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BIOSORPTION OF Cu, Zn AND Pb BY THERMOPHILIC BACTERIA – EFFECT OF BIOMASS CONCENTRATION ON BIOSORPTION CAPACITY

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Abstract

BABÁK, L., ŠUPINOVÁ, P., ZICHOVÁ, M., BURDYCHOVÁ, R., VÍTOVÁ, E.: *Biosorption of Cu, Zn and Pb by thermophilic bacteria – effect of biomass concentration on biosorption capacity.* Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 5, pp. 9–18

The aim of this work was to study the biosorption capacity of metals copper, lead and zinc by *Geobacillus thermodenitrificans* and *Geobacillus thermocatenulatus*. Solution of each metal was mixed with dry biomass and incubated at room temperature. The supernatant was taken and used for complexometric titration.

The sorption capacity for Cu^{2+} was highest when using 0.5 $g \cdot l^{-1}$ *Geobacillus thermodenitrificans* (57 ± 4 $mg \cdot g^{-1}$). The sorption capacity rapidly decreases with increased concentrations. Similarly for Zn^{2+} ions, the highest sorption capacity was for biomass concentration 0.5 $g \cdot l^{-1}$ (18 ± 3 $mg \cdot g^{-1}$) and slowly decreases. For Pb^{2+} ions, the decrease is almost linear to the biomass concentration 2 $g \cdot l^{-1}$, i.e. from 117 ± 13 $mg \cdot g^{-1}$ to 53 ± 3 $mg \cdot g^{-1}$.

The sorption capacity of Cu^{2+} ions was highest at the lowest biomass concentration of *Geobacillus thermocatenulatus* (65 ± 3 $mg.g^{-1}$), then it sharply decreased and at concentration of biomass of 1 $g \cdot l^{-1}$ did not changed. In the case of Zn^{2+} ions, we could seen a moderate drop with the increasing concentration with the range of 24 ± 3 to 12.3 ± 0.4 $mg \cdot g^{-1}$. For Pb^{2+} ions was the decrease slow, from 119 ± 8 $mg \cdot g^{-1}$ to 54 ± 4 $mg \cdot g^{-1}$.

Affinity of metals to bacteria was determined in the order $Pb^{2+} > Cu^{2+} > Zn^{2+}$. The results show, that *Geobacillus thermocatenulatus* has better sorption capabilities than *Geobacillus thermodenitrificans*.

biosorption, heavy metals, *Geobacillus thermodenitrificans*, *Geobacillus thermocatenulatus*

For several decades, scientists have engaged in options and ways to remedy of water pollution by heavy metals. Nowadays, heavy metals are usually removed using physical and chemical methods (Volesky, 2001).

Most widely used method for this is chemical precipitation. This procedure is based on the formation of insoluble compounds influence of coagulants, which are then filtered out. This is one of the cheapest and the most simple methods, but it occurs to creation a large amount of toxic waste. Next industrial using is adsorption on sorbents (e.g. activated carbon), ion exchange, in which metal ions are replaced by less dangerous ions from the resins. The method of ion exchange is effective, but quite expensive. Finally, it is possible to use oxidation and reduction with forming insoluble oxides or metals (Volesky, 2001; Ahluwalia *et al.*, 2007).

Micro-, nano- and ultrafiltration and reverse osmosis are the most commonly used physical methods in industry (Volesky, 2001). The principle is based on the separation membrane under pressure. Higher concentrations of heavy metals can be removed by electrochemical cleaning. The disadvantages of the physical processes are little efficacy at lower concentrations and the formation of secondary waste. This waste must be further processed or stored (Volesky, 2001; Ahluwalia *et al.*, 2007).

The biosorption is ability of biomass, whether microbial or plant, to bind metals from aqueous environments on cells of biomass. Biosorption is passive and is not independent on metabolism by compared with complex process of bioaccumulation. It can be carried out using inactivated or dead biomass (Ahluwalia *et al.*, 2007; Volesky, 2007).

The main advantages of biosorption over conventional methods are:

- a lower price – does not need the nutrients and biomass can be obtained as a waste product,
- high efficiency and speed – it takes in the order of minutes to hours,
- waste minimization,
- possibility of regeneration of biosorbent,
- ability to recover the metal, which is especially useful in the greater the value and quantity of metals,
- process of biosorption is not controlled by cell,
- metabolic products do not affect the amount of sorbed metal,
- dead biomass is not susceptible to the toxic effects of metals,
- do not must to maintain conditions suitable for living cells (Ahluwalia *et al.*, 2007; Ahalya *et al.*, 2003).

Biosorption is based on physical-chemical interaction between metal and functional groups present on the surface of microbial cells. The structural composition of the cell wall is complex, allowing more options for sorption. The amount of sorbed metal varies with different cell wall structure of individual organisms. These differences are shown on Fig. 1 (Volesky, 2007; Vijayaraghavan *et al.*, 2008).

Seaweeds contain large amounts of alginate and fucoidan. Mushrooms have a strong layer of chitin. The cell walls of G^+ bacteria are composed of thick layer of peptidoglycan (90 %) with teichoic and teichuronic acids. G^- bacteria have a peptidoglycan layer conversely very thin (only 10 to 20 %) and do not contain teichoic acids. They have the outer membrane of phospholipids, lipoproteins, and lipopolysaccharide (Volesky, 2007; Vijayaraghavan *et al.*, 2008).

The above-mentioned biopolymers are good sources of metal-binding functional groups. Carboxyl, sulfate, amide, phosphate and phosphodiester groups include among the most important functional groups. But their presence does not guarantee availability for biosorption, which can be influenced by the conformation and steric interference (Ahluwalia *et al.*, 2007; Volesky *et al.*, 1995).

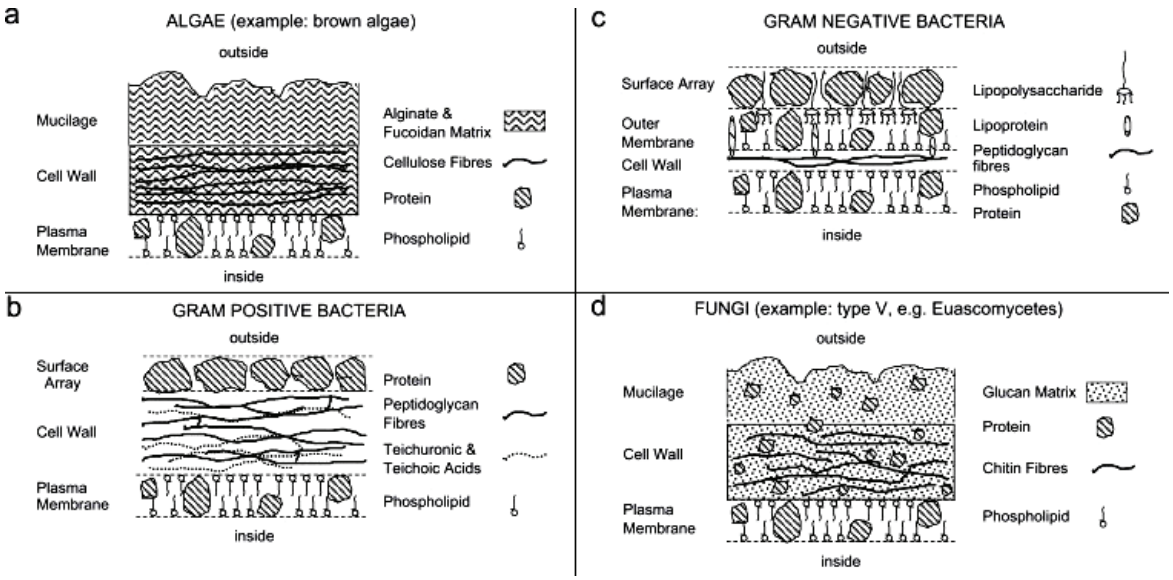
Mechanisms of biosorption

The main mechanisms of metal binding to the cell wall include ion exchange, physical adsorption, formation of complexes and chelation. Microprecipitates or capture in capillary of walls matrix are less involved in the biosorption. They operate independently and their effect is combined with total metal sorption. Their representation is quantitatively and qualitatively different depending on the strain, origin of biomass and used procedure (Volesky *et al.*, 1995).

Ion exchange and adsorption

During the experiments was obtained in a lot of knowledge to explain the course of biosorption. Principle is not exactly known even after thorough research. At biosorption is very important to have an idea about mechanism of ion exchange and adsorption (Naja *et al.*, 2007; Raize *et al.*, 2004).

Adsorption unlike ion exchange assumes that all binding sites on the sorbent are loose and



1: *Schematic representation of the cell wall (Volesky, 2007)*

immediately accessible from the sorbate solution. The principle of adsorption is showing on Eqn (1), the ion-exchange model is described by Eqn (2):

$$B^- + M^+ \leftrightarrow BM, \quad (1)$$

where B^- is the free binding site, M^+ is metal cation and *BM* is adsorbed metal on the sorbent,

$$BH + M^+ \leftrightarrow BM + H^+, \quad (2)$$

where *B* is the binding site occupied by *H* proton, which participates in metal ion exchange with M^+ cation. At sorption of divalent metal cations is necessary to adjust stoichiometry both equations (Naja *et al.*, 2007).

The rate of electrostatic attraction in biosorption depends on the type and number of binding sites in the biomass and is also influenced with ionization and occupying space with proton or other ion. Occupancy of binding sites is related to the pH and pK_a of functional groups. Amino groups are positively charged in protonated form and neutral in deprotonated form. Carboxyl, sulphate and phosphate groups are neutral in protonated form and negatively charged in deprotonated form that allowing the attraction of metal cations (Naja *et al.*, 2007; Veglio *et al.*, 1997).

Formation of complexes and chelation

The complex is compound formed from one or more central atoms (usually metal cations) surrounded by ligands that are bound to them. Ligands can be further divided by the number of groups which are able to bind to the metal. Ligands with one binding site are binding only through a coordination group and usually consists water-soluble ionic complexes. Ligands with multiple binding sites contain more than one linkage group. When ligands bind to a single central metal is called chelating agent complexes and is then called chelates. The metal ion is closed in the cycle when chelates are forming. Most of the chelate ligand contains three major donors (nitrogen, oxygen and sulphur) (Naja *et al.*, 2007; Raize *et al.*, 2004).

From basic characteristics of complex formation can be assumed that biosorption of metal also takes place with these mechanisms. Ligands with multiple binding sites are most often found on the cell surface. Chemical bond formation depends on the electronegativity, ionisation and redox potential and the radius of the metal ions. It was found that metal ion with higher electronegativity is strongly attracted to the surface (Naja *et al.*, 2007; Veglio *et al.*, 1997).

Factors influencing the biosorption

As already mentioned, biosorption may be under conditions suitable for living cells. Theoretically, the dead biomass could be used in any environment, but the process itself is influenced by several factors that may have a significant influence on the sorption capacity of the sorbent or sorption rate and thus need to be taken into account. Biosorption is mainly influenced by pH, concentration and type of biomass, the presence of other metals, temperature and initial concentration of metal (Ahalya *et al.*, 2003).

Temperature

Temperature affects the stability of the metal in solution, the configuration of the cell wall or stability of complex cells with bound metal. In general, a temperature has much less influential than other factors, especially if it is between 20–35 °C. Biosorption of some metals (uranium, copper) may even take place without restrictions in a wide temperature range (Ahalya *et al.*, 2003; Naja *et al.*, 2007; Öztürk *et al.*, 2004).

The pH

The hydrogen exponent is probably the most important factor in biosorption. It affects the solubility of metal, ionization of functional groups of cell walls and competitivenes of metals. The availability of free binding sites varies depending on the pH. These places are partially protonated at lower pH, it prevents access of positively charged metal ions. At sufficiently low pH are all protonated binding sites, and this leads to complete desorption of linked metal ions, which is used for regeneration biosorbents. On the other side, extreme pH values may damage the structure biosorbent. The cells are deformed and reduce the sorption capacity. At higher pH significantly reduces the solubility of metals, metal hydroxides formed, which collide and thus impede biosorption. For most metals are found as the optimum pH range 3–6 (Ahalya *et al.*, 2003; Naja *et al.*, 2007; Kratochvil *et al.*, 1998; Pagnanelli *et al.*, 2003).

The concentration and type of biomass

A large number of types of biomass have been studied in terms of their biosorption properties – algae, bacterial biomass, biomass of fungi and plants. It was found that depends not only on species but also on growth conditions (culture medium), physiological condition and age of biomass. If it is a concentration of biomass, it appears the use of high concentrations is very effective, while at lower concentrations leads to a higher intake of specific metal (Ahalya *et al.*, 2003; Naja *et al.*, 2007).

The presence of other metals

Biosorption of one type of metal can be reduced or even made impossible any other kind of metal present in solution. The rate of inhibition of metal biosorption depends on the strength of which the individual metals bind to biomass. Generally, light metals (alkali and alkaline earth metals) bind less strongly than heavy metals or radioactive elements. Thus the presence of very light metal does not affect on sorption of heavy metals. Between heavy metals is weakly bound zinc, which is more influenced by other metals (Naja *et al.*, 2007).

The sorption capacity of sorbent

Sorption takes place in the system of solid phase (sorbent = biological material) and liquid (usually water) containing dissolved substance which is to soak (sorbate – metal ions). Sorbate is attracted by affinity bound to the sorbent and the above-mentioned mechanisms. The process continues until equilibrium between the amount of bound sorbate and the balance in the solution. Rate affinity for sorbate decides on its distribution in the solid and liquid phase (Volesky, 2003).

The quality of the sorbent is assessed on the amount of sorbate which is on sorbent captured and that remains established. For this purpose was introduced variable sorption capacity of sorbent (q) expressed as the amount of sorbate bound with unit of the solid phase (weight or volume). Calculation of the sorption capacity is based on material balance of sorption system:

$$q = \frac{V \times (C_i - C_f)}{m_{biomass}}, \quad (3)$$

where V is the volume of solution containing the metal, C_i is the initial concentration and C_f the final (equilibrium) metal concentration in solution and $m_{biomass}$ is the amount of added biosorbent (biosorbent dry weight). The use of "wet weight" of the biomass is not appropriate, where no exact translation for the wet weight to dry weight. Different types of biomass contain different amounts of moisture, both intracellular and in intercellular space. Therefore it is appropriate to indicate conditions such as centrifugation, to compare the quality of the sorbent were as accurate as possible (Volesky, 2003).

The 20 ml solution of metal with concentration about 0.5 g·l^{-1} (for Cu^{2+}, Zn^{2+}), resp. 1 g·l^{-1} (for Pb^{2+}) was mixed with dry biomass with concentration 0.5; 1.0; 2.0 and 3.0 g·l^{-1}. The mixture was incubated at room temperature and 100 rpm for 12 hours. The biomass was harvested by centrifugation at 6 000 rpm for 10 min. The supernatant was used for complexometric titration.

For the determination of heavy metals were collected 2 ml sample, which were diluted with distilled water to a volume circa 25 ml. For Cu ions was added about 5 ml of 1 mol·l^{-1} NH_4Cl and a drop of NH_3 to value of pH circa 8. Then was added murexide as an indicator. The color change during titration was from yellow to violet. To a solution with the zinc ions were added several drops of 0.1% solution of xylenol orange and the resulting yellow solution was neutralized by 1 mol·l^{-1} NaOH to form a red color. Then was added dropwise 1 mol·l^{-1} HNO_3 to the disappearance of red color. Finally, it was added solid urotropine until the red color appeared again. The color change during titration was from red to lemon yellow. For the determination of Pb ions was added several drops of 0.2% solution of xylenol orange and piecewise solid urotropine to intense red-violet color. The color change during titration was from red to lemon yellow. All samples were titrated with 0.001 mol·l^{-1} EDTA three times.

Metal concentrations were calculated from the volumes obtained by titration. These concentrations were averaged and the confidence interval was calculated using Excel. Error of sorption capacity was calculated using the equation (4), where values of C_i, C_f, ΔC_i and ΔC_f were calculated in Excel. The value of ΔV (error of graduated cylinder) was $2.5 \cdot 10^{-4}$ l. The value of Δm_B (error of analytical balance) was $5 \cdot 10^{-5}$ g.

$$\Delta q = \sqrt{\left(\frac{\partial q}{\partial V}\right)^2 \times (\Delta V)^2 + \left(\frac{\partial q}{\partial C_i}\right)^2 \times (\Delta C_i)^2 + \left(\frac{\partial q}{\partial C_f}\right)^2 \times (\Delta C_f)^2 \times \left(\frac{\partial q}{\partial m_{biomass}}\right)^2 \times (\Delta m_{biomass})^2} \quad (4)$$

MATERIALS AND METHODS

Geobacillus culture was cultivated in a bioreactor on synthetic medium with the composition: 5 g peptone, 3 g beef extract, 0.01 g manganese sulphate monohydrate, 1 liter of distilled water. Bacterial cells were harvested by centrifugation at 6 000 rpm for 10 minutes at the end of exponential growth phase. Then the cells were washed with distilled water and centrifuged again. Washing procedure was performed twice. Washed cells were subsequently dried to stable weight.

Portions of salt were dissolved in volumetric flasks in deionized water to the desired concentration. The optimum pH for biosorption Cu^{2+} and Zn^{2+} is the value of 5.0 and for Pb^{2+} is the optimum pH value of 4.0. pH adjustment was performed using 0.1 mol·l^{-1} HNO_3 and 0.1 mol·l^{-1} NaOH. The exact concentration of the solutions was determined by complexometric titration.

RESULTS AND DISCUSSION

Table I contains all the observed results for *Geobacillus thermodenitrificans* CCM 2566. Sorption capacities depending on the concentration of biomass in the graphs were constructed (Fig. 2).

Sorption capacity was the highest in case of Cu^{2+} ions (Fig. 2a) when using 0.5 g·l^{-1} biomass, and that was 57 ± 4 mg·g^{-1}. Then capacity decreases sharply and at concentrations of 1, 2 and 3 g·l^{-1} was not changing and moves about values of 20 mg·g^{-1}. Similar was the situation with Zn^{2+} ions (Fig. 2b), where was the highest sorption capacity when using biomass concentration 0.5 g·l^{-1} (18 ± 3 mg·g^{-1}). However, in contrast to Cu^{2+} ions, influence of biomass concentration was not so expressive. At Pb^{2+} ions (Fig. 2c) was not fall so much sharp. It was almost linear until biomass concentration 2 g·l^{-1}, where falled to the value of 53 ± 3 mg·g^{-1}. Sorption

I: *The values of sorption capacity at various concentrations of biomass*

***Geobacillus thermodenitrificans* CCM 2566**

Metal	$c_{biomass}$ [g/l]	$m_{biomass}$ [g]		$C_{metal\,1}$ [mg/l]	$C_{metal\,2}$ [mg/l]	$C_{metal\,3}$ [mg/l]	Ø C_{metal} [mg/l]	q [mg/g]
Cu	0.5	0.011	initial	496.650	499.834	499.834	499 ± 2	57 ± 4
			final	464.814	467.997	467.997	467 ± 2	
	1	0.023	initial	515.752	515.752	517.344	516.3 ± 0.8	23 ± 1
			final	488.691	490.283	490.283	489.8 ± 0.8	
	2	0.041	initial	499.834	499.834	503.017	501 ± 2	19 ± 2
			final	461.630	458.446	464.814	462 ± 3	
	3	0.062	initial	515.752	515.752	517.344	516.3 ± 0.8	16.0 ± 0.6
			final	467.997	467.997	464.814	467 ± 2	
Zn	0.5	0.009	initial	509.572	509.572	511.211	510.1 ± 0.9	18 ± 3
			final	501.380	503.018	501.380	501.9 ± 0.9	
	1	0.022	initial	540.703	542.342	542.342	541.8 ± 0.9	12 ± 1
			final	529.234	527.596	527.596	528.1 ± 0.9	
	2	0.041	initial	540.703	540.703	542.342	541.2 ± 0.9	9.1 ± 0.9
			final	524.319	521.042	522.680	523 ± 2	
	3	0.061	initial	540.703	539.065	542.342	541 ± 2	7.4 ± 0.9
			final	516.126	521.042	517.765	518 ± 2	
Pb	0.5	0.010	initial	1006.930	1 006.930	1 001.739	1 005 ± 3	117 ± 13
			final	955.026	944.646	944.646	948 ± 6	
	1	0.021	initial	1 069.214	1 074.405	1 064.024	1 069 ± 5	91 ± 8
			final	965.407	980.978	975.788	974 ± 7	
	2	0.041	initial	1 069.214	1 074.405	1 064.024	1 069 ± 5	53 ± 3
			final	960.217	965.407	960.217	962 ± 3	
	3	0.062	initial	1 069.214	1 074.405	1 064.024	1 069 ± 5	54 ± 6
			final	908.313	918.694	882.361	903 ± 17	

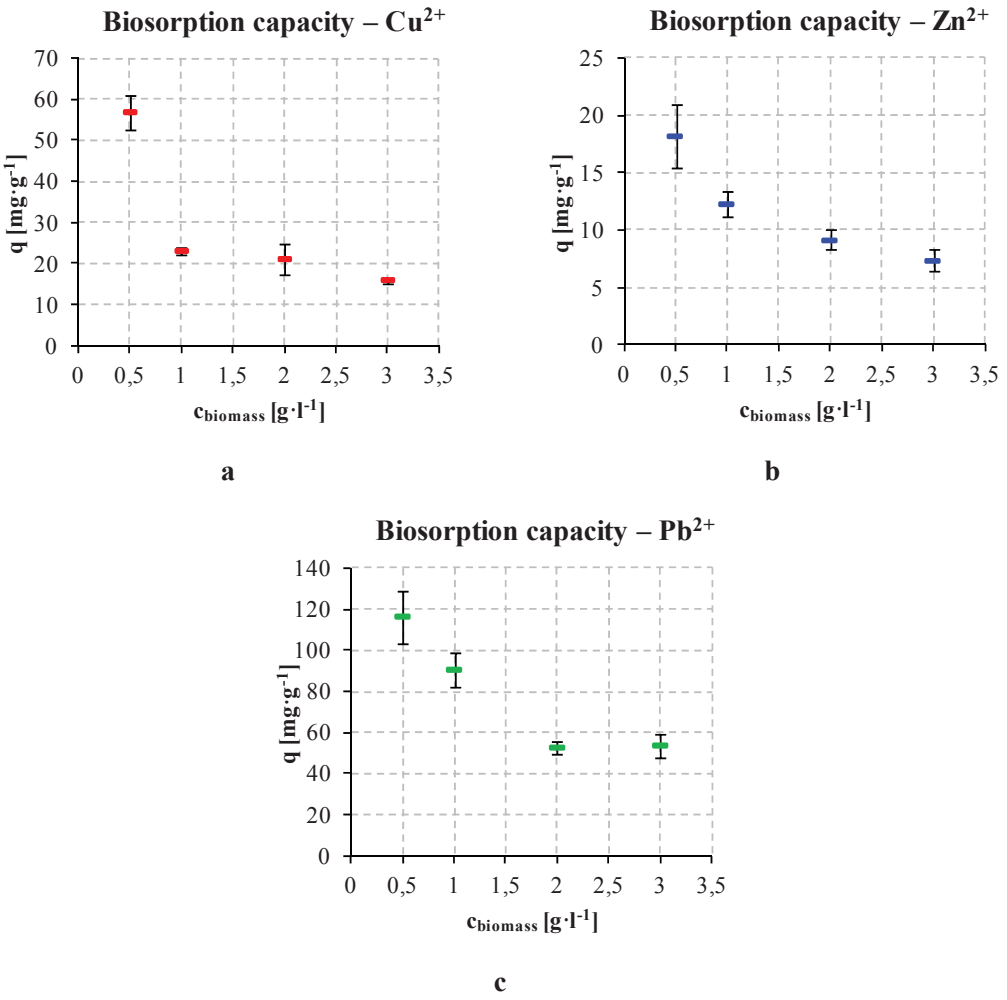
capacity was not changed with higher biomass concentration.

These results correspond with conclusions described in literature – the falling biomass concentration increases it's sorption capacity, though absolute loss of metal is increasing with increasing biomass concentration. Using lower biomass concentration is more effectively for this reason. Reasons of falling sorption capacity at higher biomass concentrations can be mutually interaction between binding sites (Ahalya *et al.*, 2003; Özdemir *et al.*, 2009).

Results reached in this study show that *Geobacillus thermodenitrificans* CCM 2566 cells have higher sorption capacity for Cu^{2+} ions than for Zn^{2+} ions. These results are according to the study of Chatterjee *et al.*, 2010, which deals with biosorption by these bacteria. In that study, sorption capacities for Zn^{2+} 48.2 mg·g^{-1} and for Cu^{2+} 50.0 mg·g^{-1} are described. Their results were determined for concentration of metal 175 mg·l^{-1}, which was highest concentration in this study. Biomass concentration 2 g·l^{-1} was used. This study didn't deal with dependence of sorption capacities of biomass concentration. Confrontation presented sorption capacities for existent concentration biomass (Cu^{2+} 50.0 mg·g^{-1} versus 19 ± 2 mg·g^{-1} and Zn^{2+} 48.2 mg·g^{-1} versus 9.1 ± 0.9 mg·g^{-1}) it is possible conclude that the high concentration metal (here around 0.5 g·l^{-1}) can influence biosorption negatively. The reasons of different sorption capacity of strains can be also their different origin. In study was used strain which was isolated from river Damodar in India, therefore his cell wall can be adapted to the presence of heavy metal (Chatterjee *et al.*, 2010).

Tab. II contains all the observed results for *Geobacillus thermocatenulatus* CCM 2809. Sorption capacities depending on the concentration of biomass in the graphs were constructed (Fig. 3).

Sorption capacity for Cu^{2+} ions by strain *Geobacillus thermocatenulatus* CCM 2809 (Fig. 3a) is highest at the lowest biomass concentration (65 ± 3 mg·g^{-1}). Then it sharply decreases and from biomass concentration of 1 g·l^{-1} does not change much. In the case of Zn^{2+} ions (Fig. 3b) again applies an inverse relationship, even if biomass concentration already did not have so expressive influence on sorption capacity. When comparise the sorption capacities for Cu^{2+} and Zn^{2+}, the strain CCM 2809 has a greater sorption capacity for copper ions. Pb^{2+} ions (Fig. 3c) show a gradual



2: *The dependence of sorption capacity of biomass concentration with using Geobacillus thermodenitrificans CCM 2566 (ions: Cu^{2+} (♦), Zn^{2+} (▲) and Pb^{2+} (■))*

decline. The lowest two concentrations (0.5 g·l^{-1} and 1 g·l^{-1}) has not shown much differences in sorption capacity, therefore concentration of 0.5 g·l^{-1} was not so effective and so convenient, because the absolute loss of metal was lower.

CONCLUSIONS

To assess the ability of biosorbent bind heavy metal ions the sorption capacity was determined. This value expresses the amount of metal which is able to bind to the given number of cells (in this study milligrams of metal per gram of cells). When studying the influence of biomass concentration on biosorption, several facts were found. First, the sorption capacity increases at the lower concentrations of cells. Furthermore, the biosorption of both bacteria was very similar. The results show that cells of *Geobacillus thermocatenulatus* CCM 2809 had higher sorption capacity for Cu^{2+} and Zn^{2+} ions. The sorption capacity of Pb^{2+} ions using biomass concentration 0.5 g·l^{-1} of *Geobacillus thermodenitrificans* CCM 2566 was higher than in the case of *Geobacillus thermocatenulatus* CCM 2809. This difference, however, is not expressive. In generally, we can say that *Geobacillus thermocatenulatus* CCM 2809 cells have higher sorption capacity for all three metals than *Geobacillus thermodenitrificans* CCM 2566 cells. It was also found that the affinity of metals was determined in the direction of $Pb^{2+} > Cu^{2+} > Zn^{2+}$ for both bacteria.

SUMMARY

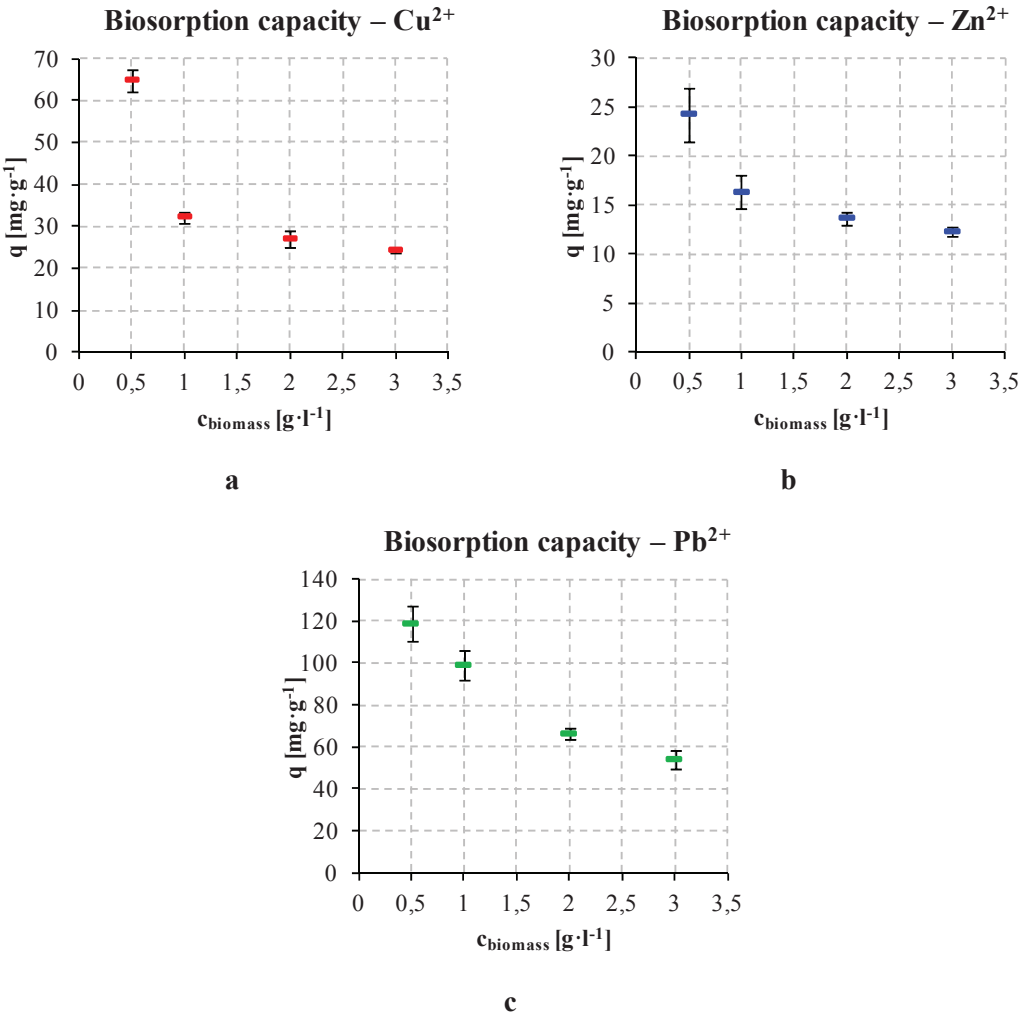
The biosorption allows the binding of metals from aqueous environments of the cells of biomass. Difference between biosorption and bioaccumulation is that biosorption is passive and is not independent on cell metabolism. It can be carried out using inactivated or dead biomass. Biosorption is mainly influenced by pH, concentration and the type of biomass, presence of other metals, temperature

II: *The values of sorption capacity at various concentrations of biomass*

***Geobacillus thermocatenulatus* CCM 2809**

Metal	$c_{biomass}$ [g/l]	$m_{biomass}$ [g]		$C_{metal\,1}$ [mg/l]	$C_{metal\,2}$ [mg/l]	$C_{metal\,3}$ [mg/l]	Ø C_{metal} [mg/l]	q [mg/g]
Cu	0.5	0.011	initial	498.242	499.834	499.834	499.3 ± 0.8	65 ± 3
			final	467.997	466.405	466.405	466.9 ± 0.8	
	1	0.023	initial	515.752	515.752	517.344	516.3 ± 0.8	32 ± 1
			final	483.915	483.915	485.507	484.4 ± 0.8	
	2	0.041	initial	515.752	515.752	517.344	516.3 ± 0.8	27 ± 2
			final	455.263	461.630	463.222	460 ± 4	
	3	0.062	initial	498.242	499.834	499.834	499.3 ± 0.8	24.2 ± 0.6
			final	423.426	426.610	425.018	425 ± 1	
Zn	0.5	0.009	initial	507.786	509.424	507.786	508.3 ± 0.9	24 ± 3
			final	497.958	496.320	497.958	497.4 ± 0.9	
	1	0.022	initial	507.786	509.424	507.786	508.3 ± 0.9	16 ± 2
			final	491.406	489.768	493.044	491 ± 2	
	2	0.041	initial	507.786	509.424	507.786	508.3 ± 0.9	13.7 ± 0.6
			final	479.940	481.578	481.578	481.0 ± 0.9	
	3	0.061	initial	507.786	509.424	507.786	508.3 ± 0.9	12.3 ± 0.4
			final	471.750	473.388	471.750	472.3 ± 0.9	
Pb	0.5	0.010	initial	1006.930	1006.930	1001.739	1005 ± 3	119 ± 8
			final	944.646	944.646	949.836	946 ± 3	
	1	0.021	initial	996.549	1006.930	1001.739	1002 ± 5	99 ± 7
			final	903.123	903.123	892.742	900 ± 6	
	2	0.041	initial	996.549	1006.930	1001.739	1002 ± 5	66 ± 3
			final	871.980	871.980	866.790	870 ± 3	
	3	0.062	initial	996.549	1006.930	1001.739	1002 ± 5	54 ± 4
			final	825.267	851.219	840.838	839 ± 12	

and initial concentration of metal. The objective of this study was to study the biosorption of copper, lead and zinc depending on concentration of biomass *Geobacillus thermodenitrificans* and *Geobacillus thermocatenulatus*. *Geobacillus* cell culture was cultivated in a bioreactor on synthetic medium with the composition: 5 g peptone, 3 g beef extract, 0.01 g manganese sulphate monohydrate and 1 liter of distilled water. Bacterial cells were harvested by centrifugation at 6 000 rpm for 10 minutes at the end of exponential growth phase. Then the cells were washed with distilled water and centrifuged again. Washing procedure was performed twice. Washed cells were subsequently dried to stable weight. The solution of metal was prepared with concentration about 0.5 $g \cdot l^{-1}$ (for Cu^{2+}, Zn^{2+}), resp. 1 $g \cdot l^{-1}$ (for Pb^{2+}). The solution with volume 20 ml was mixed with dry biomass with concentration about 0.5; 1; 2 and 3 $g \cdot l^{-1}$. The mixture was incubated at room temperature and 100 rpm for 12 hours. The biomass was harvested by centrifugation at 6 000 rpm for 10 min. The supernatant was used for complexometric titration. The sorption capacity for Cu^{2+} was highest with using 0.5 $g \cdot l^{-1}$ *Geobacillus thermodenitrificans* (57 ± 4 $mg \cdot g^{-1}$). The sorption capacity rapidly decreases with increased concentrations. Similarly for Zn^{2+} ions, the highest sorption capacity was for biomass concentration of 0.5 $g \cdot l^{-1}$ (18 ± 3 $mg \cdot g^{-1}$) and slowly decreases. For Pb^{2+} ions, the decrease is almost linear to the concentration of biomass of 2 $g \cdot l^{-1}$, i. e. from 117 ± 13 $mg \cdot g^{-1}$ to 53 ± 3 $mg \cdot g^{-1}$. The sorption capacity for Cu^{2+} ions is highest at the lowest concentration of biomass *Geobacillus thermocatenulatus* (65 ± 3 $mg \cdot g^{-1}$), then it sharply decreases and at concentration of biomass of 1 $g \cdot l^{-1}$ has not changed. In the case of Zn^{2+} ions, we could see a moderate drop with the increasing concentration over the range of 24 ± 3 to 12.3 ± 0.4 $mg \cdot g^{-1}$. For Pb^{2+} ions was the decrease slow, from 119 ± 8 $mg \cdot g^{-1}$ to 54 ± 4 $mg \cdot g^{-1}$. Affinity of metals to bacteria was determined in the order $Pb^{2+} > Cu^{2+} > Zn^{2+}$. Our results show that *Geobacillus thermocatenulatus* cells have better sorption capabilities than *Geobacillus thermodenitrificans* cells.



3: *The dependence of sorption capacity of biomass concentration with using Geobacillus thermocatenulatus CCM 2809 (ions: Cu^{2+} (♦), Zn^{2+} (▲) and Pb^{2+} (■))*

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