BIOSORPTION OF ZINC FROM AQUEOUS SOLUTION USING ALGAE AND PLANT BIOMASS

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Abstract: In the present study, the sorption capacity of plant biomass has been studied; particularly the ability of biomass algae *Chlorella vulgaris*, filamentous green algae *Spirogyra sp.* and roots, stems and leaves of an invasive plant *Reynoutria japonica* to bind up Zn^{2+} ions. The results of this biosorption study revealed that the rate and extent of uptake were affected by pH level, contact time and initial metal concentration. The maximum uptake of metal ions was obtained at pH 6.0. The equilibrium sorption data for metal system at pH 6 were described by the Langmuir isotherms model. For Zn^{2+}, sorption capacity q_{max} of 17 mg/g was achieved using biomass from leaves. Removal of Zn^{2+} with 1g of biosorbent from leaves was almost 77% when present in low concentrations, whereas it is lower at higher concentrations.

Keywords: *Chlorella vulgaris*, *Spirogyra sp.*, *Reynoutria japonica*, biosorption, zinc, isotherm

1. Introduction

Metal pollution is one of the most important environmental problem today. Three kinds of metals are of concern, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) (WANG and CHEN, 2009). Metals can be distinguished from other toxic pollutants, since are non-biodegradable and can accumulate in the living tissues, thus becoming concentrated throughout the food chain (WILLIAMS *et al.*, 1998).

By far the greatest demand for metal sequestration comes from the need for immobilizing the metals "mobilized" by and partially lost through growing and ever-intensifying human technological activities. Pollution of the environment by toxic metals arises as a result of many human activities like mining, metallurgy, electroplating, leather tanning, metal finishing, textile industry, and paper industry. Effects of these metals on ecosystems are of large economic and public-health significance (VOLESKY, 2001).

Biosorption and bioaccumulation belong to the group of biological methods suitable for heavy metal removal from wastewater (KADUKOVÁ and VIRČÍKOVÁ, 2005). The biosorption is relatively new technology and could be considered for its economic edge as a possible alternative technique for metal recovery. In recent years, biosorption has been widely studied for the removal of metal ions, especially at the concentrations ranging from 1 to 100 mg/l, due to its lower costs and higher effectiveness than the conventional methods such as chemical precipitation and ion exchange (HAN *et al.*, 2008). Different biomass have been used to adsorb metal ions from the environment. Bacteria, fungi, yeast, algae, and plants have proved to be potential metal sorbents (GUPTA *et al.*, 2000; ROMERA *et al.*, 2007). While choosing biomaterial for metal sorption, its origin is a major factor to be taken into account; it can come from a) microorganisms as a by-product of fermentation industry, b) organisms naturally available in large quantities in nature and c) organisms cultivated or propagated for biosorption purposes using inexpensive media (AHLUWALIA and GOYAL, 2007). Understanding the sorption of metal ions from aqueous solution is important for apllication in industrial water treatment.

In this study we wanted to compare biosorption of zinc by laboratorly cultivated species *Chlorella vulgaris* with ubiquitous and in large quantities in nature found species *Spirogyra sp.* and *Reynoutria japonica*. *Chlorella vulgaris* is fast–growing edible algae, cultivated on large scale, and used as a food or feed supplement (CHOJNACKA, 2007). Several studies (BISHNOI *et al.*, 2007; GUPTA *et al.*, 2006; GUPTA and SHARMA, 2008) has been reported about *Spirogyra sp.*, harvested from natural populations, which have exhibited its excellent ability to remove chromium, copper and lead from aqueous solutions. The cell wall of *Spirogyra sp.* is very similar to that of terrestrial plants because its main components are cellulose and pectin. Last species - *Reynoutria japonica* - is an invasive ubiquitous plant investigated currently as a possible energetic plant. It is also known that the species of this genus are able to accumulate heavy metals from soil.

Zinc is the fourth among metals of the world in annual consumption. It is extensively used in the automobile industry, for the production of protective coatings for iron and steel, in cosmetics, powders, ointments, antiseptics, paints, varnishes, rubber and linoleum. Zinc is also needed for manufacturing of parchment papers, glass, automobile tires, television screens, dry cell batteries and electrical equipment. The main sources of Zn in the environment are zinc fertilizers, sewage sludges and mining and smelting (BRADL *et al.*, 2005). The average human body contains about 2 grams of zinc, which is essential for the normal activity of DNA polymerization and for protein synthesis. Soluble and astringent acid salts, such as $ZnSO_4$ in large doses (about 10 g), have caused internal organ damage and death (GUPTA and SHARMA, 2003).

2. Materials and methods

The culture of unicellular algae species, *Chlorella vulgaris* (Fig.1), the culture of filamentous green algae *Spirogyra sp.* (Fig. 2 and 3) and plant *Reynoutria japonica* (Fig.4) were used for laboratory experiments.

2.1 Chlorella vulgaris cultivation

Chlorella vulgaris in log phase used in the experiments was inoculated in medium Milieu Bristol, modification 3N, at pH 7. The medium was sterilized by autoclaving at 121°C for 15 minute. Medium was stored at 4°C until inoculated. Cultures were grown in liquid media in 2 L glass Erlenmeyer flasks, aerated with filtered air, and incubated at 25°C on a light table. Samples were taken every 24 h using sterile glass pipettes. In the samples the cell counts were obtained using the Thoma cellula, and the content of pigment chlorophyll a was determined by DU®-64 Spectrophotometer (Beckman, memory PAC $^{+M}$).

2.2 Preparation of biosorbents

When *Chlorella vulgaris* cultures reached the stationary phase, they were harvested and used for metal ion biosorption experiments. *Chlorella vulgaris* was first cooled down in order to ease following centrifugation (10 minutes at 3000 rpm), and obtained biomass was extensively washed two times with water (1 L of de-ionized water per 1 g of biomass) to get rid of medium remnants. Then it was dried in the oven (at 60°C) overnight and pulverized in a grinding mortar. Acid pre-treatment was then done by washing the sorbent with 0.2 M solution of H_2SO_4 (1 L of acid per 1 g of biomass for 90 minutes), washing twice again by de-ionized water, centrifuged once more and dried 24 hours at constant temperature of 60°C.

Filamentous green algae *Spirogyra sp.* was collected from a fresh water pond. The sorbent was then prepared using the same procedure as described above.

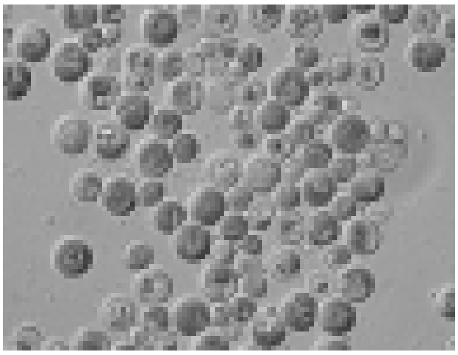

Fig. 1. *Chlorella vulgaris*

Fig. 2. *Spirogyra sp.*

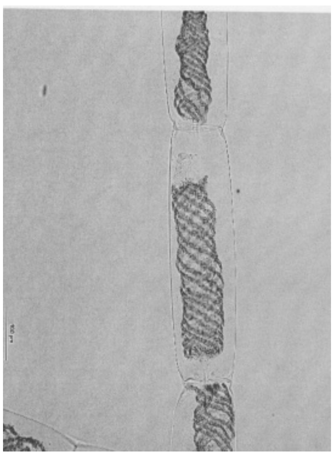

Fig. 3. *Spirogyra sp.*

Fig. 4. *Reynoutria japonica*

All samples of *Reynoutria japonica* used were collected from the same non-urban area in foothills of the Lysá hora mountain, in the area of the Moravskoslezské Beskydy. This sampling area does not have any prior history of contamination by heavy metals. Roots, stems and leaves of this plant were air-dried at room temperature. Dried samples were ground and screened using a sieve shaker; uniform particle size fraction 1-2 mm was obtained. Ion exchange resins manufactured for the same purpose generally feature particle sizes between 0.7 and 1.5 mm. Biosorbent granule size usually ranges between 1 to 2 mm. Particles of roots, stems and leaves were twice washed with 0.01 M of HCl (1 L of acid per 10 g of biomass), then with extensive volume of de-ionized water in order to remove soil or debris, and finally washed with distilled water. The biomass samples were then oven-dried at 90°C for one day.

2.3 Chemicals

Zn^{2+} ions ($ZnSO_4.7\ H_2O$) were used in this study. Test solutions containing this single ion were prepared by diluting proper amount of 1g/L stock solution of zinc ion in order to obtain desired concentrations. Chemicals used were of analytical reagent grade and therefore used without further purification.

2.4 Procedure of experiments

2.4.1 Time course

Time course of Zn^{2+} uptake by *Chlorella vulgaris*, *Spirogyra sp.* and *Reynoutria japonica* was investigated. The sorbent with the final concentration 0.5 g/L of dried biomass of algae and 1 g/L of *Reynoutria japonica* (leaves, stems, roots) was suspended in 200 ml or 500 ml of zinc ion solution. The flasks were placed on a shaker at 120 rpm at a room temperature (around 25°C) for 24 hours for algae and 6 hours for *Reynoutria japonica*. The pH value of the solutions tended to drop during the equilibration and during the sorption experiments it was adjusted with 0.1 M solution of NaOH. The temperature and pH were measured by a microcomputer meter. Samples were taken from the solution at desired intervals and were filtered through membrane filter Millipore 0.45 μm or, in the case of *R. japonica*, through filter paper AK-01 blue, dry. All samples were duplicated with the average presented in the results. The heavy metal concentrations in the resulting supernatant were measured by the Atomic Absorption Spectrophotometry (AAS) Unicam *969*, wavelength 213.9 nm.

2.4.2 Effect of pH

The experiment was conducted at concentration 100 mg/L of zinc ions for *Chlorella vulgaris* and 10 mg/L for *Spirogyra sp.* and *Reynoutria japonica*, 1 g/L biosorbent dose in 50 ml and 500 ml zinc solution for six hours with pH varying from 4.0-6.0. pH of the solution was adjusted using 0.1 M NaOH.

2.4.3 Adsorption isotherm

In the present experiment we have determinate adsorption isotherms for zinc. Amounts of 2 g/L dry acid-pre-treated biomass of algae and 1 g/L dry acid-pre-treated plant biomass (all three types: roots, stems and leaves) were suspended in samples of various concentrations (10-100 mg/L) of Zn^{2+} solutions. The pH of solutions before and during equilibration was adjusted with 0.1 M and 0.01 M solutions of NaOH. After 60 minutes of incubation, zinc samples were filtered in order to remove the biomass and metal concentration in supernatant was measured with AAS. Sorption capacity of the substrate (*q*) expressed in terms of metal amount sorbed on the unitary biosorbent mass (mg/g). This parameter has been calculated as indicated below (CIMINO *et al.,* 2000).

$$q = V(C_i - C_f)/S \quad (1)$$

where:
V – the volume of metal-bearing solution contacted with the sorbent some [L],
C_i – initial and residual concentrations of metal in the solution [mg/L],
C_f – residual concentrations of metal in the solution [mg/L],
S – the amount of the added biosorbent [g].

3. Results and discussion

3.1 Time course

The kinetics experiments of zinc ions removal from solutions showed that biosorption is the equilibrium process, in which the equilibrium is reached after about 10 minutes. The ions are bound with the biomass steadily, and the final concentration of metal ions remained unchanged for 100 hours (CHOJNACKA *et al.*, 2005).

To determine time course of biosorption of *Chlorella sp*. biomass we have chosen zinc concentration of 100mg/L, based on our experience with previous experiments with chromium (CHOVANCOVÁ, 2001). As zinc displayed different behaviour and the efficiency of its removal in high concentration was not sufficient, for following experiments with *Spirogyra sp.* and *Reynoutria japonica* we decreased concentrations to 10 mg/l. Nevertheless, for both concentrations (100 mg/L and 10 mg/L) it can be concluded that at optimal pH value 6 (Fig. 5 and 6) zinc binding onto algal and plant biomass is rapid (in the first 10 minutes). During the process, concentration of a metal ion gradually decreases and the equilibrium is reached; the concentration remains almost constant after approximately 100 minutes. Adsorption slowed down in later stages because initially a large number of vacant surface sites may be available for adsorption and after some time, the remaining vacant surface sites may be difficult to occupy due to forces between the solute molecules of the solid and bulk phase (BISHNOI *et al.,* 2007). It is, however, necessary to say that in the case of higher concentration (100 mg/L) the equilibrium status has not been reached and after about 300 minutes zinc started to be separated out back into the solution (Fig. 5).

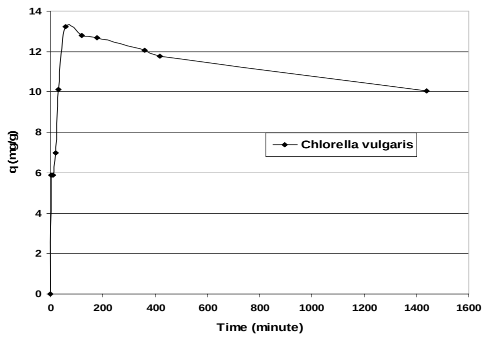

Fig. 5. Time course of metal sorption of divalent zinc by *Chlorella vulgaris*, pH 6.0, initial metal concentration 100 mg/L, room temperature 25°C.

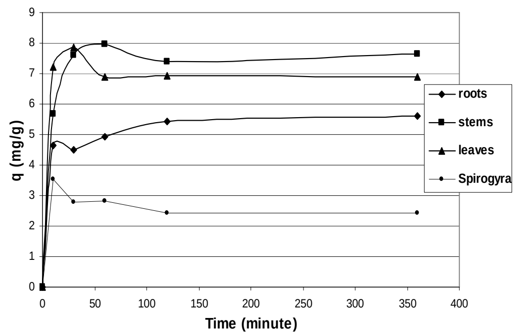

Fig. 6. Time course of metal sorption of divalent zinc by roots, stems and leaves of *Reynoutria japonica* and *Spirogyra sp*., pH 6.0, initial metal concentration 10 mg/L, room temperature 25°C.

3.2 Effect of pH

pH is one of the most important environmental factors influencing not only site dissociation, but also the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, and precipitation are strongly influenced by pH and, on the other side, strongly influence the speciation and the biosorption availability of the heavy metals (ESPOSITO *et al.,* 2002). The effect of pH on the zinc biosorption capacity of biomass has been shown in Fig. 7. The increase of sorption of zinc ions corresponds to pH values growing from 4.0 to 6.0, the latter being the optimum pH for biosorption of zinc ions by all types of biomass. Acid-washed biosorbents can be viewed as natural ion-exchange materials that primary contain weak acid and basic groups (KRATOCHVIL and VOLESKY, 1998). Number of negatively charged active sites (functional groups such as carboxyl, amine, hydroxyl and phosphate groups) at higher pH increases, facilitating a higher electrical attraction to positively charged metal ions (KLIMMEK *et al.*, 2001; ROMERA *et al.*, 2007). CHOJNACKA *et al.* (2005) identified in cyanobacteria *Spirulina sp.* different functional groups at different pH values. In pH value range 5.0-9.0, which covers pH value used in our experiments, she identified - with use of potentiometric titration - carboxyl and phosphate group to be the main functional groups. Our results of optimum pH value are in agreement with studies of ROMERA *et al*. (2001), who studied untreated algae biomass of *Spirogyra insignis*, and SALEHIZADEH *et al*. (2003) and NORTON *et al.* (2004) studying bacteria.

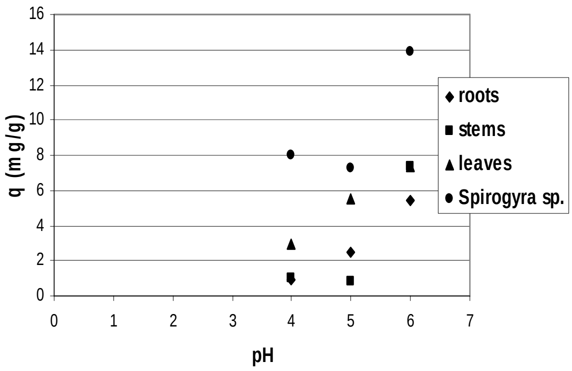

Fig. 7. Effect of pH on the sorption of Zn^{2+} by roots, stems and leaves of *R. japonica* and *Spirogyra sp*., pH 4.0-6.0, initial metal concentration 10 mg/L, room temperature 25°C.

3.3 Determination of adsorption isotherms

The Fig. 8 displays metal uptake isotherms for Zn^{2+} ions plotted against final metal concentration C_f in aqueous solutions. When the initial Zn^{2+} concentration increased from 10 to 50 mg/L, uptake by *Chlorella vulgaris* increased, and reached 15.9 mg/g.

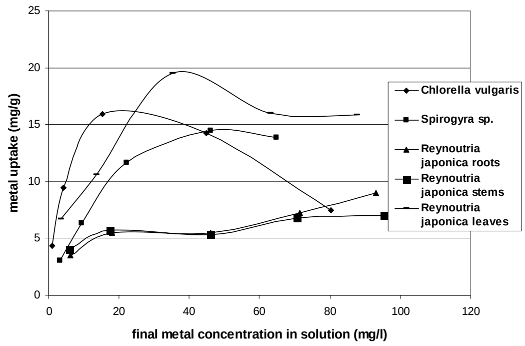

Fig. 8 Sorption isotherms for the sorption of Zn^{2+} onto *Chlorella vulgaris*, *Spirogyra sp.* and roots, stems and leaves of *Reynoutria japonica* biomass, pH 6.0, room temperature 25.0 °C.

Similar results were obtained with leaves of *Reynoutria japonica* (uptake 19.5 mg/g), and *Spirogyra sp*. biomass (uptake 14.5 mg/g), both at initial zinc concentration 75 mg/L. It is possible to conclude that in both low and high concentrations, leaves showed the best results. At initial Zn^{2+} concentration 100 mg/L, decrease of sorption in all biomass types was recorded, with the exception of roots and stems of *Reynoutria japonica*; however these two types of biomass displayed generally the lowest zinc uptake ability among all biomass investigated.

The process of zinc sorption on the biosorbent was described by the Langmuir adsorption model, which is widely used to analyze data for water and wastewater treatment applications. The Langmuir model represents one of the first theoretical descriptions of nonlinear sorption and suggests that uptake occurs on a homogeneous surface by monolayer sorption without interaction between adsorbed molecules. In addition, the model assumes uniform energies of adsorption onto the surface and no transmigration of the adsorbate (SAHMOUNE *et al.*, 2008). The Langmuir equation is given by Eq. (2)

$$q = q_{\max} bC_f / 1 + bC_f \qquad (2)$$

where:

q_{max} – maximal metal uptake [mg/g],
b – a constant related to the affinity of the binding sites [L/mg],
q – experimental metal uptake [mg/g],
C_f – residual concentrations of metal in the solution [mg/L].
q and b can be determined from the linear plot of C_f/q vs. C_f (DONMEZ *et al.*, 1999).

The main advantage of this model is the possibility of evaluation of q_{max} - maximum possible quantity of a metal ion adsorbed per gram of adsorbent, and b – parameter related to the affinity of binding sites for a metal ion (MICHALAK *et al.,* 2007). In general, for good biosorbents, high q_{max} and high b are desirable (DAVIS *et al.*, 2003). The Langmuir constants R^2 for the zinc biosorption onto biomass of *Chlorella vulgaris, Spirogyra sp*. and *Reynoutria japonica* are showed in Fig. 9 and 10. The results indicate the highest applicability of the Langmuir model for two biosorbents: *Spirogyra sp.* with the best regression coefficient $R^2 = 0.9834$, and leaves of *Reynoutria japonica* $R^2 = 0.9807$. Based on q_{max}, it can be concluded that *Spirogyra sp*. (q_{max} = 17.8 mg/g) has slightly higher adsorption capacity for zinc than leaves of *Reynoutria japonica* (q_{max} =17.0 mg/g). As for parameter b, however, *Spirogyra sp*. has significantly lower (b = 0.06) than leaves of *Reynoutria japonica* (b = 0.26). HASHIM and CHU (2004) suggest that a biosorbent with low q_{max} and high b could outperform a biosorbent with high q_{max} and low b, especially in cases where the metal ion to be removed is present at trace amounts. Therefore, higher b value of leaves of *Reynoutria japonica* indicates its higher affinity for zinc. Ranking of sorptions of all sorbents studied, based on q_{max}, can be set up as follows: *Spirogyra sp.* > leaves > stems > *Chlorella vulgaris* > roots of *Reynoutria japonica*.

Very similar results for *Spirogyra sp*. were obtained by ROMEA *et al.* (2007). In his experiments with untreated *Spirogyra sp*. biomass maximum capacity for zinc was found to be 21.6 mg/g, and parameter b = 0.04. Adsorption capacity for zinc by waste tea leaves biomass (similar type of biomass as leaves of *Reynoutria japonica*) was found to be 11.8 mg/g (TEE and KHAN, 1988).

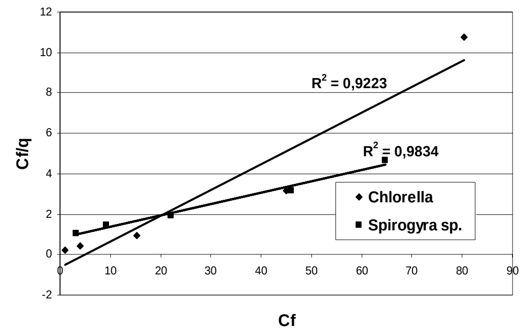

Fig. 9. Sorption isotherms for the sorption of Zn^{2+} onto *Chlorella vulgaris* and *Spirogyra sp*., pH 6.0, room temperature 25°C.

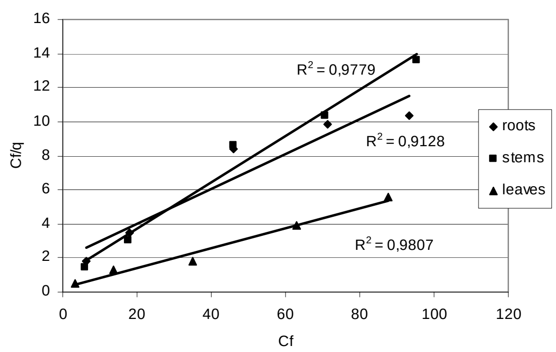

Fig. 10. Sorption isotherms for the sorption of Zn^{2+} onto roots, stems and leaves of *Reynoutria japonica*, pH 6.0, room temperature 25°C.

4. Conclusions

This work has demonstrated the possibility of utilization of biomass of algae *Chlorella vulgaris* and *Spirogyra sp*., and of a plant *Reynoutria japonica* for biosorption of zinc ions from aqueous solutions.

The binding capacity of Zn^{2+} ions to biosorbent has been shown to depend upon pH, with the highest binding at pH 6.0.

The biosorbents have good capacity for zinc adsorption, especially in low metal concentrations.

There were no major differences in biosorption of zinc between cultured *Chlorella vulgaris* and natural *Spirogyra sp.* and *Reynoutria japonica*.

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