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ROLE OF SUPERFICIAL FUNCTIONAL GROUPS IN DETERMINING PHOSPHATE BIOSORPTION EFFICIENCY ON MARINE GREEN ALGAE

BY

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Abstract. In this study, three types of marine green algae biomass (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*) were used for the biosorption of phosphate from aqueous solution. These marine green algae are abundant in the Black Sea, and can represent a cheap and easy-to-obtain source of renewable biomass. The efficiency of these three types of algae biomass was tested at different initial concentration of phosphate (24 – 95 mg·L^{-1}), and the biosorption capacity, increases in the order: *Ulva intestinalis*>*Ulva lactuca*> *Cladophora vagabunda*. This variation is mainly determined by the number and types of the functional groups on the surface of the algae biomass (analyzed using the Boehm method), and can be considered an important criterion in selecting algae biomass for phosphate biosorption. In addition to the biosorption capacity of algae biomass for phosphate, the pH, electrical conductibility and turbidity of the solutions obtained after filtration were also measured. All these parameters play an important role in selecting the appropriate type of algae biomass for phosphate removal from aqueous media.

Keywords: green marine algae biomass, superficial functional groups, phosphate, biosorption, aqueous solution.

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1. Introduction

The implementation of intensive agriculture as a solution to respond to the demand for food on the market, has led to a significant increase in the use of fertilizers based on phosphorus and nitrogen, to increase agricultural production (Hou *et al*., 2025; Ge *et al*., 2025). Unfortunately, the indications for the use of such fertilizers are not always respected in agricultural practices, which mean that significant amounts of phosphates and nitrogen compounds are leached into water sources (Culas *et al*., 2025). It is known that leakage of phosphate from agricultural land in surface water sources is responsible for their eutrophication (McDowell and Haygarth, 2024; Wahtra and Johnsson, 2025). The eutrophication processes occur as a result of the enrichment of water in nutrients (as phosphate), which cause an uncontrolled increase in the number of aquatic organisms, which use phosphate as nutrient (Mishra and Tripathi, 2023). Therefore, the quality of water sources is significantly affected, as well as the quality of the ecosystems, and this is not desirable in terms of environmental protection.

In order to minimize all these negative consequences, many studies in the literature have focused on finding adequate methods to remove phosphate from aqueous media (Zahed *et al*., 2022). Thus, various chemical methods (such as chemical precipitation, ion exchange or electrochemical processes) (Christensen *et al*., 2022; Usman *et al*., 2022), biological methods (such as microorganisms accumulation) (Abdoli *et al*., 2024) or physical methods (such as membrane related processes or adsorption) (Usman *et al*., 2022) have been tested in different experimental conditions to evaluate the phosphate removal efficiency. Among them, adsorption on biological materials (or biosorption) has been frequently used to retain phosphate from aqueous media, because it is considered high-efficiency, environmentally friendly and low-cost method (Usman *et al*., 2022; Mahmoudy*et al*., 2024). Numerous materials of biological origin (such as agro-industrial wastes, biopolymers, biochar, etc.), (Fan and Zhang, 2018; Fatima *et al*., 2021; Konneh *et al*., 2021), have been reported in the literature for the retention of phosphate from aqueous media, and the advantages of using these biosorbents have been clearly presented in these studies. The efficiency of these materials in phosphate retention processes depends on the number and nature of the superficial functional groups of the biosorbent, but also on the initial pH of aqueous solution and the concentration of phosphate (Liu and Zhang, 2015; Zhang *et al*., 2022). However, the use of materials of biologic origin as biosorbents also has some disadvantages that must be mentioned. Most often, when biological materials are added to aqueous media, they release certain organic compounds from their composition, resulting in a substantial increase in the chemical oxygen demand of the effluent obtained after biosorption, and a significant deterioration in its quality (Anggreini *et al*., 2025). Moreover, many biologically derived materials (leaves, stems, peels, etc.) that are widely available are already used in animal husbandry or as raw materials in the food,

pharmaceutical, and cosmetics industries (Lee *et al*., 2021; Sandoval *et al*., 2024), which limits their use as biosorbents. The use of green marine algae biomass as biosorbents for the retention of various pollutants from aqueous media significantly limits many of these drawbacks. First, the release of organic compounds from the composition of green algae biomass is quite low (they do not contain water-soluble pigments) (Farobie *et al*., 2022), which means that changes in the chemical oxygen demand of the effluent resulting from the biosorption is insignificant. Also, the use of this type of biomass in other industrial processes is limited. The few attempts to obtain biologically active compounds from green algae biomass in our country (Sîrbu *et al*., 2019; Cadar *et al*., 2023), are more research directions than actual industrial applications. Consequently, all these aspects qualify the biomass of marine green algae as a suitable material for biosorption applications, and their wide availability represents an additional advantage for their use in environmental remediation processes.

In the coastal area of the Black Sea, numerous species of marine green algae are present, among which *Ulva lactuca*, *Ulva intestinalis*, and *Cladophora vagabunda* are the most widespread (Marin and Filimon, 2024). In addition to their high natural availability, these green marine algae offer significant advantages for use as biosorbents due to the low cost and the ease of preparation, which makes them suitable for the development of efficient and sustainable solutions for treating contaminated aqueous effluents (Younis and Almutairi, 2023). However, when assessing their biosorptive performance, in addition to low cost and ease of preparation, the availability of superficial functional groups to participate in biosorption processes, as well as the values of some quality parameters (such as: pH, electrical conductibility, turbidity) of the effluents resulting from biosorption, must be also taken into account.

Based on these considerations, in this study, three types of green marine algae (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*) were used for phosphate retention from aqueous media. In addition to evaluating their biosorption capacities for phosphate, the number and nature of superficial functional groups were determined using the Boehm titration method. These values, together with the rapid characterization of the effluent quality obtained after biosorption (through pH, electrical conductivity, and turbidity), can provide sufficient information for selecting the most suitable green algae biomass for phosphate removal from aqueous media.

2. Materials and Methods

The three types of algae used in this study (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*) were collected from the Black Sea coast (Costinești, 2023). After sampling, the algae were washed several times with tap water and

distilled water, dried in air (3-4 days, until a constant mass) at room temperature, ground, and sieved. The algae biomass was stored in airtight containers.

The stock solution of HPO_4^{2-} (1190 mg·L^{-1}) was prepared by dissolving an appropriate amount of K_2HPO_4 (purchased from Chemical Company, Iași) in distilled water, and working solutions were obtained by dilution. All other chemical reagents (NaOH, $NaHCO_3$, Na_2CO_3, HCl, etc.) used in the experimental studies were of analytical grade and were used without further purification.

All biosorption studies were performed in batch systems by mixing 25 mL of solution (pH = 6.5, containing different phosphate concentrations (23.79, 47.58, and 95.16 mg·L^{-1}) with a given amount of biosorbent (biosorbent dose = 4 g/L) for 24 h (sufficient to reach the equilibrium), at room temperature (21 ± 1°C). After filtration (quantitative filter paper), the phosphate concentration was determined spectrophotometrically using ammonium molybdate (Digital Spectrophotometer S 104D, λ = 650 nm, 1-cm glass cell, linear dynamic range = 1.65 – 6.60 mg·L^{-1}, detection limit = 0.021 mg·L^{-1}), and used to calculate the biosorption capacity (q, mg/g) and removal percent (R, %), according to the equations:

$$q = \frac{(c_0 - c)\cdot V}{m} \quad (1)$$

$$R = \frac{c_0 - c}{c_0} \cdot 100 \quad (2)$$

where: c_0 and c are initial and equilibrium concentration of phosphate in solution (mg·L^{-1}), V is the volume of solution (L), and m is the amount of algae biomass (g).

The aqueous solutions obtained after filtering the algae biomass were then used to determine pH (Mettler Toledo pH/ion-meter, equipped with a combined glass electrode), electrical conductibility (JENWA conductometer, 4590-type), and turbidity (Hanna Instruments turbidimeter, HI88703 type).

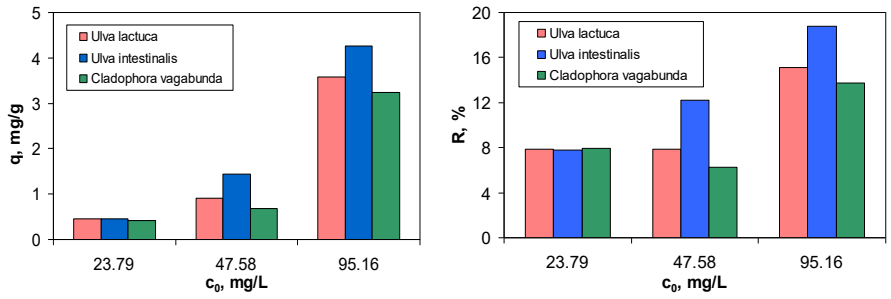
The determination of the number and types of functional groups on the surface of each algae biomass was carried out using the Boehm titration method (Danushika *et al*., 2025). Briefly, four samples of 0.5 g from each algae biomass (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*) were mixed with 50 mL of 0.1 N $NaHCO_3$, Na_2CO_3, NaOH and HCl solutions, respectively, and kept in contact for 24 h (with intermittent stirring), followed by filtration. The excess $NaHCO_3$, Na_2CO_3 and NaOH in the filtrate were titrated with 0.1 N HCl using phenolphthalein as indicator, while the excess HCl was titrated with NaOH using the same indicator. The concentration of carboxylic groups (in the presence of $NaHCO_3$), lactonic groups (in the presence of Na_2CO_3), alcoholic/phenolic groups (in the presence of NaOH), and basic groups (in the presence of HCl) was calculated using the following relationship:

$$n = \frac{(v_t - v_{t,0})\cdot 0.1}{m} \quad (3)$$

where: n is the concentration of functional groups (meq·g^{-1}), v_t is the volume of solution (HCl or NaOH) used for titration of excess $NaHCO_3$, Na_2CO_3, NaOH and HCl respectively from the solutions obtained after biomass filtration (mL), v_{t0} is the volume of solution (HCl or NaOH) used for titration of blank samples (without addition of algae biomss) (mL), 0.1 is the normal concentration of HCl or NaOH solutions (N), m is the amount of algae biomass. All the experiments, including titrations were performed in triplicate, and average values were used for calculations and graphical representations.

3. Results and discussion

To select the most suitable green algae biomass for phosphate removal from aqueous media, biosorption studies were carried out using *Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda* as biosorbents. These experimental investigations were conducted at pH = 6.5 (which ensures the presence of phosphate in solution predominantly as HPO_4^{2-} ions) and at various initial phosphate concentrations, and the obtained biosorption capacities and removal percent are presented in Fig. 1.



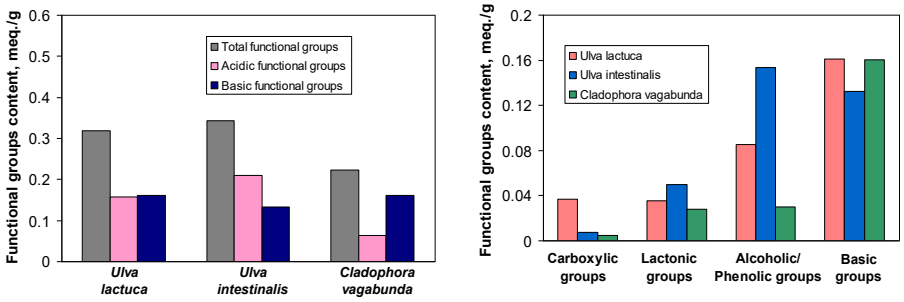
(a) (b)

Fig. 1 – Variation of biosorption capacity (a) and removal percent (b) for phosphate biosorption on the three types of marine green algae biomass (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*). (Experimental conditions: pH = 6.5, algae biomass dose = 4 g·L^{-1}, contact time = 24 h, temperature = 21± 1°C).

As shown in Fig. 1, increasing the initial concentration of the phosphate leads to an increase in the biosorption parameters (q and R), regardless of the type of algae biomass used as biosorbent. Such a variation is frequently observed in biosorption processes (Mahmoudy *et al*., 2024), and suggests that the retention of phosphate on the studied algae biomasses involves the presence of physico-chemical interactions, in which the superficial functional groups play an important role. Therefore, determining the number and nature of the functional groups on the surface of each type of algae biomass may represent an important criterion in selecting the biosorbent with the highest efficiency.

Detailed analysis of the experimental results (Fig. 1a) shows that, while at low initial phosphate concentrations (23.79 $mg \cdot L^{-1}$) the biosorption capacities of the three algae biomasses are relatively similar, increasing the phosphate concentration (47.58 and 95.16 $mg \cdot L^{-1}$) leads to much more pronounced differences between the values of this parameter. Thus, for an initial concentration of 47.58 $mg \cdot L^{-1}$, the q value obtained for *Ulva intestinalis* is 36% higher than that obtained for *Ulva lactuca* and 52% higher than that obtained for *Cladophora vagabunda*, while for the initial concentration of 95.16 $mg \cdot L^{-1}$, the q value obtained for *Ulva intestinalis* is 16 % higher than that obtained for *Ulva lactuca* and 24% higher than that obtained for *Cladophora vagabunda*. The removal percents follow the same order, *Ulva intestinalis* > *Ulva lactuca* > *Cladophora vagabunda* (Fig. 1b**)**, although the differences between the values of this parameter do not exceed 10%, regardless of the initial phosphate concentration.

In order to explain the differences between the experimentally obtained biosorption capacities for the three types of algae biomasses (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*), the superficial functional groups were analyzed using the Boehm titration method. The quantitative distribution of acidic and basic functional groups for each algae biomass (Fig. 2) provides relevant information regarding the specific interactions that are important for phosphate biosorption from aqueous media.



(a) (b)

Fig. 2 – Total content of functional groups (a) and types of functional groups (b) on the surface of the three marine green algae biomass (Experimental conditions: algae biomass dose = 4 $g \cdot L^{-1}$, contact time = 24 h, temperature = 21± 1°C).

As can be observed from Fig. 2, all types of algae biomass exhibit surface rich in acidic and basic functional groups, which suggest that the surface of these biomasses have a high degree of polarity and may provide active sites for biosorption processes. *Ulva intestinalis* exhibits the highest total content of functional groups (0.3436 $meq \cdot g^{-1}$), followed by *Ulva lactuca* (0.3188 $meq \cdot g^{-1}$) and *Cladophora vagabunda* (0.2232 $meq \cdot g^{-1}$), and also the highest content of acidic groups (0.2109 $meq \cdot g^{-1}$) compared with *Ulva lactuca* (0.1580 $meq \cdot g^{-1}$) and

Cladophora vagabunda (0.0628 meq·g^{-1}) (Fig. 2a). In the case of basic functional groups, the highest content was determined for *Ulva lactuca*(0.1607 meq·g^{-1}), closely followed by *Cladophora vagabunda* (0.1603 meq·g^{-1}), and then *Ulva intestinalis* (0.1326 meq·g^{-1}) (Fig. 2a).

Among the acidic functional groups, *Ulva intestinalis* exhibits the highest content of lactonic (0.0499 meq·g^{-1}) and alcoholic/phenolic (0.1534 meq·g^{-1}) groups, whereas *Ulva lactuca*shows the highest content of carboxylic groups on its surface (0.0371 meq·g^{-1}). *Cladophora vagabunda* displays the lowest amount of acidic groups, regardless of their nature (Fig. 2b).

The differences in the content of functional groups determined by the Boehm method for each type of algae biomass (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda)* are also supported by the pH values measured in the aqueous solutions obtained after filtering the biomass, in the absence of phosphate. As shown in Fig. 3a, for a phosphate concentration of 0 mg·L^{-1}, the lowest pH value (pH = 5.94) was recorded for *Ulva lactuca*, where the content of carboxylic groups is the highest (Fig. 2b), while the highest pH value (pH = 6.72) was measured for *Cladophora vagabunda*, which has the highest content of basic groups (Fig. 2b). In the case of *Ulva intestinalis*, the pH value measured under these conditions is 6.44 (Fig. 3a), which once again confirms that the superficial functional groups of this type of green marine algae are dominated by acidic functional groups (Fig. 2a).

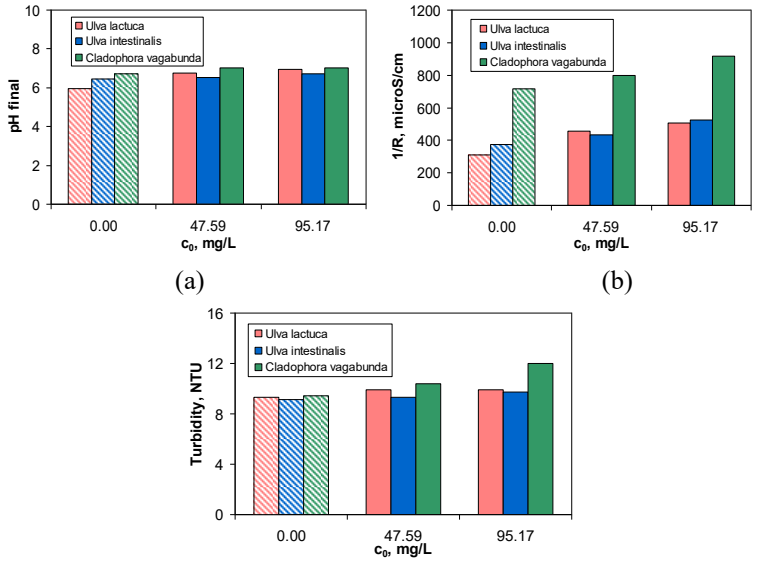


Fig. 3 – pH (a), electrical conductibility (1/R) (b) and turbidity (c) measured in the aqueous solutions obtained after filtering the algae biomass at different initial phosphate concentrations (*Ulva lactuca*, *Ulva intestinalis* and *Cladophora vagabunda*).

Among the acidic groups, lactonic and alcoholic/phenolic groups are the most abundant in the composition of this algae biomass (Fig. 2b), and moreover, these groups do not ionize, but in aqueous solution they can easily protonated.

The correlation between the content of superficial functional groups (Fig. 2b) and the experimentally determined phosphate biosorption capacities (Fig. 1a) indicates that the number and availability of lactonic and alcoholic/phenolic groups play a much more important role in the biosorption process than the basic groups. If the availability of functional groups is considered to be approximately the same for all three types of algae biomasses (due to their identical preparation procedure), then the greater the number of these functional groups, the higher the resulting biosorption capacities will be. Therefore, in the biosorption process, the presence of such weakly acidic functional groups (lactonic and alcoholic/phenolic), which remain protonated even under neutral conditions (Sîrbu *et al*., 2019), constitutes the active centers to which phosphate can bind on the algae biomass surface, predominantly through electrostatic interactions or hydrogen bonding.

Thus, at pH = 6.5, phosphate (present in aqueous solution predominantly as HPO_4^{2-}) will be electrostatically attracted to such protonated functional groups (Fig. 4) on the surface of the algae biomass (interaction (1)). Unfortunately, this type of electrostatic interaction is necessary, but not sufficient. For phosphate to be retained on the algae biomass surface through biosorption, these electrostatic interactions must be complemented by the formation of hydrogen bonds (interaction (2)), which can occur only when functional groups are positioned in geometrically favorable configurations (Fig. 4). Therefore, the number of such functional groups is important and contributes directly to enhancing the efficiency of the biosorption process.

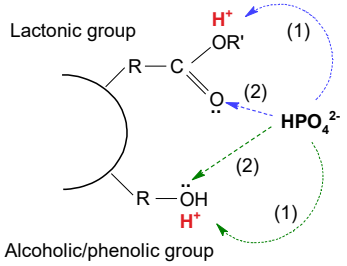


Fig. 4 – Schematic illustration of the possible interactions that occur during phosphate biosorption onto green marine algae biomass.

This is the reason why *Ulva intestinalis*, which contains the highest number of lactonic and alcoholic/phenolic groups (Fig. 2b), allows for more efficient retention of phosphate (Fig. 1) compared with *Ulva lactuca* (which due to the higher content of dissociated carboxylic groups (under these experimental conditions) generates electrostatic repulsion and limits the achievement of

type (1) interactions) and *Cladophora vagabunda* (where basic groups predominate, which prevent the formation of hydrogen bonds (type (2) interactions) (Fig. 4).

The retention of phosphate on the surface of the algae biomass through electrostatic interactions accompanied by hydrogen bonding is also supported by the experimentally measured values of pH, electrical conductivity (1/R), and turbidity at different initial phosphate concentrations (Fig. 3). Thus, it can be observed that, regardless of the type of algae biomass, increasing the phosphate concentration from 47.59 to 95.17 $mg \cdot L^{-1}$ (Fig. 3a) leads to an insignificant increase in the pH of the solution obtained after filtering the biosorbent. Thus, the pH increases by 0.99 units in the case of *Ulva lactuca*, by 0.29 units in the case of *Ulva intestinalis*, and by 0.32 units in the case of *Cladophora vagabunda***,** which further highlights that no interactions involving proton exchange occur during the phosphate biosorption process. Also, the small increase in electrical conductibility (1/R) by approximately 200 $\mu S \cdot cm^{-1}$ (200 $\mu S \cdot cm^{-1}$ for *Ulva lactuca*, 151 $\mu S \cdot cm^{-1}$ for *Ulva intestinalis*, and 201 $\mu S \cdot cm^{-1}$ for *Cladophora vagabunda*) (Fig. 3b) is most likely due to the higher concentration of ions in solution as the initial phosphate concentration increases, and is not a consequence of the biosorption process. Moreover, the insignificant variation in the turbidity of the solutions, regardless of the type of algae biomass or the phosphate concentration in solution (Fig. 3c), indicates that no secondary processes occur during the phosphate biosorption process (such as phosphate precipitation or the release of organic compounds from the algae biomass), and therefore no additional precautions are required.

All these experimental considerations show that green algae biomass can be used as a biosorbent for phosphate removal from aqueous solutions, and the main criterion for selecting the type of algae biomass that will ensure enhanced biosorption efficiency is the content of lactonic and alcoholic/phenolic groups in the biomass composition.

4. Conclusions

Three types of marine green algae biomass (*Cladophora vagabunda*, *Ulva lactuca* and *Ulva intestinalis*) were used for the biosorption of phosphate ions from aqueous solution, in this study. The evaluation of the three green algae biomasses at different initial phosphate concentrations (24-95 $mg \cdot L^{-1}$) demonstrates that the efficiency of the biosorption process depends on the type of algae biomass, increasing in the order: *Ulva intestinalis* > *Ulva lactuca* > *Cladophora vagabunda.* This variation of the biosorption capacity is mainly determined by the number and types of the functional groups on the surface of the algae biomass. Thus, the experimental results showed that the number and availability of lactonic and alcoholic/phenolic groups play a much more important role in the biosorption process than carboxylic or basic groups.

The presence of these functional groups on the surface of the algae biomass allows phosphate retention through electrostatic interactions accompanied by hydrogen bonds, a hypothesis also supported by the experimentally measured values of pH, electrical conductibility and turbidity, at different initial phosphate concentrations. All these results highlight one more time that green algae biomass can be used as a biosorbent for phosphate removal from aqueous solutions, and the main criterion for selecting the type of algae biomass that will ensure increased biosorption efficiency is the content of lactonic and alcoholic/phenolic groups in the biomass composition.

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ROLUL GRUPĂRILOR FUNCȚIONALE SUPERFICIALE ÎN DETERMINAREA EFICIENȚEI DE BIOSORBȚIE A FOSFAȚILOR PE ALGE VERZI MARINE

(Rezumat)

În acest studiu, trei tipuri de biomasă de alge verzi marine (*Ulva lactuca*, *Ulva intestinalis* și *Cladophora vagabunda*) au fost utilizate pentru biosorbția fosfatului din soluție apoasă. Aceste alge verzi marine sunt abundente în Marea Neagră și pot reprezenta o sursă ieftină și ușor accesibilă de biomasă regenerabilă. Eficiența celor trei tipuri de biomasă algală a fost testată la diferite concentrații inițiale de fosfat (24–95 $mg \cdot L^{-1}$), iar capacitatea de biosorbție crește în următoarea ordine: *Ulva intestinalis* > *Ulva lactuca* > *Cladophora vagabunda*. Această variație este determinată în principal de numărul și tipurile grupărilor funcționale de pe suprafața biomasei algale (analizate prin metoda Boehm) și poate fi considerată un criteriu important în selectarea biomasei de alge pentru biosorbția fosfatului. Pe lângă capacitatea de biosorbție a biomasei de alge pentru fosfat, au fost măsurate și pH-ul, conductivitatea electrică și turbiditatea soluțiilor obținute după filtrare. Toți acești parametri joacă un rol important în selectarea tipului adecvat de biomasă de alge pentru îndepărtarea fosfatului din medii apoase.