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The Use of Algae in the Process of Cadmium and Lead Ions Removal from Wastewater

Katarzyna Cygnarowska
Faculty of Infrastructure and Environment,
Czestochowa University of Technology, Poland
https://orcid.org/0000-0002-2700-8569

corresponding author's e-mail: katarzyna.cygnarowska@pcz.pl

Abstract: The study presents the possibility of using chlorophyta in the removal of cadmium and lead ions from industrial wastewater produced after the washing of equipment used in the manufacture of battery and batteries. The process was conducted with the use of two algal cultures: *Raphidocelis subcapitata* produced in laboratory conditions, and a mixed chlorophyta population collected from a natural, eutrophicated water reservoir with heavy metal ions present in the water and sludge. The study showed that the effectiveness of a pure algal culture is comparable to that of a mixed chlorophyta population, characterized by greater diversity of functional groups at binding sites and higher resistance to stress that may occur in the wastewater environment. The maximum effectiveness of ions sorption was 64% for cadmium (mixed algal population) and 60% for lead (*Raphidocelis subcapitata*).

Keywords: algae, heavy metals, cadmium, lead, biosorption, *Raphidocelis subcapitata*

1. Introduction

Currently, there is growing interest in biological methods of removing heavy metal ions from wastewater. The most popular methods are biosorption and bioaccumulation. Biosorption takes place on the surface of a cell and involves the exchange of substances diffusing into the cell and metabolites transferred out of it. Dynamic exchange of substances present within the cell with the ones around it determines constant regeneration of the cell surface and the ability to sorb new molecules. This process may be equally efficient with live and dead matter (Romera et al. 2007, Jayakumar & Govindaradjane 2017).

Bioaccumulation involves the accumulation of ions or chemical compounds, such as chlorinated hydrocarbons or heavy metal salts, inside a cell. It often has a detrimental effect on the cell organelles, e.g., leading to chlorophyll loss, lower enzymatic activity, or prolonged regeneration time. As a result of gradual degradation, the cell decomposes and releases the accumulated substances to the environment (Filipiuk et al. 2006). Bioaccumulation requires the supply of metabolic energy, so it only occurs with the participation of living organisms.

The most commonly used biological method of wastewater treatment is biosorption. For many years, this process has been subject to many laboratory studies, which have proved beyond doubt that some groups of microorganisms, such as fungi, yeast, bacteria and algae, may sorb various dangerous substances, i.a., heavy metal ions, from the environment (Tab. 1) (Wang & Chen 2009).

Table 1. Effectiveness of different groups of organisms removing ions of selected heavy metals

Metal	Biosorbent	Organism	Metal content [µmol/g $_{d.m.}$]
Cd^{2+}	Brown algae	*Ascophyllum nodosum*	1900
	Aquatic plants	*Potamogeton luceus*	1100
	Bacteria	*Bacillus sp.*	900
	Yeast	*Candida tropicalis*	530
	Fungi	*Rhizopus arrhizus*	240
Cu^{2+}	Aquatic plants	*Potamogeton luceus*	1280
	Bacteria	*Bacillus sp.*	2400
	Yeast	*Candida tropicalis*	1270
	Fungi	*Rhizopus arrhizus*	253
Ni^{2+}	Brown algae	*Ascophyllum nodosum*	680
	Yeast	*Candida tropicalis*	340
	Fungi	*Rhizopus arrhizus*	305
Pb^{2+}	Brown algae	*Saragassum natans*	1300
	Aquatic plants	*Potamogeton luceus*	1360
	Bacteria	*Bacillus sp.*	2110
	Fungi	*Absidia orchidis*	1700
Zn^{2+}	Aquatic plants	*Potamogeton luceus*	1000
	Bacteria	*Bacillus sp.*	2110
	Yeast	*Candida tropicalis*	460
	Fungi	*Rhizopus arrhizus*	310

(Filipiuk et al. 2006)

The phenomenon of biosorption can be compared to the process of ion exchange. The sorption of metals occurs thanks to the presence of i.a., various polysaccharides containing functional groups (carboxyl, phenol, hydroxyl, etc.) on the cell surface (Ramrakhiani et al. 2011). Other mechanisms that may stimulate this process are complexation, microprecipitation or oxidation – reduction reactions, and physical adsorption. (Jayakumar & Govindaradjane 2017, Mata et al. 2008).

The main advantages of biosorption used in removing metal ions from wastewater are the ease of separating the biomass from the solution, and the possibility to regenerate and use the same biomass several times. As for disadvantages, the mechanical strength of microorganisms is rather poor.

The process of biosorption of heavy metals from wastewater is based on the contact between the solid body and the liquid, which can be static or dynamic. In both methods, biomass is mixed with the solution to be purified, and after a specific incubation time, it is separated from the purified solution and regenerated via the desorption of metal ions. If the process is carried out in several steps, its effectiveness is greatly improved (Filipiuk et al. 2006).

The sorption capacity of freshwater and seawater algae is one of the highest out of all microorganisms used in biosorption. Their high effectiveness in the removal of heavy metal ions has been proved by many researchers (Bădescu et al. 2017, Flouty & Estephane 2012). The doubtless advantages of most kinds of algae are their common occurrence, easy culture, and high affinity for various metal ions. The biosorption process with the use of algae allows for the recovery of the sorbed metal ions and easy disposal (Ibrahim et al. 2018, He & Chen 2014).

Algae are effective as biosorbents, especially in removing low concentrations of heavy metal ions. The removal of concentrations higher than several mg/dm^3 is less effective. The aim of the work is to present the possibility of using chlorophyta in the removal of cadmium and lead ions from industrial wastewater produced after the washing of equipment used in the manufacture of batteries. The research was conducted with the use of a model solution and wastewater.

2. Methodology and course of the study

2.1. The origin of algae

The experiments were carried out with the use of algae from two cultures: *Raphidocelis subcapitata* proliferated in laboratory conditions from a lyophilized pure culture, and a mixed chlorophyta population from a natural water reservoir.

In culture 1, i.e., a pure *Raphidocelis subcapitata* culture, there were few cells of *Scenedesmus quadricauda* (< 0.1%), which got to the environment despite the sterilization of the medium. *Raphidocelis subcapitata* algae are commonly found in natural surface standing waters, mostly in temperate climate conditions.

Culture 2 was obtained from the highly eutrophicated Poraj dam reservoir, located at 763.3 km of the Warta River in the southern part of Poland. Each year, there are algal blooms in that reservoir, mainly including chlorophyta, whose abundant development causes water alkalization in the process of photosynthesis. High pH fosters the precipitation of metal ions. Tests of water and sludge from the reservoir confirmed the presence of metals such as nickel (15-59 mg Ni/kg), cadmium (1.5-2.3 mg Cd/kg), and copper (3.3-7.5 mg Cu/kg)

(Rosińska & Dąbrowska 2008). Culture 2 is a mixed population of chlorophyta, mostly made up of *Tetrasporales*, *Chlorosarcinales*, *Chlorococcales*, and *Volvocales* obtained from the water of the reservoir.

2.2. The culture medium

The culture medium used in the culture process was prepared in accordance with the applicable regulation (Commission Directive No. 92/69/EEC of 31.07.1992). The stock solutions contained the following salts:

- Solution I: 1.5 g NH_4Cl, 1.2 g $MgCl_2 \times 6H_2O$, 1.8 g $CaCl_2 \times 2H_2O$, 1.5 g $MgSO_4 \times 7H_2O$ and 0.16 g KH_2PO_4,
- Solution II: $FeCl_3 \times 6H_2O$, 0.08 g and disodium edetate (Na_2EDTA) $\times 2H_2O$, 0.1 g,
- Solution III: 0.185 g H_3BO_3, 0.415 g $MnCl_2 \times 4H_2O$, 0.003 g $ZnCl_2$, 0.0015 g $CoCl_2 \times 6H_2O$, 0.00001 g $CuCl_2 \times 2H_2O$ and 0.007 g $Na_2MoO_4 \times 2H_2O$,
- Solution IV: 50 g $NaHCO_3$.

Four stock solutions were prepared by entering weighted amounts of the selected salts and complementing them with distilled water up to the volume of 1 dm^3. The solutions were sterilized and then kept in dark bottles at 4°C.

The stock solutions were used to prepare the culture medium for the culture of algae. 10 cm^3 of stock solution I was entered into a 1 dm^3 volumetric flask, and then 1 cm^3 samples of the remaining stock solutions (II, III and IV) were added; finally, it was complemented with deionized water up to the volume of 1 dm^3.

2.3. Origin of wastewater

The wastewater used in the experiment had a weakly acidic reaction (pH = 6.1) and contained the following concentrations of heavy metal ions: Cd – 21.7 mg/dm^3, Ni – 72.4 mg/dm^3, Zn – 84.1 mg/dm^3, Cu – 36.2 mg/dm^3, Pb – 58.4 mg/dm^3. The wastewater came from battery production industry, in particular, from the washing of equipment used in manufacture technology. Samples of wastewater were collected to plastic containers and kept at the temperature of 4°C. The sampling took place just after the process of washing of the equipment, directly from the outlet to the treatment plant.

2.4. Model study: determination of metal ions in algae and in the culture medium

Before the sampling of algae, the cultures were mixed, and algae samples were entered into a number of bioreactors with 50 cm^3 culture medium. Then, specific amounts of heavy metal ions were entered so as to achieve the desired concentration. After the assumed exposure time, the samples were centrifuged for 5 minutes at 5,000 rpm. Finally, the culture medium and the biomass were separated.

2.4.1. Determination of metal ions in the model solution

The model solution was the centrifuged culture medium (section 2.4.). 30 cm^3 of medium was taken from each centrifuged sample and filtered through a qualitative filter; afterwards, each sample of the filtrate was placed in a tight, sterile plastic container. Next, each sample was acidified with concentrated HNO_3 up to pH of approx. 2 and kept at 4°C until the assay with the flame atomic absorption spectroscopy method (AAS) in accordance with the standard PN-81/C-04570/01.

2.4.2. Preparation of biomass

The biomass obtained after centrifugation (section 2.4.) was placed in a quartz vaporizer and then dried up to dry matter at 105°C, ground in a mortar and weighed. Then, the samples were subject to mineralization in accordance with PN – EN 14084:2004 in a VELP DK20 mineralizer. The samples were transferred to the mineralizer reaction vessels and flooded with 12.5 cm^3 of aqua regis (HCl 38% and HNO_3 65% at the ratio of 3:1). The procedure was carried out in three successive temperature ranges (I – 20 minutes at 70°C, II – 40 minutes at 100°C, III – 30 minutes at 140°C). After the procedure, the hot samples were filtered through qualitative filters to measuring cylinders and complemented with distilled water up to 50 cm^3. Then, the samples were placed in sterile plastic containers and kept at 4°C until the determination of heavy metal ions content with the atomic absorption spectroscopy method (AAS). The experiment was performed in three iterations.

2.5. Experiment using wastewater: determination of metal ions in algae and wastewater

The experiment was carried out with the use of the same algae that were used in the model study. A number of samples containing algae and culture medium (50 cm^3) were collected and then centrifuged to separate the medium from the biomass. The culture medium was removed and the remaining biomass (approx. 7 cm^3) was washed several times with redistilled water and then entered into reactors containing wastewater (43 cm^3). After the assumed exposure time, the samples were centrifuged and the wastewater and biomass were separated.

In the subsequent stages of the study, the procedure was the same as in the case of preparing the model solution (section 2.4.1) and biomass (section 2.4.2) in the model study.

2.6. Algal culture

Both cultures had similar conditions of development. They were kept at the temperature of 25°C (±2°C) and continuously lit with fluorescent lamps. The number of algal specimens developing in the culture medium was determined with the use of a microscope and a Sedgwick-Rafter Counting Cell. The experiment began after obtaining the culture with the density of 2,500,000 specimens in 1 cm^3 of the medium.

2.7. Procedure of the experiments

2.7.1. Model study

The concentrations of metal ions used in the model study were adjusted to the contents of heavy metal ions in the wastewater. Thus, it was possible to compare the results of the experiment done with the model solution and the experiment using wastewater. For the same reason, the pH of the culture medium was adjusted to 6.1, i.e., the pH of the wastewater. In order to maintain the same conditions of procedures with the use of wastewater and the model solution, all five heavy metal ions present in the wastewater were entered into the model solution, although this study only focuses on the assessment of effectiveness of biosorption of ions of cadmium and lead.

Solutions of metal compounds in the amounts corresponding to their concentrations in the wastewater were entered into 50 cm^3 of culture medium containing the algae *Raphidocelis subcapitata* (culture 1). Cadmium was entered in the form of salt $Cd(NO_3)_2 \times 4H_2O$ in the amount corresponding to 21,7 mg/dm^3, and lead – in the form of $Pb(NO_3)_2$ – in the amount of 58,4 mg/dm^3. The control solution contained the culture medium and *Raphidocelis subcapitata* algae without the added metals.

The experiments were carried out for six exposure times: 1, 10, 30, 60, 120 minutes and 24 hours. After the lapse of the each time of contact between metals and the algal biomass, the contents of cadmium and lead ions were determined in the model solution and in the algal biomass (section 2.4).

A similar experiment was carried out for culture 2, containing a mixed chlorophyta population. The experiment was performed in three iterations.

2.7.2. Experiment using wastewater

Algae *Raphidocelis subcapitata* were entered into the samples of wastewater containing metals (culture 1). The time of contact of metals in the wastewater with the algae was 1, 10, 30, 60, 120 minutes or 24 hours, and after the lapse of each time, the contents of cadmium and lead ions in the biomass were determined. A similar experiment was carried out for culture 2, containing a mixed chlorophyta population. The experiment was performed in three iterations.

3. Results and discussion

Before the study, the contents of cadmium and lead in both cultures were determined. The population of *Raphidocelis subcapitata* did not contain any Cd or Pb ions. Cadmium ions (< 0.01 mg Cd/$g_{d.m.}$) and lead ions (0.01mg Pb/$g_{d.m.}$) were observed in the mixed algal population collected from a natural water reservoir. After specific times of contact between algae and metals, the process was controlled by determining the concentrations of metal ions in the algal biomass and in the model solution or wastewater. The effectiveness of the process was assessed with reference to the control samples.

3.1. Model study

The model study of cadmium ions showed an increase in the content of this element in the algal biomass of both cultures (Fig. 1). The degree of cadmium ions removal by *Raphidocelis subcapitata* (culture 1) was higher and achieved much more quickly than in the case of a mixed chlorophyta population (culture 2). Even in the first minute, the sorption of cadmium in the biomass amounted to 6.33 mg/$g_{d.m.}$, while the mixed population sorbed 1.38 mg/$g_{d.m.}$ after one minute of exposure. The maximum saturation for culture 1 was achieved after 60 minutes of contact (8.26 mg/$g_{d.m.}$), and retained at least for 60 minutes thereafter. After 24 hours, cadmium ions were partially desorbed (6.42 mg/$g_{d.m.}$). For culture 2, maximum saturation was never achieved. The sorption of Cd ions by a mixed chlorophyta population was growing for the whole duration of the experiment. After 24 hours, it was 8.16 mg/$g_{d.m}$.

In the initial stages of the process, the effectiveness of cadmium ions removal from the model solution was several times higher with the use of *Raphidocelis subcapitata*. After 24 hours, the situation changed and the effectiveness of Cd ions removal by the mixed chlorophyta population did increase, but the time needed for this to occur was much shorter in the case of culture 1.

The dynamics of lead ions removal shows an increase in the content of that element in biomass for both cultures (Fig. 1). From the 10th minute to the end of the experiment, the culture of *Raphidocelis subcapitata* (culture 1) displayed relatively unchanged, high effectiveness of sorption of Pb ions (10.0-10.8 mg/$g_{d.m.}$). This proves that doing the experiment for longer than 10 minutes is useless and ineffective. The mixed chlorophyta population (culture 2) displayed much lower effectiveness of lead ions removal than did *Raphidocelis subcapitata* after shorter exposure times (up to 30 minutes). However, from the 60th minute on, it was approx. 15% more effective (12.5-12.9 mg/$g_{d.m.}$).

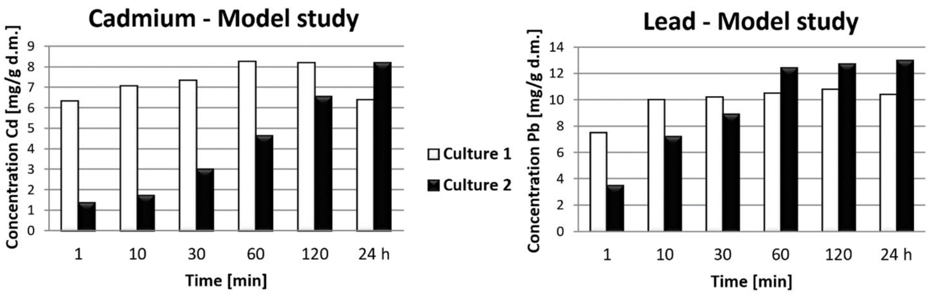

Fig. 1. Changes in cadmium and lead concentrations in the algae biomass depending on the time of exposure

To conclude, for shorter exposure times, *Raphidocelis subcapitata* (culture 1) displayed much better affinity for cadmium and lead ions removal than a mixed chlorophyta population with greater diversity of binding sites.

3.2. Experiment using wastewater

The experiment using wastewater proved the higher effectiveness of cadmium ions sorption by the mixed algal population than by *Raphidocelis subcapitata* (culture 1) (Fig. 2). The effectiveness of a mixed population (culture 2) was on average two times higher for nearly all the times of contact between the biomass and Cd ions. After 24 hours, it was more than 4.5 times higher, because cadmium ions in culture 1 were desorbed. The best effect of cadmium ions removal for the mixed algal population was achieved in the 60th minute (8.02 mg/$g_{d.m.}$), and for *Raphidocelis subcapitata* in the 120th minute (4.07 mg/$g_{d.m.}$).

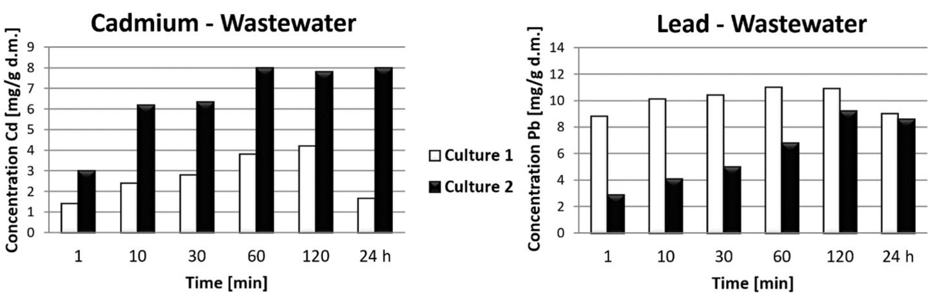

Fig. 2. Changes in cadmium and lead concentrations in the algae biomass depending on the time of exposure

In the case of *Raphidocelis subcapitata*, the effectiveness of lead ions removal from the wastewater was comparable to the effectiveness of sorption in the model study. Culture 1 achieved the maximum saturation (Fig. 2) after 60 minutes (11.05 mg/$g_{d.m.}$). For shorter exposure times, the mixed algal population displayed almost 3 times lower effectiveness of removing lead ions from the wastewater. It only achieved the maximum effectiveness after two hours (9.22 mg/$g_{d.m.}$).

On the basis of the results of cadmium and lead ions removal from wastewater, it was proved that the use of a mixed algal culture makes it possible to achieve a higher level of sorbing ions of both elements in the biomass than the use of *Raphidocelis subcapitata*. The mixed algal population displays high effectiveness of removing cadmium ions from wastewater and is only 15% less effective in removing lead ions (after 120 minutes).

The experiment showed a varied degree of removal of cadmium ions, depending on the medium, exposure time, and the used algal culture (Fig. 3). Cadmium was most effectively removed from the model solution by*Raphidocelis subcapitata* (67%). A similar degree of removal was achieved with the use of the mixed algal population but only after 24 hours of exposure. An opposite trend was observed in the wastewater, where the mixed algal population displayed at least two times higher effectiveness of sorbing cadmium ions than did *Raphidocelis subcapitata* for all exposure times (after 120 minutes, culture 1: 34%, culture 2: 64%).

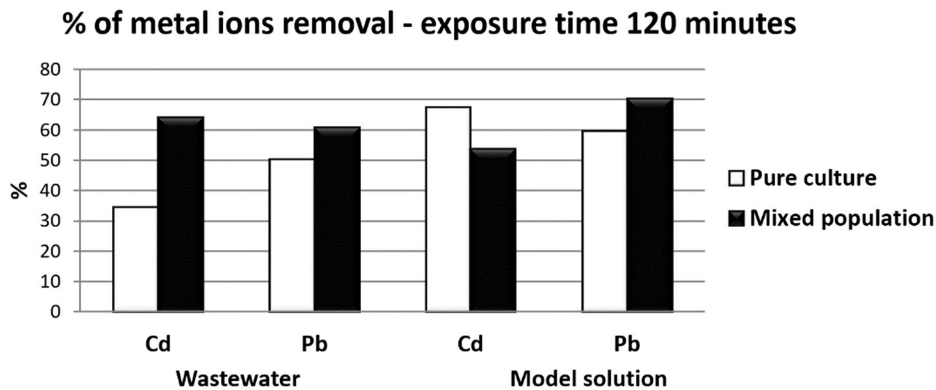

Fig. 3. Percentage removal of cadmium and lead ions after 120 min.

The obtained results proved that the effectiveness of removing lead ions is relatively high in the case of culture 1 and around 57-61% in the model study and in the wastewater. *Raphidocelis subcapitata* displayed high affinity for lead ions removal in the selected pH conditions (6.1) and resistance to various substances present in the wastewater.

In the model study, the process of lead ions removal with the use of a mixed chlorophyta population was slower. Finally, the mixed population proved to be a 16% more efficient biosorbent of lead, but only after 60 minutes of exposure (71% removal). In the case of culture 2, lead ions were removed less efficiently from the wastewater than from the model solution. The mixed population achieved the maximum saturation after 120 minutes of contact, which corresponded to 50% effectiveness of lead ions removal.

Raphidocelis subcapitata are microorganisms used in toxicity tests. They are the subject of very few scientific works concerning the issue of biosorption, although they are common in the temperate climate. The procedure with the use of a mixed chlorophyta population from a natural water reservoir is not popular with many researchers, either, because microorganisms occur in waters quite randomly and it is difficult to reconstruct the culture. However, if metal ions need to be removed from wastewater with diverse composition, a mixed population may be more effective than a pure culture.

Before the experiment, it was assumed that a mixed algal population collected from a natural, highly eutrophicated water reservoir with the content of heavy metal ions (several or over a dozen of mg/dm^3) would be a much more effective biosorbent than the culture of *Raphidocelis subcapitata* produced in laboratory conditions. Greater diversity of functional groups at binding sites and the necessity to develop and adapt in a more toxic environment were in favor of the mixed algal population. Hence, better effects of biosorption in the mixed chlorophyta population were expected, especially when the process involved natural wastewater.

However, *Raphidocelis subcapitata* proved to be much more resistant to additional substances present in the wastewater (e.g., surfactants), which were not taken into consideration in the model solution (it included the elements of the culture medium, section 2.2.). They displayed better effectiveness of removing lead from the wastewater and cadmium from the model solution than did the mixed population, and their effectiveness was only slightly lower (by 15%) when sorbing lead from the model solution. In all these cases, *Raphidocelis subcapitata* worked much more quickly than the mixed population. They achieved 70% of the maximum saturation even in the first minute of the experiment. The mixed chlorophyta population needed much more time (at least 60 minutes) to achieve the results comparable to those achieved by *Raphidocelis subcapitata.* The only case in which the mixed population was more effective (2 times and more) was the removal of cadmium ions from the wastewater.

4. Conclusions

The aim of the study was to present the possibility of using chlorophyta in the removal of cadmium and lead ions from industrial wastewater produced after the washing of equipment used in the manufacture of batteries. The experiment proved that *Raphidocelis subcapitata* and a mixed chlorophyta population can be effectively used to remove heavy metal ions from wastewater. The process of biosorption had different intensities, mostly depending on the time of exposure and the used biomass. The maximum effectiveness of the process was 64% for cadmium and 60% for lead. The effect of purification is not sufficient to safely channel the wastewater to waters or to the ground (providing the content of Cd and Pb ions). Still, the effects are promising, and conducting the procedure in several steps would probably lead to much better effects.

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