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The use of algae to remove copper and lead from industrial wastewater

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Abstract: The aim of the research was to evaluate the effectiveness of the removal of Cu and Pb ions by algae. The experiments were carried out in the presence of two algal populations: a pure culture of *Raphidocelis subcapitata*, and a mixed chlorophyta population. The research involved a model study, experiments in the presence of wastewater from the manufacture of batteries, and the study of process kinetics. The wastewater pH was 4.0, and the initial concentrations of metal ions in the wastewater were 95.4 mg/L for Pb and 48.3 mg/L for Cu, respectively. The maximum sorption capacity of the pure *Raphidocelis subcapitata* culture was 14.8 mg/g d.m. for Pb, corresponding to the removal of 72% of lead, and 6.1 mg/g d.m. for Cu, corresponding to the removal of 43% of copper from the wastewater. The best ion sorption efficiency in the case of the mixed chlorophyta population was 7.0 mg/g d.m. for Pb, i.e., 61% removal of lead, and 12.8 mg/g d.m. for Cu, i.e., 69% removal of copper ions from the wastewater. The optimum duration of the process was found to be 1 hour, since the majority of biomass samples reached the maximum saturation after that time. On the basis of the obtained results (Lagergren models), it was found that the dominant mechanism of the process was chemisorption.

Keywords: algae, sorption, heavy metals, lead, copper, *Raphidocelis subcapitata*

INTRODUCTION

The awareness of ecotoxicological effects and the stricter legal regulations concerning the reduced emission of industrial heavy metal ions call for new methods of wastewater treatment. In accordance with the current knowledge, it is impossible to eliminate the problem of environmental pollution, including water, with heavy metal ions. All attempts to isolate them or bind them into non-reactive complexes are only temporary solutions (Karaouzas et al. 2021). Changes i.a., in the environmental conditions, redox potential, or pH may cause metal ions to form more reactive chemical compounds, which might contaminate entire ecosystems. The effects of polluting ecosystems with heavy metals are particularly dangerous because they can accumulate at various trophic levels (Escudero et al. 2018).

Nearly all companies dealing with metal processing, mining, and many other industries represent point sources of heavy metals emissions. Conventional methods of the removal of those heavy metals, based on physical, chemical, and physico- or electrochemical processes, are often expensive, burdensome, and may lead to secondary pollution of the environment. For example, electroflotation is an effective method of removing lead ions with the concentration of 100 mg/L from wastewater (>98% metal removal), but it requires the presence of sulfates or a polymetal solution, as well as a power source (Merzouk et al. 2009); chemical precipitation is also effective (>99% lead removal) but it requires the presence of

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synthetic iron sulfide as the adsorbent (Özverdi & Erdem 2006); ultra- and nanofiltration are very effective methods of removing high concentrations of lead ions, too (>99% metal removal), but they require periodic cleaning or replacement of membranes and a power source, which generates some additional expense (Katsou et al. 2011, Gherasim & Mikulášek 2014).

For many years, researchers have been trying to find how to replace conventional methods of removal and recovery of heavy metal ions with methods based on using organic matter, which are more environment-friendly, often cheaper than the conventional ones, and less burdensome (Volesky 1990, Gunatilake 2015, Shamim 2018). The development of heavy metal ions biosorption using fungi, bacteria or algae has long been the object of considerable interest because of their potential use in the treatment of polluted soils and waters. Biosorption makes use of the biomass of microorganisms as the sorbent. When the appropriate conditions are ensured, the process may go on efficiently with the use of live or dead (inactive) biomass. It has been proven that inactive biomass may be more effective than live since it does not require special conditions (conditions for growth and development, medium, or light) (Mata et al. 2008, Flouty & Estephane 2012, Bădescu et al. 2017). Understanding the mechanisms of the development of tolerance to the presence of heavy metal ions in different species is of key importance for any potential use of that capacity in cleaning the environment.

The sorption abilities of many species of micro and macro algae, such as e.g.:

- *Scenedesmus quadricauda* – the maximum lead sorption capacity in pH 5.0 was 333.3 mg/g d.m. (Mirghaffari et al. 2015),
- *Chlorella vulgaris* – the maximum lead sorption capacity in pH 7.0 was 178.5 mg/g d.m. for Pb (Edris et al. 2014),
- *Spirogyra* sp. – the maximum lead sorption capacity in pH 5.0 was 140 mg/g d.m. for Pb (Gupta & Rastogi 2008),
- *Turbinaria conoides* – the maximum lead sorption capacity in pH 4.5 was 438.4 mg/g d.m. for Pb (Senthilkumar et al. 2007),

have already been studied and documented. However, researchers are still looking for new species of algae that ensure low costs when used as biosorbents as well as displaying a high affinity to remove metal ions from the solution and are easy to recover and regenerate (Nateras-Ramírez et al. 2022).

This study aims to present the possibility of using chlorophyta to remove copper and lead ions from industrial wastewater produced after washing equipment in the manufacture of batteries.

EXPERIMENTAL METHODOLOGY AND COURSE OF THE STUDY

The aim of the experiment was to compare the efficiency of removing copper and lead ions from industrial wastewater and a model solution.

Origin of algae

The study was conducted using two algal cultures. The first one was a pure culture of *Raphidocelis subcapitata* produced in laboratory conditions (culture 1). *Raphidocelis subcapitata* microalgae commonly occur in the fresh waters of temperate climate rivers and lakes. The second culture was a mixed population of chlorophyta collected from a natural water reservoir (culture 2). It included various kinds of algae, with a clear predominance of *Tetrasporales*, *Chlorosarcinales*, *Chlorococcales*, and *Volvocales*. Culture 2 was obtained from the highly eutrophicated Poraj dam reservoir, located in the south of Poland. The presence of Pb, Cu, Ni, and Cd ions (in concentrations from several to more than 10 mg/L) was observed in the reservoir sediments (Rosińska & Dąbrowska 2008).

Origin of wastewater

Wastewater produced after the washing of equipment for battery manufacture was collected for the study. The wastewater was characterized by pH = 4.0, and the contents of heavy metal ions in the wastewater were: 10.7 mg/L for Cd, 48.3 mg/L for Cu, 79.4 mg/L for Ni, 95.4 mg/L for Pb, and 88.2 mg/L for Zn.

Model study – determination of Cu and Pb ions in algae and the culture medium

The model solution was a culture medium (four basic solutions) with the following composition:

- solution I was: NH_4Cl, $MgSO_4{\cdot}7H_2O$, KH_2PO_4, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$.

– solution II was: $FeCl_3·6H_2O$, $Na_2EDTA·2H_2O$.
– solution III was: H_3BO_3, $MnCl_2·4H_2O$, $ZnCl_2$, $CoCl_2·6H_2O$, $CuCl_2·2H_2O$, $Na_2MoO_4·2H_2O$.
– solution IV was $NaHCO_3$.

The aim of the model study was to determine the content of Cu and Pb ions sorbed by the algae and the ions remaining in the culture medium (model solution). Samples of the culture medium (50 cm^3 each) containing algae were placed in a number of bioreactors. Then, appropriate amounts of heavy metal ions were introduced and left on magnetic stirrers for the scheduled contact time. Next, the samples were centrifuged at the speed of 6,000 rpm for 5 minutes, and the culture medium was decanted from above the biomass. The model solution was filtered though a filter, acidified with concentrated HNO_3, and then the contents of copper and lead ions were assayed with the flame atomic absorption spectrometry method (AAS) in compliance with PN-ISO 8288:2002. The biomass was dried up, weighed (0.2011–0.2197 g) and mineralized. The produced solutions were filtered through qualitative filters (65 g/m^3), and then the Cu and Pb ion contents sorbed in the biomass were assayed with AAS.

All of the assays were carried out three times and the result is the average value.

Experiment using wastewater – determination of Cu and Pb ions in algae and the wastewater

The experiment using wastewater assumed isolating algal biomass from the culture medium, washing it with redistilled water, and introducing it to wastewater samples (50 cm^3 each) containing heavy metal ions. The samples were left on magnetic stirrers for the assumed time of exposure to metals, and then the sorbed contents of copper and lead ions in biomass and wastewater were assayed as in the model study.

All of the assays were carried out three times and the result is the average value.

The model study was conducted in similar pH conditions and with the use of analogical concentrations of ions of all the five heavy metals detected in the wastewater. This made it possible to compare the contents of copper and lead ions in algal biomass, in wastewater, and in the model solution for each of the six exposure times: 1, 10, 30, 60, 120 minutes, and 24 hours. The control samples were wastewater and culture medium (for the model study). The experiment was conducted with the use of both algae cultures: a pure culture of *Raphidocelis subcapitata*, and a mixed chlorophyta population.

Equations used

The adsorption capacity of the biosorption process was calculated by means of:

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

where C_0 and C are the initial and equilibrium concentration [mg/L] of metal ions in the solution of volume V [cm^3], which contains the specified mass of the biosorbent m [g].

The parameters of the process and its rate were determined by using the Lagergren equations:

– pseudo-first-order (PFO):

$$\ln\left(q_{eq} - q\right) = \ln q_{eq} - k_I t \tag{2}$$

where q is the amount of metal bound by the adsorbent [g], q_{eq} is the equilibrium value of q [g], t is the time to equilibrate [min], and k_I is the model velocity constant [min^{-1}];

– pseudo-second-order (PSO):

$$\frac{t}{q} = \frac{1}{k_{II} q_{eq}^2} + \frac{1}{q_{eq}} t \tag{3}$$

where k_{II} is the model velocity constant [g/(mg·min)].

The removal efficiency of heavy metal ions from the solution was calculated with:

$$\eta = 100 \cdot \left(1 - \frac{C}{C_0}\right) \tag{4}$$

where C and C_0 are the final and initial concentrations of metal ions in the solution [mg/L].

RESULTS AND DISCUSSION

The concentrations of copper and lead ions assayed before the experiment in both algal populations and in the culture medium were low (below 0.1 mg/g d.m. for Cu and below 0.01 mg/g d.m. for Pb), so they were not taken into consideration when calculating the results.

The model study was conducted at a pH of 4.0 (the same as in the wastewater). Wastewater produced as a result of washing equipment for the manufacture of batteries has variable pH values, mostly depending on the amount of water, the cleaning agents used, and the manufacture technology. Every batch of wastewater may have a different composition and reaction, unlike post-manufacture wastewater, which usually has a similar composition and pH close or equal to 0, because of the high contents of sulfates used in the manufacture.

The concentrations of copper and lead ions used in the model study (48.3 mg/L for Cu and 95.4 mg/L for Pb) were similar to the concentrations determined in the wastewater collected for the experiment.

Model study

The study showed that a pure culture of *Raphidocelis subcapitata* (culture 1) displayed much better (by more than 70%) sorption properties in the initial minutes of the process than the mixed chlorophyta population (culture 2) (Fig. 1). Culture 1 reached the maximum lead ions sorption capacity (10.58 mg/g d.m.) after one hour of exposure to the metal. The dynamics of the removal of lead ions by culture 2 showed increasing efficiency as the time of the experiment increased. The process slowed down after an hour of contact with lead ions, although maximum saturation was obtained after 24 hours (14.12 mg/g d.m.).

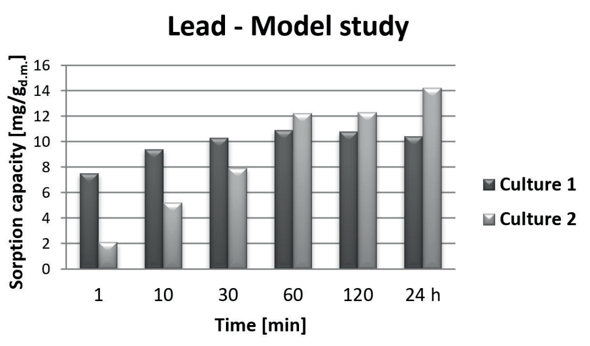

Fig. 1. Efficiency of lead ion sorption depending on the contact time

Copper sorption (Fig. 2) was more efficient for the entire time of the experiment with the use of a mixed algal population (only the desorption of Cu ions was observed after 24 hours). The maximum saturation for both cultures was observed after one hour of exposure to the metal. The efficiency of the process using culture 2 was 25% higher than in the case of culture 1.

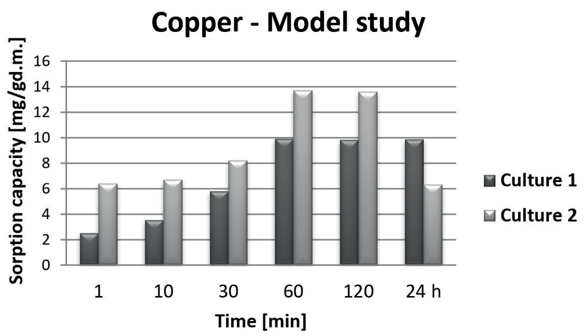

Fig. 2. Efficiency of copper ion sorption depending on contact time

In the model study, a better efficiency of the process was observed after a longer time of contact between the biomass and copper and lead ions with the use of a mixed chlorophyta population. After one hour of exposure, the desorption of copper was observed in both cultures, and of lead ions in the pure culture. On that basis, it was concluded that a one-hour duration of contact between algae and metal ions was the optimum amount.

Experiment using wastewater

Much better efficiency of lead ions biosorption from the wastewater was obtained with the use of culture 1 (Fig. 3). In the chosen process conditions, *Raphidocelis subcapitata* displayed much higher (even by more than 60%) affinity at removing Pb than the mixed chlorophyta population. The near-maximum saturation was obtained as early as in the 10^{th} minute of contact (13.7 mg/g d.m.). The trend of process changes is similar to the model study. However, the process going on in wastewater is more efficient. Culture 2 obtained the maximum sorption capacity after 2 hours of exposure to the metal (9.1 mg/g d.m.). The desorption of lead ions was observed in both cultures after 2 hours of exposure to the metal.

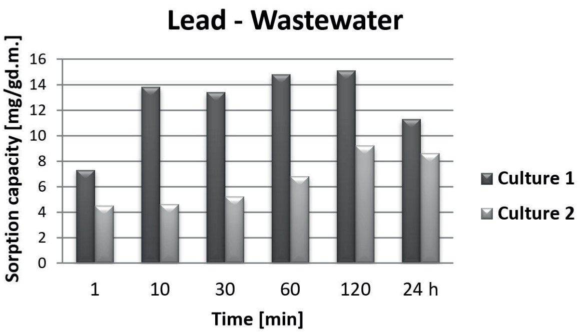

Fig. 3. Efficiency of lead ion sorption depending on contact time

In the case of removing copper ions, the opposite trend was observed (Fig. 4). Higher efficiency (by over 50%) was observed when using the mixed chlorophyta population. For both cultures, the maximum sorption capacity was achieved after 1 hour of contact (*Raphidocelis subcapitata* – 6.1 mg/g d.m., mixed chlorophyta population – 12.4 mg/g d.m.)

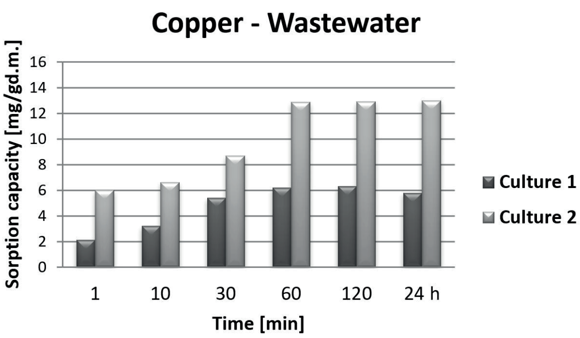

Fig. 4. Efficiency of copper ion sorption depending on contact time

The process of removing lead ions from wastewater was much more efficient with the use of a pure culture of *Raphidocelis subcapitata*, whereas copper was more efficiently sorbed in the presence of mixed chlorophyta population.

The contact time of 1 hour was adopted for comparing the experimental results, sincee most algae samples reached maximum saturation with heavy metal ions after this time (Fig. 5). After this point, the equilibrium between ions that had been sorbed and those that remained in the solution was also disturbed in many samples, and the natural desorption of copper and lead ions was observed. The study showed that the efficiency of removing lead ions from wastewater using *Raphidocelis subcapitata* was the highest out of all the studied samples for both metals and was 72% (61% in the case of the mixed chlorophyta population). In the model study, an opposite situation was observed: the pure culture removed lead less efficiently than the mixed population (culture 1 – 51%, culture 2 – 69%) (Fig. 5A). Copper was removed with a similar degree of efficiency (68–71%) by the mixed chlorophyta population, both from the wastewater and from the model solution. The worst effects were obtained when removing copper from wastewater using *Raphidocelis subcapitata* (43%) (Fig. 5B).

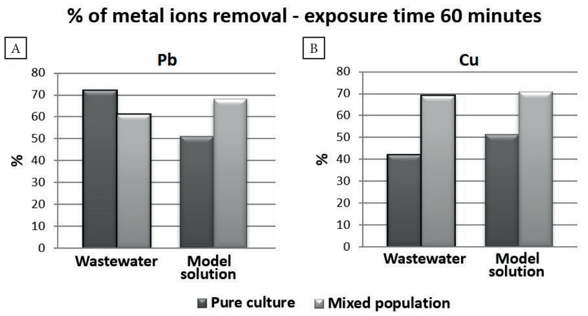

Fig. 5. Removal of Pb (initial concentration 95.4 mg/L) (A) and Cu (initial concentration 48.3 mg/L) (B) ions after one hour

The mixed population was collected from a natural, strongly eutrophicated water reservoir polluted with heavy metals and had a greater diversity of microalgae (and hence, binding sites, which influence i.a., sorption of metal ions) than pure *Raphidocelis subcapitata*. population. Therefore, theoretically it should display better efficiency and speed of the process and greater "tolerance" of microorganisms i.a., to heavy metals, through mechanisms that have been developing for many generations. This assumption was confirmed in the model study and in removing copper ions from wastewater. 69% reduction of Cu ions was achieved in wastewater at pH 4.0 and with the initial concentration of 48.3 mg/L for Cu. A mixed algal population, one which was not laboratory controlled, is hard to reproduce, so experiments using it are rare. However, it may be more efficient that a uniform population in removing substances from a solution with varied composition.

Raphidocelis subcapitata algae showed greater affinity than the mixed population for removing lead from wastewater in the chosen experimental conditions. Very few researchers carrying out experiments on the biosorption of heavy metal ions consider *Raphidocelis subcapitata* to be an interesting biosorbent. There are other, more popular and better known microalgae that can be better sorbents of copper or lead ions, removing bigger amounts of the metal even at higher initial concentrations. One example is *Spirogyra* sp., which in the conditions of pH 5.0 and the initial concentration of 200 mg/L for Pb is able to sorb 80% Pb ions (Gupta & Rastogi 2008), or *Chlorella vulgaris*, achieving 94.74% saturation with lead ions (Peng et al. 2017). Red algae *Gelidiella acerosa* are able to remove 96.36% copper at pH 5.31 with the initial concentration of 23.87 mg/L (Dulla et al. 2020), whereas *Chlorella vulgaris*, 88% (Ansilago et al. 2021). There are few publications concerning biosorption with the use of *Raphidocelis subcapitata*, and the ones that exist do not refer to heavy metals (Carvalho et al. 2015, Hom-Díaz et al. 2022), so their ability to remove metal ions, optimum pH conditions or potential substances that could facilitate the process are not yet well known. This microorganism is used most often in toxicity tests. However, the result of removing 72% lead ions from wastewater with the initial concentration of 95.4 mg/L for Pb can be regarded as an encouraging sign for the continuation of research on this microalga. The author has previously published a sighting study concerning the biosorption of lead ions with the use of *Raphidocelis subcapitata* and achieved 94% removal from wastewater produced after the manufacture of batteries with an initial concentration of 98.4 mg/L and pH 4.5 (Kipigroch 2020). The present experiment was conducted at pH 4.0. Its results prove that apart from the initial concentration, pH value has the greatest impact on the efficiency of the process (Sulaymon et al. 2013). Biomass concentration is also very significant for the proper course of the experiment (Chojnacka 2009). Research results show that if the biomass concentration is too low, it does not ensure a sufficient number of binding sites for metal ions which could potentially be sorbed. However, if the concentration is too high, it is also unfavorable, because binding sites are limited and blocked as a result of biomass particles clumping. Al-Dhabi and Arasu showed that, when removing lead ions from wastewater (initial concentration of 99.8 mg/L for Pb), the optimum concentration of sea algae biomass necessary for the optimum process efficiency (over 99.8%) should be 16.2 g/L in pH 5.0 (Al-Dhabi & Arasu 2022). The biomass concentration used in the discussed study was approx. 40 g/L. Perhaps a lower biomass concentration would improve the efficiency of the process.

The aim of this study was to compare the efficiency of the removal of copper and lead ions from wastewater and the model solution with the use of two algal cultures. The comparison of the efficiency of lead ions biosorption from wastewater by both cultures showed that after one hour of exposure, the difference in process efficiency was 11% (pure culture: 72%, mixed population: 61%). In the case of copper, there was a greater difference – 27% (pure culture: 42%, mixed population: 69%).

Biosorption kinetics

Pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetic models were tested for both cultures. Calculations of kinetic parameters read from the graphs (Figs. 6, 7), are presented in Table 1.

PSO adsorption parameters q_{eq}, and k_{II} were determined by plotting t/qt versus t.

PFO and PSO kinetic models were matched with the kinetic experimental data (Equations (2) and (3)). The PSO model is often perceived as the best fitting only at the beginning of the process (Sadaf & Bhatti 2014). In the discussed study, the pseudo-second order kinetics better fits the presented experiment. This is shown by the similar results obtained experimentally and the calculations of q_{eq} value combined with a high degree of correlation (R^2 > 0.95). On the basis of the PSO model it can be concluded that the speed of the process can be limited by the speed of chemical reactions occurring between the sorbent and the sorbate (Maurya et al. 2014), and the dominant biosorption mechanism is chemisorption.

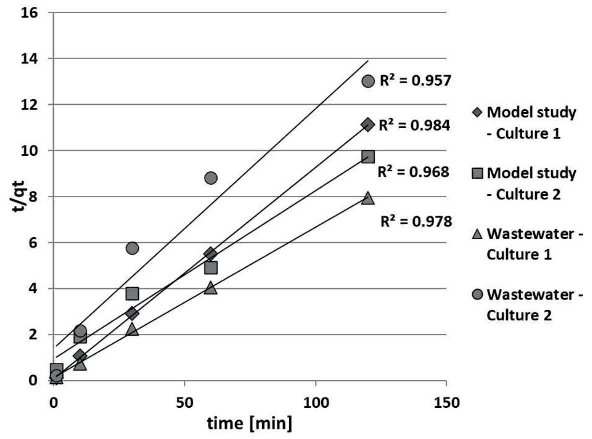

Fig. 6. PSO model for lead biosorption

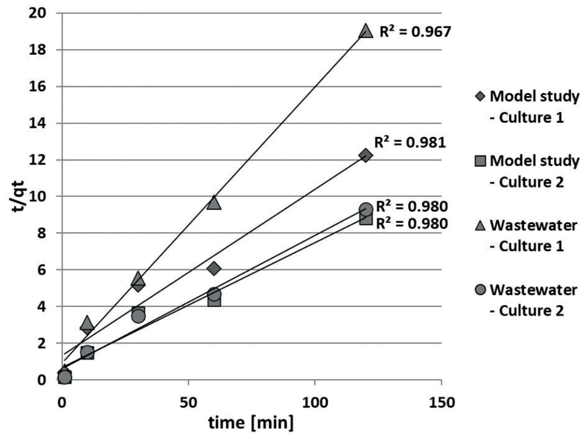

Fig. 7. PSO model for copper biosorption

Table 1
Kinetics parameters for the sorption of ions copper and lead

	q_{exp} [mg/g]	PFO			PSO		
		q_{eq} [mg/g]	k_I [min^{-1}]	R^2	q_{eq} [mg/g]	k_{II} [g/(mg·min)]	R^2
Lead							
Model study – Culture 1	10.91	7.12	0.057	0.652	10.74	0.069	0.984
Model study – Culture 2	12.20	8.52	0.068	0.698	11.81	0.123	0.968
Wastewater – Culture 1	14.83	9.21	0.123	0.621	14.51	0.148	0.978
Wastewater – Culture 2	6.83	4.23	0.197	0.619	6.54	0.268	0.957
Copper							
Model study – Culture 1	9.90	6.18	0.033	0.624	9.71	0.071	0.981
Model study – Culture 2	13.72	8.74	0.079	0.637	13.45	0.254	0.980
Wastewater – Culture 1	6.21	4.92	0.038	0.792	6.01	0.231	0.967
Wastewater – Culture 2	12.86	10.12	0.112	0.786	12.61	0.173	0.980

CONCLUSIONS

The research was conducted with the use of two algal cultures: a pure culture of *Raphidocelis subcapitata*, and a mixed chlorophyta population. The experiment involved a model study and a study using wastewater produced after the washing of equipment used in battery manufacture. The maximum sorption capacity of the pure culture was 14.8 mg/g d.m. for Pb, i.e., 72% removal of lead ions, and 6.1 mg/g d.m. for Cu, i.e., 43% removal of copper from the wastewater. The best ion sorption efficiency in the case of the mixed chlorophyta population was 7.0 mg/g d.m. for Pb, i.e., 61% lead removal, and 12.8 mg/g d.m. for Cu, i.e., 69% removal of copper ions from the wastewater. The optimum duration of the process was 1 hour, after which time most of the biomass samples reached full saturation. The study confirms that a longer experiment time ensured greater efficiency in terms of biosorption.

In the author's opinion, the efficiency of removing copper and lead ions from wastewater is insufficient. The process conditions (pH, initial concentrations) were not adjusted during the experiment and were the same as the conditions in the collected wastewater, whereas previous research (Cygnarowska 2021) suggests that changing the pH could improve the process efficiency. The studied population of *Raphidocelis subcapitata* gives promising results, but further research is necessary to discover their full potential.

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