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Sorption of copper (II) and cadmium (II) ions with the use of algae

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ABSTRACT

The study involved initiating the process of metal biosorption with the use of live algae by means of administering cadmium and copper. Cultures of algae from two different environments were used. *Pseudokirchneriella subcapitata* algae bred in laboratory conditions from a pure culture were dominant in culture no. 1. Culture no. 2 was a mixed population of chlorophyta from a natural reservoir. The process was evaluated regarding changes in metal concentrations in the algal biomass after 10 min, and then, after 1, 2, 4, and 24 h of exposure. Changes of Cd(II) and Cu(II) concentrations in the culture medium were also determined. The research proved that the studied populations were good biosorbents. Mixed algal population from a natural water reservoir sorbed Cd(II) and Cu(II) more effectively than the population of algae from a laboratory culture. Mixed algal population retained nearly ten times more Cd(II) and over three times more of Cu(II) than the population bred from a pure culture of *P. subcapitata*.

Key words: Biosorption; Cadmium; Copper; Microprecipitation; *Pseudokirchneriella subcapitata*; Algae

1. Introduction

In water environment, heavy metals may occur in a molecular form (e.g. colloids) or a dissolved one (such as free ions, complex ions, and chelated metals with organic and inorganic ligands). Ions of Cu, Cd, Pb, Hg, Zn, Mn, Ni, Cr, Sn, and Co may be accumulated on and in living organisms [1]. Copper (II) and especially cadmium (II) present ecological threat to water organisms even in low concentrations. Hence, it is important to find methods of removing heavy metals from water environment [2,3]. The highest acceptable concentration of cadmium and its compounds in surface waters depends on the content of calcium carbonate in the water, but does not exceed 1.5 μg/dm^3. This value applies to waters with hardness higher than or equal to 200 mg $CaCO_3$/dm^3. The chemical condition of waters for which the content of cadmium and its compounds exceeds the acceptable norm is referred to as "less than good" [4].

The conventional methods of removing ions of heavy metals from water include mostly: chemical

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precipitation, electrochemical processes, reversed osmosis, ion exchange, and adsorption. The majority of those methods are expensive and troublesome, and what is more, they generate dangerous waste [2]. Biosorption is an innovative technology of removing hazardous substances from water. The process takes advantage of the ability of organic matter to accumulate ions of heavy metals through the processes of metabolic or physicochemical uptake [5–7].

Researchers have proved in many studies that the algae which are available in virtually unlimited amounts, make an effective group of organisms taking up metal ions [8,9]. Besides, they have proved that the cation-exchange capacity for cyanobacteria (algae belonging to *Procariota*) is much higher than that for filiform eukaryotic algae. The concentration factor—expressed as the ratio of metal ion concentration in algae (mg/g d.m.) to the concentration in the surrounding water—may assume values from a few hundred to a few dozen thousand. In the cells of cyanobacteria, heavy metal ions are accumulated in cytoplasm and in structures called polyphosphate granules [1].

Factors affecting the process of biosorption are mainly: the pH of the solution, the ionic strength, the concentration of the biosorbent, the temperature, and the initial concentration of heavy metals [10,11]. The pH plays a vital role in the process, as it is decisive for the speciation form of the metal and chemical activity of the biomass functional groups [8]. Ionic strength is another important parameter, because it influences, among others, the activity of metals and competition between ions. The temperature recommended for the process is 20–35˚C. Higher temperatures have a positive impact on the effectiveness of the process by means of increasing surface activity and kinetic energy [10]. Biomass concentration also has an influence on the effectiveness of biosorption. Too high biomass concentration may contribute to blocking active sites responsible for the uptake of metals, which, in turn, may lead to lowering the effectiveness of the process [8].

The biosorption process may occur both with the use of live and dead biomass. Dead algae may well be used to take up metal ions, but the use of live biomass is more favorable due to its continuous growth and simultaneous bioaccumulation [12]. Therefore, not only the selection of the proper kind but also of the proper biosorbent weight is significant in the process of removing ions.

Algae have many qualities stimulating the accumulation and selective removal of metal ions. These include among others: high tolerance of selected species to the presence of heavy metals, susceptibility to genetic modifications, high surface-area-to-volume ratio, and the presence of phytochelatins. Phytochelatins are produced by algal cells in response to penetration of ions of heavy metals such as: As, Ag, Cd, Cu, Hg, Sn, and Zn.

Cadmium (II) is the strongest inductor of phytochelatins among them. For example, the synthesis of phytochelatins begins at the moment of cadmium (II) penetrating into the cell and stops when the metal ions are bound. None other group of compounds induces synthesis of phytochelatins in algal cells.

It has been proved that selected algae species have high resistance to the presence of metals. Differences in resistance may also occur within the same species. They emerge when algae are adapted to life in habitats characterized by different contents of heavy metal ions. That indicates good adaptation abilities of selected algae species [13].

The use of natural biosorbents should be particularly taken into consideration because of the low cost at which they may be obtained as well as simple waste management. Algae biomass is characterized by a virtually unlimited number of binding sites, high affinity to various metals, and the possibility of their recovery [14–17].

2. Aim of the research

The aim of the research was to determine the capacities of sorption of Cu(II) and Cd(II) with the use of live algal culture bred in laboratory conditions as well as mixed-algal population obtained from a water reservoir.

3. Methodology of the research

3.1. Origin of the algae

The algae used in the study came from two cultures. *Pseudokirchneriella subcapitata* algae bred in laboratory conditions from a lyophilized pure culture were dominant in culture no. 1. It must be noted that the culture of *Pseudokirchneriella Subcapitata* was not kept in sterile conditions. That was the reason for the presence of negligible amounts of other chlorophyta, mainly *Scenedesmus quadricauda*. Culture no. 2 was a mixed population of algae (mainly chlorophyta) with dominant forms belonging to *Chlorococcales*—single forms and living in various colonies alike. Culture no. 2 was obtained from the Poraj dam reservoir, localized on the Warta River (Poland).

3.1.1. The Poraj dam reservoir

In the waters of the reservoir, mass algal bloom is observed every year. The blooms result in increased

photosynthesis process, which leads to alkalization of the environment. The increase of pH promotes the precipitation of heavy metals from the depths to the sludge [18]. Presence of ions of heavy metals such as nickel (II) 15–59 mg/kg, cadmium (II) 1.5-2.3 mg/kg, and copper (II) 3.3–7.5 mg/kg were found in the sludge coming from the reservoir. In the mobile fractions of the sludge, cadmium (II) was predominant [19].

3.2. The culture medium

The culture medium was prepared in accordance with the applicable regulation (Commission Directive No. 92/69/EEC of 31/07/1992).

3.2.1. Composition of the basic solutions

- Solution I was: NH_4Cl—1.5 g; $MgCl_2x6H_2O$—1.2 g; $CaCl_2x2H_2O$—1.8 g; $MgSO_4x7H_2O$—1.5 g; KH_2PO_4—0.16 g.
- Solution II was: $FeCl_3x6H_2O$—0.08 g; $Na_2EDTAx2H_2O$—0.1 g.
- Solution III was: H_3BO_3—0.185 g; $MnCl_2x4H_2O$—0.415 g; $ZnCl_2$—0.003 g; $CoCl_2x6H_2O$—0.0015 g; $CuCl_2x2H_2O$—0.00001 g; Na_2MoO_4 $x2H_2O$—0.007 g.
- Solution IV was $NaHCO_3$—50 g.

3.2.2. Preparation of the basic solutions

Weighted amounts of appropriate salts were entered to each of four volumetric flasks and complemented with distilled water up to the volume of 1 dm^3. The solutions were sterilized. The basic solutions were kept in dark bottles at 4 ℃.

3.2.3. Preparation of the culture medium

About 10 cm^3 of basic solution I was entered into a 1 dm^3 volumetric flask, followed by adding 1 cm^3 of each of the other basic solutions (II, III, and IV); finally, it was complemented with deionized water up to the volume of 1 dm^3.

3.2.4. Determination of metal ions in algal biomass

First, the algal biomass was mineralized (in accordance with PN—EN 14084:2004).

Weighed out dry mass (d.m.) of algae (0.038–0.058 g) was entered to 100 cm^3 test tubes. About 10 cm^3 of chloroazotic acid was added to each of the prepared samples. Next, the process was conducted in VELP DK20 digestion unit in three successive temperature ranges: I—20 min at temperature 70 ℃, II—30 min at temperature 100 ℃, and III—20 min at temperature 140 ℃. After cooling, the solutions were dripped through qualitative filters. Then, Cd(II) and Cu(II) were assayed with the AAS method according to PN-ISO 8288/2002 using the novAA 400 spectrometer from Analytik Jena Company, with atomization in the flame.

4. Procedure

4.1. Breeding the algae

The cultures were carried out simultaneously in two tanks, 120 dm^3 each, in the temperature 24 ℃ (±2 ℃) lit with cool white fluorescent lights L36W/840 in a continuous mode. After entering the culture medium prepared in accordance with the procedure described in 3.2.3. to the tanks, previously multiplied culture of *P. subcapitata* algae was entered in the culture referred to as no. 1, and algae obtained from natural environment, to culture no. 2 (item 3.1). So as to keep the algae suspended, the cultures were slightly mixed. The pH in both cultures was kept at the level of 7.5–7.8. The control of the process involved systematic measurements of the number of algae occurring in the culture medium. The number of algae (specimens) was determined with the use of a microscope and a Sedgwick-Rafter Counting Cell.

4.2. Procedure of the experiments

About 200 cm^3 of the culture medium grafted with known numbers of algae sampled from culture no. 1 was entered into each of 11 glass 500 cm^3 bioreactors. Then, solutions of cadmium and copper compounds were entered in amounts corresponding to the concentrations of those ions in the water of the Poraj dam reservoir [20].

- Bioreactors 1–5 — a solution of cadmium prepared from the $CdCl_2 \times H_2O$ salt was entered, giving the concentration of 0.142 mg/dm^3.
- Bioreactors 6–10 — a solution of copper prepared from the $CuCl_2$ salt was entered, giving the concentration of 0.142 mg/dm^3.
- Bioreactor 11 — culture medium including *P. subcapitata* algae was the control sample.

The process was carried out for five reaction times: 10 min, 1, 2, 4, and 24 h.

After the lapse of a specific incubation time, the whole content of bioreactors was centrifuged for 5 min at the speed of 3,000 rpm. The centrifuged algae were dried to solid mass in the temperature 105˚C, and then ground down in a mortar. Next, the obtained biomass samples were mineralized and then the concentration of the cadmium and copper ions was assayed.

From each sample of the decanted culture medium, 20 cm^3 was taken through a qualitative filter and acidified with 1 cm^3 of concentrated HNO_3 to pH approx. 2. The samples were kept at temperature 4˚C until the moment when the concentration of cadmium and copper ions was determined.

All the assays (including the control sample) were carried out twice.

Subsequently, the experiment was performed for algae coming from culture no. 2. In order to obtain comparable results, the number of algae in both cultures was equalized before entering the solutions of Cu(II) and Cd(II) (in the same concentration as for culture no. 1).

5. Results and discussion

5.1. Changes of cadmium (II) and copper (II) concentration in the algal biomass

The biosorption process was controlled by determination of the concentration of ions of metals in the algal biomass and in the culture medium after a specified incubation time. The process was evaluated in relation to the control culture without any compounds of Cu(II) or Cd(II).

Before entering compounds of Cu(II) or Cd(II) to the culture medium, it was proved that the participation of Cd(II) in the biomass of algae multiplied from the pure culture (culture no. 1) was 0.023 mg/g d.m., and in the biomass of algae coming from the Poraj reservoir (culture no. 2), it was 0.007 mg/g d.m. It was also determined that the participation of copper in the biomass of algae from culture no. 1 was 0.041 mg/g d.m., and from culture no. 2 was 0.017 mg/g d.m. (Table 1). It was proved that even in the initial phase of the experiment, cadmium (II) and copper (II) occurred in low concentrations in the biomass of algae coming from different environments. It is thought that the presence of ions of copper in the biomass is justified. Copper (II) is one of microelements and was part of the culture medium. As for the ions of cadmium, it is supposed that they had been entered there incidentally. It might have happened as a result of slight (regarding the number of the specimens entered) pollution of the culture with *S. quadricauda*. It must also be emphasized that the concentration of Cu(II) and Cd(II) ions in the biomass of algae coming from culture no. 1 was approximately two times higher.

In further studies on the dynamics of removing metal ions, it was noted that the concentration of cadmium (II) in the biomass after the initial increase from 0.023 to 0.110 mg/g d.m. (culture no. 1) and from 0.007 to 1.251 mg/g d.m. (culture no. 2) in the second hour of incubation lowered to 0.057 and 0.663 mg/g d.m., respectively (Table 1, Fig. 1), and grew again in the subsequent period. Cadmium (II) concentration after 4 h was 0.134 (culture no. 1) and 0.726 (culture no. 2) mg/g d.m. After extending the time of incubation up to 24 h, it was proved that the highest concentration of cadmium (II) in the biomass of algae coming from culture no. 1 was 0.134 mg/g d.m. It was found that the concentration had stabilized even in the 4th hour of the culture. In the algal biomass from culture no. 2, the highest concentration of cadmium (II) was 1.561 mg/g d.m., which occurred after 24 h of incubation.

Comparing the effectiveness of the sorption process for both populations, it was found that the biomass of algae coming from natural environment retained nearly 10 times more cadmium (II) in comparison with laboratory-bred algae *P. subcapitata*.

Table 1
The dynamics of changes in cadmium and copper ions concentrations within 24 h exposure

	Cadmium (II) concentration in algae [mg/g d.m.]		Copper (II) concentration in algae [mg/g d.m.]	
Time of exposure	Culture no. 1	Culture no. 2	Culture no. 1	Culture no. 2
0	0.023	0.007	0.041	0.017
10 min	0.047	0.652	0.206	0.875
1 h	0.110	1.251	0.271	1.657
2 h	0.057	0.663	0.654	1.939
4 h	0.134	0.726	0.541	1.252
24 h	0.131	1.561	0.148	1.162

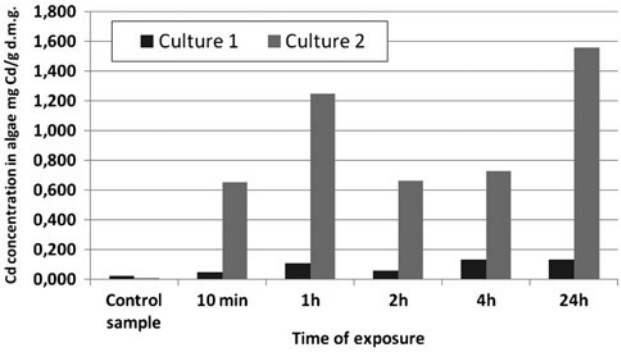

Fig. 1. Change in concentration of cadmium (II) in the algae biomass depending on the time of exposure.

In studies on the dynamics of copper (II) sorption process, it was noted that in the initial incubation period (Table 1, Fig. 2), the concentration of Cu(II) in the algal biomass gradually increased. For example, after the lapse of two hours, an increase from 0.041 to 0.654 mg/g d.m. (culture no. 1) and from 0.017 to 1.939 mg/g d.m. (culture no. 2) was observed. The next period of the study showed that in the fourth and then in the 24th hour of the process, the copper (II) concentration only decreased gradually. After four hours of incubation, the concentration of copper (II) was 0.541 mg/g d.m. (culture no. 1) and 1.252 mg/g d.m. (culture no. 2). After 24 h of incubation it was shown that cadmium (II) concentration in algae from culture no. 1 was 0.148 mg/g d.m., and from culture no. 2 was 1.162 mg/g d.m. It was found that the process of copper (II) ions biosorption was most evident in the case of algae coming from the natural environment in the second hour of the experiment (1.939 mg/g d.m.). The concentration of copper (II) in the biomass of algae from culture no. 2 after two hours of exposure was more than three times higher than the concentration in the biomass of algae from culture no. 1.

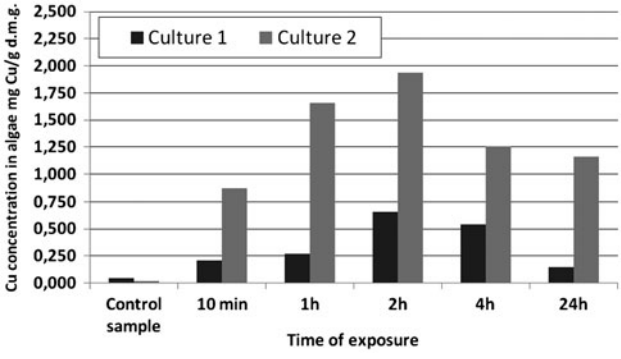

Fig. 2. Change in concentration of copper (II) in the algae biomass depending on the time of exposure.

5.2. Changes of cadmium (II) and copper (II) concentration in the culture medium

The dynamics of changes in cadmium (II) concentration and the initial and final copper (II) concentration in the culture medium during the sorption process is presented in Table 2. The rate of removal of those ions is considered to be very high (99%). After 24 h of the biosorption process, there was a change of concentration in the culture medium from 0.142 mg/dm^3 for cadmium (II) to 0.08 mg/dm^3 and for copper (II), to 0.04 mg/dm^3.

5.3. Summary

The current knowledge allows to distinguish a few mechanisms responsible for the uptake of metal ions in the process of biosorption. The most important of them are: complex reaction, chemisorption, chelation, physical adsorption, and microprecipitation. Thus, the process of biosorption is complex and difficult to evaluate, since the above-mentioned mechanisms may occur simultaneously and with different efficiencies. The process depends to a great extent on the kind of algae, physicochemical properties of the water environment, and on the kind of functional groups present in the cell walls of the algae [7].

It must be emphasized once again that the process of sorption was carried out in such a way that the only difference in the experiment was the kind of algae used. Therefore, comparing the final concentrations in biomass of algae from culture no. 1 and no. 2: for cadmium (II) 0.131 and 1.561 mg/g d.m. and for copper (II) 0.148 and 1.162 mg/g d.m., as well as taking into consideration the final concentrations of cadmium (II) and copper (II) in the culture solution no. 1, it was found that certain losses of the ions had occurred. The final concentration for culture no. 1 was up to 0.08 mg Cd(II)/dm^3 and for copper (II), up to

Table 2
Changes in Cd(II) and Cu(II) ions concentration within 24 h exposure in the culture medium

Time of exposure	Cd(II) concentration in the medium [mg/dm^3] Culture no. 1	Cu(II) concentration in the medium [mg/dm^3] Culture no. 2
0	0.01	0.01
10 min	0.05	–
1 h	0.08	–
2 h	0.10	–
4 h	0.07	–
24 h	0.08	0.04

0.04 mg Cu(II)/dm^3. Too little concentration of cadmium (II) and copper (II) in algal biomass and very high rate of their removal from the culture medium indicate that another process was at work besides sorption, leading to such effective removal of cadmium (II) and copper (II).

It is thought that the observed changes in concentrations of Cd(II) and Cu(II) in the medium in combination with changes in the algal biomass are difficult to explain in detail without the knowledge of changes in concentration of the other indicators, including biogenes (N and P).

However, the fact that the study of sorption was carried out in oxidizing conditions was taken into consideration. The photosynthesis process occurred over the whole experiment period, what is also connected with the increase of the biomass. That is why there is a high probability that changes in contents of heavy metal ions in the culture medium might also have been caused by the process of microprecipitation connected with the increase of pH. That statement is in agreement with the process of keeping carbonate equilibrium and taking CO_2 in the process of photosynthesis.

6. Conclusions

The study proved that algae were a beneficial biosorbent for ions of cadmium and copper. The process occurred with various intensities depending on the time of contact between the biomass and a given metal. The mixed population of chlorophyta taken from a natural water reservoir sorbed much better both Cd(II) (almost 10 times more effectively) and Cu(II) (over three times more effectively) in comparison with *P. subcapitata* bred in laboratory conditions. Biodiversity of algae had a favorable influence on the effectiveness of Cu(II) and Cd(II) removal process.

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