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BIOSORPTION OF HEAVY METALS WITH THE USE OF MIXED ALGAL POPULATION

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Abstract: This work presents the results of a study whose aim was to determine the influence of algal blooms on precipitation of heavy metals. The scope of the study covered culture of a mixed population made up of *Scenedesmus* and *Pseudokirchneriella* algae in experimental conditions and initiating a metal biosorption process with the use of culture biomass by administering ions of Zn(II) and Ni(II). The process was controlled by assessing the level of biosorption of metals entered at a one-off basis in the form of Zn(II) and Ni(II) salts or in the form of mixture of both ions, in comparison to the control sample, at different exposure times (2 hours and 24 hours). The presence of metals was determined both in the biomass and in the culture medium. The presented results of the study confirm the effectiveness of Chlorophyta in the process of zinc and nickel biosorption. A phenomenon of competitiveness between the metals was observed when they were administered at the same time.

INTRODUCTION

The problem of contamination of waters with heavy metals, occurring for many years, indicates real dangers for the environment and for humans. Therefore, solutions are sought which would be both safe for the environment and effective in the function of cleaning waters of a reservoir from heavy metals.

One of the directions of study connected with this issue is to determine how the biosorption process occurs on the example of selected organisms. Biosorption of heavy metals is a process of their removal in cooperation of microorganisms. Ions of metals enter a cell or are bound by function groups of the cell wall. Whether the metal will be accumulated in the cell wall or diffuse into the cell depends on the form in which the metal is accessible for microorganisms [6]. Thanks to the complexing properties of the cell wall, the accumulation of metal on the surface of microorganisms is possible. For the metal to be accumulated inside the cell, metabolic energy is necessary, as well as efficient system of transport through the cytoplasmic membrane and activity of enzymatic systems. The effectiveness of metal biosorption is also affected by whether the cell is alive or dead [3].

The biosorption process allows for concentration of little volumes of metals and is undoubtedly one of the cheaper methods of eliminating metals from contaminated waters. It is characterized by high efficiency and short time of reaction. The microorganisms which demonstrate the possibility of biosorption of heavy metals from degraded environments are, among others, algae. Chlorophyta, commonly occurring in surface waters, may cause changes of metal contents in water reservoirs. It is also thought that algal blooms, common in spring and summer in water reservoirs, may be favourable for transfer of heavy metal ions from waters to bottom sediments [6]. That phenomenon is promoted by higher metal concentrations occurring in the surface microlayer than in deeper waters [10]. That allows the blooming algae to sorb greater amounts of heavy metals, which as a result will be accumulated in bottom sediments.

There are many studies conducted whose aim is to develop new technologies of treatment of wastewaters contaminated with heavy metals. Many species of algae resistant to heavy metals are successfully used in experiments aimed at biological sewage treatment (e.g. *Spirogyra* – removal and recovery of mercury from sewage) or recovery of metals from contaminated waters (e.g. mining wastewaters) [9]. The use of natural biosorbents should be particularly taken into consideration due to their relatively low production costs, resulting from the biological origin. The advantages of biomass include virtually non-limited number of binding sites, high affinity to various metals and the possibility of their recovery. What is more, biological methods making use of biomass are characterized by simple disposal of waste, e.g. by burning [3, 5, 7].

AIM AND SCOPE OF THE STUDY

The aim of the study was to find out to what extent ions of heavy metals, introduced on a one-off basis in the form of Zn(II) and Ni(II) salts or in the form of a mixture of Zn(II) and Ni(II), will be biosorbed by algae biomass and what concentrations of them will still remain in the solution.

The scope of the study covered a culture of mixed algal population in laboratory conditions (so as to obtain material for study), followed by initiating the process of precipitation of Zn(II) and Ni(II) ions with the use of the culture biomass, as well as evaluating the process with consideration of the level of biosorption of both metals.

METHODOLOGY OF THE STUDY

Characteristics of the algae used in the study

The object of the study was a mixed population of *Pseudokirchneriella subcapitata* and *Scenedesmus quadricauda* Chlorophyta.

Pseudokirchneriella subcapitata are numbered among colonial organisms, which usually occur as unicellular organisms in a culture. Cells of *Pseudokirchneriella subcapitata* have the shape of crescents, 8−12 μm long. They multiply through zoospores, at the rate of 4−8 cells a day. Those algae are a common element of phytoplankton of warm temperate climate fresh waters. They occur in eutrophicated waters [1]. They are used as plant bioindicators for chronic toxicity tests Algaltoxkit F™. Although the culture medium used for multiplication of *Pseudokirchneriella subcapitata* was sterilized, various kinds of Chlorophyta occurred in the culture, mainly as the *Scenedesmus quadricauda*

dominant. Those algae are commonly found in surface waters, usually fresh ones. The width of the cells is 6−18 μm and length – 14−25 μm. They have a cylindrical shape and often create four-cell cenobia [4].

The culture medium

The medium for algae culture was prepared in accordance with Commission Directive No. 92/69/EEC of 31/07/1992. Four basic solutions were prepared, on the basis of which the medium was created:

– solution I: NH_4Cl – 1.5 g; $MgCl_2 \times 6H_2O$ – 1.2 g; $CaCl_2 \times 2H_2O$ – 1.8 g; $MgSO_4 \times 7H_2O$ – 1.5 g; KH_2PO_4 – 0.16 g;
– solution II: $FeCl_3 \times 6H_2O$ – 0.08 g; $Na_2EDTA \times 2H_2O$ – 0.1 g;
– solution III: H_3BO_3 – 0.185 g; $MnCl_2 \times 4H_2O$ – 0.415 g; $ZnCl_2$ – 0.003 g; $CoCl_2 \times 6H_2O$ – 0.0015 g; $CuCl_2 \times 2H_2O$ – 0.00001 g; $Na_2MoO_4 \times 2H_2O$ – 0.007 g;
– solution IV: $NaHCO_3$ – 50 g.

The salts were weighed out to four volumetric flasks in the amounts mentioned above, and then complemented with distilled water up to 1 dm^3. The basic solutions were sterilized and then kept in the temperature of 4°C, in sterile, dark bottles. The prepared solutions were used for preparation of culture medium. In order to make it, 10 cm^3 of basic solution I was entered into a 1 dm^3 volumetric flask and samples of 1 cm^3 of basic solutions II, III and IV were added; finally, it was complemented with deionized water to the volume of 1 dm^3 [8].

Measurement of metal content in algal biomass

The algal biomass was mineralized in accordance with PN – EN 14084:2004.

Weighed out, dry mass of algae was entered to seven threaded test tubes (0.2 g to each). 10 cm^3 of chloroazotic acid was added to each tube. The samples were left for 24 hours. Then mineralization was performed in a NANOCOLOR VARIOZ thermoblock. The process lasted for 45 minutes in the temperature of 120°C. Next, the content of tubes was filtered through a qualitative filter (65 g/m^3). The AAS-assay was performed for the solutions.

The metal content in the culture media and biomass was determined with the method of Atomic Absorption Spectrometry (AAS) in the flame, in accordance with PN-81/C-04570/01 and with the use of MOVAA 400 spectrometer from Analityk Jena.

THE COURSE OF THE STUDY

Keeping the culture

The culture was initially multiplied in 1 dm^3 round-bottommed flasks and then closed with cotton wool stoppers. The freshly-prepared medium was aerified for 30 minutes, and then the pH was adjusted to the value of 8.3 ± 0.2, after which a graft of clean *Pseudokirchneriella subcapitata* culture was introduced. In order to prevent precipitation of salts included in the medium, the flasks were located on magnetic stirrers working in low gear. The culture was kept in the temperature of 30°C and lit with fluorescent lights L36W/840 in a continuous mode. After obtaining 6 dm^3, the culture was moved to a water tank for further multiplication. After obtaining 14 dm^3 of culture with density of 2,400,000 specimens in 1 cm^3 of the medium, the experiment was started.

Doing the experiment

1.5 dm^3 samples of medium grafted with algae were entered into seven 2 dm^3 beakers, constituting bioreactors. In order to prevent precipitation of metals entered, before their introduction to the algal culture, the pH was adjusted to 7.5. Next, metal ions were entered in the following order (Fig. 1):

– to bioreactors I and IV, $ZnCl_2$ was entered in the amount increasing Zn concentration in the solution of 0.1 mg/dm^3,
– to bioreactors II and V, $NiCl_2 \times 6H_2O$ was entered in the amount increasing Ni concentration in the solution of 0.3 mg/dm^3,
– to bioreactors III and VI, both zinc and nickel ions were entered in the amounts increasing Zn and Ni concentration in the solution of 0.1 mg Zn/dm^3 and 0.3 mg Ni/dm^3,
– the reactor VII was a control culture.

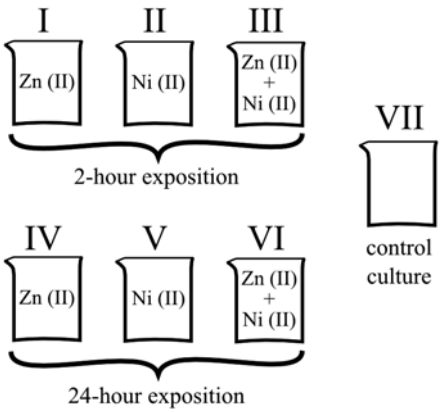

Fig. 1. Distribution of heavy metals in the bioreactors

The introduced metal concentrations were determined with the use of information on the contents of nickel and zinc in the Poraj dam reservoir located on the Warta River. Concentrations of metals used in the experiment were assumed on the basis of an average content of Ni(II) and Zn(II) in the waters of the reservoir in the year 2009 [2].

After the lapse of 2 hours from the moment of administering metals, the contents of bioreactors I, II and III were centrifuged in a centrifugal separator for 5 minutes at the speed of 4,000 rotations per minute. Then, the culture medium was decanted from above the algae. The centrifuged algae were dried to solid mass in the temperature of 105°C, and then ground down in a mortar. The dry mass of algae in each bioreactor amounted to approx. 0.2 g. Mineralization of the 3 obtained samples of biomass was performed, and then the content of Zn(II) and Ni(II) was determined with the use of AAS (see "Measurement of metal content in algal biomass").

From each sample of decanted culture medium, 20 cm^3 was taken through a qualitative filter and acidified with concentrated HNO_3 to pH approx. 2. The medium samples were kept in the temperature of 4°C until the moment of determination of metal content with AAS.

An analogous process was performed for the content of bioreactor VII, containing the control sample, and after 24 hours from the moment of administering metals, the same was done to the contents of bioreactors IV, V and VI.

The cultures of algae to which metals had been administered were incubated in the temperature of 30°C and lit for 2 hours or 24 hours.

RESULTS AND DISCUSSION OF RESULTS

The control of biosorption process was done by determination of changes in metal concentrations after 2 and 24 hours from the moment of their administering. Both the algae biomass and the culture medium were tested (Table 1). Evaluation of the process was performed in comparison to the control culture (not treated with metals).

Table 1. Values of concentrations of Zn(II) and Ni(II) determined with the AAS method for particular samples in the medium and in algae biomass

Type of culture	Time of exposure to heavy metal ions [h]	**Zn**			**Ni**		
		In the culture medium [mg/dm^3]	In biomass [mg/dm^3]	[mg/mg of d.m. of algae]	In the culture medium [mg/dm^3]	In biomass [mg/dm^3]	[mg/mg of d.m. of algae]
Control culture	0	0.0362	13.34	0.095	0.0099	0.5847	0.004
Treated with Zn(II)	2	0.0654	18.47	0.131	-	-	-
Treated with Ni(II)	2	-	-	-	0.1911	8.32	0.056
Treated with Zn(II) +Ni(II)	2	0.0792	71.40	0.489	0.1745	2.642	0.018
Treated with Zn(II)	24	0.0584	20.12	0.128	-	-	-
Treated with Ni(II)	24	-	-	-	0.1468	11.075	0.072
Treated with Zn(II) +Ni(II)	24	0.0548	90.25	0.564	0.1641	10.83	0.068

In the culture medium (Fig. 2), the highest Zn(II) concentration was after 2 hours of exposure, from the moment of introducing Zn(II) and Ni(II) at the same time. The highest concentration of Ni(II) was achieved after 2 hours of exposure, from the moment of administering nickel ions. In both cultures, after 24 hours there was a decrease of both Zn(II) and Ni(II) concentrations in comparison to the concentration observed after 2 hours of exposure.

When comparing direct changes of concentrations of Zn(II) and Ni(II) to the control sample, it was observed that they were higher for Ni(II) than for Zn(II). It must be noted

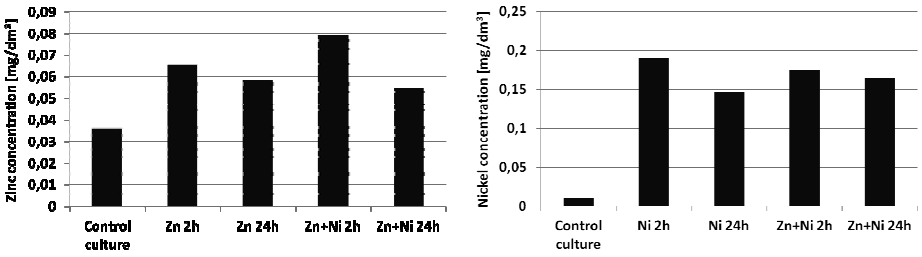

Fig. 2. Change in concentration of Zn(II) and Ni(II) in the culture medium depending on the time of exposure (2 hours and 24 hours)

that it resulted from administering three times higher concentrations of nickel than those of zinc.

When observing the process of biosorption in algae biomass (Fig. 3), a clear mutual correlation between zinc and nickel was found. In samples after 2 and 24 hours, to which both ions were administered, the concentration of Zn(II) was three times higher than in biomass taken from the culture after 2 and 24 hours of exposure if only Zn(II) was administered. In the culture treated with zinc, after 2 hours of exposure the concentration of Zn(II) in biomass amounted to 18.47 mg Zn/dm^3 (after 24 hours of exposure – 20.12 mg Zn/dm^3), and for the culture additionally enriched with Ni(II), the concentration of zinc rose to 71.40 mg Zn/dm^3 (after 24 hours of exposure – 90.25 mg Zn/dm^3). The presence of nickel improved biosorption of Zn(II) in algae biomass.

The effectiveness of biosorption process in the case of administering individual metals was much higher for nickel than for zinc in comparison to the control sample. The concentration of Ni(II) after 2 hours of exposure (8.32 mg Ni/dm^3), was 14 times higher in comparison to the control sample (0.5847 mg Ni/dm^3), and after 24 hours, nearly 19 times higher (11.075 mg Ni/dm^3).

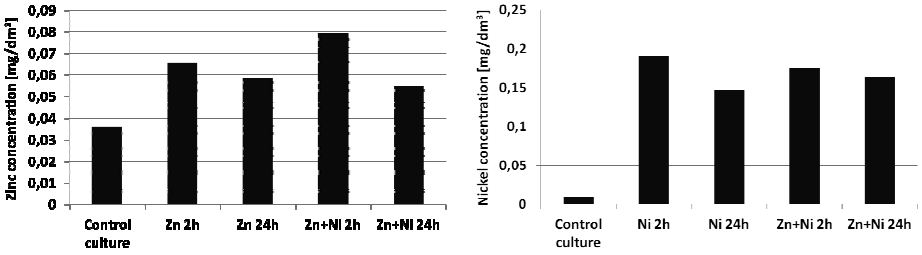

Fig. 3. Change in concentration of Zn(II) and Ni(II) in the algae biomass depending on the time of exposure (2 hours and 24 hours)

When administering both metals at the same time, biosorption of nickel in biomass was much less intensive. The content of Ni(II) after 2 hours of exposure was 2.64 mg Ni/dm^3 in the culture treated with both metals, which is a result over 3 times lower

than in the culture treated with nickel alone (8.32 mg Ni/dm^3). As for concentration of Ni(II) after 24 hours of exposure, it was comparable in cultures fed with zinc and nickel (10.83 mg Ni/dm^3) and nickel alone (11.075 mg Ni/dm^3). A longer exposure time positively affects the effectiveness of nickel biosorption when administering both metals at the same time.

CONCLUSIONS

The results of this study, presented in the work, prove that a mixed Chlorophyta population is a favourable biosorbent for ions of Zn(II) and Ni(II). The process occurred with various intensity depending on the time of exposure of biomass to a given metal and presence or absence of another metal in the environment. A longer time of exposure helped improve the process of biosorption in algae biomass, at the same time lowering the content of metals in the culture medium.

It was observed that nickel was faster accumulated than zinc. After two hours of exposure, the concentration of nickel in biomass was 14 times higher than in the control sample. Biosorption of zinc was faster when there was nickel in the environment, and nickel was faster sorbed alone. The presence of zinc in the environment inhibited the sorption of nickel.

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BIOSORPCJA METALI CIĘŻKICH Z UDZIAŁEM MIESZANEJ POPULACJI GLONÓW

Niniejsza praca prezentuje wyniki badań, których celem było określenie wpływu zakwitów glonowych na wytrącanie metali ciężkich. Zakres badań obejmował hodowlę mieszanej populacji złożonej z rodzajów *Scenedesmus* i *Pseudokirchneriella* w warunkach eksperymentalnych, a następnie zainicjowanie procesu biosorpcji

metali przy udziale hodowlanej biomasy, poprzez dawkowanie jonów Zn(II) i Ni(II). Kontrola procesu polegała na dokonaniu oceny stopnia biosorpcji metali, wprowadzonych jednorazowo w postaci soli Zn(II) lub Ni(II) lub w postaci mieszaniny obu jonów, w porównaniu do próbki kontrolnej przy różnym czasie ekspozycji (2 oraz 24 godziny). Oznaczeniom na obecność metali poddano zarówno biomasę, jak i podłoże hodowlane. Przedstawione wyniki badań potwierdzają skuteczność zielenic w procesie biosorpcji cynku oraz niklu. Zaobserwowano zjawisko konkurencyjności pomiędzy metalami, gdy były dawkowane jednocześnie.