UNIVERSITY OF PÉCS

Doctoral School of Chemistry

Heavy metal biosorption on microorganisms

PhD Thesis

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1. Introduction

The penetration of heavy metal contamination into soil, surface water, groundwater and drinking water by anthropogenic processes causes significant environmental problems. The heavy metals are valuable raw materials essential for industry and everyday life. Some of them have biocatalytic function, so they also play important role in the organisms. Because of bioaccumulation and the potential toxic effects of metal ions in living systems, contaminated water can be dangerous, but its treatment causes technological difficulties.

Bacteria and algae, as *biosorbents* are readily available, inexpensive, reproducible and resistant against environmental effects. They play a very important role in the preservation of the healthy environment. Quick and cost-effective biotechnological methods using the significant heavy metal binding capacity of the microorganisms could be developed to reduce the concentration of heavy metals in contaminated waters.

Based on publications, effective procedures were elaborated using suspension of microorganisms, but they are time-consuming and not cost effective. Finding the right carrier is essential. Intensive research has been performed to combine the high adsorption capacity of carriers and immobilized microorganisms. This research attracts great interest in the industry.

2. Objectives

The biosorption process by several bacteria (*Pseudomonas aeruginosa* PAO1, *Pseudomonas fluorescens* BME, *Escherichia coli* ATCC25922, *Escherichia coli* D31 m3) and algae (*Chlorella vulgaris, Spirulina (Arthrospira) platensis-maxima*) strains was studied to develop a cost-effective adsorption system, which has high adsorption capacity for heavy metal ions and can be regenerated.

The objectives of the dissertation are the followings:

1) Selection of the optimum conditions for the biosorption processes.

2) Assay of metal-binding ability of freeze-dried biomass of several bacteria and algae strains (*Pseudomonas aeruginosa* PAO1, *Pseudomonas fluorescens* BME, *Escherichia coli* ATCC25922, *Escherichia coli* D31 m3, *Chlorella vulgaris, Spirulina (Arthrospira) platensis-maxima*), using solutions with Pb^{2+}-, Cd^{2+}-, Cu^{2+}- and Zn^{2+}-ion content.

3) Ranking the biosorbents based on their metal-binding capacities and applicability.

4) Characterization and evaluation of the immobilization procedures of the selected biomass.

5) Creation of a system that combines the metal adsorption capacity of the selected biosorbent and the immobilizing agent.

3. Materials and methods

Lyophilized **bacterial cells**: *Pseudomonas aeruginosa* PAO1, *Pseudomonas fluorescens* BME, *Escherichia coli* ATC25922, *Escherichia coli* D31 m3 (University of Pécs, Medical School, Department of Medical Microbiology and Immunology) and **algae**: *Chlorella vulgaris* and *Spirulina (Arthrospira) platensis-maxima*) (Academy of Sciences of the Czech Republic, Czech Republic) were studied in free and immobilized form to develop an effective biosorbent.

Characterisation of the cell surface. The electrokinetic properties of the cells were evaluated with ζ-potential measurements; the specific cell surface charge of *P. aeruginosa, C. vulgaris, S. platensis-maxima* cells was measured by titration using surfactant solution. The surface groups of *P. aeruginosa, C. vulgaris, S. platensis-maxima* cells, which are presumably involved in the heavy metal adsorption, were demonstrated by FT-IR spectroscopy.

Measurements under static conditions. The efficiency of the biosorption process can be influenced by several parameters: biosorbent dosage, pH, kinetics of the process, temperature, heavy metal concentration of the solutions, viability of the cells and surface pretreatment. The optimal experimental conditions were chosen by testing these factors under “batch” conditions.

Measurements under dynamic conditions. For immobilization the *S. platensis-maxima* algae cell mixture was used to prepare gel beads. Alginate and chitosan were chosen as carrier materials. The size, immobilizing agent and algae concentrations of the beads were optimized under static conditions. In column studies the effect of flow rate, column size, initial heavy metal ion concentration and regeneration of the beads were investigated. The residual Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} ion concentration of the effluent was determined with Atomic Absorption Spectrometer.

4. Results

Characterization of the biosorption process for heavy metal ions under static conditions:

1. High adsorption efficiency of (tested) biosorbents can be achieved for the removal of heavy metal ions in aqueous suspension at pH 5-6 using 1 g/dm^3 biomass concentration.

2. The adsorption capacity of various biosorbents was evaluated for heavy metal ions and microorganisms:

Pseudomonas aeruginosa: $Pb^{2+} = Cd^{2+} < Cu^{2+} < Zn^{2+}$

Pseudomonas fluorescens: $Pb^{2+} = Cd^{2+} < Cu^{2+} < Zn^{2+}$

Escherichia coli: $Pb^{2+} = Zn^{2+} < Cd^{2+} < Cu^{2+}$

Escherichia coli D31: $Pb^{2+} < Cd^{2+} < Cu^{2+} < Zn^{2+}$

Chlorella vulgaris: $Pb^{2+} < Cd^{2+} < Zn^{2+} < Cu^{2+}$

Spirulina platensis-maxima: $Pb^{2+} = Cd^{2+} < Zn^{2+} < Cu^{2+}$

Pb^{2+}*:* *E. coli* strains < Activated carbon < *P. fluorescens* < *Candida tropicalis* < *C. vulgaris* < *P. aeruginosa* < *S. platensis-maxima*

Cd^{2+}: *E. coli* strains < *Pseudomonas* strains < Activated carbon < *Candida tropicalis* < *C. vulgaris* < *S. platensis-maxima*

Cu^{2+}: Activated carbon < *Pseudomonas* strains < *E. coli* strains < *C. vulgaris* < *Candida tropicalis* < *S. platensis-maxima*

Zn^{2+}: *E. coli* < Activated carbon < *E. coli* D31 < *Pseudomonas* strains < *Candida tropicalis* < *C. vulgaris* < *S. platensis-maxima*

3. The cell surface charge of the tested microorganisms is negative above pH 4 in aqueous suspension. The theoretical cation-exchange capacity and specific surface charge of cells has been determined. These results refer to the adsorption of metal ions and to the role of surface groups. The adsorbed amounts of metal ions by *P. aeruginosa* bacterial cells were significantly lower than the amounts of the

exchangeable ions, which indicate physical adsorption processes. The adsorbed amounts of metal ions by algae cells were significantly higher than the amounts of exchangeable ions, which mainly refer to chemical adsorption processes. The impact of ion exchange, complexation and other ion retention mechanisms may also be important.

4. The biosorption equilibrium has been characterized by Langmuir, Freundlich, Temkin, Frumkin and Langmuir-Freundlich adsorption models using non-linear least-squares estimation. Estimated adsorption isotherms show very good fitting to the experimental data. The biosorption process using various microorganisms is very rapid and spontaneous in each system, which is confirmed by the calculated negative values of free enthalpy change (ΔG) from Langmuir and Frumkin constants. The adsorbed amounts of heavy metals decrease with increasing temperature, which indicates exothermic process in nature. This has been proven by the calculated negative values of enthalpy change (ΔH) from van't Hoff equation and Temkin model.

Characterization of the biosorption process for heavy metal ions under dynamic conditions:

5. For immobilization in alginate and chitosan *S. platensis-maxima* cells were selected due to their high adsorption capacities. The diameter of the alginate gel beads was 2 mm, the alginate and the algae cell concentrations were 20 g/dm^3 and 1 g/dm^3, respectively. The diameter of the chitosan gel beads was 2 mm, the chitosan and the algae cell concentrations were 40 g/dm^3 and 1 g/dm^3. Deformity and algae cell leakage of the resistant gel beads were not observed after rinsing with solutions containing metal ions.

6. The maximum adsorbed amounts of metal ions were calculated from the breakthrough curves using 2 cm^3/min flow rate, 30 cm column height and 100 mg/dm^3 initial metal ion concentration. The alginate and chitosan gel beads can also be used as biosorbents due to their high adsorption capacities. The effect of algae cells immobilization is the slight decrease in the maximum adsorbed amounts of metal ions by alginate beads.

7. The adsorption capacities of the chitosan gel beads are lower for Pb^{2+}-, Cd^{2+}- és Cu^{2+}-ions, while it is equal to that of alginate gel beads for Zn^{2+}-ion. The immobilized *S. platensis-maxima* cells increase the adsorption capacity of chitosan gel beads by fifty percent.

8. The gel beads containing *S. platensis-maxima* cells could be regenerated with mineral acid solutions. The beads were able to adsorb the same amounts of metal ions in five sorption periods.

5. Summary

The adsorption potentials of various bacterial and algae cells (*Pseudomonas aeruginosa* PAO1, *Pseudomonas fluorescens* BME, *Escherichia coli* ATCC25922, *Escherichia coli* D31 m3, *Chlorella vulgaris, Spirulina (Arthrospira) platensis-maxima*) for heavy metal ions were compared. The *S. platensis-maxima* strain was selected which is able to remove heavy metal ions with high efficiency from aqueous solutions having low concentration (< 100 mg/dm^3). Column packing materials were developed with the immobilization of *Spirulina platensis-maxima* cells. These low-cost biomaterials can be regenerated and used as sorbent with high efficiency.

The conditions were optimized for the adsorption process. The optimum conditions were - 1 g/dm^3 biomass concentration and pH 5-6 – appropriate high adsorption efficiency for Pb^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+} removal in each biosorbent system. The biosorption process is very rapid even at room temperature, and raising the temperature is not favourable because of decrease of the adsorbed amounts.

Between the examined biosorbents the *Spirulina platensis-maxima* blue algae cells have the highest adsorption capacity for metal ions. The adsorption capacities of *Chlorella vulgaris* green algae cells were lower. *Pseudomonas* bacterial cells are able to adsorb higher amounts of metal ions, than the *Escherichia* strains. The *Escherichia* strains have the lowest adsorption capacity compared to other studied microorganisms.

Column packing materials were prepared from gel beads using *Spirulina platensis-maxima* cells immobilized in alginate and chitosan. The chitosan beads have high mechanical strength. The adsorption capacities of the immobilizing agent and gel beads containing algae cells with optimized composition were compared under dynamic conditions. The alginate gel beads represent the highest adsorption potential for Pb^{2+}, Cd^{2+} and Cu^{2+} ions normalized to the dry weight content. The chitosan gel beads containing *Spirulina platensis-maxima* cells have greater adsorption capacity for Zn(II) ions than the chitosan gel beads. However these beads have lower capacity for Zn(II) ions than that of freely suspended algae cells. The gel beads can be regenerated in five adsorption/desorption cycles using dilute inorganic acids.

6. Publications

Publications related to the thesis

Anikó Kőnigné Péter, Béla Kocsis, Alizabeta Hegedüsova, Tímea Pernyeszi: Bioadsorption of lead(II) and cadmium(II) ions into the lyophilized cell surface of *Pseudomonas aeruginosa* in aqueous suspension
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