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The Effect of Biomass (*Chlorella vulgaris, Scenedesmus armatus*) Concentrations on Zn^{2+}, Pb^{2+} and Cd^{2+} Biosorption from Zinc Smelting Wastewater

Wpływ stężenia biomasy (*Chlorella vulgaris, Scenedesmus armatus*) na biosorpcję Zn^{2+}, Pb^{2+} i Cd^{2+} ze ścieków z ocynkowni

Non-modified and chemically modified algal biomass of *Chlorella vulgaris* and *Scenedesmus armatus* were chosen to examine their Zn^{2+}, Pb^{2+} and Cd^{2+} sorption activity. The dry biomass was chemically modified with 1M HNO_3 in order to determine the influence of acid-treatment on the heavy metal ions removal rate. The heavy metal uptake process was found to be rapid and very efficient. The sorption process of heavy metals is highly pH-dependent. It influences heavy metal forms in the solution, and functional groups located on the cell surface. The effectiveness of biosorption depends also on the biomass concentration and its modifications. The highest adsorption capacities of both, *Chlorella vulgaris* and *Scenedesmus armatus* towards zinc, lead and cadmium ions were found for the lowest sorbent concentrations. Batch experiments showed that the differences between uptake by non-modified and acid-modified biomass are relatively small. It can be due to the fact that non-modified algal biomass exhibits high sorption capacity.

Keywords: **algae, *Chlorella vulgaris*, *Scenedesmus armatus*, heavy metal ions, sorption, modified microalgal biomass**

Introduction

The increasing contamination of the environment by heavy metals is a worldwide problem. This situation is a result of industrial activities which are related to the population growth, urbanization and industrialization [1, 2]. The toxic effect of heavy metals on the environment and living organisms is observed even at low concentrations, due to their accumulation in the food chain [3, 4]. Thus, scientists focus on finding the proper method for such contamination removal.

Many conventional processes are known and used for the heavy metals removal from industrial effluents. They include precipitation, ion exchange, membrane separation, adsorption, electrochemical treatment, solvent extraction, evaporation and reverse osmosis. However, most of these processes are expensive and not environmentally friendly, since waste products and toxic sludge are produced, which have to be disposed [3-8]. Hence, the development of efficient and inexpensive methods for heavy metals removal may be crucial.

The use of biological material is a potential technology for heavy metals removal from industrial effluents, due to the low cost of the sorbent and environmentally friendly manner of the process [3, 6]. Microorganisms which have been tested for active (bioaccumulation) or passive (biosorption) heavy metals accumulation include bacteria, algae, fungi or yeast [3, 5, 9-12]. Microalgae, as well as living or dead forms are being increasingly used as metal biosorbents [13-15]. This is due to the rapid uptake of metal ions, low cost and high efficiency of the process, energy and time saving, fast growth rate of the biomass and the possibility of reuse. The heavy metal ions uptake by algal biomass is connected to the ion exchange process [1, 4, 9, 16, 17]. The used algal biomass could be used as a feedstock for e.g. biofules production [18].

Some studies have shown that the removal of heavy metals from industrial wastewater can be enhanced by different biomass pre-treatments. They include treatment with solutions of different acids, alkalis, organic solvents and other chemicals [5, 19, 20]. However, the information on the performance of chemically modified algae for smelter wastewater treatment has not been investigated previously. Therefore, the aim of this study was to investigate the influence of chemical modification and algal biomass concentration on heavy metals removal from the industrial wastewater.

These studies were carried out in order to: (i) examine the hypothesis that the chemical modification of algal biomass would enhance the removal efficiency of heavy metals, (ii) to determine the most suitable biomass concentration for heavy metals removal, and (iii) to verify if there was a potential possibility of *C. vulgaris* and *S. armatus* application as effective biomaterial for the development of Zn^{2+}, Pb^{2+} and Cd^{2+} removal procedure on the industrial scale.

1. Materials and methods

1.1. Substrates

The experiments were conducted using industrial wastewater acquired from zinc smelter. The initial metal concentrations for Zn^{2+}, Pb^{2+} and Cd^{2+} were 87.0, 0.725 and 8.35 mg/L, respectively. The pH of the wastewater equaled 10.2.

The strains of unicellular green microalgae *C. vulgaris* and *S. armatus* were obtained from the Culture Collection of Baltic Algae (CCBA), Institute of Oceanography, University of Gdansk, Poland. In the exponential phase of algal growth, the biomass of *Chlorella vulgaris* and *Scenedesmus armatus* were harvested by sedimentation and centrifugation (4000 g for 15 min). Dry biomass was obtained by drying in an oven at 80°C for 24 h. Then, it was grounded in a mill in order to unify the grains diameter. The resulting biomass powder was used as the raw biomass, or initially subjected to acid chemical treatment in this research.

The algal biomass was subjected to acid-modification with 1M HNO_3 to enhance the metal sorption capacity [19]. A sample of 20 g of the raw powdered biomass was modified with 800 mL of treating agent for 24 h under slow stirring. The modified cells were washed several times with deionized water. Then, the biomass was dried in an oven at 80°C for 24 h.

1.2. Batch experiment

The biosorption experiments were performed in 250 mL Erlenmayer flasks. The amount of industrial wastewater in each flask equaled 100 mL. The initial pH of the wastewater was adjusted to 6 with the use of 1% HCl. The sorbent in the concentration of 50, 10 and 1 g/L in the form of raw or modified biomass was added to the wastewater samples and then, using the flask shaker the samples were stirred for 30 mins. Next, the samples were subjected to sedimentation for another 30 min.

The literature and preliminary studies conducted by the authors showed that this is the proper time to reach equilibrium [3, 5, 19]. After that time the pH values were measured and afterwards the biomass was removed by filtration through a 0.45 μm membrane filter. The supernatant was acidified with 10% HNO_3 to the pH of 2 and analyzed for heavy metal: Zn^{2+}, Pb^{2+} and Cd^{2+} concentrations by inductively coupled plasma spectrometer (ICP-OES).

Heavy metal uptake by biomass was calculated according to the formula:

$$\text{Uptake} = \frac{C_0 - C_k}{C_0} \cdot 100, \ \% \tag{1}$$

where:
C_0 - the initial metal concentration, mg/L,
C_k - the final metal concentration, mg/L.

Sorption capacity of biomass was calculated according to the formula:

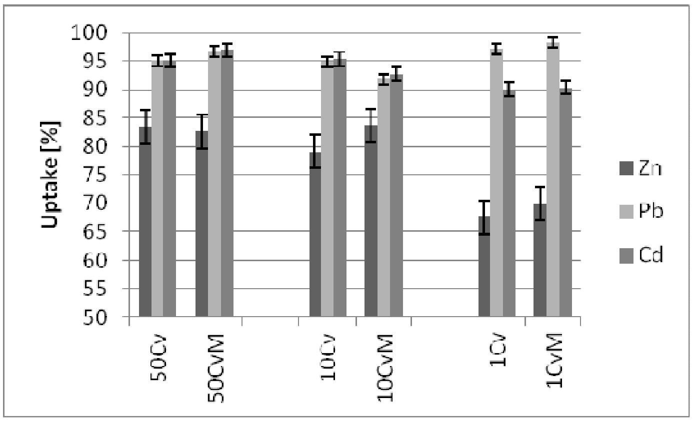
$$A = \frac{(C_0 - C_k)}{m} V, \ \frac{\text{mg}}{\text{g}} \tag{2}$$

where:
V - volume of the sample, L,
m - mass of the sorbent, g.

2. Results and discussion

2.1. Influence of heavy metal concentration on uptake them by biomass

Chong et al. found that cadmium ions are better sorbed than zinc ions by algae biomass [21]. Figure 1 shows that the same trend was also observed in the present experiment. However, the highest initial concentration of Zn^{2+} (87.0 mg/L) that was 10-fold as Cd^{2+} (8.35 mg/L) and 120-fold as Pb^{2+} (0.725 mg/L) influenced the lowest efficiency of uptake of that heavy metal by biomass. The uptake of Cd^{2+} by was of 89÷96%, nevertheless, the modified or non-modified biomass of both algal strains was tested.



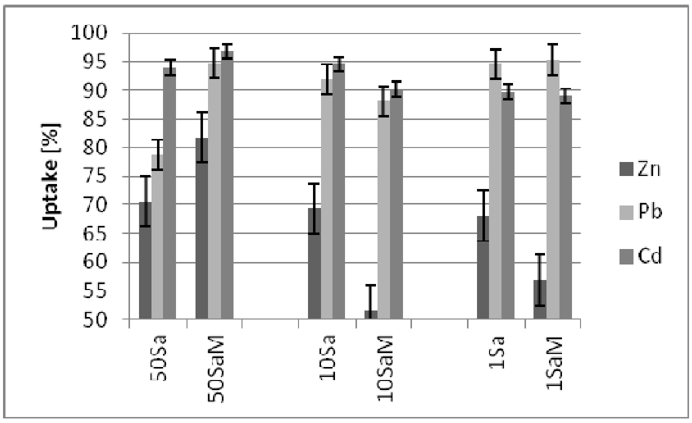


Fig. 1. Heavy metal uptake by non-modified and modified (M) *Chlorella vulgaris* (Cv) and *Scenedesmus armatus* (Sa) biomass, and the final pH of the solution

The heavy metals concentration as well as sorbent concentration influence the efficiency of sorption. It is due to the availability of binding sites responsible for metal ions removal [3]. The lowest uptake of Zn^{2+}, that was observed for all sorbent concentrations, can be also the result of the biomass selectivity to metal ions.

Sekhar et al. determined that the higher initial concentration of zinc ions, the lower the percentage of others metal removal [22]. However, the increase in the initial metal concentration to a certain level increases the sorption capacity due to the lower availability of the binding sites and higher amount of heavy metal sorbed per unit biomass. To obtain the highest uptake of heavy metals, the biomass concentration can be increased only to a certain level [22].

The highest uptake of Zn^{2+} was found by *Chlorella vulgaris* than for *Scenedesmus armatus* and the maximum sorption of Zn^{2+} was achieved at 10 g/L (83%) with no significant increase at 50 g/L biomass concentration. For Sa strain, non-modified biomass sorption reached maximum uptake of 68÷70% at all biomass concentration tested. Treated biomass showed inconsistent results.

For the initial concentration of *Chlorella vulgaris* and *Scenedesmus armatus* of 1 and 10 g/L, the maximum uptake of Pb^{2+} by was 88÷98% nevertheless, the modified or non-modified biomass was used. The initial biomass concentration of 50 g/L showed inconsistent results.

2.2. Sorption capacity of *Chlorella vulgaris* and *Scenedesmus armatus* towards heavy metals ions

Table 1 shows the sorption capacity of *Chlorella vulgaris* and *Scenedesmus armatus* towards Zn^{2+}, Pb^{2+} and Cd^{2+}. It was found that sorption capacity highly depends on the biomass concentration and the pH value of the treated wastewater.

Table 1. **The sorption capacity of non-modified and modified (M) *Chlorella vulgaris* (Cv) and *Scenedesmus armatus* (Sa) biomass, and the final pH of the solution**

Sorbent	Sorbent concentration g/L	Sorption capacity mg/g			pH
		Zn^{2+}	Pb^{2+}	Cd^{2+}	
Cv	50	1.45	0.01	0.16	8.68
CvM	50	1.44	0.01	0.16	7.56
Cv	10	6.88	0.07	0.80	8.64
CvM	10	7.28	0.07	0.77	7.65
Cv	1	58.80	0.70	7.52	8.43
CvM	1	60.80	0.71	7.54	7.80
Sa	50	1.23	0.01	0.16	8.61
SaM	50	1.42	0.01	0.16	7.84
Sa	10	6.03	0.07	0.78	8.63
SaM	10	4.49	0.06	0.74	7.77
Sa	1	59.20	0.69	7.39	8.45
SaM	1	49.40	0.69	7.33	7.88

The sorption capacity grows with the decrease in the sorbent concentration. This phenomenon was observed for *Chlorella vulgaris* and *Scenedesmus armatus*, and the differences between the values for modified and non-modified biomass were insignificant.

For both, *Chlorella vulgaris* and *Scenedesmus armatus,* the highest sorption capacities for Zn^{2+} (49.40÷60.80 mg/g), Pb^{2+} (0.69÷0.71 mg/g) and Cd^{2+} (7.33÷7.54 mg/g) were observed for 1 g/L of the sorbent. Similar trends were observed for cadmium and lead ions removal with the use of *Chlorella vulgaris* as biosorbent by Edris et al. [4]. They found that Cd^{2+} and Pb^{2+} are preferably sorbed by smaller biomass concentration.

At very high biomass concentrations, biomass aggregation is often observed. The result is the reduction of the surface area available for sorption, the blocking of the binding sites and in consequence, the decrease in heavy metal uptake per unit biomass [16]. Thus, the sorption capacity in the present experiment decreased with the increase in the biomass concentration.

2.3. Influence of biomass pre-treatment

The use of acid modification on algal biomass results in lowering the pH and desorbing metals and other impurities back to the solution. However, acids can also cause damage of the algal binding sites due to the hydrolysis of polysaccharides which are present on the cell wall surface [16].

In the present study, the differences between sorption capacity of non-modified and modified biomass are relatively small. When algal biomass cell wall is not occupied by cations or this amount is negligible, the differences in uptakes by non-modified and modified biomass can be insignificant. Thus, acid modification cannot influence the removal efficiency. Furthermore, the biomass always has a finite number of binding sites. Consequently, the modification does not create new sites, but only releases the occupied ones [5, 16, 19].

Mehta and Gaur have found that acid-pretreatments enhanced the uptake of heavy metals by algae [20]. However, HCl for some algal species increased the sorption capacity more efficiently than HNO_3. Therefore, the probable explanation for the limited influence of algal biomass modification in the current experiment can be also related to the acid which has been chosen. Similarly, the concentration of HNO_3 in this research was 1M [19], while Mehta and Gaur have used 0.1 mM solution [20]. Moreover, Abu Al-Rub et al. [1] used different concentration of acid solution in their experiment, which equaled 0.1M HCl. They used *Chlorella vulgaris* in sorption/desorption cycles to remove heavy metal ions [1].

Unfortunately, the problem is that acid modification causes biomass losses. In the present experiment, the treatment with 1M HNO_3 caused 20% mass loss. A similar result was obtained by Park et al. In their experiment the mass loss equaled 17% [23]. This can affect the economic aspects of the biosorption process of heavy metals removal.

2.4. The pH influence

Heavy metal sorption by algal biomass is highly pH-dependent [24, 25]. It influences both, heavy metal forms in the solution, and the functional groups located on the cell surface [16]. The algal cell wall contains alginate that leads to very good sorption properties due to its hydroxyl and carboxyl groups [24]. Figure 2 presents the pH value changes for modified and non-modified algal biomass at different concentrations. It can be observed that the pH value of the samples with modified biomass is lower than the samples with non-modified biomass. However, the addition of biomass in both forms to the wastewater increases the pH value.

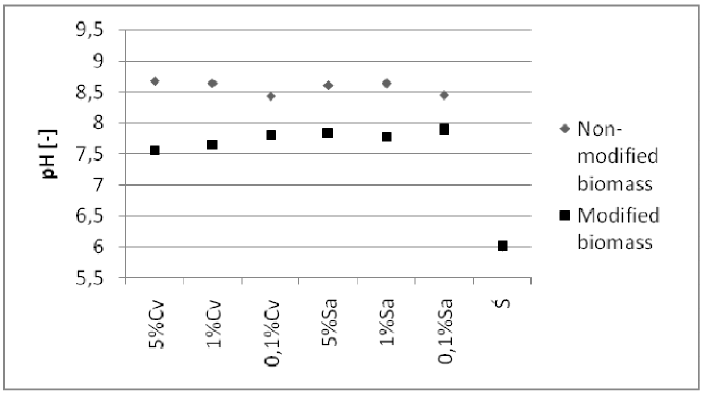


Fig. 2. Changes in the pH values for non-modified and modified (M) *Chlorella vulgaris* (Cv), *Scenedesmus armatus* (Sa) biomass and wastewater (S) pH value. The wastewater (S) was the blank sample

With the increase in acidity, the solubility and mobility of heavy metals increases. The heavy metals chemistry analysis at different pH values is crucial to understand the sorption mechanism [19]. Zinc, cadmium and lead are present in the solution almost completely in the cationic forms at the pH of 6, < 7 and < 5, respectively [26, 27]. Hence, the pH of wastewater in the present experiment has been reduced to pH = 6 before their contact with sorbents. This slight acidification can influence the increase in solubility of heavy metals and bioavailability of them to the algal biosorbent.

The industrial wastewater contains carbonates, phosphates and sulfur. At the pH above 7, the solubility of zinc is reduced, and the presence of cationic form significantly decreases [26]. Thus, the uptake of zinc was not as high as the uptake of cadmium and lead.

Cadmium is considered the most mobile. Likewise, the presence of chlorine, which is found in the analyzed wastewater, enhances the assimilation of cadmium due to the formation of cadmium compounds [27, 28]. Therefore, a very effective uptake of this heavy metal by both *Chlorella vulgaris* and *Scenedesmus armatus* was obtained.

At low pH values (below 2), the cell surface is charged positively as the cell wall ligands are associated with hydronium ions [19, 16]. Thus, repulsive forces decrease the possibility of binding between heavy metal ions and algae biomass. The property of the metal ions release from the binding sites by lowering the pH value is often used for the regeneration of the biosorbent and the recovery of heavy metals. The advantage of such opportunity is the multiple reuse of algal biomass and thus, better economy of the biosorption process [24]. A big advantage of acid-treatment is also the fact that due to washing, more binding sites become available for metal ions in the further process [19].

With the increase in the pH values, the deprotonation of functional groups and the increase in their negative charge are observed. More ligands, such as amino, carboxyl, phosphate and imidazole groups could be removed, and that would result in the attraction between these charges and metal ions. The adsorption is hence increased at high pH [4, 16, 24, 29].

But pH can be increased only to certain level, because at higher pH values the problem of metals precipitation occurs. Thus, it is very important to find the optimal pH for the sorption process [4, 19, 29-31].

Conclusions

In this study, the ability of zinc, lead and cadmium biosorption by non-modified and acid-modified *Chlorella vulgaris* and *Scenedesmus armatus* biomass at the concentrations of 50, 10 and 1g/L has been demonstrated. In general, the process of heavy metal sorption from the industrial wastewater was very quick and efficient. It should be also noticed that almost all the uptakes of lead and cadmium by both, modified and non-modified *Chlorella vulgaris* and *Scenedesmus armatus* biomass were at max of 90%. However, treated biomass showed somehow inconsistent results.

The highest sorption capacity of *Chlorella vulgaris* for the removal of zinc, lead and cadmium ions of 60.8, 0.71 and 7.54 mg/g was found for the lowest sorbent concentration of 1 g/L. The results were achieved for modified algal biomass. The highest sorption capacity of *Scenedesmus armatus* for the removal of zinc, lead and cadmium ions of 59.2, 0.69 and 7.39 was found also for the lowest sorbent concentration of 1 g/L. These results were obtained for non-modified algal biomass.

In order to deeply explain the relationship between biomass concentration and heavy metal removal there is a need to provide investigations on for biomass concentration between 1÷10 g/L.

In conclusion, the results achieved during the biosorption study have confirmed the possibility to remove heavy metals from the industrial wastewater by algal biomass. It is possible to obtain over 90% of the removal efficiency, but several conditions have to be fulfilled, i.e. the optimal pH, biomass concentration or suitable biomass modification. The process efficiency can also vary due to the presence of competitive metal ions and other chemicals which are present in the industrial wastewater, e.g. sulphur compounds, carbonates and phosphates.

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Streszczenie

Celem badań była ocena wydajności biosorpcji Zn^{2+}, Pb^{2+} i Cd^{2+} pochodzących z ocynkowni przez modyfikowaną i niemodyfikowaną biomasę glonów z rodzaju *Chlorella vulgaris* i *Scenedesmus armatus*. Powietrznie suchą biomasę glonów poddano chemicznej modyfikacji przy użyciu 1M HNO_3 w celu oceny wpływu kwaśnej modyfikacji na szybkość usuwania wybranych jonów metali ciężkich. Stwierdzono, że proces usuwania jonów metali ciężkich zachodził z dużą wydajnością w krótkim czasie. Wydajność sorpcji jonów metali ciężkich zależała od odczynu środowiska, który miał wpływ na dostępność grup funkcyjnych na powierzchni sorbentu. Efektywność procesu biosorpcji zależała również od stężenia biomasy i jej modyfikacji. Najwyższą pojemność sorpcyjną odnotowano po użyciu sorbentu w najniższym stężeniu. Na podstawie uzyskanych rezultatów badań stwierdzono, że zastosowanie biomasy modyfikowanej i niemodyfikowanej nie wpłynęło znacząco na różnice w wydajności sorpcji jonów metali ciężkich. Mogło to być spowodowane tym, że biomasa niemodyfikowana posiadała wyjściowo dużą pojemność sorpcyjną.

Słowa kluczowe: ***Chlorella vulgaris*, *Scenedesmus armatus*, jony metali ciężkich sorpcja, modyfikowana biomasa glonów**