

Bioleaching of Ni from contaminated sediments of the water reservoir Ružín I using autochthonous bacteria and *Bacillus megaterium*

KATARÍNA JABLONOVSKÁ, ZUZANA PÁLLOVÁ and IVETA ŠTYRIAKOVÁ

Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45,
SK-043 53 Košice, Slovakia; jablonov@saske.sk

Abstract

Water reservoir Ružín I on the territory of Eastern Slovakia is rich in sediments being contaminated with several heavy metals. They come from the mining and metallurgical industrial activities carried out near the water reservoir. This work is dealing with the study of the mobility of Ni between the liquid phase and sediment matrix. During the bioleaching processes carried out in the laboratory the conditions given by the environment of the dam were imitated and preserved also in the flasks. Bacterial species *Bacillus megaterium* were used to increase the efficiency of Ni bioleaching. The obtained results were compared with the activation of autochthonous microflora. Kinetics of bioleaching was measured for 40 days. The bioleaching of Ni in the presence of autochthonous microflora achieved higher intensity and effectivity as in the case of the application of *Bacillus megaterium*. Because of the known mobility of Ni in the sedimentary environment there is important to assess the risk of its inadvertent release into the aqueous solution. Bacterial dissolution of Fe was simultaneously monitored during the bioleaching processes, because the most of the Ni was present in the Fe oxides and hydroxides.

Key words: heavy metals, nickel, iron, bioleaching, heterotrophic bacteria, *Bacillus megaterium*

Introduction

Water reservoir Ružín I in the inlet of the Hornád river is one of the most affected localities in the Eastern Slovakia by the contamination by heavy metals (Hg, Pb, Cu, Ni, Co, Zn, As, Sb, Cd) because of the intensive mining and metallurgical activities in this area (Brehuv et al., 2005). Bottom sediments of this water reservoir even after the attenuation of the industrial activity present the important receptors of the pollution. The amount of the contaminated sediment deposited annually onto the bottom of the tank moreover brings about a risk in term of its functions' restrictions. The issue of its work out and the next use means a poser also for experts. Contaminated sediments could be considered as a special waste classified into the 832 02 category by EWC (European Waste Catalogue and Hazardous Waste List). This category includes wastes inadvisable for disposal by thermal and physical-chemical processes. Treatment of these sediments is possible after their relocation to the dumping site, where the leaching and removal of undesirable metals could be ensured with biological processes. After the application of bioremediation technologies sediments are reusable for example in agriculture and building industry. Bioleaching of heavy metals from the contaminated sediments is affected by several physical-chemical and biological factors: character of contaminated particles, temperature, O₂ and CO₂ concentrations, pH, redoxpotential, composition of the

medium, bacterial genus involved in the bioleaching process and its concentration (Chen et al., 2001). Depending on the character of the sediment particles, metals can be bound with them in various chemical forms, which in term of their mutual chemical interactions, mobility, bioavailability and potential toxicity, exhibit different properties (Singh et al., 2005). For the evaluation of the mobility of elements in water-sedimentary system usually the fractionation analysis of sediments called sequential extraction procedures is used (Vojteková et al., 2006). Sequential extraction method was proposed by Tessier et al. It allows to determine the fractionation of metals and to control their deposition into the environment and therefore to assess their potential impact on the aquatic biota and also to propose appropriate remediation techniques for their elimination (Korfali et al., 2004). This sequential extraction method includes the distribution of heavy metals into the following fractions: ionic exchange fraction, carbonate fraction, fraction of iron and manganese oxides, fraction of organic matter and sulphides and residual fraction containing aluminosilicate components of the sediment (Tessier et al., 1979). Most of the metals presented in the sediment usually occur in the residual fraction. Under the normal experimental conditions, metals presented in this fraction are insoluble and with solid bindings. Therefore their presence in this form is considered as an indicator of the rivers' system contamination (Singh et al., 2005). Oxides of iron and manganese, which usually occur in the form of films and

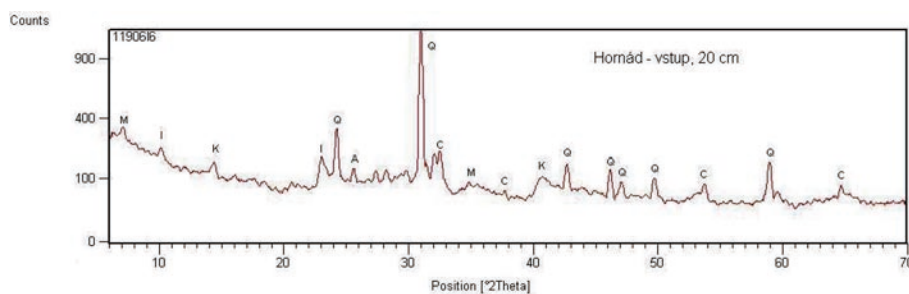


Fig. 1. Mineralogical composition of the sediment sample, depth 20 cm, X-ray analysis. Legend: C – chlorite, I – illite, K – kaolinite, A – albite, M 15 – montmorillonite 15A, M 22 – montmorillonite 22A, Q – quartz.

new-precipitated secondary minerals together with organic matter and clays, represent also an important component. Stated oxides preferentially adsorb or co-precipitate heavy metals occurring in the water column. From this point of view, they suppose to be the most effective surface-active phase of sediments (Hartley et al., 2004; Lombi et al., 2004).

The reactions of metals, which are important from an environmental point of view, take place in anoxic environment natural for the activity of dissimilatory chemoautotrophic and heterotrophic microorganisms using metals as an analogue of O_2 . The process of the reduction of iron and manganese by microorganisms, through reduction solubility of solid Fe (III) and Mn (IV) oxides, affects the conversion of the mineralogical composition of sediments and provides energy necessary for the growth of microorganisms (Lloyd, 2003). As a result of the release of Fe (III) as electron acceptors come to the influence of the mobility of toxic metals which are bonded with the stated minerals (Lovley et al., 2000). The changes of the mobility of metals remit to a certain degree of instability in an aquatic environment caused by the influence of microflora and the possibility of their entry and accumulation in the food chain links.

The purpose of this work is to promote understanding of the process of nickel mobility as one of the contaminants of sedimentary environments of the dam Ružín, depending on the mineralogical composition of sediment and activities of autochthonous microflora, or selected bacterial species in the laboratory conditions. Understanding, exploitation and regulation of this process in in-situ conditions provides a purposeful remediation method of sediments contaminated with heavy metals.

Material and methods

The samples of the sediment were collected from the sediments' surface layer, depth 20 cm, in the coastal zone Ružín I Hornád – entry. Obtained samples were stabilized by deep-freezing ($-15\text{ }^\circ\text{C}$) with an allowance to preserve the integrity of samples in accordance with ISO 5667-12.

The mineralogical composition of the samples was determined by X-ray diffraction analysis (Xpert Philips – Germany) and the content of Ni and Fe in the sediment matrix by atomic absorption spectrometry (AAS OPTION – spectrometer, AA – 30, Australia).

Effect of bacterial cultures and observed leaching medium for the extraction of heavy metals from the sediment was monitored during the bioleaching processes

carried out in Erlenmeyer flask with the volume 1 000 ml at $25\text{ }^\circ\text{C}$ for 40 days partially aerobically in the static conditions. Erlenmeyer flasks' content consisted of 30 g sediment, 600 ml of sterile nutrient medium and bacterial inoculum with a concentration of 10^{10} cfu/ml. Gradual changes in the metal concentrations observed in the nutrient media were monitored in four day intervals by the atomic absorption spectrometry (AAS Varian AA-Z 240 with GTA-120 and AA 240-FS, Australia). The monitoring of the bioleaching processes revealed the kinetics of Fe and Ni extraction and also the activity of bacterial strains and as demonstrated below it allows the precise timing of the subsequent repeated exchanges of bioleaching medium. The purpose of this procedure was to achieve the highest removable metal leaching from the matrix sediment with bacterial activity.

Bacterial species *Bacillus megaterium* used in the bioleaching process has been isolated from the samples of sediments and identified by BBL identification system (Becton – Dickinson, USA). Bacterial cells were cultivated on nutrient agar No. 2 (Imuna Šarišské Michalany) at $28\text{ }^\circ\text{C}$ for 18 hours and centrifuged at 5 000 rpm for 15 minutes. Consequently the cell suspension was washed by physiological solution and added at concentration 10^{10} into the Ashby's solution (glucose 2g/100 ml $(NH_4)_2SO_4$, K_2HPO_4 , $MgSO_4 \cdot 7 H_2O$, NaCl). The changes in pH during the bioleaching process were measured by potentiometry at PHM 210 METERLAB (France).

The sequential extraction of metals from sediments was performed according to Tessier et al. (1975). Homogenized sediment samples of the quantity of 1 g were dried at $105\text{ }^\circ\text{C}$ for 5 hours. They were subsequently agitated with the extractant and centrifuged at 5 000 rpm for 10 min. Supernatant was purified by decantation. Aroused pellet was purified with the distilled water. This process was repeated for using different extraction reagents. The essential characteristic of each extraction solution presents

Tab. 1
Extraction agent used by sequential extraction method according to Tessier (Tessier et al., 1979; Lake, 1987; Pickering, 1986)

Fraction	Extraction solution
Exchangeable	1M $MgCl_2$
Carbonates	1M NaOAc, HOAc
Iron and manganese oxides	0.04 M $NH_2OH \cdot HCl$ in 25 % HOAc
Organic matter	0.02 M HNO_3 in 30 % H_2O_2
Residual	mixture 5 : 1 HF and $HClO_4$

Tab. 2
Physical – chemical characteristics of the sediment sample

Sample	TOC (%)	pH	Eh (mV)	Dry weight (%)
Ružín I	4.05	7.13	341.4	57.02

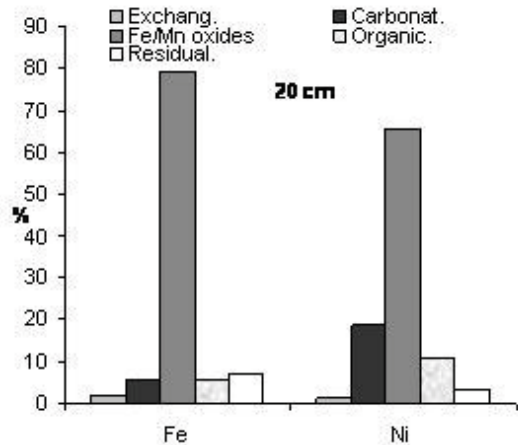


Fig. 2. The effectivity of Fe and Ni extraction by using a sequential extraction by Tessier.

its ability to minimize the dissolution of other fraction, than it is intended. Extraction reagents used during the sequential extraction of metals from sediments are listed in Tab. 1.

Results and discussions

The mineralogical composition of sediment shown in Fig. 1 confirms its aluminosilicate character with a dominance of quartz and amorphous phases indicating the presence of Fe oxides and hydroxides. From the mineralogical point of view there was found, that the composition of the sediment represents an environment offering optimal conditions for the growth of so-called “silicate bacteria” including *Bacillus megaterium*.

The top layer of the sediment represents the contact zone between the liquid and solid phases of the sedimentary environment. It reflects the current load of sediments with occurring contaminants. Depending on changes of the physical-chemical properties of the environment, the top layer of the sediment represents a risk metal release into the liquid phase and their subsequent incorporation into the food chain links. The total content of the organic matter, representing 4.05 % (Tab. 2) is able to adsorb a certain amount of Ni that is reversibly releasable from this phase back into the environment. Humic substances enter by the activity of indigenous species of heterotrophic bacteria into the biochemical reactions with an indirect mechanism of the reduction of insoluble Fe (III) to Fe (II). Anaerobic conditions, pH values close to the neutral and the presence of a sufficient amount of organic material could admit the

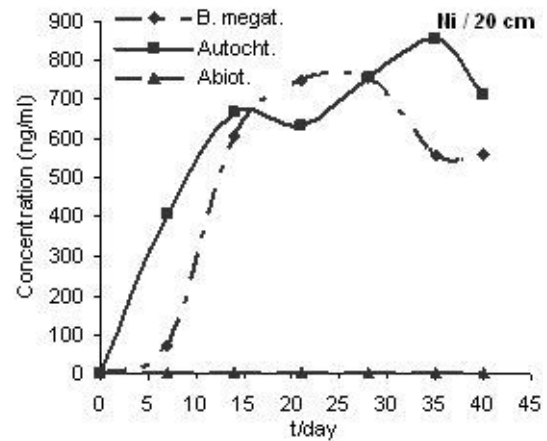


Fig. 3. The kinetics of the bioleaching of Ni.

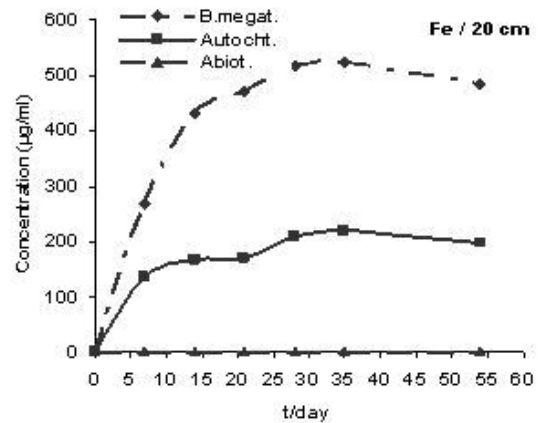


Fig. 4. The kinetics of the bioleaching of Fe.

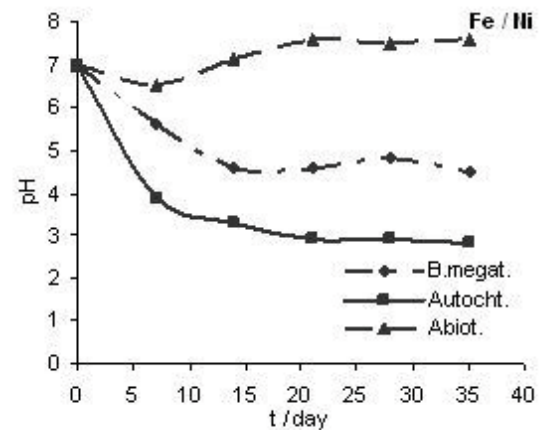


Fig. 5. The kinetics of pH in the bioleaching system.

activity of bacterial species supporting the mobility of Ni in the sedimentary environment.

Fig. 2 describes the binding options of Ni in particular fractions of the sediment and demonstrates its dominant presence (66.36 %) in the fraction of Fe and Mn oxides and hydroxides. These oxides are found in the sediment between particles of other minerals, either as a thin

film or as individual particles. In anoxic conditions they are characterized by thermodynamic instability. They have an excellent ability to bind several heavy metals preferentially in their structure. The activity of Fe (III) reducing microorganisms causes changes in the structure of aluminosilicate components and also in the structure of amorphous Fe oxides, which is leading to the release of Ni into the liquid phase. Understanding the process of mobilization of Ni and the possibility of its regulation permits the utilization of indigenous or inoculated bacterial species for the bioleaching processes of Ni. The task of the experiment was to determine the effectivity of the bacterial species *Bacillus megaterium* in the releasing of Ni and Fe from the sediment matrix in comparison with using the autochthonous microflora. The maximum concentration of Ni in solution was recorded in 27th day at bioleaching by bacterial species *Bacillus megaterium*, and 35th day at bioleaching by autochthonous microflora. Kinetics of Ni and Fe bioleaching processes in term of the time did not correspond to each other. For the Fe in both cases the maximum concentration was reached in 27th day of bioleaching. Comparing the overall extraction efficiency, the maximum efficiency was achieved by using bacterial species *Bacillus megaterium* by bioleaching of Ni and activation of autochthonous microflora was efficient by bioleaching of Ni (Figs. 3 and 4). The decrease of Ni and Fe concentration in the bioleaching medium was related to the accumulation of metabolites of heterotrophic bacterial species (fatty acids), which reduced the pH of the bioleaching system. It causes a decrease in activity of presented microflora and also the extraction of metals into the solution. The decrease in pH, as shown in Fig. 5 refers to the production of fatty acids in to bioleaching process.

Conclusions

The determination of bioleaching kinetics in a 45-day interval is important for the finding of the time of the maximum activity of the presented microflora in the process of metal leaching in specified conditions and also in the term of time for the bioleaching medium exchange at the subsequent discontinuous bioleaching before the decrease of the extraction.

Bioleaching system without bioaugmentation (Control) and without addition of bioleaching medium showed at absence of Ni and Fe in the liquid phase (Figs. 3 and 4). Control in Fig. 5 showed the buffering capacity of sediment during the monitoring of metals mobility. Ashby's mineral medium with the addition of glucose as organic

carbon source led to an increasing mobility of Ni and Fe in bioleaching system caused by the activity of indigenous heterotrophic bacterial species. The inoculation of bacterial species *Bacillus megaterium* in symbiosis with the autochthonous microflora in stated conditions showed the highest efficiency in bioleaching of Fe from the sediment. The bioleaching of Ni was efficiently by using of autochthonous microflora. According to these results, bioleaching of the sediments could be still an actual issue for the next studies.

Acknowledgements. This work was supported by the Science and Technology Assistance Agency under the contract No. APVV-0472-07 and No. APVT-51-006304 and VEGA No. 2/0049/08

References

- BREHUV, J., BOBRO, M., HANČULÁK, J., ŠPALDON, T. & SLANČO, P., 2005: Obsah vybraných prvkov vo vode a v nánosoch nádrže vodného diela Ružín I v ostatnom období. In: *Zbor. prednášok z konferencie Sedimenty vodných tokov a nádrží, Bratislava (11. – 12. 5. 2005)*, 91 – 96.
- HARTLEY, W., EDWARD, R. & LEPP, N. W., 2004: Arsenic and heavy metal mobility in iron oxide amended contaminated soils as evaluated by short- and long-term leaching tests. *Environmental Pollution*, 131, 495 – 504.
- CHEN, S. Y. & LIN, J., 2001: Bioleaching of heavy metals from sediment: Significance of pH. *Chemosphere*, 44, 1 093 – 1 102.
- KORFALI, S. I. & DAVIES, E. B., 2004: Speciation of metals in sediment and water in a river underlain by limestone: Role of carbonate species for purification capacity of rivers. *Advances in Environmental Research*, 8, 599 – 612.
- LOVLEY, D. R. & ANDERSON, R. T., 2000: The influence of dissimilatory metal reduction fate and metal contaminants in the subsurface. *Hydrology*, 8, 77 – 88.
- LLOYD, J. R., 2003: Microbial reduction of metals and radionuclides. *FEMS Microbiology Reviews*, 27, 411 – 425.
- LOMBI, E., SLETTEN, R. S. & WENZEL, W. W., 2004: Remediation of polluted soils. *Encyclopedia of soil in the Environment, RE Hamon*, 379 – 385.
- SINGH, P. K., MOHAN, D., SINGH, K. V. & MALIK, A., 2005: Studies on distribution and fractionation of heavy metals in Gomti river – a tributary of the Ganges, India. *J. Hydrobiology*, 312, 14 – 27.
- TESSIER, A., CAMPELL, P. G. C. & BISSON, M., 1979: Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, 51, 844 – 851.
- VOJTEKOVÁ, V. & KRAKOVSKÁ, E., 2006: Frakcionačná analýza sedimentov – Limitácie selektivity sekvenčného lúhovania. *Chemické listy*, 100, 1 096 – 1 104.

*Rukopis doručený 22. 6. 2010
Rukopis akceptovaný red. radou 7. 9. 2010
Revidovaná verzia doručená 19. 10. 2010*