

Biosorption of Zn by dried biomass of *Euglena gracilis* from aqueous solutions

Linda Machalová¹, Tomáš Lempochner¹, Patrícia Marková², Martin Valica¹, Vanda Adamcová¹, Lenka Hutárová², Juraj Krajčovič², Miroslav Horník^{1,✉}

¹Institute of Chemistry and Environmental Sciences, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Nám. J. Herdu 2, Trnava 91701, Slovak Republic

²Institute of Biology and Biotechnology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Nám. J. Herdu 2, Trnava 91701, Slovak Republic

✉ Corresponding author: hornik@ucm.sk

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Abstract

The aim of this work was to evaluate the possibility to utilize the dried biomass of *Euglena gracilis* var. *bacillaris* as a biosorbent applied for the removal of Zn from aqueous solutions. For these purposes, experiments involving solutions spiked by ⁶⁵ZnCl₂ were carried out under the conditions of batch systems. The prepared biosorbent in the form of dried biomass of *E. gracilis* (< 300 μm) was characterized in terms of the presence of functional groups (COOH, PO₃H₂, OH, and NH₂), the concentration of binding sites c_{An} and the value of $pH_{zpc} = 6.6$ using the modelling program ProtoFit. From the kinetics of Zn biosorption, it can be assumed that the Zn removal was a rapid process, in which the concentration equilibrium $[Zn]_{\text{solution}} : [Zn]_{\text{biosorbent}}$ was stabilized in the first 5 min of interaction. In individual experiments, the effect of solution pH, initial biosorbent or Zn concentration were evaluated. Based on the MINEQL+ speciation modelling program, we predicted the decrease in the abundance of free Zn²⁺ cations in the presence of different concentrations of EDTA as a complexing agent in the solution. It was found that the Zn biosorption decreased linearly with the decreasing the proportion of Zn²⁺ in solution. The biosorption data expressed as equilibrium values of the remaining Zn concentration in solution C_{eq} (μmol.dm⁻³) and equilibrium values of specific adsorption Q_{eq} (μmol.g⁻¹; d.w.) were well fitted to the Langmuir model of adsorption isotherm in comparison with the Freundlich model. The maximum adsorption capacity of the dried biomass of *E. gracilis* for the biosorption of Zn reached the value $Q_{max} = 0.53 \pm 0.05$ mmol.g⁻¹ or 34.7 ± 3.4 mg.g⁻¹ (d.w.), respectively.

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Introduction

Heavy metal pollution poses serious health risks to living organisms and leads to deterioration of environmental quality as a result of the high toxicity of some heavy metals combined with their non-biodegradability, mobility, and ability to accumulate in living organisms and the food chain (Cheng *et al.* 2019). Heavy metals enter the water bodies from a variety of industrial activities, such

as minerals extraction, water treatment, metal molding, metal coating, batteries, agriculture, and power generation (Kamarudzaman *et al.* 2015). Huge amounts of toxic metals, such as As, Cd, Cu, Cr, Ni, Pb, and Zn are dumped into water assets without proper treatment (Dobrowolski *et al.* 2019). The highest permitted concentrations of Cd, Pb, Cu in drinking water is limited to 0.003, 0.01, and 2 mg.dm⁻³, respectively, by the World Health Organization (WHO 2011), and the concentration of Zn should not exceed 1 mg.dm⁻³ (Li *et al.* 2022). Among the heavy metals, zinc is of special interest, because of its high toxicity at certain concentrations and its wide range of industrial applications, such as electroplating, metal-finishing, mining and foundry activities, paint, pigments, ceramic industries, photographic paper, accelerators for rubber vulcanization, textiles, fertilizers, batteries, and accumulator manufacturing (Parab *et al.* 2006). Therefore, it is very important to treat the wastewater released from industries before it discharges into the environment and satisfy the environmental regulations for various bodies of water (Shanmugaprakash and Sivakumar 2015).

Traditionally, many technologies are available for zinc removal, including chemical precipitation, conventional coagulation, reverse osmosis, ionic exchange, membrane-based separation, and carbon adsorption, but these are frequently inefficient when applied for the removal of metal ions in low concentrations, and require costly equipment and high-cost operation and energy (Maiti *et al.* 2009). These methods often involve high capital and operational costs and may also be associated with the generation of secondary wastes, which present treatment problems. This has resulted in a need for innovative treatment technologies for trace metal removal (Parab *et al.* 2006).

The adsorption process has reached comparatively higher efficiency for the removal of contaminants. However, high costs of adsorbents have made the adsorption a use-limited method for industrial application (Gupta and Suhas 2009). This process also has several advantages, such as the ability to treat heavy metals that are present in low concentration (1 – 100 mg.dm⁻³), low operational costs, and regeneration ability (Mathew *et al.* 2016). Past research studies have discussed the use

of a range of adsorbents with different treatment capabilities (Pathirana *et al.* 2021).

One of the promising alternative methods and sustainable strategies is biosorption for cleaning up water that has been contaminated with heavy metals by anthropogenic activities and/or by natural processes, as it is effective, cheap, and eco-friendly (Kratochvil and Volesky 1998; Suazo-Madrid *et al.* 2011). Biosorption can be defined as the removal of metal or metalloid species, compounds, and particulates from solution by biological material (Gadd 1993). Different physico-chemical mechanisms involved in biosorption include microprecipitation, chelation, complexation, ion exchange, and physical adsorption (Cheng *et al.* 2019). The technology might overcome the disadvantages of conventional physico-chemical technologies with the advantages of reusability, low operating cost, short operation time, and without secondary pollution (Anastopoulos and Kyzas 2015; Ekere *et al.* 2016). During the last decade, a lot of reviews have been published that discuss the advantages and disadvantages of the application of biosorption in the removal of heavy metals (Beni and Esmaili 2020; Elgarahy *et al.* 2021; Priyadarshane and Das 2021; Ubando *et al.* 2021; Nateras-Ramírez *et al.* 2022; Syeda *et al.* 2022). Recently, there has been considerable interest in the use of microorganism-based biosorbent materials (Wang and Chen 2009). Various types of biosorbents have been developed from different raw biomass, such as bacteria (e.g. Xu *et al.* 2020), fungi (e.g. Lu *et al.* 2020), and algae (e.g. Liu *et al.* 2021).

Among the biosorbents, algae-based biosorbents in particular have been a focus of attention owing to their outstanding uptake capacity, the absence of toxic waste generation, low cost, time and energy saving, year-round occurrence, ease of handling, and great natural abundance in the environment, and renewability (Cheng *et al.* 2019). Microalgae are recognized as a multi-functional solution to contemporary concerns, such as carbon dioxide (CO₂) emissions (Xu *et al.* 2019), energy production (Suparmaniam *et al.* 2019), and wastewater treatment as well (García-Galán *et al.* 2020). Review studies focusing on various wastewater treatment capabilities of microalgae have been conducted as

elaborated in the following works (Abdel-Raouf *et al.* 2012; Souza *et al.* 2012; Acién *et al.* 2016; Ubando *et al.* 2021). It is generally known that the functional groups present in algae play an important role in the selective surface attachment of the solute ions (Jayakumar *et al.* 2022). There are significant differences in the adsorption capacity between microalgae species (Nateras-Ramírez *et al.* 2022). These differences are attributed to many factors, such as cell wall strength, polysaccharides, pectins, glycoproteins, shape, size, and functional groups that interact with pollutants (Kumar *et al.* 2015). Moreover, researchers in recent years have attempted to produce novel algal biosorbents by using molecular, chemical, and extraction approaches to improve metal-binding capacity and elucidate the inherent mechanisms (Cheng *et al.* 2019). In the scientific literature, we can find several papers that point out the possibility of algae-derived biosorbents using in the removal of Zn from model solutions (Areco *et al.* 2018; Giarikos *et al.* 2021; Gu and Lan 2021).

Only a limited number of papers have addressed the application of *Euglena* biomass in heavy metal removal. But these works have shown that *Euglena gracilis*, a free-living photosynthetic protist, is a promising model for heavy metal removal from polluted environments (García-García *et al.* 2016; Jasso-Chávez *et al.* 2021).

In the previous papers, the bioaccumulation and biosorption characteristics of dead and living non-growing biomass of bacteria isolated from spent nuclear fuel pools in binding ^{137}Cs , ^{60}Co , Cd, and Mn were studied and compared (Tišáková *et al.* 2013; Machalová *et al.* 2015; Pipiška *et al.* 2018). The main objective of this work was to characterize the dried biomass of *Euglena gracilis* var. *bacillaris* as a biosorbent for the removal of Zn from wastewaters or contaminated solutions. For these purposes, radiolabelling of solutions using the radioisotope ^{65}Zn and scintillation gamma-spectrometry were used. In the first step, the characterization of the prepared biosorbent was carried out using potentiometric titration and the modelling program ProtoFit. The effect of contact time, pH, biosorbent dose, and initial Zn concentration were investigated on the biosorption

efficiency for Zn removal. In individual experiments, the effect of Zn speciation changing in the solution given by the addition of EDTA- Na_2 and predicted using the modelling program MINEQL+ on the biosorption of Zn was also evaluated. The biosorption equilibrium and the maximum specific biosorption capacity were analysed by the application of Langmuir and Freundlich models of adsorption isotherms using a non-linear regression.

Experimental

Microalgal culture

Culture of *Euglena gracilis* var. *bacillaris* was statically cultivated in Hutner's medium (Hutner *et al.* 1956), pH 3.5 during 21 d under permanent illumination ($30 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and at 26 °C. The medium was sterilized at 121 °C for 20 min before use. After cultivation, the cells were collected by removing the medium by centrifugation (5 min at 4,500 rpm). To obtain the defined biomass with a particle size of $<300 \mu\text{m}$, the biomass was washed 5-times in deionized water ($0.05 \mu\text{S}\cdot\text{cm}^{-1}$) and allowed to dry at 60 °C for 48 h. Subsequently, the biomass was homogenized by grinding in a mortar with a pestle and sieving through the standardized sieves.

Biosorption experiments

Dried biomass of *E. gracilis* with a particle size $<300 \mu\text{m}$ (5 or 10 mg; d.w.) was added to a series of 50 cm^3 Erlenmeyer flasks containing 20 cm^3 of ZnCl_2 solution of known concentration in deionized water spiked with known initial activity of $^{65}\text{ZnCl}_2$ (40 – 50 $\text{kBq}\cdot\text{dm}^{-3}$). Incubation was carried out on a shaker (250 rpm) at 25 °C. Evaporation of water from the surface of the solution was prevented by covering the neck of the flask with parafilm. At time intervals and at the end of the exposure, samples of the solution were taken from the flasks. After separation of the biomass by centrifugation (5 min at 4,500 rpm) and subsequent filtering through the syringe filters (13 mm diameter; $0.45 \mu\text{m}$ permeability), the residual Zn concentration in the solution was calculated through the residual ^{65}Zn activity determined by scintillation gamma-

spectrometry. The amount of Zn adsorbed by the dried biomass of *E. gracilis* was calculated using Eq. 1:

$$Q = (C_0 - C_t) * \frac{V}{M} \quad (1)$$

where: Q – is the amount of Zn adsorbed by dried biomass of *E. gracilis* per unit mass of biomass [$\mu\text{mol.g}^{-1}$; d.w.]; C_0 and C_t – are the initial concentrations of Zn in the solution in time t_0 and at the time of sampling t [$\mu\text{mol.dm}^{-3}$]; V and M – is the volume of the solution [dm^3] and the amount of dried biomass of *E. gracilis* [g; d.w.].

In the evaluation of the effect of Zn speciation on its biosorption by dried biomass of *E. gracilis*, the molar amounts of the disodium salt of ethylenediaminetetraacetic acid (EDTA- Na_2) were added to 20 cm^3 of a solution containing 20 $\mu\text{mol.dm}^{-3}$ of ZnCl_2 to obtain the proportion of the Zn^{2+} form in the solution of 0 %, 20 %, 40 %, 60 %, 80 %, and the maximum achievable amount of Zn^{2+} at pH 4.0. The calculation of the molar addition of EDTA- Na_2 was carried out based on the prediction of the chemical speciation of Zn in the solution using the modelling program MINEQL+ ver. 4.62.3.

Mathematical models for the description of Zn biosorption

The adsorption isotherms of Langmuir (1918) and Freundlich (1906) were used to describe the biosorption of Zn by dried biomass of *E. gracilis*.

Langmuir model of adsorption isotherm is given by Eq. 2:

$$Q_{eq} = \frac{b * Q_{max} * C_{eq}}{1 + b * C_{eq}} \quad (2)$$

Freundlich model of adsorption isotherm is described by Eq. 3:

$$Q_{eq} = K * C_{eq}^{\frac{1}{n}} \quad (3)$$

where: Q_{eq} – is the equilibrium amount of sorbate (metal) bound on the surface of the adsorbent [$\mu\text{mol.g}^{-1}$; d.w.]; C_{eq} – is the equilibrium

concentration of sorbate (metal) in the solution [$\mu\text{mol.dm}^{-3}$]; Q_{max} – is the Langmuir constant characterizing the maximum adsorption capacity of the adsorbent for a given sorbate (metal) [$\mu\text{mol.g}^{-1}$; d.w.]; b – is the Langmuir constant describing the affinity between sorbate (metal) and adsorbent [$\text{dm}^3/\mu\text{mol}$]; K – is the Freundlich constant related to the adsorption capacity of the adsorbent for a given sorbate (metal) [dm^3/g ; d.w.]; n – is the Freundlich constant characterizing the adsorption intensity (dimensionless constant).

Potentiometric titration and prediction of pH_{zpc} values

Potentiometric titration was used to characterize the possible functional groups present on the surface of *E. gracilis* biomass and involved in metal biosorption following the procedure described by Zhang *et al.* (2010). The dried biomass (0.3 g) was quantitatively transferred into an Erlenmeyer flask containing 100 cm^3 of a mixture of a 0.1 mol.dm^{-3} HCl and NaCl and allowed to agitate on a shaker (250 rpm) at 25 °C for 2 h. After centrifugation (5 min at 4,500 rpm), the biomass was quantitatively transferred to 100 cm^3 of 0.1 mol.dm^{-3} NaCl solution and left to stir again under the above conditions. After 2 h of stirring, potentiometric titration was carried out directly in this suspension by the gradual addition of a solution of a mixture of 0.1 mol.dm^{-3} NaOH and NaCl. After each addition of this solution and after equilibrium reaching, the pH of the suspension was measured using a glass electrode (three-point calibration with solutions – pH 4.000, 7.000, and 10.000) over a pH range of 2.00 – 11.00 and at 25 °C. The relationship between the volume of titration solution added and the measured pH value was analysed using the modelling program ProtoFit ver. 2.1 (Turner and Fein 2006) to predict the pKa values of each functional group and the concentrations of binding sites (C_{An}) corresponding with the predicted functional groups on the biomass surface. This procedure also allowed the estimation of the pH_{zpc} (pH value of Zero Point of Charge) value which represents the pH at which the biomass has a net zero surface charge.

Cation Exchange Capacity

The determination of the cation exchange capacity (CEC) of dried biomass of *E. gracilis* was carried out by the procedure specified for the determination of soil CEC (STN ISO 11260). In the first step, 3 cm³ of 0.1 mol.dm⁻³ BaCl₂ was added to 0.250 g of dried biomass and the resulting suspension was allowed to stir (250 rpm) for 1 h at 25 °C. Subsequently, the biomass was centrifuged (5 min at 4,500 rpm) and the supernatant was decanted.

This procedure involves the addition of 3 cm³ of 0.1 mol/dm³ BaCl₂, agitation, centrifugation of the biomass, and decantation of the supernatant was repeated 2 more times. To the barium-saturated biomass sediment, 3 cm³ of 0.025 mol.dm⁻³ BaCl₂ was added, and the suspension was left to stir (250 rpm) for 19 h at 25 °C. Then, the biomass was centrifuged (5 min at 4,500 rpm) and the supernatant was decanted. To the centrifuged biomass, 3 cm³ of 0.02 mol.dm⁻³ MgSO₄ was added, and the resulting suspension was allowed to agitate (250 rpm) for 19 h at 25 °C. After centrifugation of the biomass (5 min at 4,500 rpm), Mg concentration in the obtained supernatant was determined chelatometrically using 0.02 mol.dm⁻³ EDTA-Na₂ (standardization with CaCO₃ and eriochrome black T as an indicator at pH 10.0 achieved with ammonia buffer).

The CEC value of the biomass was calculated using the following Eq. 4:

$$CEC = \frac{(M_0V_0 - M_SV_S) * F}{10^{-3}} \quad (4)$$

where: *CEC* – is the cation exchange capacity [meq/100 g]; *M*₀ – is the concentration of Mg added to the sample [mol.dm⁻³]; *V*₀ – is the volume of Mg solution added to the sample [dm³]; *M*_S – is the concentration of Mg determined in the supernatant [mol.dm⁻³]; *V*_S – is the volume of the supernatant [dm³]; *F* – is the conversion factor (in the case of divalent ion and 0.250 g weight, *F* = 800 meq (100 g.mol⁻¹)).

Scintillation gamma-spectrometry

A gamma-spectrometric scintillation detector 76BP76/3 (Envinet, Czech Republic) with well type crystal NaI(Tl) was used for the determination

of ⁶⁵Zn activity in the sampled solutions and in the biomass of *E. gracilis*. Spectrometric analysis and calculation of activity were carried out by the data processing software ScintiVision-32 (Ortec, USA). To calibrate the energies and efficiencies of the ⁶⁵Zn activity measurements, a library including characteristic γ -photon peaks of the following radionuclides was built: ¹⁰⁹Cd (*E* _{γ} = 88.04 keV), ¹³⁷Cs (*E* _{γ} = 661.66 keV), and ⁶⁵Zn (*E* _{γ} = 1,115.52 keV). For the calibration, standard solutions of the above radionuclides with known activities to the date of determination of the samples with respect to their half-lives (¹⁰⁹Cd – *T*_{1/2} = 462.6 d, ¹³⁷Cs – *T*_{1/2} = 11,019 d, and ⁶⁵Zn – *T*_{1/2} = 243.9 d) were used.

Prediction of Zn speciation and statistical analysis of the data

To calculate the percentage proportion of Zn²⁺ form in the model solutions, the speciation modelling program MINEQL+ ver. 4.62.3 (Environmental Research Software, USA) was used. This program, within the mathematical iterations, applies the USEPA database of thermodynamic constants and predicts the distributions of individual chemical forms of metals in solution depending on the solution pH, temperature, ionic strength, concentration of cations or anions, and for the carbonate system naturally occurring in equilibrium with atmospheric CO₂ (pCO₂ = 38.5 Pa).

For graphical evaluation of the results and statistical analysis of the data obtained, the program OriginPro ver. 8.5 (OriginLab Corporation, USA) was applied.

The estimation of the goodness of fit of the applied models of adsorption isotherms (Eq. 2 and Eq. 3) to the obtained data was evaluated using coefficients of determination (*R*²) (Eq. 5) and the corrected Akaike information criterion (*AIC*_C) (Eq. 6).

$$R^2 = 1 - \frac{\sum_{i=1}^N (Q_{pred} - Q_{exp})^2}{\sum_{i=1}^N (Q_{pred} - \bar{Q}_{med})^2} \quad (5)$$

$$AIC_C = N \cdot \ln \left(\frac{\sum_{i=1}^N (Q_{exp} - Q_{pred})^2}{N} \right) + 2p + \frac{2p(p+1)}{N-p-1} \quad (6)$$

where: N – is the number of obtained data; Q_{exp} – is the obtained experimental Q values [$\mu\text{mol.g}^{-1}$; d.w.]; Q_{pred} – is the predicted Q values by the models of adsorption isotherms [$\mu\text{mol.g}^{-1}$; d.w.]; Q_{med} – is the mean of the obtained experimental Q values [$\mu\text{mol.g}^{-1}$]; p – is the number of fit parameters.

Results and Discussion

Biosorbent characterization

Before the realization of the biosorption experiments, we focused on the physico-chemical characterization of dried biomass of *E. gracilis* as a potential metal biosorbent. From the point of view of the biosorption of metal cations or cationic xenobiotics, it is important to identify the active binding sites in the form of the quantitative and qualitative representation of negatively charged functional groups present on the adsorbent surface that are responsible for cations binding. To find out the qualitative presence of functional groups on the surface

of biosorbents, FT-IR analysis is often applied in published scientific papers (see e.g. Giri *et al.* 2021; Ajala *et al.* 2022). However, there are many papers in which authors have either used potentiometric titration or applied this method in combination with FT-IR analysis in order to identify functional groups on the surface of biosorbents that play an important role in biosorption processes (Martín-Lara *et al.* 2016; Wang *et al.* 2022).

For the qualitative characterization of the functional groups and to determine the concentrations of binding sites c_{An} for each predicted functional group type on the surface of the dried biomass of *E. gracilis*, potentiometric titration with the application of the modelling program ProtoFit ver. 2.1 was used. Fig. 1A shows a curve describing the dependence between the volume of 0.1 mol.dm^{-3} NaOH solution added and the measured pH value of the solution containing the dried biomass of *E. gracilis*. As can be seen, in the first stage of the titration, there was a significant consumption of NaOH solution with minimal change in the pH value of the solution. This points to the fact that significant dissociation

of functional groups in the form of reactions, such as $\text{COOH} \rightarrow \text{COO}^- + \text{H}^+$ or $\text{R-O-PO}_3\text{H}_2 \rightarrow \text{R-O-PO}_3\text{H}^- + \text{H}^+$, lead to the release of H^+ cations and occur in a given pH range. From $\text{pH} \sim 4$, a significant change in the pH of the solution can be observed with the addition of NaOH solution. It indicates the significant presence of relevant, especially acidic functional groups, present only in the form of free dissociated groups. The potentiometric titration expressed in the form of the first derivation according to Gran (Fig. 1B) revealed 4 inflection points representing the pH values at which a significant dissociation of functional groups occurs.

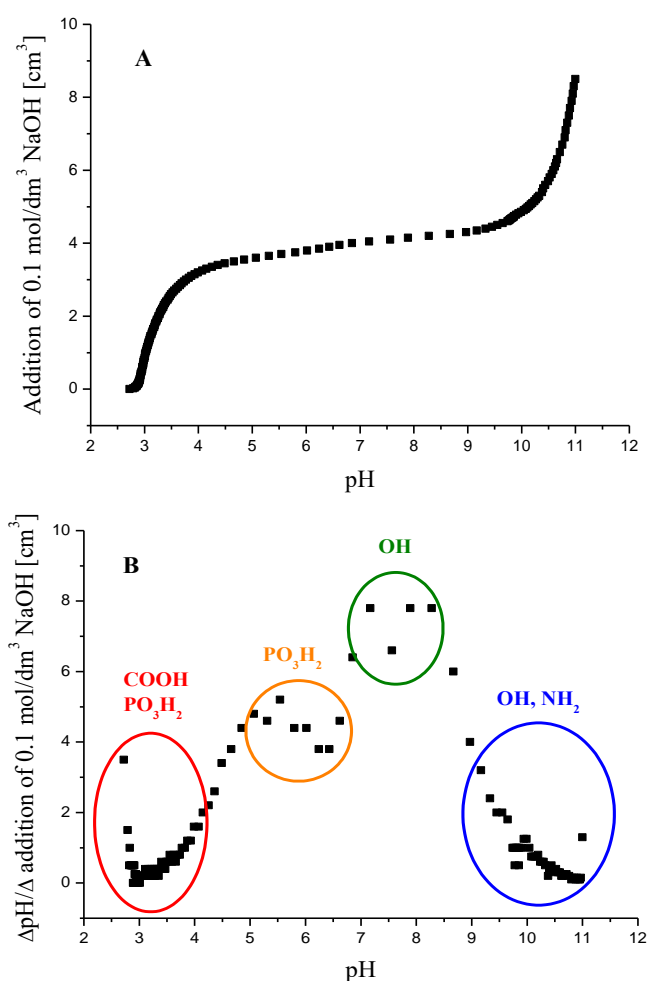


Fig. 1. **A** – Curve describing the dissociation of functional groups on the surface of dried biomass of *E. gracilis* (3.0 g.dm^{-3}) carried out by potentiometric titration through the sequential addition of 0.1 mol.dm^{-3} NaOH, at the background electrolyte of 0.1 mol.dm^{-3} NaCl and $25 \text{ }^\circ\text{C}$. **B** – Potentiometric titration expressed as first derivation according to Gran with identification of potential functional groups on the surface of dried biomass of *E. gracilis*.

Based on the pH values of these inflection points, functional groups such as COOH, PO₃H₂, OH, and NH₂, can be predicted. The mentioned potentiometric curve provides the necessary primary data characterizing the nature of the surface of dried biomass of *E. gracilis*, which in the next step of prediction of the functional groups as well as the concentration of binding sites c_{An} using the modelling program ProtoFit were applied.

The ProtoFit program also allowed for prediction of the pH_{zpc} value representing the pH value, at which the net surface charge of the biosorbent is equal to zero in terms of the dissociation of the relevant functional groups. It is valid that the adsorbent or biomass surface has an overall positive charge if the pH of the environment is lower than the pH_{zpc} value. Conversely, if $pH > pH_{zpc}$, the surface of the adsorbent or biomass shows an overall negative charge (Sun *et al.* 2011). Table 1 summarizes the predicted functional groups and concentrations of binding sites c_{An} for each type of functional group occurring on the surface of dried biomass of *E. gracilis*, as well as the predicted pH_{zpc} value using the modelling program ProtoFit.

Table 1. Prediction of the presence of functional groups, concentration of binding sites c_{An} and pH_{zpc} values for the particle surface (< 300 μm) of dried biomass of *E. gracilis* using the modelling program ProtoFit ver. 2.1. For details see Fig. 1.

Functional group	c_{An} [mmol.g ⁻¹]	pH_{zpc}
COOH, PO ₃ H ₂	0.209	6.57
PO ₃ H ₂	0.079	
OH	0.016	
NH ₂ , OH	0.204	

Another important parameter that describes the ability of the adsorbent to bind ionic forms of organic compounds or metals is the determination of the cation exchange capacity (CEC) value. CEC values are commonly determined for soil samples in terms of assessing the binding and mobility of metal cations and are commonly expressed in milliequivalents of exchangeable cations per 100 grams of dry sample. Thus, it quantitatively describes the ability of the given material to bind the cations. In the case of clay-rich or organic soils, it takes values up to 20 meq/100 g. For sandy soils, which have a low

capacity to bind cations, it is only 2 to 3 meq/100 g. In the case of the dried biomass of *E. gracilis*, it was found that the CEC value was 9.8 meq/100 g (d.w.).

Biosorption experiments

Contact time and pH

In general, it is known that the removal of contaminants by biological systems can occur through three different processes, namely (i) biosorption, a metabolism-independent process of contaminants binding onto the cell surface, (ii) bioaccumulation (entry of contaminants into the cell interior mediated by passive or active transport mechanisms), and (iii) biodegradation (degradation of organic compounds by extracellular or intracellular enzymes). The latter two mechanisms are metabolism-dependent.

Since biosorption is a time-dependent process, the evaluation of the time course as well as the determination of the equilibrium time i.e., the time when equilibrium is established, is one of the essential steps in the description of biosorption processes. This is also due to the fact that in the evaluation of the effects of various factors on biosorption processes, it is necessary to carry out experiments under concentration equilibrium conditions $[\text{sorbate}]_{\text{biosorbent}}: [\text{sorbate}]_{\text{solution}}$.

Fig. 2A shows the kinetics of Zn biosorption by dried biomass of *E. gracilis* under conditions of batch systems. The kinetic data were fitted using a pseudo- n^{th} -order kinetic model (Özer 2007) in the form of the following equation (Eq. 7):

$$Q_t = Q_{eq} - \left[(n - 1) * k_n * t + Q_{eq}^{(1-n)} \right]^{1/(1-n)} \quad (7)$$

where: Q_t – is the amount of sorbate (metal) bound on the surface of the adsorbent at the time of sampling t [μmol.g⁻¹; d.w.]; Q_{eq} – is the equilibrium amount of sorbate (metal) bound on the surface of the adsorbent [μmol.g⁻¹; d.w.]; n – order of the adsorption reaction; k_n – rate constant of pseudo- n^{th} -order equation [kgⁿ⁻¹g¹⁻ⁿ/min]; t – adsorption time [min].

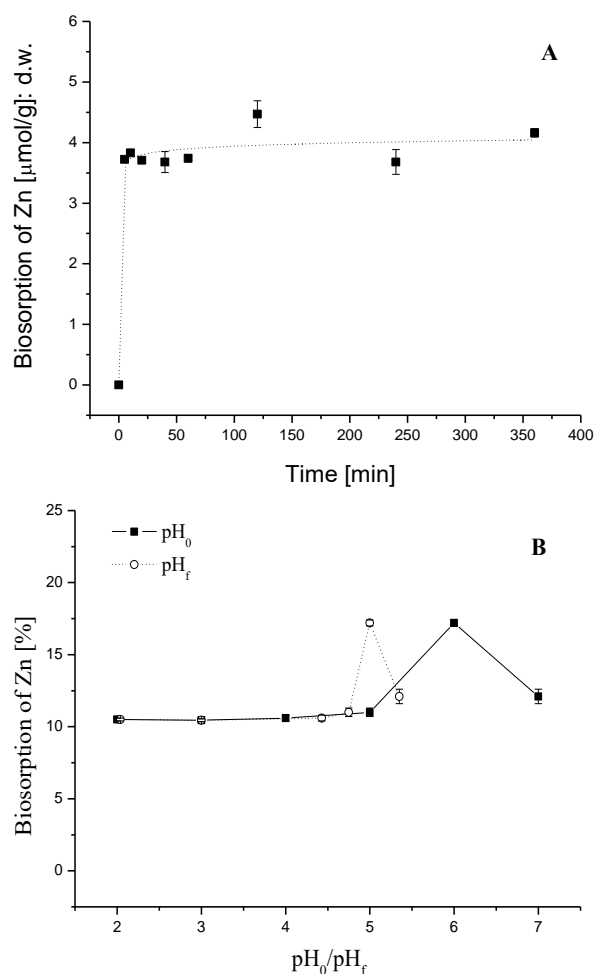


Fig. 2. A – Kinetics of Zn biosorption by dried biomass of *E. gracilis* (biomass concentration $C_B = 0.5 \text{ g.dm}^{-3}$) from solutions of $11.6 \mu\text{mol.dm}^{-3} \text{ ZnCl}_2$ in deionized water (pH 4.0). **B** – Effect of initial (pH_0) and final (pH_f) pH values on Zn biosorption by dried biomass of *E. gracilis* ($C_B = 0.5 \text{ g.dm}^{-3}$) from solutions of $14.8 \mu\text{mol.dm}^{-3} \text{ ZnCl}_2$ in deionized water after 2 h of exposure. The description of the kinetic data was carried out using a pseudo- n^{th} -order equation. Error bars represent standard deviation of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

From the kinetics of Zn biosorption, it is evident that it was a rapid process, and during the exposure time of 360 min presented typical saturation kinetics with two distinguishable phases: (i) fast initial phase (up to the first 5 min of exposure to Zn^{2+}), and (ii) phase of reaching the concentration equilibrium $[\text{Zn}]_{\text{solution}} : [\text{Zn}]_{\text{biosorbent}}$ (5 – 360 min of exposure to Zn^{2+}). After only 5 min of interaction, 16 % ($3.72 \pm 0.01 \mu\text{mol.g}^{-1}$; d.w.) of the total initial amount of Zn in the solution was adsorbed by dried biomass. Similar results were obtained by Wang *et al.* (2021) when monitoring the kinetics of Cd biosorption by the *Chlorella vulgaris* K-01

strain. Also, Pozdniakova *et al.* (2016) found that concentration equilibrium for the kinetics of Zn biosorption by biomass of 4 species of brown seaweeds (*Laminaria hyperborea*, *Pelvetia canaliculata*, *Ascophyllum nodosum*, *Fucus spiralis*) at pH 6.0 and $25 \text{ }^\circ\text{C}$ was established at times of 14, 19, 23, and 42 min, respectively.

An important factor affecting biosorption processes is the pH value of the environment, which has an effect on the adsorption capacity of a biosorbent in terms of changing the dissociation of functional groups on its surface. It can be assumed that at lower pH values, the biomass surface will have a net positive charge due to the protonation of functional groups, while the H^+ ions present will act competitively to the adsorption of positively charged molecules or metal cations. Conversely, at higher pH values, deprotonation or dissociation, especially of negatively charged functional groups (e.g., COOH , PO_3H_2), will occur to a greater extent. In this case, the biosorbent will show a negative charge and a higher adsorption capacity for positively charged molecules or metal cations.

The effect of initial pH_0 in the range 2 – 7 on Zn biosorption by dried biomass of *E. gracilis* expressed as a percentage of the total Zn in solution is depicted in Fig. 2B. It was found that the highest values of Zn biosorption ($4.13 \pm 0.05 \mu\text{mol.g}^{-1}$; d.w.) were observed at pH 6, while lower values of Zn biosorption ($2.60 \pm 0.04 \mu\text{mol.g}^{-1}$; d.w.) were confirmed at other applied pH values. A similar dependence was confirmed by Ferreira *et al.* (2011) when investigating the effect of pH values ranging from 3 to 6 on the biosorption of Ni^{2+} , Zn^{2+} , and Pb^{2+} by dried biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*. They found that the maximum values of specific biosorption were described for both organisms at pH 5. Li *et al.* (2022) also reported that the biosorption capacities of Cd^{2+} , Pb^{2+} , Cu^{2+} , and Zn^{2+} by three biosorbents (derived from the biomass of three phosphorus-accumulating organisms: *Ochrobactrum cicero*, *Stenotrophomonas maltophilia*, and *Pseudomonas putida*) ascended with increasing pH from 2.0 to 5.0 and then reached a stable level when pH further increased to 6.0.

The decrease in Zn biosorption by dried biomass of *E. gracilis* at the highest pH 7 can be explained based on two facts. The first is the fact that the

dried biomass of *E. gracilis* shows a pH_{zpc} value of about 6.6, which would suggest that at this pH value, the biomass exhibits zero surface charge. A second explanation of this phenomenon is the speciation of Zn, defined as the occurrence or proportion of the various chemical forms of Zn in solution under the given conditions. It is well known that in the biosorption of metals, their speciation plays an important role, with M^{2+} cationic forms representing the most available forms for their binding to the relevant functional groups occurring on the biosorbent surface. Based on the speciation modelling program MINEQL+ ver. 4.62.3, it was found that under the above conditions, Zn was dominantly present in the Zn^{2+} form (>98 % of the total Zn amount), with a minor occurrence of $ZnOH^+$ or $Zn(OH)_2$ forms. However, it should be noted that in the prediction of Zn speciation, the possibility of the release of substances showing the complexing properties for the formation of organic or inorganic complexes with Zn from the dried biomass of *E. gracilis* was not taken into account.

Fig. 2B also shows the effect of the equilibrium (final) pH_f values i.e., the solution pH values measured at the time of reaching the biosorption equilibrium, on the Zn biosorption. As can be seen, the initial value of $pH_0 = 6 - 7$ decreased towards the equilibrium value of $pH_f = 5$ during the experiments. On the other hand, for an initial $pH_0 = 4$, the pH value increased towards the equilibrium value of $pH_f = 5$. This phenomenon can be explained by the release of substances from the dried biomass of *E. gracilis* that have the ability to buffer the surrounding environment. This fact also correlates with the predicted value of $pH_{zpc} = 6.6$.

Zn speciation

As it was mentioned, the pH of the solution also has effect on the Zn speciation. The evaluation of this factor is particularly important because, in the case of real contaminated waters or wastewaters, it is necessary to take into account the existence of different chemical forms of metals. It is due to the presence of a wide variety of elements, but especially substances that can show

complexing properties and form more or less soluble complexes with target metal ions.

To confirm the effect of Zn speciation on its biosorption, the design of the experiment consisted of a series of solutions containing different proportions of Zn^{2+} form. For this purpose, the complexing (chelating) agent EDTA- Na_2 (disodium salt of ethylenediaminetetraacetic acid) was added in such molar amounts to $20 \mu\text{mol}/\text{dm}^3$ $ZnCl_2$, at which it was possible to achieve the proportion of Zn^{2+} form in the solution of 0 %, 20 %, 40 %, 60 %, 80 %, and the maximum achievable amount of Zn^{2+} at pH 4.0. The calculation of the molar addition of EDTA- Na_2 was carried out based on the prediction of the chemical speciation of Zn in solution using the modelling program MINEQL+ ver. 4.62.3 for pH = 4.0 and 25 °C.

Fig. 3A depicts the dependence between the molar addition of EDTA- Na_2 and the proportion of each chemical form of Zn occurring in a solution containing $20 \mu\text{mol}/\text{dm}^3$ $ZnCl_2$ at pH 4.0 and 25 °C. It was found that the proportion of Zn^{2+} form decreased linearly with increasing the concentration of EDTA- Na_2 in the solution, in favour of the formation of $Zn-H_2EDTA$, $Zn-EDTA^{2-}$, $Zn-HEDTA^-$ or $ZnCl^+$ complexes, respectively. The significance of the percentage of the mentioned Zn complexes in the solution decreased in the order: $Zn-EDTA^{2-} \gg Zn-HEDTA^- > Zn-H^2EDTA \geq ZnCl^+$.

The data describing the effect of increasing EDTA- Na_2 concentration on Zn^{2+} proportion in solution and Zn biosorption by dried biomass of *E. gracilis* indicate that the biosorption of the free ionic form of Zn^{2+} will be the dominant fraction in terms of Zn binding onto the biosorbent used (Fig. 3B). It was found that as the concentration of EDTA- Na_2 increased, the percentage of Zn^{2+} in solution decreased linearly, which led to a linear decrease in Zn biosorption. At almost 100 % initial presence of the Zn^{2+} form in solution, a maximum biosorption of $17.9 \pm 3.4 \mu\text{mol}/\text{g}^{-1}$ (d.w.) was observed in this series. At the minimum presence of Zn^{2+} (0.7 %) in solution, a more than 3-fold lower value of specific Zn biosorption ($5.5 \pm 1.0 \mu\text{mol}/\text{g}^{-1}$; d.w.) was found. Similar findings were described by Šušnovská *et al.* (2016), when studying

the effect of Cd speciation or percentage of Cd^{2+} form in model solutions on Cd biosorption by dried biomass of the moss *Vesicularia dubyana*. They found that with the increasing addition of NaCl to the solution, the initial presence of Cd^{2+} in the solution decreased in favor of CdCl^+ formation, which also resulted in a decrease in Cd biosorption.

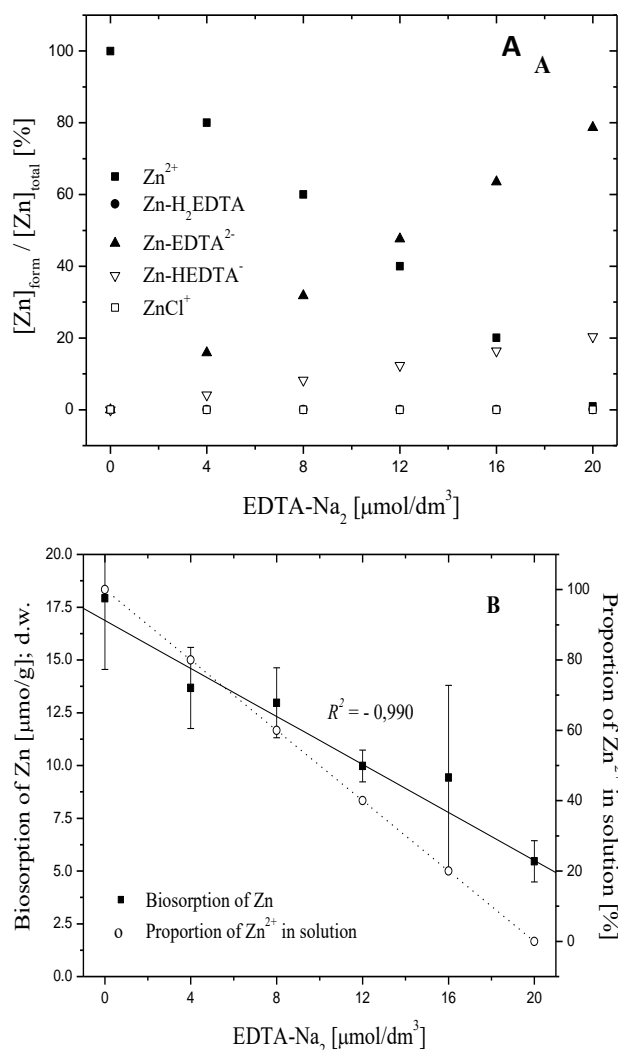


Fig. 3. A – Proportion of chemical forms of Zn in model solutions containing $20 \mu\text{mol}\cdot\text{dm}^{-3}$ ZnCl_2 without or with the addition of 4, 8, 12, 16, or $20 \mu\text{mol}\cdot\text{dm}^{-3}$ EDTA- Na_2 . **B** – Effect of Zn speciation on Zn biosorption by dried biomass of *E. gracilis* ($C_B = 0.5 \text{ g}\cdot\text{dm}^{-3}$) from model solutions containing $20 \mu\text{mol}\cdot\text{dm}^{-3}$ ZnCl_2 without or with the addition of 4, 8, 12, 16, or $20 \mu\text{mol}/\text{dm}^3$ EDTA- Na_2 after 2 h of exposure. The decrease in Zn biosorption as a function of Zn speciation is described by a linear regression. Error bars represent standard deviation of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

Biosorbent concentration

Another parameter evaluated was the effect of a biosorbent concentration amount in the range of $0.25 - 2.5 \text{ g}\cdot\text{dm}^{-3}$ on Zn biosorption by dried biomass of *E. gracilis*. From the results in Fig. 4, it can be seen that the specific biosorption of Zn expressed in $\mu\text{mol}\cdot\text{g}^{-1}$ (d.w.) decreased with increasing biosorbent concentration, while the amount of Zn adsorbed expressed as % of the initial amount of Zn in solution increased slightly with increasing biosorbent concentration. Mehta and Gaur (2005) explain this phenomenon by several explanations, namely, (i) limiting the sorbate (metal) concentration; (ii) increasing electrostatic interactions or interferences between active binding sites on the biosorbent surface; or (iii) decreasing the mixing efficiency of the batch system at higher biosorbent concentrations. According to Kumar and Porkodi (2007), in systems with higher concentrations of biosorbent particles can be also observed, the negative aggregation and change in the specific surface area ($\text{m}^2\cdot\text{g}^{-1}$; d.w.) as well as change in the conditions for its effective mixing.

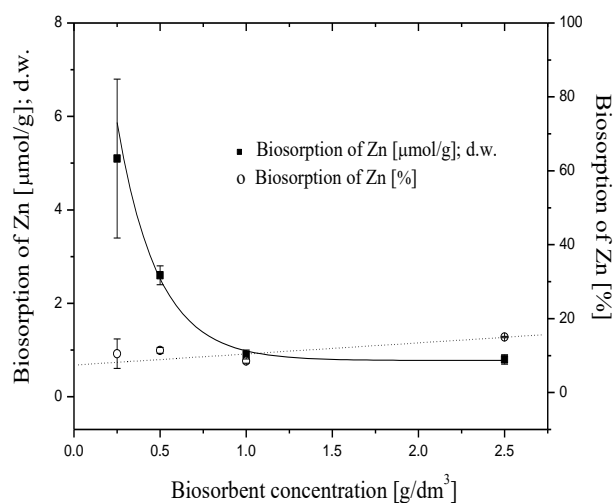


Fig. 4. Effect of biosorbent concentration in the batch system on Zn biosorption by dried biomass of *E. gracilis* from solutions of $12.2 \mu\text{mol}\cdot\text{dm}^{-3}$ ZnCl_2 in deionized water (pH 4.0) after 2 h of exposure. The description of the biosorption data was carried out using an exponential decay function (first order) and linear regression, respectively. Error bars represent standard deviation of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

Initial concentration of Zn

The biosorption processes, in terms of adsorption capacities, are also strongly affected by the initial concentration of metal in solution. For this reason,

a series of experiments aimed at monitoring the biosorption of Zn by dried biomass of *E. gracilis* at an initial Zn concentration ranging from 2.45 to 738 $\mu\text{mol}\cdot\text{dm}^{-3}$ were realized. Fig. 5 shows that as the initial Zn concentration in solution increased, the percentage of Zn adsorbed from its initial amounts (biosorption efficiency) decreased significantly. On the other hand, the specific biosorption expressed in μmol of Zn per gram of dried biomass increased with the initial Zn concentration in solution and reached a saturation value. Similar results were observed by Ferreira *et al.* (2011) when investigating the effect of initial Ni^{2+} , Zn^{2+} , and Pb^{2+} concentrations in the range of 0.5 – 3 $\text{mmol}\cdot\text{dm}^{-3}$ on the biosorption of metal cations by dried biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*. For both microorganisms, the amount of Ni^{2+} , Zn^{2+} , and Pb^{2+} adsorbed and expressed in % of the total metal in solution decreased with increasing initial metal concentration, but specific biosorption expressed in $\text{mmol}\cdot\text{g}^{-1}$ (d.w.) increased. In this respect, it is known that at lower Zn concentrations, practically all metal ions present in solution can interact with sufficient active binding sites, and therefore biosorption increases. At higher metal concentrations, lower biosorption efficiencies are observed due to the saturation of the active binding sites, which is characterised by the presence of relevant functional groups.

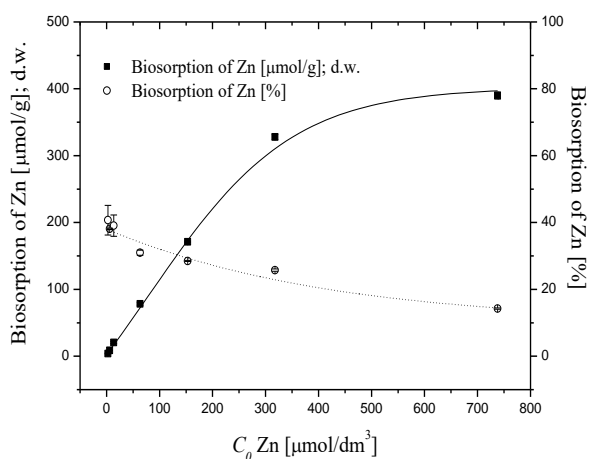


Fig. 5. Effect of initial Zn concentration (C_0) on Zn biosorption by dried biomass of *E. gracilis* ($C_B = 0.25 \text{ g}\cdot\text{dm}^{-3}$) from ZnCl_2 solutions in deionized water (pH 4.0) after 2 h of exposure. The description of the biosorption data was carried out using a Boltzmann sigmoidal equation. Error bars represent standard deviation of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

Adsorption isotherm models

The previous experiments were carried out under conditions of concentration equilibrium. Therefore, the results obtained evaluating the effect of initial Zn concentration on Zn biosorption by dried biomass of *E. gracilis* can be expressed as equilibrium values of the remaining Zn concentration in solution C_{eq} ($\mu\text{mol}\cdot\text{dm}^{-3}$) and equilibrium values of specific adsorption Q_{eq} ($\mu\text{mol}\cdot\text{g}^{-1}$; d.w.) and described by the adsorption isotherms. Equilibrium adsorption isotherms play an important role in predictive modelling in order to analyse the biosorption processes and design the biosorption systems. Adsorption isotherms are considered appropriate to understand the way that metal ions bind on the surface of adsorbents (Ahmed *et al.* 2021). Parameters obtained from mathematical models provide useful information on the affinity of biosorbent to sorbate and allow the quantification of the adsorption capacity of the biosorbent used. For the mathematical description of Zn biosorption by dried biomass of *E. gracilis* the two most commonly used adsorption isotherms, namely the Langmuir model and the Freundlich model of adsorption isotherms (Fig. 6) in their non-linear forms (Eqs. 2 and 3), were applied. The Langmuir adsorption model describes monolayer adsorption on the adsorbent, and the Freundlich model depicts heterogeneous, non-ideal, and multi-layer adsorption (Li *et al.* 2019).

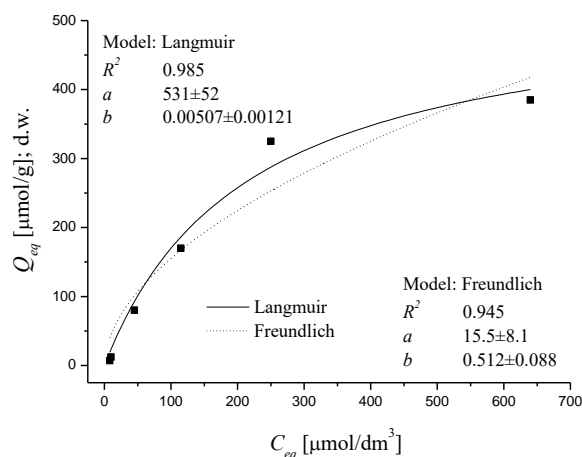


Fig. 6. Description of Zn biosorption by dried biomass of *E. gracilis* ($C_B = 0.25 \text{ g}\cdot\text{dm}^{-3}$) using adsorption isotherms according to Langmuir (1918) and Freundlich (1906). Details of the experiments are described in Fig. 5. Error bars represent standard deviation of the arithmetic mean of the results of three independent experiments ($\pm\text{SD}$; $n = 3$).

As stated by most authors, the use of adsorption isotherms in their non-linear forms is preferable to the description of the obtained data using their linearized forms, due to the fact, that

the linearization of the isotherm equations may change the distribution of errors after transforming the data to the linear form (Ncibi *et al.* 2009).

Table 2. The obtained parameters of adsorption isotherms according to Langmuir (1918) and Freundlich (1906). Details of the experiment are described in Fig. 5.

Langmuir adsorption isotherm	Q_{max} [$\mu\text{mol.g}^{-1}$]	b [$\text{dm}^3.\mu\text{mol}^{-1}$]	R^2	AIC_C	Akaike's weight
	531 ± 52	0.00507 ± 0.00121	0.985	53.83	0.9822
Freundlich adsorption isotherm	K [$\text{dm}^3.\text{g}^{-1}$]	n	R^2	AIC_C	Akaike's weight
	15.5 ± 8.1	1.95 ± 0.31	0.945	61.85	0.0178

In the evaluation of the suitability of the given adsorption isotherm model, the coefficients of determination R^2 and the corrected Akaike information criterion AIC_C were used (Table 2). The adsorption isotherm model with the higher R^2 value and the lower AIC_C value is considered most likely to be correct. As can be seen from Fig. 6, the Zn biosorption process was better described by the Langmuir isotherm ($R^2 = 0.985$; $AIC_C = 53.83$) compared to the Freundlich isotherm ($R^2 = 0.945$; $AIC_C = 61.85$). More suitability of the Langmuir model than the Freundlich model indicates a monolayer adsorption of Zn on the dried biomass of *E. gracilis* as a homogeneous surface.

Table 2 shows the parameters obtained from both non-linear adsorption isotherm equations.

The most interesting of these is the parameter from the Langmuir equation of adsorption isotherm Q_{max} ($\mu\text{mol.g}^{-1}$; d.w.), which characterizes the maximum achievable specific biosorption. The mentioned parameter is important from the point of view of the comparison of biosorbents and of the evaluation of the affinity of different biosorbents for the sorbates.

Application potential of prepared biosorbent

The maximum removal capacity Q_{max} value of the dried biomass of *E. gracilis* for the biosorption of Zn was compared with the results reported by other authors using different biosorbents and is presented in Table 3.

Table 3. Comparison of Q_{max} values quantifying Zn biosorption by biosorbents derived from bacterial, algal or moss biomass and by activated carbon as a standard adsorbent.

Sorbent	Biosorption		Reference
	[mmol.g^{-1}]	[mg.g^{-1}]	
<i>Chlorella vulgaris</i> (microalgae)	0.66	43.2	Ferreira <i>et al.</i> (2011)
<i>Ochrobactrum cicero</i>	0.97	63.1	Li <i>et al.</i> (2022)
<i>Stenotrophomonas maltophilia</i>	0.98	64.0	
<i>Pseudomonas putida</i> (bacteria)	0.96	62.9	
<i>Euglena gracilis</i> (microalgae)	0.53 ± 0.05	34.7 ± 3.4	This work
<i>Sargassum</i> sp. (seaweed – macroalgae)	0.50	32.7	Sheng <i>et al.</i> (2004)
<i>Polytrichum commune</i> (moss)	0.40	26.4	Smolyakov <i>et al.</i> (2017)
XSBLAC	1.59	103.8	Zhang <i>et al.</i> (2017)

*XSBLAC – mesoporous activated carbon prepared from *Xanthoceras Sorbifolia Bunge* hull via chemical activation (Zhang *et al.* 2017).

From this comparison, it can be concluded that the dried biomass of *E. gracilis* shows a great potential for the removal of Zn from aqueous solutions. It shows good sorption characteristics not only in comparison with other biosorbents, but also with activated carbon as a standard adsorbent. At this point, it should be mentioned that in the experiments performed, we worked with very high ratios between the applied amount of biosorbent and the volume of the model solution (1 : 2,000 to 1 : 4,000). Despite this fact, we achieved a considerable percentage removal of Zn.

The blooming interest in algae as a biosorbent is mainly due to their widespread availability, renewability, sustainability, absence of arable land requirements, and deemed good cost-effectiveness (Sandau *et al.* 1996; Sudhakar and Viswanaathan 2019; Fabris *et al.* 2020). As Carreira *et al.* (2023) stated in their review, studies on algae screening for metal sorption are prompted by their distinct characteristics, which ultimately lead to different metal sorption capacities.

The scientific community has only minimally addressed (no more than ten papers) the application of algal biomass of the genera *Euglena* as a metal biosorbent, much less in the removal of Zn. This work provides pioneering results in this field. The clear confirmation of the advantages of the application of *E. gracilis* biomass as a biosorbent for zinc and other metals in practice will require further detailed research in the future, which will focus in particular on the following points: (i) recycling and reuse of the biosorbent; (ii) evaluation of the selectivity for Zn removal or interaction with other metals, especially in the environment of real wastewater or contaminated water; and (iii) application of the biosorbent under the conditions of continuous flow column systems, which are in practice more effective and more frequently used.

Conclusion

In this work, the biosorption characteristics of the dried biomass of *E. gracilis* for the removal of Zn from aqueous solutions were investigated through batch biosorption experiments. In the first step, the prepared biosorbent (dried biomass of *E. gracilis* with a particle size of <300 μm) was subjected to

physico-chemical characterization. Based on potentiometric titration and using ProtoFit software, it was found that the surface of prepared biosorbent shows the significant presence of functional groups, such as COOH, PO₃H₂, OH, and NH₂. Suitable adsorption properties were also confirmed by the values of the parameters $pH_{zpc} = 6.6$ and CEC = 9.8 meq/100 g (d.w.). Biosorption of Zn by dried biomass of *E. gracilis* was evaluated as a function of exposure time, solution pH, and initial biosorbent or Zn concentration. Separate attention has been focused on the effect of Zn speciation on its biosorption. It was found that as the concentration of EDTA-Na₂ increased, the percentage of Zn²⁺ in solution decreased linearly, in favour of the formation of Zn-H₂EDTA, Zn-EDTA²⁻, Zn-HEDTA⁻, or ZnCl⁺ complexes, and this led to a corresponding linear decrease in Zn biosorption. The biosorption data were well fitted to the Langmuir model of adsorption isotherm in comparison with the Freundlich model. The maximum adsorption capacity of the dried biomass of *E. gracilis* for the biosorption of Zn reached the value $Q_{max} = 0.53 \pm 0.05 \text{ mmol.g}^{-1}$ or $34.7 \pm 3.4 \text{ mg.g}^{-1}$ (d.w.), respectively. The obtained results demonstrated that dried biomass of *E. gracilis* could be used as an effective and environment-friendly biosorbent for the removal of Zn from wastewaters or contaminated waters.

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Conflict of interest

The authors declare that they have no conflict of interest.

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