussions

3.1. Characterization of marine green algae biomass

One of the most important advantages of using marine green algae biomass (*Ulva lactuca sp.*) as a biosorbent for the retention of contaminants in aqueous media is the ease of preparation. As mentioned in the previous section (see section 2.1), after sampling, the algae leaves only need to be washed, dried and ground to obtain the necessary materials for biosorption studies (Fig. 1).



Fig. 1 – Marine green algae *Ulva lactuca sp.*, after sampling (a) and after biomass preparation (b).

In order to identify the nature of the functional groups on the surface of marine green algae biomass, the FTIR spectrum was recorded (Fig. 2), while SEM images (Fig. 3) were used to analyze the morphology of biomass surface.

The broad band from 3429 cm⁻¹ corresponds to the symmetric stretching vibration of O–H from hydroxyl groups and N–H from aliphatic amines from water and proteins. The bands from 2954-2856 cm⁻¹ can be attributed to the C–H bonds from the saturated hydrocarbon radicals and alcohols of lipids.

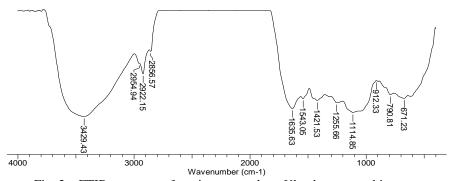


Fig. 2 - FTIR spectrum of marine green algae Ulva lactuca sp. biomass.

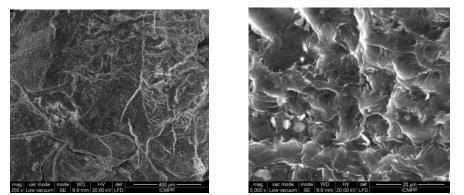


Fig. 3 – SEM images of marine green algae Ulva lactuca sp. biomass.

The band at 1635 cm⁻¹ indicates the stretching vibration of C=O and C=N bonds in proteins on the cell membranes. The bands at 1543 cm⁻¹ is characteristic to C–H and N–H bonds from proteins. The band at 1421 cm⁻¹ (C–O bond) indicates the presence of free carboxyl groups, while the band at 1114 cm⁻¹ is characteristic to the C–O bond from cellulose (Murdock and Wetzel, 2009).

Therefore, all these structural features show that marine algae biomass (*Ulva lactuca sp.*) contains numerous functional groups (with O and N atom donor) on their surface that can function as binding sites for cationic and anionic chemical species in aqueous solution. In addition, the non-uniform morphology of the surface, proven by the SEM images (Fig. 3), is another argument in favour of using of this biomass as a biosorbent.

3.2. Biosorption of Cu(II) and HPO4²⁻ ions on Ulva lactuca sp. biomass

To test the efficiency of marine green algae biomass (*Ulva lactuca sp.*) in biosorption processes, two ionic species, Cu(II) (cation) and HPO₄²⁻ (anion) were selected. These ions are considered "models" for biosorption studies, due to their high solubility and stability in aqueous media. The biosorption experiments were performed at three different values of initial concentration, but the initial solution pH (6.53), biosorbent dosage (4.0 g·L⁻¹), contact time (1440 min) and temperature ($20 \pm 2^{\circ}$ C) were kept constant. The obtained values of the biosorption capacity and the removal percent are illustrated in Fig. 4.

As expected, the biosorption capacity of marine green algae biomass increases with increasing initial concentration, for both inorganic ions (Fig. 4a). At low initial concentrations (26.36 mg·L⁻¹), the obtained value of this parameter is higher in the case of Cu(II) ions (1.32 mg·g⁻¹), compared to HPO₄²⁻ ions (0.49 mg·g⁻¹), while at high initial concentrations (105.31 mg·L⁻¹), the biosorption capacity is higher for HPO₄²⁻ ions (6.54 mg·g⁻¹) than for Cu(II) ions (6.14 mg·g⁻¹). On the other hand, the removal percents (Fig. 4b) are much higher in the case of Cu(II) ions (82.05 – 82.74%) than in the case of HPO₄²⁻ ions (8.63-

27.25%), over entire studied range of initial concentrations. These variations show that there are enough functional groups on the surface of marine algae biomass to bind inorganic ions from aqueous solution, but that, under the given experimental conditions, they have a higher affinity of Cu(II) ions (positively charged) than for HPO₄²⁻ ions (negatively charged).

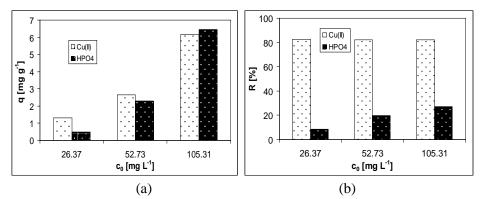


Fig. 4 – Variation of biosorption capacity (a) and removal percent (b) of marine green algae biomass (*Ulva lactuca sp.*).

In order to obtain a quantitative assessment of the efficiency of marine green algae biomass in the studied biosorption processes, the experimental data were analysed using the Langmuir model (Rangabhashiyam *et al.*, 2014):

$$\frac{1}{q} = \frac{1}{q_{max}} + \frac{1}{q_{max} \cdot K_L} \cdot \frac{1}{c}$$
(3)

where: q is biosorption capacity at equilibrium [mg·g⁻¹], q_{max} is maximum biosorption capacity [mg·g⁻¹], K_L is Langmuir constants [L·g⁻¹] and c is concentration of Cu(II) ions and HPO₄²⁻ ions in aqueous solution [mg·L⁻¹].

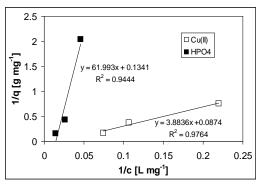


Fig. 5 – Linear representations of the Langmuir model for the biosorption of Cu(II) ions and HPO₄²⁻ ions on marine green algae biomass.

The linear representations of the Langmuir model are presented in Fig. 5, and the characteristic parameters are summarized in Table 2.

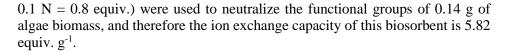
Table 2		
Langmuir model parameters calculated for the biosorption of $Cu(II)$ ions and HPO_4^{2-}		
ions on marine green algae biomass		

Parameter	Cu(II)	HPO ₄ ²⁻
R ²	0.9764	0.9444
$q_{max} [mg \cdot g^{-1}]$	11.44	7.45
$K_L [L \cdot g^{-1}]$	0.0225	0.0021

The results obtained from the modelling of the experimental data allow us to say that: (i) the experimental data are well described by the Langmuir model ($R^2 > 0.94$), therefore the retention of Cu(II) ions and HPO₄²⁻ ions takes place on the biomass surface until a monolayer coverage is formed, (ii) the maximum biosorption capacity (q_{max}) is 53% higher for Cu(II) ions than for HPO₄²⁻ ions (Table 2), and (iii) the significant differences in Langmuir constant (K_L) support the hypothesis that marine green algae biomass has higher affinity for Cu(II) ions than for HPO₄²⁻ ions (Table 2).

These differences are mainly due to the nature of the functional groups on the surface of marine green algae biomass. The much more efficient retention of Cu(II) ions compared to $HPO_4^{2^-}$ ions (Table 2) shows that most of the superficial functional groups of this biosorbent are acidic and dissociated, at this pH value (6.53). Such functional groups (negatively charged or with a high electron density at the donor atom) will exert an electrostatic attraction towards the positive ions in the aqueous solution (such as Cu(II)), while towards the negatively charged ions (such as $HPO_4^{2^-}$ ions), the interactions will be of electrostatic repulsion. Consequently, Cu(II) ions will be much more stronger bond to the biosorbent surface (trough ion exchange interactions), while the retention of $HPO_4^{2^-}$ ions will be predominantly through physical interactions (hydrogen bonds or van der Waals forces). This different behaviour of the two ionic species (Cu(II) ions and $HPO_4^{2^-}$ ions) is supported by the calculated values of the Langmuir constants (Table 2).

The presence on the surface of marine green algae biomass of the functional groups with O donor atom (donor atom with high electron density) was proved by the absorption bands in the FTIR spectrum (Fig. 2a). To test the capacity of these functional groups to ionize, the marine green algae biomass (0.14 g) was treated with 25 mL of NaOH solution (0.1 N), and the excess of NaOH was then titrated (by potentiometric and conductometric methods) with a HNO₃ solution (0.1 N). The titration curves used for the calculations (based on the law of equivalents) are shown in Fig. 6. It can be seen from Fig. 6 that the volume of NaOH solution consumed to neutralize the functional groups of the biosorbent is 8 mL (25-17 = 8 mL). This means that 0.8 equiv. of NaOH (8 mL x



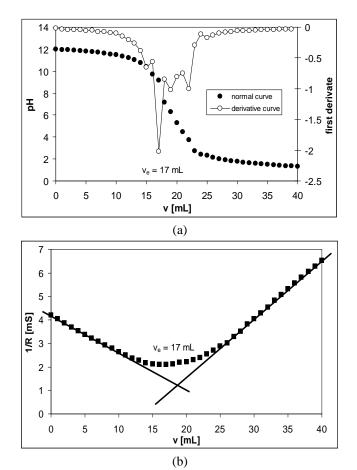


Fig. 6 - (a) Potentiometric titration curve and (b) conductometric titration curve.

This value of the ion exchange capacity obtained for marine green algae biomass is much lower than the values obtained for ion exchange resins $(10^2 \text{ equiv. g}^{-1})$ (Botelho *et al.*, 2019), but highlight the possibility of using this material for the removal, in particular, of cationic species from aqueous media.

In addition (Fig. 6), most of the functional groups on the biomass surface can dissociate easily even at pH values in the slightly acidic to neural range, which ensures the existence of active centres for the biosorption of positively charged ions form the aqueous solution. All these observations allow us to say the marine green algae biomass has all the necessary structural characteristics to be used as a biosorbent in biosorption processes.

4. Conclusions

In this study, marine green algae biomass (Ulva lactuca sp.) was used as a biosorbent for the removal of Cu(II) (inorganic cation) and HPO_4^{2-} (inorganic anion) from aqueous media. The biosorption experiments were performed in batch systems, and the maximum biosorption capacities obtained from Langmuir model, were 11.44 mg·g⁻¹ for Cu(II) ions and 7.45 mg·g⁻¹ for HPO₄²⁻ ions. The characterization of marine green algae biomass (by FTIR spectrometry, SEM microscopy, and potentiometric and conductometric titration) shows that most of the superficial functional groups of this biosorbent are acidic and dissociated. This causes the functional groups to exert an electrostatic attraction to positive ions (such as Cu(II)), and an electrostatic repulsion to negatively charged ions (such as HPO₄²⁻ ions), in aqueous solution. Consequently, Cu(II) ions will be much more stronger bond to the biosorbent surface (trough ion exchange interactions), while the retention of HPO₄²⁻ ions will be predominantly through physical interactions (hydrogen bonds or van der Waals forces). Therefore, the marine green algae biomass has all the necessary structural characteristics to be used as a biosorbent in biosorption processes, and its efficiency is higher in the case of cations than in the case of anions.

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EVALUAREA PERFORMANȚELOR DE BIOSORBȚIE A BIOMASEI DE ALGE MARINE VERZI (*ULVA LACTUCA SP.*) ÎN ÎNDEPĂRTAREA POLUANȚILOR ANORGANICI

(Rezumat)

Contaminarea surselor de apă cu poluanți anorganici (cationi sau anioni) este încă o problemă serioasă de mediu, pentru care se caută în continuare soluții. Prin urmare, găsirea unui biosorbent care poate reține atât cationi, cât și anioni reprezintă o soluție la această problemă. În plus, dacă un astfel de biosorbent are un cost de preparare scăzut, se recomandă utilizarea lui în procesele de decontaminare. Pe baza acestor considerații, în acest studiu, biomasa de alge verzi marine (Ulva lactuca sp.) a fost utilizată ca biosorbent pentru îndepărtarea Cu(II) (cationul anorganic) și HPO₄²⁻ (anionul anorganic) din mediile apoase. Experimentele au fost efectuate în sisteme discontinue, în funcție de concentrația initială a fiecărui ion anorganic, la pH (6,53), doza de biosorbant (4,0 g·L⁻¹), timpul de contact (1440 min) și temperatură (20 ± 2°C) constante. Capacitățile maxime de biosorbție obținute din modelul Langmuir în aceste condiții experimentale, au fost de 11,44 mg·g⁻¹ pentru ionii Cu(II) și, respectiv, 7,45 mg·g⁻¹ pentru ionii HPO₄²⁻. Pentru caracterizarea biomasei de alge au fost utilizate spectrometria FTIR, microscopia SEM și titrarea potentiometrică și conductometrică. Particularitățile structurale evidențiate de aceste metode arată că acest tip de biomasă are pe suprafata sa grupări funcționale care pot interacționa atât cu cationi, cât și cu anioni din medii apoase. Prin urmare, utilizarea sa ca biosorbent în procesele de decontaminare este o soluție viabilă.