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Biosorption of lead from municipal wastewater by alginate beads, free and alginate-immobilized *Chlorella vulgaris*

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ABSTRACT

In this study freely suspended, Ca-alginate immobilized *Chlorella vulgaris* cells and blank alginate beads were used for removal of Pb(II) from municipal wastewater. Experimental data showed that biosorption capacity of algal cells dependent on the operational condition such as pH, dosages, contact time and temperature. The maximum biosorption of Pb(II) was achieved with Ca-alginate immobilized algal cells at an optimum pH of 5, algal dosage of 3 g/L and contact time of 30 min at room temperature. Results revealed that biosorption rate initially increased rapidly, the optimal removal efficiency was reached within about 30 min. Increasing initial pH resulted in an increase in ions uptake. These results showed that immobilized algal cells in alginate beads could potentially enhance the biosorption of considered metal ions than freely suspended cells. Moreover, the biosorbent has significantly removed lead ions from municipal wastewater at the optimized condition. Immobilized algae in alginate beads were more effective than free cells. Consistently high ions removal was achieved.

Keywords: Municipal wastewater; Heavy metals; Freely suspended cells of *Chlorella vulgaris*; Immobilized *Chlorella vulgaris* cells; Blank alginate beads

1. Introduction

Pollution of the natural environment by heavy metals has become a serious problem. Heavy metals enter the environment through wastewater streams from industrial processes such as mining, metallurgical processes, or plastic manufacturing [1]. According to the WHO, the metals of the most concern are Al, As, Cd, Cr, Co, Cu, Fe, Pb, Hg, Ni, and Zn [2]. Due to their non-biodegradability and hazardous characteristics, heavy metals pose a great threat to the health of the environment. Aquatic organisms receive heavy metals from contaminated water or through food chain. Moreover, prolonged exposure of soils to heavy metals may result in a marked decrease in soil enzyme activities. The release of heavy metals as a result of human activities will seriously damage the ecosystem. There are some of the conventional methods used for water purification such as chemical precipitation, ion exchange, adsorption, and purification by membranes [1].

Algae, a renewable natural biomass exhibit different affinities towards different metals and therefore, are considered as a good biosorbent material [3–5]. These are the less expensive treatment for the removal of pollutants than conventional methods. It has also been reported that metal uptake capacities of certain algae are much higher than activated carbon, natural zeolite and synthetic ion-exchange resin [1,6]. Microalgae play an important role in the secondary/tertiary treatment of municipal wastewater. They enhance the removal of nutrients, heavy metals, and pathogens [7].

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The most important to the biosorption mechanism is the presence and chemistry of the algal cell wall. Biosorption in algae has mainly been attributed to the cell wall properties where both electrostatic attraction and complexation are important. Typical algal cell walls of many Chlorophyta are comprised of a fibrillary skeleton (main cellulose) and an amorphous embedding matrix. The algal cell wall is made up of polysaccharides (alginic acid, chitin, xylan), which provide functional groups known to act as metal-binding sites. Metal ion binding sites on the algal surface include sulfhydryl, hydroxyl, phosphate, sulfate, imidazole, amine, carboxyl groups. The metal uptake mechanism of algae is a bonding of metal ions with the surface followed by internalization. Either of the two mechanisms in algal biosorption is involved: (1) ion-exchange method where ions present on algal surface Ca, Mg, Na, K they are displaced by metal ions, (2) complexation between functional groups and metal ions [3]. Carboxyl and sulfate groups are involved in covalent bonding between the metal ion and functional groups. In response to metal ions, phytochelatins are produced inside the algal body [3,8].

Physical and chemical wastewater treatments may be ineffective or extremely expensive when the metal concentration in wastewater is in the range 10–100 mg/L [9,10]. The use of biological processes for this purpose may be the cost-effective removal of metals. In this context, the accumulation of metals by microorganisms, such as algae, has been known for a few decades. However, it has received interest because of its potential for application in environmental protection [9,11]. However, small particle size and low density and strength can limit the choice of a suitable reactor and make biomass or effluent separation difficult. Therefore, the potential is to provide technology using immobilized biomass to remove and recover heavy metals from wastewater [12].

Biosorption can occur as a passive process (use of dead biomass) and as an active process (bioaccumulation by live microorganisms). It is believed that biosorption is both extracellular and intracellular metal uptake by microorganisms. This process is related to metabolic activity of microorganisms and does not depend on metal thickening [13–15].

The mechanism of binding and concentration of heavy metals with natural sorbents is not clear. Research published so far indicates that sorption of metal ions occurs due to the presence of functional groups on the surface of the sorbent, such as carboxyl, phenol, hydroxyl, etc. Chemical bonding of heavy metals with functional groups can be accomplished by ion exchange or complexation [16,17].

The lead was chosen for biosorption studies for its wide variety of use in commercial applications. Lead in the environment is commonly originated from various kinds of industrial activities such as mining, smelting and metal plating, battery manufacturing, ammunition production, and paper and pulp processing. It has determined effects on the human nervous system, blood circulation system, excretory system, and reproductive system. The maximum allowable concentration of lead (Pb^{2+}) introduced into the natural environment may not exceed 0.1 mg/dm^3 (industrial wastewater), while for other wastewater 0.5 mg/dm^3 [18,19].

The objective of the study was to analyze the potential of algae for the removal of lead from municipal wastewater. Free-living algae, dead algae, immobilized algae (in alginate beads), and alginate blank beads were used for the removal of lead (Pb^{2+}) from municipal wastewater. The influence of contact time, pH, temperature, and biosorbent dose on the sorption process was studied.

2. Materials and methods

2.1. Substrate

The substrate for the examination was municipal wastewater from the wastewater treatment plant in Poland. This plant treated both domestic and industrial wastewaters by the traditional activated sludge method. The wastewaters were filtered through a 0.22 μm pore size membrane and flow into Erlenmeyer flasks. The characteristics of wastewater are listed in Table 1.

2.2. Organism and culture media

Chlorella vulgaris BA0002 was obtained from the Culture Collection of Baltic Algae (CCBA). The microalga was grown in BG11 medium at 25°C ± 1°C in 300 mL Erlenmeyer flask and was illuminated with light flux density 60 μmol/m^2 S, with 16 h light/8 h dark photoperiod. Freshwater microalgae *C. vulgaris* cells were centrifuged (2,500 rpm for 10 min) and harvested at the exponential growth phase (O.D.$_{750\text{ nm}} \approx 0.9$).

2.2.1. Bead formation and immobilization of algae

Chlorella vulgaris cells were killed by heat in the autoclave or kept alive. In order to obtain dry biomass it was dried at 60°C for 12 h and pulverized. The treated algal cells were mixed with equal volumes of 4% sodium alginate, and the alginate mixture was dropped in a 2.5% $CaCl_2$ solution, then the beads were rinsed in deionized water and stored at 4°C before being used. Each algal bead had an algal density of $3{\cdot}10^5$ cells/bead or $3{\cdot}10^6$ cells/bead according to the method of experiment. Similar procedures were replaced by an equal volume of deionized water to obtain beads without algae (alginate blank beads).

2.3. Research methodology

The heavy metals concentration in wastewater was determined before the experiment and at specified intervals: 1, 3, 5, 10, 20, 30, 60 min, 1, 2, 3, 24 h. The pH of the wastewater samples was measured at all of the intervals.

The batch biosorption experiments were conducted in 300 mL conical Erlenmeyer flasks using an orbital shaker. The flasks were in a quivering condition at an agitating speed of 150 rpm. It was conducted with light flux density of 60 μmol photon/m^2 S^1 with 16 h light/8 h dark photoperiod, at a temperature approximately 25°C. Each flask was filled with a known volume (V = 50 mL) of the sample (wastewater). Each cycle consisted of adsorption with the cell: heavy metal ratio ($3{\cdot}10^7$ cells/mL). Free algal cells, either killed or alive, were added to these flasks. Five hundred alginate algal beads, prepared from either killed or live

Table 1
Physico-chemical parameters of wastewater

pH	7.65
COD (mg/L)	424.6 ± 2.67
NH_4–N (mg/L)	30.8 ± 0.12
TN (mg/L)	202.6 ± 2.33
TP (mg/L)	3.2 ± 0.02
Lead Pb^{2+} (mg/L)	3.44 ± 0.03

cells and alginate blank cells as control were also placed in wastewater to give a density of 10 beads/mL.

The samples were withdrawn from the shaker at predetermined time intervals, filtered, and analyzed for the heavy metals concentration. At the same time, the pH was the measure. The treatment was varied between 0 and 1,440 min for wastewaters and dosage of microalgae. Each treatment was conducted with three replicates. The heavy metal solution was separated from the free algal cells by centrifugation (2,500 rpm for 10 min). The supernatant (wastewater) was collected and analyzed for residual lead concentration using inductively coupled plasma mass spectrometry (ICP-EOS) analyser (Thermo Scientific, IRIS Intrepid II XSP with duo plasma configuration). For analysis, these metal samples were acidified by addition of about 1 mL of concentrated suprapur nitric acid. All the analyses were carried out adopting standard methods of analysis.

The percentage of heavy metal removed was calculated as [2]:

$$R\% = \frac{C_i - C_e}{C_i} \times 100 \tag{1}$$

where R – bioremoval efficiency (%), C_i – initial concentration of metal in aqueous solution (mg/L), C_e – equilibrium concentration of metal in aqueous solution (mg/L).

The metal uptake (q) was calculated from the initial concentration (C_i) and the analysed final concentration (C_f) of the metal in solution according to the following equation [12]:

$$q = \frac{V\left(C_i - C_f\right)}{M} \tag{2}$$

where V – the liquid sample volume (L); M – the starting sorbent weight.

The pH was measured by the potentiometric method to the standard PN-EN 12176:2004.

Effect of pH (pH = 3.0, 5.0, 7.0) for lead removal from municipal wastewater was conducted by a series of experiments. The pH was first adjusted to a designed value: 3.0, 5.0, 7.0 with 0.1 M NH_4OH or 0.1 to 0.5 M HNO_3. Then it was measured hourly and maintained steady throughout the experiment. The resulting solution was analysed with ICP-MS analyzer. The sorption temperature was kept at approximately 25°C due to the fact that sorption of lead by algae tends to slightly increase in the range 4°C–55°C [13].

3. Results and discussion

3.1. Effect of biosorbent dose

Biosorbent concentration is one of the most important factors affecting biosorption. The batch experiments with different biosorbent dosages (0.5, 1.0 and 3.0 g/L) were conducted to determine their effect on the removal of Pb^{2+}. Freely suspended, Ca-alginate immobilized *Chlorella vulgaris* cells, and blank alginate beads have been investigated for their potential use for the removal of lead from municipal wastewater. The effect of the biomass loading of the adsorption of Pb(II) by *Chlorella vulgaris* cells is shown in Fig. 1, from which it could be seen that the value of biomass concentration influenced the uptake capacity. Data in Fig. 1 show that free cells, immobilized cells, and blank beads were all able to remove lead from solution with different efficiencies. The percentages of lead removal in each of these treatments varied depending on the dosage of biomass. The immobilized algae and the alginate blank beads remove more lead than free cells in two biomass dosages, and achieved the maximum removal efficiency for the highest dosage (3 g/L). It was 89%, 72%, and 68% for immobilized live algae, immobilized dead algae and blank beads, respectively. The free algal biomass was not as effective as the immobilized alginate beads in removing lead from wastewater. The highest removal efficiency for the free live cells was 85% for the dosage of 3 g/L. Nevertheless, it was lower than with immobilized algal beads.

Data presented in the study by Tsezos [15] revealed that the biosorption efficiency of Pb(II) ions on *Anabaena sphaerica* was affected by the dose of biomass in the solution. The biosorption of lead ions was increased with subsequently increasing the biosorbent dose (from 0.025 to 0.25 g/100 mL) and almost became constant at dosage 0.2 g/100 mL for Pb(II) (83%). Similar results were obtained in the presented paper for immobilized live algae with dosage 1.0 g/L (82%). However, the removal of Pb(II) ions

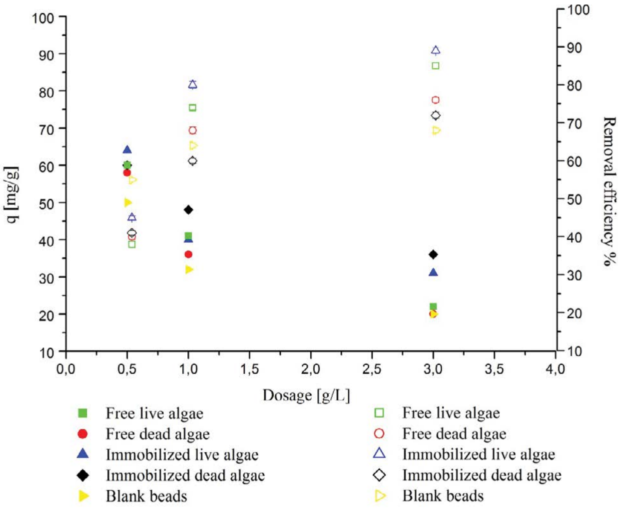

Fig. 1. Adsorption capacities of *Chlorella vulgaris* in dependence on the biomass concentration. Empty symbols (e.g., Δ) represent the removal in percentage and full symbols (e.g., ▲) represent the sorption capacity.

efficiency, higher than 85%, was obtained for free live and immobilized live cells.

Also, data presented in the study by Ahmad et al. [3] confirmed that biosorption efficiency depended on biosorbent dosage. The data revealed that the biosorption of Fe(II), Mn(II) and Zn(II) ions on freely suspended and Ca-alginate immobilised biomass of *Chlorella vulgaris* was significantly affected by the dosage.

This behavior could be explained by the formation of aggregates of the biomass at higher doses, which decreases the effective surface area for biosorption [3].

Nevertheless, maximum sorption capacity (q_{max}) corresponded to the lowest biomass concentration 0.5 g/L). Similar results were obtained with six different algae: *Codium vermilara*, *Spirogyra insignis*, *Asparagopsis armata*, *Chondrus crispus*, *Fucus spiralis* and *Ascophyllum nodosum* [16].

3.2. Effect of pH

The replicates of 50 cm^3 municipal wastewater were put in flasks at pH values of 3.0, 5.0 and 7.0 at room temperature (approximately 25°C), respectively. Biomass was added to these flasks for the purpose of determining the removal rate of cations. pH is one of the most important parameters of the biosorption of heavy metals [17]. The biosorption of lead at different pH values is illustrated in Fig. 2. The removal rate was mainly controlled by the pH of a solution. At all of the studied probes, it was a similar tendency that the lowest removal of lead was at the lowest pH (3.0). The removal efficiency of Pb(II) by free live algae increased from 56% to 89%, respectively, while the pH increased from 3.0 to 7.0. However, the dried biomass reached the highest level at pH 5.0 (78%) than it was decreasing to 65% at pH 7.0. Immobilized algal beads had a similar tendency. Removal efficiency increased at pH 5.0 and decreased at pH 7.0. It was different in the case of blank beads. The removal efficiency of lead at pH 5.0 was about 30 percentage points higher than that at pH 3.0. Moreover, the removal efficiency was still increasing and achieved a level of 70% at pH 7.0. This indicates that the optimal pH for removal of Pb(II) was 5.0 for dried biomass, and pH 7.0 was the best for free-living biomass and blank beads.

The sorption process decreased with any further increase of pH that may be related to the precipitation of metals in the form of metal hydroxide because of the reaction of metal ions with OH (amount of OH increases at high pH). Moreover, when the pH value is low (pH = 3.0), the H$^+$ concentration is high, so H$^+$ will competitively exchange cations on the surface of algae. When the pH increases the metal ions will start to replace hydrogen ions. So, when the pH of the adsorbing medium increased from 3.0 to 5.0, there was an increase in the de-protonation of the adsorbent surface, leading to a decrease in H$^+$ ions on the adsorbent surface. As the pH increases, this creates more negative charges on the adsorbent surface and the adsorption of positively charged species is more favored. Furthermore, at a pH higher than 3.0–4.0, carboxylic groups (–COOH) are de-protonated and negatively charged, so the attraction of cations would be enhanced [17,18]. According to the literature, the pH values in the range of 4.0 to 7.0 were adequate for binding lead [14]. Furthermore, the positive results of lead biosorption using *C. vulgaris* were obtained at pH adjusted to 7.0 [17]. On the other hand, it was proven that a greater heavy metal uptake was observed at higher pH. With increasing pH value, amino and carboxyl groups, are exposed and thus negative charges are produced. The attraction between these negative charges and the metals ions would increase the biosorption onto the cell surface [3,20,21].

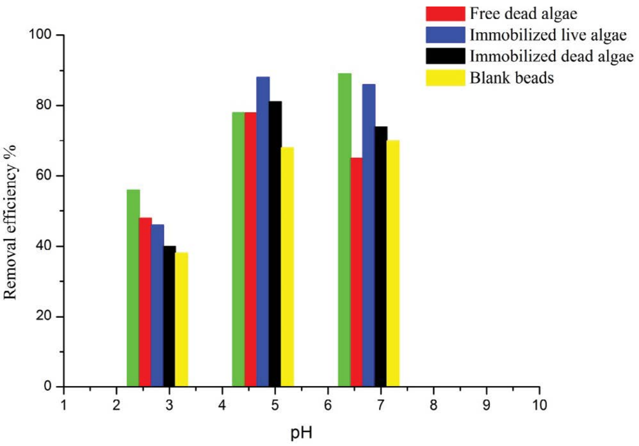

Fig. 2. Effect of pH on the removal of lead ions in contact time 24 h, adsorbent dose: 3 g/L of *Chlorella vulgaris*.

Protonation and deprotonation of functional groups are controlled by the pH of the medium, which affects the biosorption capacity, at low pH, carboxylic groups, being acidic, exist in a protonated state due to the presence of excess H$^+$ and H$_3$O$^+$; therefore, repulsive forces of these protonated groups with positively charged lead ions are responsible for the lower biosorption capacity at low pH. With the increase in pH, functional groups such as amine, carboxyl and hydroxyl groups are exposed by deprotonation. Such behavior enhances electrostatic attraction with lead ions due to a negative charge. The high increase in pH leads to the formation of hydroxide anionic complexes and precipitation is reported to be the reason for the low biosorption capacity [22–24].

According to the study by Ahmad et al. [3], the removal efficiency of Fe(II), Mn(II) and Zn(II) ions increased upon increasing the pH values and the maximum sorption reached at pH 6. However, the biosorption of mentioned metals on freely suspended algal biomass was lower than for immobilized algal biomass of *Chlorella vulgaris*. The same results for lead biosorption from municipal wastewater were obtained for immobilized biomass at pH = 5.0. Similarly, in the present study, lead adsorption by biomass increased with pH increase from 3 to 7 [23,24].

According to the data from the study by Greene and Darnall [25], the optimum pH for the Pb adsorption by *Chlorella vulgaris* was at 6 with 99.4% removal efficiency. Moreover, the results presented in the study by Goher et al. [26] on biosorption of Ni(II) onto alga *Oedogonium hatei* showed that negligible biosorption was found at lower pH range 2–4. The increase in Ni(II) biosorption was observed at higher pH values (5–6).

In general, at pH > 6.0–7.0, metal adsorption rate decreases to a great extent [27].

3.3. Effect of retention time

The contact time of the biosorbent influences total biosorption. An increase in contact time up to the optimum contact time increases biosorption. Occupancy of all active sites causes saturation of biomass, leading to an equilibrium state. The optimum time is different for different types of biosorbents (Fig. 3).

According to the figure, the adsorption of lead on the algae biosorbent is fast in the first 30 min and the rate is then slow. The variations of metal removal percentage at a different retention time with *Chlorella vulgaris* are shown in Fig. 3. Microalgal cells were added to flasks of 50 cm^3 of wastewater at pH 5. The rapid reduction of the concentration of Pb in the solution was at the first 5 min for free-living microalgae and up to 30 min, whereby the removal rate reached up to 89%. However, the best removal efficiency was achieved with immobilized live algae and reached up to 92% in the first 5 min. The lowest level of removal efficiency was achieved by blank beads. It reached up to only 68% and the level was maintained nearly during all time of the experiment. In the case of non-living *Chlorella vulgaris* (free and immobilized), the fast removal at the beginning may be attributed to a larger biosorbent surface area being available for adsorption of lead ions as well as a high number of adsorptive sites. The adsorption kinetics changed rapidly along with the contact time, after that they decreased [28,29].

Also, other researchers reported that the optimum time was different for different types of biosorbents such as 60 min for red macroalgae [30] and 300 min for immobilized algal mass (240 min for free suspended mass) [3].

The data presented by the study by Greene and Darnall [25] and Aun et al. [31] were similar to those presented in this paper and showed that lead adsorption increased rapidly with the contact time up to 30 min. Then, the biosorption rate becomes slower till approaching equilibrium.

Likewise, the results presented by Tsezos [15] indicated that biosorption of Pb(II) using *Anabaena sphaerica* was rapid in the first 20 min then was gradually increased till the equilibrium was attained at 90 min and became constant.

4. Conclusion

This study indicated that green algae *Chlorella vulgaris* can be used as an efficient biosorbent material for the removal of Pb(II) from wastewater. This ability was dependent on pH, time of contact, the dosage of algae, and the form of its dosage (free-living, immobilized, or blank beads). Sorption capacity depended on the pH and the biomass concentration. The optimum sorption pH for Pb(II) was from 5.0 to 7.0.

Reducing the biomass concentration increased the sorption capacity, and in many cases values were higher at the lowest concentration (0.5 g/L). The removal of lead ions was increased with subsequently increasing the biosorbent dose. Biosorption of lead by the algae means that these algae can be used for heavy metal removal from the effluent before being discharged into the environment. This will reduce environmental pollution. The best removal efficiency was achieved by immobilized live algae and reached up to 92% in the first 5 min. In conclusion, the alginate beads (with or without algae) can be used effectively to remove lead from wastewater. The alginate matrix contributed significantly in adsorbing lead than the algal biomass, with little enhancement effects from the biomass either live or killed.

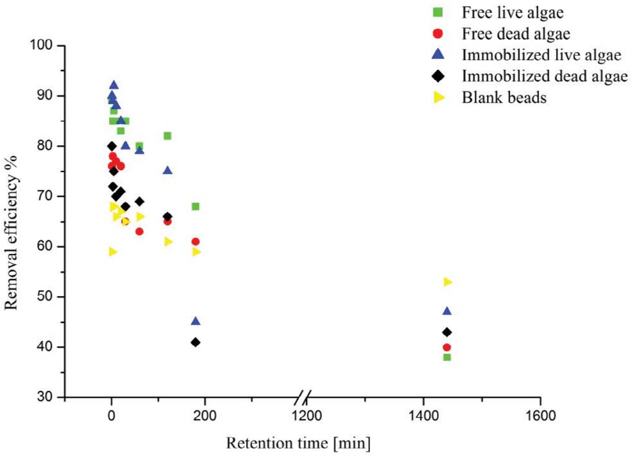

Fig. 3. Effect of contact time on the removal of lead ions, adsorbent dose: 3 g/L of *Chlorella vulgaris*.

The results showed that immobilized algal biomass in alginate beads could enhance the biosorption of lead(II) as compared with freely suspended biomass. The mechanisms and kinetic study of Pb(II) ions depended on pH, the dosage of biomass and contact time. The biosorption capacity of Pb(II) for freely suspended *C. vulgaris* biomass q_{max} = 60 mg/g, while immobilized algal biomass q_{max} = 65 mg/g was obtained. Moreover, the biosorbent has significantly removed heavy metals from real industrial effluent at the specific optimized condition.

The concentration of biomass is directly proportional to the metal uptake. Metal uptake depends on binding sites. More biomass concentration or more metal ions restricts the access of metal ions to binding sites.

Acknowledgments

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