Biotechnology & Metals

Košice 2014

Faculty of Metallurgy of Technical University in Košice
Institute of Geotechnics of Slovak Academy of Sciences

Book of Abstracts

3rd International Scientific Conference on Biotechnology and Metals

Editors:
Kaduková Jana, Luptáková Alena, Velgosová Oksana

ISBN: 978-80-553-1787-8

September 17 – 19, 2014, Košice, Slovakia

INTERNATIONAL SCIENTIFIC COMMITTEE

Ljudmilla Bokányi	University of Miskolc, Hungary
Vladimír Čablík	VŠB–TU Ostrava, Czech Republic
Nicolas Kalogerakis	Technical University of Crete, Greece
Mária Kušnierová	Slovak Academy of Sciences, Kosice, Slovakia
Anna Kinga Nowak	Cracow University of Technology, Poland
Neil Rowson	University of Birmingham, Great Britain
Mariola Saternus	Silesian University of Technology, Poland
Alexandra Šimonovičová	Comenius University, Bratislava, Slovakia
Miroslav Štofko	Technical University in Košice, Slovakia
Stefano Ubaldini	National Research Council, Roma, Italy

ORGANIZING COMMITTEE

Chair
Jana Kaduková Technical University in Košice

Vice-chair:
Alena Luptáková Slovak Academy of Sciences, Košice

Members

Technical University in Košice
Martin Fujda
Štefan Nižník
Oksana Velgosová
Anna Mražíková
Renáta Marcinčáková

Slovak Academy of Sciences, Košice
Slavomír Hredzák
Jana Jenčárová
Eva Mačingová
Ingrida Kotuličová
Mária Praščáková
Oľga Šestinová

REVIEWERS

Jana Kaduková
Oksana Velgosová
Anna Mražíková
Renáta Marcinčáková
Alena Luptáková

ISBN: 978-80-553-1787-8

Preface

Dear collegues and friends,

it is honour to welcome you at the conference Biotechnology and Metals.

What may seem a disadvantage of biotechnology, such as its multidisciplinary character, which forces us to leave established way of thinking, to learn new things and connect knowledge from several areas, is becoming its advantage. We can discover undiscovered and explore unexplored. The beauty of this work is also its connection with practice. Not in all scientific areas a scientist can see the results of his/her work applied to a real life in a relatively short time.

This conference aims to create a space for the meeting of two ostensibly unconnected areas – biology and technology, whether focused on metal recovery from raw materials and wastes, removal of toxic metals from the environment or processing of various materials.

Connection of scientific areas reflects well on people connection. The result of one of such connection is also long-term cooperation between Faculty of Metallurgy, Technical University in Kosice and Institute of Geotechnics, Slovak Academy of Sciences, which has brought not only lots of scientific knowledge but also several friendships. We believe that this conference will have the same contribution in scientific areas and in friendship as well.

We hope you will enjoy meeting in the conference and visiting Kosice as well. We are looking forward to see you again at the conference Biotechnology and Metals held in the next three years.

Jana Kaduková
Alena Luptáková

Biotechnology & Metals

Košice 2014

CONTENT

Biotechnology & Metals Košice 2014

3rd International Conference
Biotechnology and Metals
17th and 19th of September, 2014,
Košice, Slovakia

Conference Programme

Venue: University Library of the Technical University in Košice

WEDNESDAY, SEPTEMBER 17th, 2014	
9:00 – 10:00	***Registration of participants***
10:00 – 10:30	***Welcome and keynote speech***
10:30 – 12:30	***Invited Lectures*** **Pristaš P.** - Non-ferrous metal industry waste disposal sites as a source of polyextremotolerant bacteria **Bokányi L.** - State-of-art and Perspectives of the Bioleaching and Biosorption in Waste Treatment **Mandl M.** - Impact of Oxygen Demand on Aeration in *Acidithiobacillus ferrooxidans* Cultures: Biotechnological Implications **Saternus M. –** Dark Site of Metals
13:00 – 14:00	***Lunch***
14:00 – 15:30	**Session 1:** **Chair-persons:** Bokányi L. (HU) and Bálintová M. (SK)
	Kaduková J. – Biometallurgical research at the Faculty of Metallurgy **Luptáková A.** - Past, present and future of biohydrometallurgy at the Institute of Geotechnics of Slovak Academy of Sciences **Pipíška M., Tišáková L., Godány A., Horník M., Augustín J.** - Bacteria from Extreme Environment and their Potential in Metal and Radionuclide Bioremediation **Kupka D., Kovařík M., Václavíková M, Dakos Z., Jáger D.** - Reduction of Soluble Fe^{3+} Sulfate to Fe^{2+} Sulfate by Acidophilic Heterotroph *Acidiphilium* SJH **Kotuličová I., Luptáková A., Bálintová M., Demčák Š.** - Bacterial Regeneration of Barium Sulphate to Barium Sulphide
18:00 – 22:30	**Welcome party**

THURSDAY, SEPTEMBER 18th, 2014	
9:00 – 10:30	**Session 2: Biosorption and sorption** **Chair- persons:** Mandl M. (CZ) and Pipíška M. (SK)
	Remenárová L., Pipíška M., Augustín J., Soja G. - Biochar Derived from *Triticum aestivum*: Study of Character and Mechanism of Metal Ions Removal **Paulovics J., Bokányi L.** - Development of Biosorbents Applying *Undaria pinnatifida* Macro Algae **Urík M., Gardošová K., Bujdoš M., Matúš P.** - Humic Acid Sorption Interactions with Biological Surfaces and Heavy Metals **Ivánová D., Kavuličová J., Kaduková J.** - Application of Biosorbents and Ion-Exchanger for Copper Ions Removal
	Poster section 1: **Didyk-Mucha A., Sadowski Z.** - Bioleaching of Lead-Bearing Ashes **Willner J., Fornalczyk A., Saternus M.** - Possibilities of Using Cyanogenic Microorganisms in the Recovery of Precious Metals from Waste Materials **Hagarová I., Bujdoš M., Urík M., Mackových D.** - Surfactants of Different Origin Used for Extraction of Heavy Metals from Contaminated Soils and Sediments **Herková, M., Čablík, V., Čablíková, L.** - The Biosorption of Chromium(III) Cations by *Reynoutria japonica* **Šestinová O., Findoráková L., Dolinská S., Špaldon T., Kurbel T.** - Earthworms as a tool of toxicity determination of sediment polluted by mining activity on the territory of Eastern Slovakia
10:30 – 11:00	***Coffee break***
11:00 – 12:30	**Session 3:** **Chair- persons:** Sadowski Z. (PL) and Beolchini F. (I)
	Dakos Z., Kupka D., Kovařík M., Briančin J. - Ochreous Precipitates from Smolník Abandoned Mine **Jenčárová J., Luptáková A.** - The Ways of Polution Elimination from Mine Drainage Waters **Fonti V., Dell'Anno A., Beolchini F.** - Biogeochemical Interactions in the Application of Biotechnological Strategies to Marine Sediments Contaminated with Metals **Vojtková H., Šimonovičová A., Čerňanský S., Kučerová R., Dirner V., Pauditšová E., Švanová P.** - Inhibition Effects of Sediments from the Černý Příkop Site on Growth and Bioleaching of Microscopic Filamentous Fungi **Oroszová L., Štefušová K., Václavíková M.** - Composite Material for Arsenic Removal from Waters

13:00 – 14:00	***Lunch***
15:00	**Tokaj wine cellar visit**
FRIDAY, SEPTEMBER 19th, 2014	
9:00 - 10:30	**Session 4: Bioleaching** **Chair-persons:** Šimonovičová A. (SK) and Saternus M. (PL)
	Baranska J.A., Sadowski Z. - Hydrodynamic Study of Column Bioleaching Processes **Semerád M., Čerňanský S., Šimonovičová A., Kubátová A., Takáčová A.** - Fungal Bioleaching of Electronic Waste **Marcinčáková R., Kaduková J., Velgosová O., Mražíková A.** - Lithium Bioleaching from Lepidolite using *Rhodotorula rubra* **Mražíková A., Marcinčáková R., Kaduková J., Velgosová O., Bálintová M.** - Influence of Used Bacterial Culture on Zinc and Aluminium Bioleaching from Printed Circuit Boards
	Poster section 2: **Hruška M.** - Stabilization of Contaminated Soil by Using Elementary Iron Nanoparticles and Their Interactions with Soil and Bacteria **Mačingová E., Luptáková A.** - Biological Removal of Inorganic Pollutants from Acid Mine Drainage **Sobariu D.L., Zimmermann Y-S., Müller S., Gavrilescu M., Lenz M.** - Selective Recovery of Antimony from Wastewaters **Willner J., Fornalczyk A., Saternus M.** - Selective Recovery of Copper from Solutions after Bioleaching of Electronic Waste
10:30 – 11:00	***Coffee break***
11:00 – 12:15	**Session 5:** **Chair-persons:** Čablík V. (CZ) and Václavíková M. (SK)
	Luptáková A., Eštoková A., Kovalčíková M., Števulová N. - Study of Concrete Materials Biocorrosion Caused by Sulphuretum Activity **Kavuličová J., Kaduková J., Ivánová D.** - The Effects of Copper on Seed Germination and Early Seedling Growth of *Raphanus sativus* **Kaduková J., Velgosová O., Mražíková A., Marcinčáková R., Tkáčová E.** - In Vitro Toxicity Assessment of Silver Nanoparticles Synthesized using *Parachlorella kessleri* **Šimonovičová A., Jesenák K., Čerňanský S.** - Inhibition of Fungal Pellet Growth by Montmorillonite **Václavíková M.** - Water and Soil Clean-up from Mixed Contaminants
12:15 – 12:30	***Closing Ceremony***
13:00 – 14:00	***Lunch***

HYDRODYNAMIC STUDY OF COLUMN BIOLEACHING PROCESSES

Joanna A. Baranska, Zygmunt Sadowski
Wroclaw University of Technology, Faculty of Chemistry, Department of Chemical Engineering, Wybrzeze Wyspianskiego 27, Wroclaw 50-370, Poland

ABSTRACT
The principal bioleaching techniques for metal ions recovery include tank and heap or dump leaching. Heap leaching is cheaper and easy to construct and operate. Laboratory simulation of heap bioleaching involves the use of column bioreactor with recycled leaching solution. The main bioleaching parameters can be tested using such equipments. The progress in the mathematical modelling of column bioreactor is connected to the packed bed characteristics.
The shape and size of leaching particles that make up the bed bear responsibility for a pore size distribution. The pore sizes determine the velocity of leaching solution flows through a packed bed. The velocity of leaching solution through the bed is determined by Darcy’s law. One of the critical parameter considered is the distribution of the leaching solution throughout the column and its relation to the flow rate.
The small particles made a compressed bed with, very low bed porosity with results in different pressure drops. The bed porosity increases, then the more the shape of particles deviates from the spherical shape are presented. The structure of packed bed can be improve by the addition of inertial material (polyethylene and glass big particles) to the bed. The considerations regarding the optimum structure of packed bed in the column are studied. The aim of the present work is therefore to study the effect of inertial support materials on the leaching of copper ore (black shale).

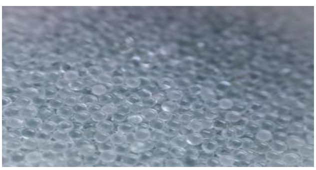
Fig. 1a Gass sheres

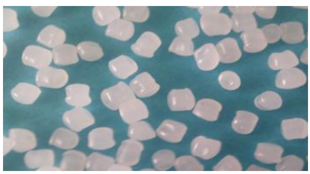
Fig. 1b Polyethylene beads

Two types of support particles were used. Glass beads (hydrophilic) and polyethylene (hydrophobic) particles were used as the support materials. Fig. 1a and Fig. 1b. Leaching liquid flowing through porous abundance is wasting the energy. The slippage is proportional to a difference is hurling between upper but bottom with layer of the deposit. According to the Ergun theory the drop of the pressure in the porous deposit the L in length is equal:

$$\frac{\Delta p}{L} = aU + bU^2 \qquad (1)$$

where: a=150; b=1.75

The porosity represents the pore volume of a representative sample (bed) divided by its bulk volume. Permeability characterizes the ease with which a leaching solution may be flow through the porous bed. The relationship between permeability and porosity is Kozeny-Carman equation.

Different types of packing have been employed. The properties of plastic + ore beds are summarized in Tab. 1.

Tab. 1 The properties of plastic + ore beds

Polymer/ore ratio [g/g]	Flow [ml/s]	Bed porosity	Bed surface [m^2/g]	Degree of dispersion	Hydraulic diameter x 10^{-3}
0/80	0.050	0.5542	4.128	3.414	6.49
15/80	0.034	0.4298	3.489	26.328	4.40
30/80	0.036	0.4195	3.024	46.358	2.606
50/80	0.020	0.3389	2.571	34.920	1.552

The results presented at Fig. 2 indicate that the copper recovery is very similar for glass spheres and polyethylene beads.

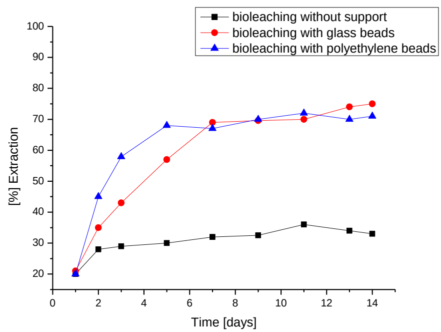

Fig. 2 Copper recovery

In conclusion, support material strongly affected the copper recovery during the bioleaching process.

Keywords: packed bed, leaching solution flow, bioleaching, *Acidithiobacillus ferrooxidans*, Ergun equation, support material.

OCHREOUS PRECIPITATES FROM SMOLNÍK ABANDONED MINE

Zuzana Dakos, Daniel Kupka, Michal Kovařík, Jaroslav Briančin
Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001Košice, Slovak Republic

ABSTRACT

Metal sulfides of rock origin disrupted by mining activities exposed to oxidation conditions (contact with water and atmospheric oxygen) cause start of processes producing Fe, other metals, sulfides and acidity. The result of oxidizing weathering of iron sulfides is the production of AMD and the consequent formation of ochreous precipitates in drainage systems and in the surroundings of AMD seepage on the surface. Apart from these facts, the genesis of AMD is facilitated by indigenous chemolithotrophic iron and sulphur oxidizing bacteria, especially of genus *Acidithiobacillus* and *Leptospirillum*.

The long-term monitoring of AMD waters collected at the shaft Pech (Smolník) point at the enduring risk of contamination of particular components of environment in mining area Smolník. Elemental analysis, X-ray diffraction, Mössbauer spectroscopy and scanning electron microscopy of the ochreous precipitates formed in Smolník AMD stream revealed Schwertmannite as the dominant solid phase in the precipitates (Fig.1 and 2) The chemical analysis of AMD effluents and the elemental composition of related sediments indicated considerable scavenging potential of the ochreous precipitates towards arsenic, aluminium, lead and other metal species.

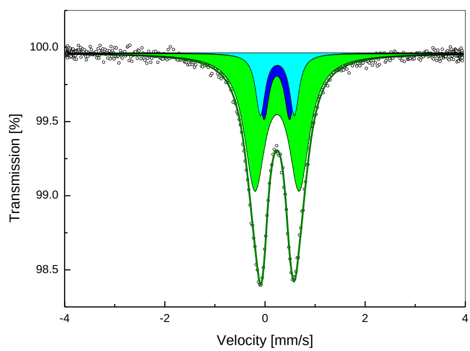

Fig 1. Fitted ^{57}Fe Mössbauer spectrum of natural schwertmannite

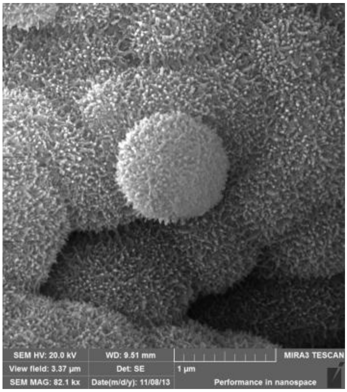

Fig 2. SEM micrographs of natural schwertmannite

Keywords: acid mine drainages, ochreous precipitates, schwertmannite

Acknowledgement: the work has been supported by Slovak Grant Agency VEGA Grant No. 2/0166/11; Slovak Research and Development Agency within the project APVV-0252-10/WATRIP as well as within the Marie Curie Programme FP7-People-IAAP-612250.

BIOLEACHING OF LEAD-BEARING ASHES

Agnieszka Didyk-Mucha, **Zygmunt Sadowski**
Wroclaw University of Technology, Faculty of Chemistry, Department of Chemical Engineering, Wybrzeze Stanislawa Wyspianskiego 27, Wroclaw 50-370, Poland

ABSTRACT
Nowadays, the global economy develops very fast. Every year several branch of industry use a large amount of raw materials. As a result of that, the number of rich deposits of metals is systematically decreasing. Moreover, access to the rich ores is declining. Depletion of ores, enrichment problems, rising cost of energy used in the separation and recovery processes force mining industry to search a new ways of metals obtaining. Good solutions can be mineral bioprocessing. Mineral bioprocessing such as biohydrometallurgy is environmentally friendly and decrease costs of waste disposal. One of biohydrometallurgy method is bioleaching.
It is a process of obtaining valuable metals from ores using microorganisms. Bioleaching is very complex. It is the results of several enzymatic, chemical and electrochemical reactions. Leaching leads to dilution of solid and transfer ionic form of extracted component (components) into the solution. Leaching is electrochemical process and it occurs only when one or more components have different oxidation degree in solid and solution. Schematically, this process can be described as follows:

$$\text{solid} + \text{leaching agent} \rightarrow \text{metal ions in solution}$$

Bioleaching can use a several species of ferrous and sulfur oxidizing bacteria, including *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Bioleaching (also known as biooxidation process) require presence of oxygen. Oxygen is use by microorganism to oxidation Fe^{2+} ferrous ions. Form in this process Fe^{3+} ions are used to oxidize the ore. Energy from bioleaching process is used by bacteria to carbon dioxide bonding.
In the following work, the results of bioleaching of lead-bearing ashes from copper smelter (Glogow, Poland) were presented. The bioleaching kinetic was monitored by pH and Eh values measuring. Moreover, protein, lead and iron concentration was monitored. Lead-bearing ashes bioleaching were carried in the presence of: pyrite, sulfur, sphalerite and galena.

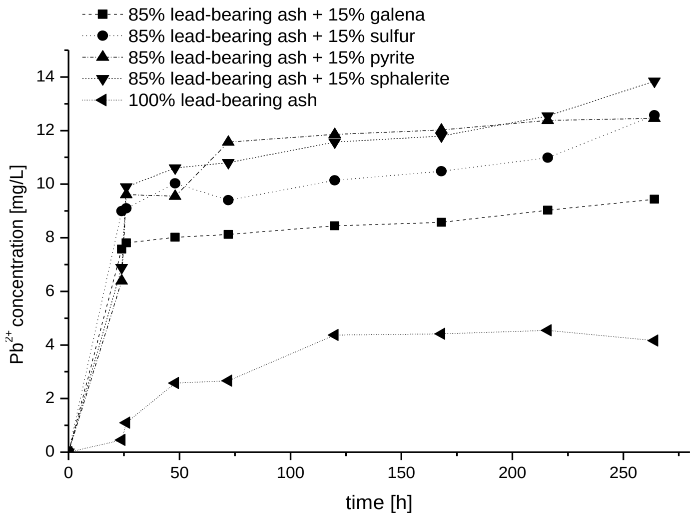

Fig. 1 Bioleaching kinetics of lead-bearing ashes

Influence of these additives on bioleaching recovery was investigated. The typical experimental data are presented at Fig.1. Result shows that additives improve bioleaching kinetic and recovery. The best effects were obtained for bioleaching in the presence of sphalerite.

Keywords: bioleaching, lead-bearing ashes, *Acidithiobacillus ferrooxidans*, sphalerite, galena, pyrite.

BIOGEOCHEMICAL INTERACTIONS IN THE APPLICATION OF BIOTECHNOLOGICAL STRATEGIES TO MARINE SEDIMENTS CONTAMINATED WITH METALS

Viviana Fonti, Antonio Dell'Anno, Francesca Beolchini
Department of Life and Environmental Sciences Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

ABSTRACT
The study deals with biotechnological strategies applied to marine sediments contaminated with metals. Inoculated microbial strains (autotrophic vs. mix heterotrophic and autotrophic), energy source amendment (iron, elemental sulphur, glucose), sediment geochemistry and metal partitioning in sediment, were the main factors of the investigation. The solubilization efficiency of the metals in the presence of active autotrophic Fe/S oxidizing bacteria was highly variable, with the highest for Zn (40%-76%, Fig. 1) and the lowest for Pb (0%-7%).

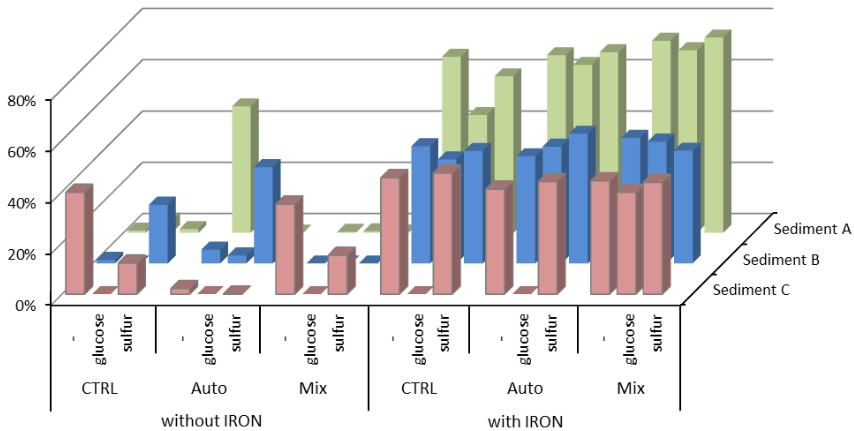

Fig. 1 Zinc solubilisation efficiencies during bioleaching, for three sediments with different geochemical properties (20% sediment, room temperature)

The role of the bacteria resulted to be mainly associated with the production and recycling of leachant chemical species, mainly as protons and ferric ions. Metal solubilization appeared to be strictly associated to the maintenance of acid and oxidative conditions, to the chemical behavior in aqueous environment of each metal species and to the geochemical characteristics of sediment.

As a whole, the results have allowed the development of a conceptual model describing the biogeochemical interactions occurring during the application of bioleaching to contaminated marine sediments (Fig. 2).

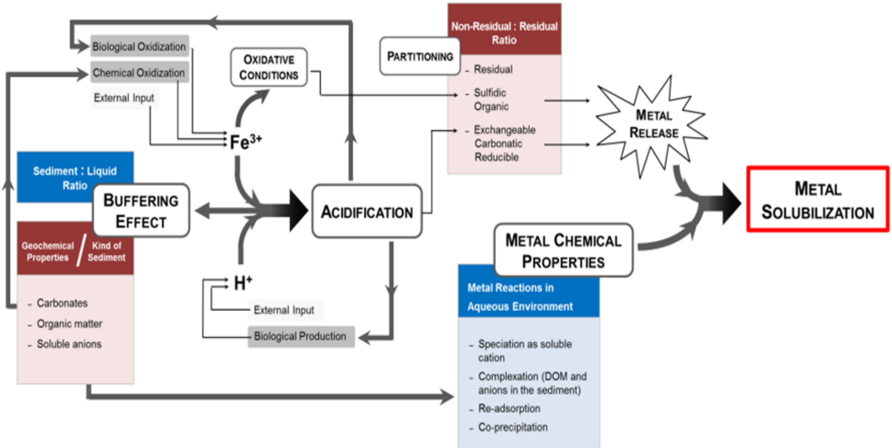

Fig. 2 Conceptual model of biogeochemical interactions involved during the application of bioleaching to marine sediments

Keywords: bioleaching, marine sediment, metal contamination, conceptual model

SURFACTANTS OF DIFFERENT ORIGIN USED FOR EXTRACTION OF HEAVY METALS FROM CONTAMINATED SOILS AND SEDIMENTS

Ingrid Hagarová[a], Marek Bujdoš[a], Martin Urík[a], Daniela Mackových[b]

[a] *Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Mlynská dolina G, Bratislava 84215, Slovak Republic*

[b] *State Geological Institute of Dionýz Štúr, Geoanalytical Laboratories, Markušovská cesta 1, Spišská Nová Ves 05240, Slovak Republic*

ABSTRACT

In this work, synthetic non-ionic surfactants such as Triton X-100 and Triton X-114 (*octylphenoxypolyethoxyethanol* with PEO chain length of 9.5 and 7.5, respectively), biosurfactant saponin (isolated from *Quillaja saponaria*), and metabolites of microscopic fungi (*Aspergillus niger*) were compared for extraction of heavy metals (such as Cu, Pb, Ni, and Zn) from contaminated soils and sediments. The first sampling site was situated near open quartzite mine Šobov (Banská Štiavnica), the second one was situated in the region of Pezinok which is famous for its important ore deposits in the Malé Karpaty Mts., the third one (sediment Richnava) was situated in alluvium of Hornád, the fourth one (sediment Jaklovce) was situated in alluvium of Hnilec. Total content of heavy metals in these samples can be seen in Table 1.

Table 1 Total content of heavy metals in studied samples

Sample	Cu (mg/kg)	Pb (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
Soil Šobov	37.0 ± 0.8	99.4 ± 2.5	5.3 ± 0.3	92.5 ± 2.2
Soil Pezinok	72.3 ± 2.0	48.7 ± 1.8	72.0 ± 2.2	210 ± 4.0
Sediment Richnava	376 ± 2.8	116 ± 2.9	56.2 ± 1.5	425 ± 2.8
Sediment Jaklovce	304 ± 2.6	70.0 ± 3.4	40.1 ± 1.8	430 ± 3.2

For extraction procedure, batch experiments were conducted using a sample to an extracting solution ratio of 1:5. While non-ionic surfactants and metabolites of *Aspergillus niger* have shown weak capability to remove the studied metals (removal efficiency less than 6 % in all cases), natural plant-based biosurfactant saponin can be considered as a promising biodegradable agent for remediation of Cu, Ni, and Zn from contaminated soils and sediments. The removal percentage was in the range of 14 – 46 % (see Fig. 1). Maximal removal yields were achieved at saponin concentration of 2 % (m/v) and removal incubation time of 24 hours. Higher concentrations and longer incubation times have shown no improving in removal efficiencies.

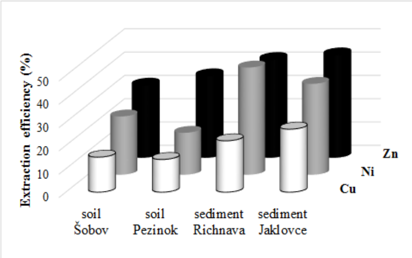

Fig. 1 Removal of heavy metals from contaminated soils and sediments by using quillaja saponin

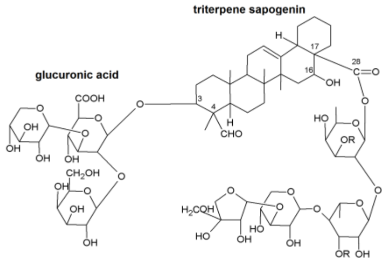

Fig. 2 Chemical structure of quillaja saponin according to Chen et al., Process Biochem. 43 (2008) 488

In the literature, there is a little information about the biosurfactant-metal interactions. In such kind of interactions, the structure, size, and charge of biosurfactants are expected to play an important role. By using quillaja saponin (chemical structure see in Fig. 2), a three-step mechanism of micellar washing of basic surface contaminated with acidic metal ions was proposed (Chen et al. Process Biochem. 43 (2008) 488). Schematic description can be seen in Fig. 3.

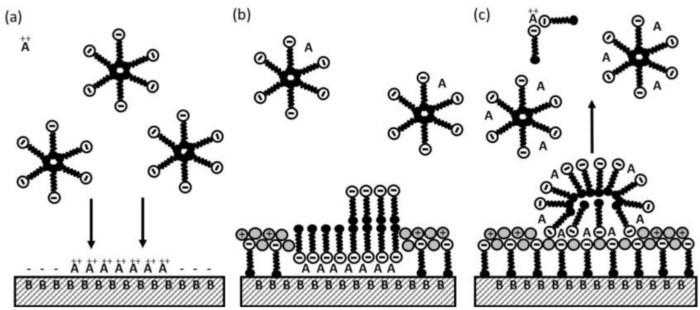

Fig. 3 Three-step mechanism of micellar washing of basic particles (anionic surface B^-) contaminated with acidic metal ions (cations A^{++}) in an anionic surfactant solution above the critical micellar concentration

Keywords: Heavy metals, synthetic surfactant, biosurfactant, soil, sediment.

Acknowledgement

The work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences under the contract Nos. VEGA 1/0274/13 and VEGA 1/0203/14.

THE BIOSORPTION OF CHROMIUM(III) CATIONS BY *Reynoutria japonica*

Mariana Herková[1], Vladimír Čáblík[2], Lucie Čáblíková[2]

[1] *VŠB-Technical University of Ostrava, Faculty of Mining and Geology, Institute of Environmental Engineering, Czech Republic*

[2] *VŠB-Technical University of Ostrava, Faculty of Mining and Geology, Institute of Clean Technologies for Extraction and Utilization of Energy Resources, Czech Republic*

ABSTRACT

This paper focuses on sorption of trivalent chromium onto biosorbent prepared from plant *Reynoutria japonica*. In laboratory, the sorption capacity, the balance parameters, the effect of pH, method of preparation of plant- physical and chemical pretreatment for sorption of heavy metal ions were investigated Based on the obtained results the sorption properties of the studied sorbents were defined. Analyses of sorption capacity was carried out for 60min until equilibrium between the solution and the sorbent was reached. From measured results were compiled Langmuirs and Freundlichs models. It has been shown that the method of preparation of plant biomass and parameter of pH has an impact on the sorpion properties.

Keywords: trivalent chromium, biosorption, *Reynoutria japonica*

ACKNOWLEDGEMENT: This study was supported by the research project SGS SP2014/7 and project Institute of clean technologies for mining and utilization of raw materials for energy use, reg. no. CZ.1.05/2.1.00/03.0082 supported by Research and Development for Innovations Operational Programme financed by Structural Founds of Europe Union and from the means of state budget of the Czech Republic

STABILIZATION OF CONTAMINATED SOIL BY USING ELEMENTARY IRON NANOPARTICLES AND THEIR INTERACTIONS WITH SOIL AND BACTERIA

Miroslav Hruška
Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Department of Geoenvironmental sciences, Kamycká 1176, 165 21 Praha 6 – Suchdol, Czech Republic

ABSTRACT

Extensive attention has been paid towards the risky elements found massively in soil. These soils are so called contaminated. However, soil remediation methods such as sanation using elementary nanoparticles are useful technique against such contaminated soils. Nano particles are intensively investigated method with great potential of sanation. Elementary iron nanoparticles (nZVI) are able to quickly and effectively remove chlorinated hydrocarbons and immobilize metals and metalloids in contaminated soils.

In present scenario we are using elementary iron nanoparticles (nZVI) for in-situ remediation of contaminants present in soil. The ability of nZVI to stabilize metals in contaminated soil was being studied by using batch experiments (Fig. 1). After the addition of 0.5 % and 1 % of nZVI into the contaminated soil we observed that Zinc (Zn) content in the soil solution reduced by 8 to 80 % after 14 and 30 days (Fig.2). It was also found that the content of Plumbum (Pb) in the soil solution was reduced by 20-50 % after 7 and 14 days (Fig.3). The content of Cadmium (Cd) was found to be reduced by 20 to 80 % after 14 and 30 days (Fig. 4).

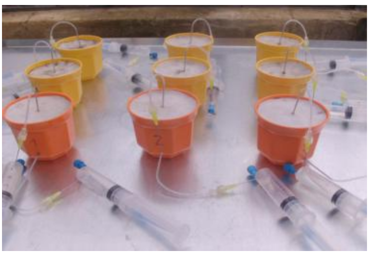

Fig. 1 The stabilization of contaminated soils using nZVI (batch experiment)

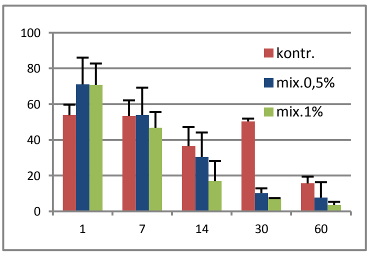

Fig. 2 Effect of nZVI on Zn stabilization in contaminated soil

For studying the effect of bacteria on nZVI we used bacteria of the genus *Acidithiobacillus ferooxidans*. The addition of 0.1% mass amount of nZVI into the cultivation medium has a positive effect on the growth of bacteria which is faster as compared to control without nZVI (Fig. 5). Bacteria varied the oxidation number of nZVI (Fig. 6). Finally, we concluded that there is some interaction between soil and iron nanoparticle nZVI and nZVI with bacteria. However, the ultimate aim is to study the interactions between the contaminated soils, nZVI and bacteria.

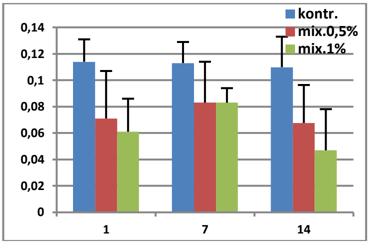

Fig. 3 Effect of nZVI on Pb stabilization in contaminated soil

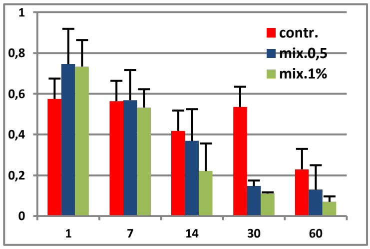

Fig. 4 Effect of nZVI on Cd stabilisation in contaminated soil

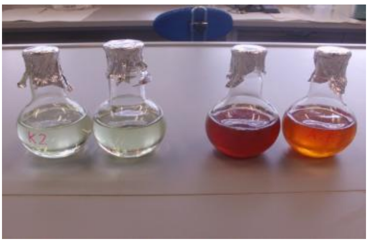

Fig. 5 Effect of nZVI on the bacteria growth of the genus *Acidithiobacillus ferooxidans*

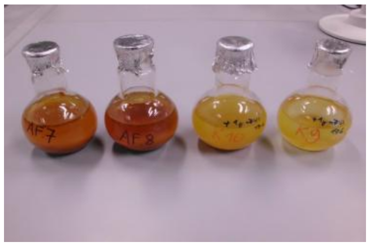

Fig. 6 Effect of bacteria *Acidithiobacillus ferooxidans* to change the oxidation state of nZVI

Keywords: elementary iron nanoparticles, contaminated soil, *Acidithiobacillus ferooxidans*

Acknowledgement
Dissertation work is directly related to the project, which got a university grant CIGA 2014th.

APPLICATION OF BIOSORBENTS AND ION-EXCHANGER FOR COPPER IONS REMOVAL

Dana Ivánová[a], **Jana Kavuličová**[a], **Jana Kaduková**[b]
[a] *Technical University in Košice, Faculty of Metallurgy, Department of Chemistry, Park Komenského 19, Košice 04200, Slovak Republic*
[b] *Technical University in Košice, Faculty of Metallurgy, Department of Material Science, Park Komenského 11, Košice 04200, Slovak Republic*

ABSTRACT
Copper ions removal by different sorbents from model aqueous solutions with initial concentration 16 mg l^{-1} and 160 mg l^{-1} was realized. The decrease of copper ions concentrations after addition of biosorbents prepared from mosses *Sphagnum sp.*, *Polytrichum commune* and a green alga *Parachlorella kessleri* was compared to the decrease after application of commercial cationic exchanger Amberlite IR 120 (Fig. 1, 2).

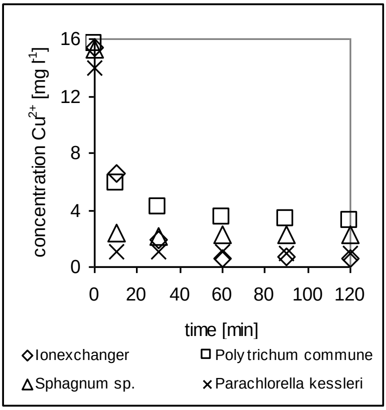

Fig. 1 Decrease of copper ions concentration after addition of studied sorbents, c_0=16 mg l^{-1}

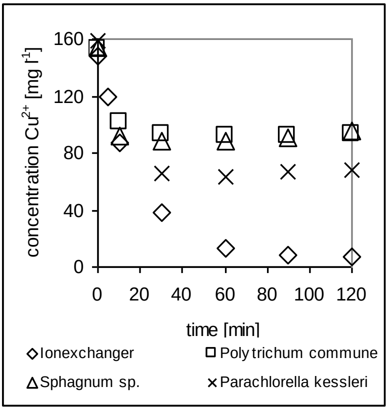

Fig. 2 Decrease of copper ions concentration after addition of studied sorbents, c_0=160 mg l^{-1}

The experimental data revealed that maximum amount of copper adsorbed by *Parachlorella kessleri* and *Sphagnum sp.* from both model solutions increased rapidly with contact time up to 10 min. *Polytrichum commune* achieved maximum sorption capacity at higher concentration of solution up to 30 min, at lower concentration of solution up to hour. The maximum decrease of the copper (II) concentrations by ion-exchanger was detected after one hour. After

10 min of the process ion-exchanger removed 57.6 % of copper ions from solution with Cu^{2+} concentration 16 mg.l^{-1} and 41.1% of copper ions from solution with Cu^{2+} concentration 160 mg.l^{-1}, respectively.

Table 1 The equilibrium sorption capacity and efficiency for studied sorbents, with initial concentration of Cu^{2+} 16 mg l^{-1} or 160 mg l^{-1}

sorbent	c_0=16 mg l^{-1}		c_0=160 mg l^{-1}	
	qe (mg g^{-1})	Q_e (%)	qe (mg g^{-1})	Q_e (%)
Ion-exchanger	7.43	96.1	70.99	95.3
Parachlorella kessleri	6.51	92.8	45.80	57.5
Sphagnum sp.	6.52	85.4	28.98	37.7
Polytrichum commune	6.20	79.5	29.85	39.0

From the comparison in Tab. 1 is visible that in the equilibrium the best sorbent with 96.1% and 95.3 % removal of copper ions is commercially produced ionexchanger for both model solutions. It seems that mosses and green algae with the biosorption efficiency from 79.5% to 92.8% are suitable to remove copper ions mainly from solution with lower metal content. Biosorbents have lower copper ions removal efficiency from solution with higher concentration against ion-exchanger. The maximum decrease of the copper concentration was detected 57.5% for *Parachlorella kessleri*, 39% for *Polytrichum commune* and 37.7% for *Sphagnum sp*.

The biosorption equilibrium was reached by all studied sorbents up to 2 hours. But biosorbents needed less than 30 minutes (generally 10-15 min) to reach the equilibrium while ion-exchanger more than 1 hour. This fact makes biosorption more attractive process for the use in columns. High biosorption capacity, high efficiency, high biosorption rate and cost-effectiveness assign biosorbents as economical alternative materials for metal removal from solution at lower metal concentrations.

Keywords: *Polytrichum commune, Sphagnum sp., Parachlorella kessleri,* ionexchanger, copper (II) sorption

Acknowledgement

This work was supported by the Slovak Grant Agency VEGA, Grant No. 1/0235/12.

THE WAYS OF POLUTION ELIMINATION FROM MINE DRAINAGE WATERS

Jana Jenčárová[a], Alena Luptáková[a]
[a] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovakia*

ABSTRACT
Water pollution by metals, originated especially in various industry sectors as final industrial effluents components, represent today important environmental problem because increased concentrations of metals in waters are dangerous for all living organisms. Conventional methods for removing metal ions from aqueous solutions, such as chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, can be alternate by biological-chemical methods.

The objectives of the present work are to utilize some biological-chemical processes for the mine drainage water treatment. These waters are often characterized by typical high concentrations of sulphates and metals as a consequence of the mining industry of the sulphide minerals. The studied principles of the metals and sulphates elimination from mine drainage waters include sulphate reduction, bioprecipitation by the application of sulphate-reducing bacteria (SRB) and sorption by the biogenic sulphides.

Biological-chemical process based on the ability and activity of these bacteria to reduce sulphates results in hydrogen sulphide creation which binds with metal cations in solutions to insoluble precipitate forms. The concentration of the sulphates in the solutions was determined by ion chromatography. The properties and composition of biogenic precipitated materials synthesized under specific laboratory conditions and modified growth media was examined by EDX and XRD.

The sorbent preparation in form of biogenic iron sulphides was realized at 30°C in anaerobic conditions. The culture of bacteria (genera *Desulfovibrio*) was isolated from the mineral spring Gajdovka (Košice, Slovakia) using Postgate C medium.

The elimination of heavy metals from waters was examined using prepared sorbent samples and mine drainage waters from Slovak localities Banská Štiavnica and Smolník, which composition is in Table 1. Sorption experiments were realized during 24 hours. Concentrations of metal ions in solutions were determined by AAS.

Table 1 The analysis of mine drainage water

Sample	pH	Sulphates (mg/l)	Fe (mg/l)	Zn (mg/l)	Cu (mg/l)
Banská Štiavnica (BŠ)	6.15	980	<0.05	5.7	0.1
Smolník (Sm)	3.80	1690	206.9	6.3	1.0

Keywords: metals, mine drainage water, bacteria

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the contract SRDA-0252-10 and by the Scientific Grant Agency under the contract 2/0166/11.

IN VITRO TOXICITY ASSESSMENT OF SILVER NANOPARTICLES SYNTHESIZED USING *Parachlorella kessleri*

Jana Kaduková[a], Oksana Velgosová[a], Anna Mražíková[a], Renáta Marcinčáková[a], Eva Tkáčová[b]

[a]*Technical University in Košice, Faculty of Metallurgy, Department of Material Science, Park Komenského 11, Košice 04200, Slovak Republic*

[b] *Regional Office of the Public Health, Ipeľská 1, 04011 Košice, Slovakia, detached office Department of Environmental Microbiology, Roosveltova 8, 04001 Košice, Slovakia*

ABSTRACT

Nowadays, silver nanoparticles (AgNPs) are used in a wide range of applications. Rapid developments in the manufacture and use of engineered nanoparticles have also led to an urgent need for assessing their possible risk to humans and the environment, but environmental risk assessment significantly lags behind invention and today's global consumption volumes. Silver and silver nanoparticles are known for their antimicrobial properties which are mostly studied using common tests with bacteria, rarely by tests involving algae or higher plants. As there are some assumptions that biologically produced nanoparticles are more compatible with organisms the aim of the article was to focus on the toxic effects of biologically produced AgNPs on G^- and G^+ bacteria, microscopic algae and higher plants (seeds of *Sinapis alba*).

Biologically prepared nanoparticles expressed stronger toxic effects on plant seed germination and root growth than on the growth of bacteria (Fig. 1). Inhibition of seed germination and root growth was visible from the concentration of 20 mg/l of silver nanoparticles on.

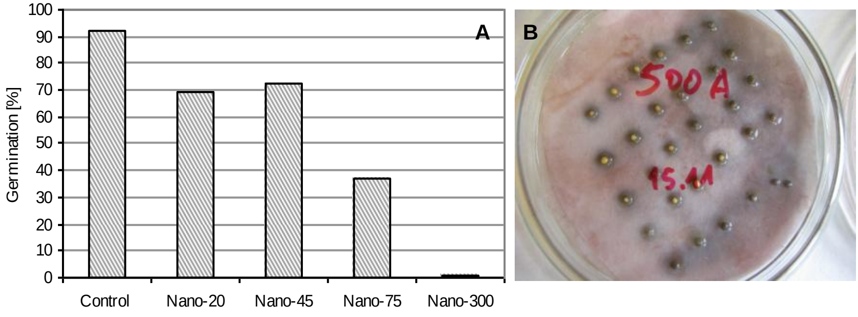

Fig. 1 The influence of increasing Ag NPs concentration on *Sinapis alba* seed germination (A), appearance of *S. alba* seeds treated with 300 mg/l AgNPs (B).

Only 39 and 1% of seeds germinated at 75 and 300 mg/l, respectively. Even more significant toxic effects were observed for root growth. Silver ions were more toxic than silver nanoparticles at all studied concentrations. The preliminary IC50s for Ag^+ and silver nanoparticles were calculated to be 5.2 mg/l and 42.8 mg/l, respectively.
The toxicity against microscopic green alga *Parachlorella kessleri* was not clear although slight inhibition of algal growth on agar plates was observed at concentration 75 mg/l and visible inhibition zones were reported at 300 mg/l of nanoparticle concentration. Again silver ions exhibited stronger toxicity effect than silver nanoparticles.
Toxicity of silver nanoparticles against G^- *E. coli* and G^+ *Staphylococcus aureus* was observed at concentration 195 mg/l. Silver ions from concentration 140 mg/l on were toxic for mentioned bacteria (Fig. 2).

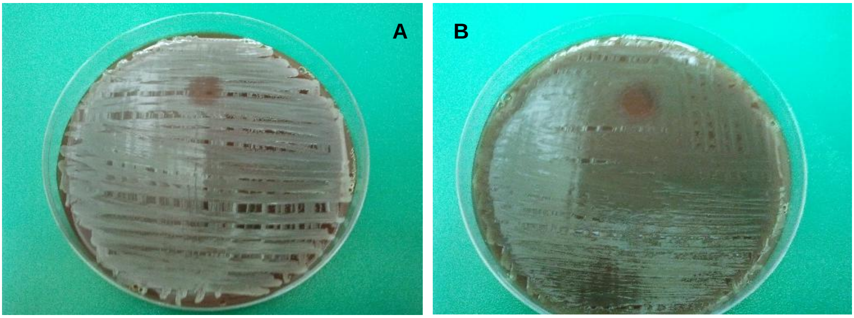

Fig. 2 Agar plate with grown culture of *S. aureus* after 24 hour (A) and *E. coli* after 24 hour cultivation (B) with silver nanoparticle concentration 195.3 mg/l.

Many authors claim that the toxicity effects depend on nanoparticle sizes but according to published studies it is clear that the silver nanoparticles toxicity is not so easy to evaluate and that more factors not only silver concentration (and even not only the size of nanoparticles) are probably responsible for that as data from different studies are significantly different for the same studied species.

Keywords: silver nanoparticles, toxicity, *Sinapis alba*, *Parachlorella kessleri*, *E. coli*, *Staphylococcus aureus*

Acknowledgement
This work was supported by the Slovak Grant Agency VEGA, Grant No. 1/0235/12.

THE EFFECTS OF COPPER ON SEED GERMINATION AND EARLY SEEDLING GROWTH OF *Raphanus sativus*

Jana Kavuličová[a], Jana Kaduková[b], Dana Ivánová[a]
[a]*Technical University in Košice, Faculty of Metallurgy, Department of Chemistry, Park Komenského 19, Košice, 042 00, Slovak Republic*
[b]*Technical University in Košice, Faculty of Metallurgy, Department of Material Science, Park Komenského 11, Košice, 042 00, Slovak republic*

ABSTRACT
Increasing application of organic manures and phytochemicals, such as fungicides, pesticides and insecticides, results in progressive accumulation of copper in soil. Copper (Cu) is essential mineral nutrient for plant growth and development at low concentrations, which plays key roles in photosynthetic and oxidative stress protection. However, excessive quantities of Cu can lead to phytotoxicity such as leaf chlorosis and growth inhibition.
Germination assay is a basic procedure to determine heavy metals toxic effects on plants. Seed germination and the early seedling growth are more sensitive to metal pollution so the study gives the picture about the metal toxicity.
Standard seed germination and root elongation test according to the protocol of Slovak standard method was carried out on filter paper and agar in May and September. In laboratory conditions, effects of different concentrations of copper in the form of $CuSO_4$ (Tab. 1) on germination and hypocotyl growth of seedlings of *Raphanus sativus* after 8d were investigated.

Table 1 Experimental design

Cu^{2+} content (mM)	0	0.002	0.02	0.2	1	2
Sample	R0	R1	R2	R3	R4	R5

May: Compared to the zero-Cu control, the germination capacity of *R. sativus* was decreased by 30% at 0.002 mM Cu^{2+} (R1) on filter paper. Interestingly, a slight stimulation of germination (10%) at 0.02 and 0.2 mM of Cu^{2+} was observed. This could be attributed to an overproduction of reactive oxygen species (ROS) in seeds by copper, resulting in a slightly enhanced level of oxidative stress that stimulates germination [9]. The germination capacity of *R. sativus* was decreased by 30% and 50% at 1 and 2 mM Cu^{2+} (R4 and R5) on agar (Fig. 1a).
September: At the all concentrations of Cu^{2+} the germination capacity of seeds on filter paper was almost constant (>95%). Only a slight decrease was observed at 2 mM Cu^{2+} (R5) treatment. On the contrary, on agar the germination capacity of radish seeds ranged from 60% to 25% at 0.2 to 2 mM

Cu^{2+} (R3-R5) compared to zero-Cu control. Surprisingly the total germination of *R. sativus* seeds, germination of filter paper was influnced by the year period, too. In this case, enhancing of germination was observed in September (Fig. 1b).

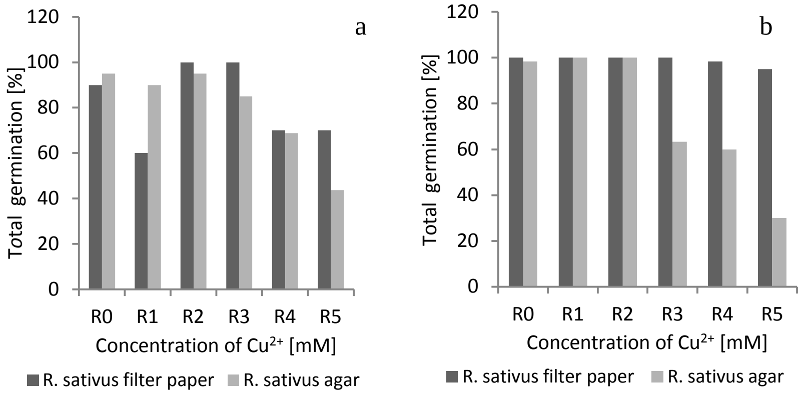

Fig. 1 Total germination capacity of *R. sativus* at Cu treatment in May (a) and September (b).

From the comparison of results between hypocotyl length tests of *R. sativus* growing on filter paper and agar is visible that hypocotyl length was much negatively affected in response to Cu^{2+} on filter paper what is opposite to abovementioned results of germination. The effect on the early seedlings growth was influenced by the year period in a greater extent in comparison with seed germination.

The seed germination and root elongation test is one of the simplest methods of environmental biomonitoring. Germination test gives the picture about the metal toxicity. From our results is visible that the choice of substrate can influence the outcome of germination tests. During testing on filter paper the influence of year period was observed but this would need further testing and confirmation.

Keywords: Germination, hypocotyl length, copper, Raphanus *SATIVUS*

Acknowledgement

This work was supported by the Slovak Grant Agency VEGA, Grant No. 1/0235/12.

BACTERIAL REGENERATION OF BARIUM SULPHATE TO BARIUM SULPHIDE

Ingrida Kotuličová[a], **Alena Luptáková**[a], **Magdaléna Bálintová**[b], **Štefan Demčák**[b]

[a] *Slovak Academy of Sciences, Institute of Geotechnics, Department of Mineral Biotechnologies, Watsonova 45, 040 01 Košice, Slovak Republic*

[b] *Technical University of Košice, Civil Engineering Faculty, Institute of Environmental Engineering, Vysokoškolská 4, 042 00 Košice, Slovak Republic*

ABSTRACT

Acid Mine Drainage (AMD) is a big problem in mining industry. It causes surface water pollution, which may impact aquatic life as well as whole ecosystem. Acid mine waters are characterized by low pH and high content of heavy metals and sulphates.

Methods that are used to remove sulphates are:

- Precipitation of sulphates with lime and limestone
- Precipitation using barium salts
- Sulphate precipitation in the presence of aluminium and calcium ions
- Sulphate removal using aluminous cement
- Membrane processes
- Ion exchange process
- Sorption
- Biological sulphate removal methods using sulphate reducing bacteria.

Principle of the methods using barium salts is sulphate precipitation in form of barium sulphate (insoluble compound). Barium chloride, barium hydroxide and barium sulphide are used for precipitation:

$$BaCl_2 + H_2SO_4 \rightarrow BaSO_4 + 2HCl \quad (1)$$

$$Ba(OH)_2 + H_2SO_4 \rightarrow BaSO_4 + 2H_2O \quad (2)$$

$$BaS + H_2SO_4 \rightarrow BaSO_4 + H_2S \quad (3)$$

Because of high investment costs of barium salts, barium sulphate precipitate is recycled to the form of barium sulphide. It can be recycled chemically by thermic reduction of barium sulphate with carbon in the form of coal at a temperature of 1200 °C to barium sulphide:

$$BaSO_4 + 2C \rightarrow BaS + 2CO_2 \quad (4)$$

The energy involved during the chemical reduction is factors affecting significantly the economy of this process. Bacterial reduction of barium sulphate, to obtain barium sulphide by sulphate-reducing bacteria (SRB), could be used as an alternative and more economical method.

SRB represent a very specialized group of bacteria that uses sulphates as a terminal electron acceptor for their metabolism. These bacteria utilize organic compounds which range from simple organic acids (acetate and lactate) and ethanol to long-chained fatty acids and certain aromatic compounds.
The aim of our study was to recycle $BaSO_4$ using SRB to BaS. $BaSO_4$ arising out of the sulphate removal from AMD by barium chloride was used. We assumed the biochemical reaction initiated by SRB in the presence of sodium lactate as an electron donor and $BaSO_4$ as an electron acceptor under anaerobic conditions according to equations (5) and (6):

$$Na\text{-}lactate + BaSO_4 \xrightarrow{SRB} biomass + BaCO_3 + BaS + CO_2 + H_2S \qquad (5)$$

$$BaSO_4 + 2H_2S \longrightarrow BaS + SO_2 + 2H_2O + S \qquad (6)$$

In the experiment a culture of SRB (genera *Desulfovibrio* and *Desulfotomaculum*) was used. It was obtained from drinking mineral water Gajdovka (locality Kosice-north, Slovak Republic). SRB were grown at 30 °C under static and anaerobic conditions. The inoculum of SRB was 10% (v/v). The abiotic controls were carried out without the SRB application at the same conditions. The ion-chromatography method was used to determine the concentration of sulphates using chromatograph Dionex ICS 5000. Soluble sulphide was analysed using a methylene blue method based on a colorimetric determination of dissolved H_2S and HS^- using *N*,*N*-dimethyl-*p*-phenylenediamine.

Keywords: Acid Mine Drainage, Sulphate-reducing bacteria, barite, bacterial reduction

Acknowledgements: the work was supported by the Slovak Research and Development Agency under the contract No. SRDA-0252-10 and Slovak grant agency for project No. 2/0166/11.

REDUCTION OF SOLUBLE Fe^{3+} SULFATE TO Fe^{2+} SULFATE BY ACIDOPHILIC HETEROTROPH *Acidiphilium SJH*

Daniel Kupka, Michal Kovařík, Miroslava Václavíková, Zuzana Dakos, Dávid Jáger

Institute of Geotechnics of the Slovak Academy of Sciences,Watsonova 45, 043 53 Kosice, Slovak Republic

ABSTRACT

Acidophilic heterotrophic iron-reducing bacterium *Acidiphilium SJH* was grown in mineral salt medium with glucose as carbon and energy source. Bacterial activity was monitored by respirometric measurements of oxygen consumption and carbon dioxide production rates.

Soluble ferric iron sulfate was added as alternative electron acceptor. At pH values below 2.5 ferric iron is dissolved in the media and abiotic oxidation of ferrous iron to ferric iron with molecular oxygen is slow. Redox potential of Fe^{3+}/Fe^{2+} ion pair at pH = 2 is 0.77 V. This value is very close to redox potential of O_2/H_2O which is 0.82 V. Ferric iron thus can serve as suitable alternative electron coupled to bacterial oxidation of organic or inorganic compounds.

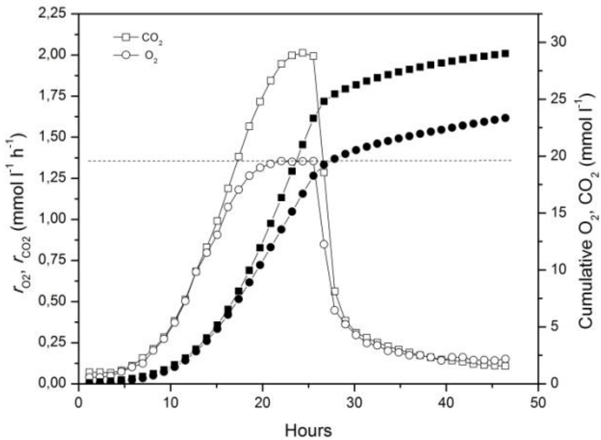

Fig. 1 CO_2 production rate (squares) and O_2 consumption rate (circles) and cumulative O_2 and CO_2 (closed symbols) from bacterial oxidation of glucose under condition with limited oxygen supply

Our work was aimed to study the bacterial dissimilatory iron respiration at *Acidiphilium SJH* under different incubation conditions. Cultures incubated in fully aerobic conditions showed exponential growth and oxygen-coupled respiration with equimolar CO2 production to O2 consumption ratio (RQ) from the glucose oxidation. Under conditions with limited oxygen transfer rate (Fig. 1), carbon dioxide production exceeded the oxygen consumption at the expense of concurrent ferric iron-coupled respiration. Under strictly anaerobic conditions (N2) growth-uncoupled respiration of ferric iron was observed.

Keywords: Acidiphilium, bacterial iron reduction, respirometry

Acknowledgement
This work has been supported by Slovak Research and Development Agency within the project APVV-0252-10/WATRIP as well as within the Marie Curie Programme FP7-People-IAAP-612250 and Slovak Grant Agency VEGA 2/0166/11.

STUDY OF CONCRETE MATERIALS BIOCORROSION CAUSED BY SULPHURETUM ACTIVITY

Alena Luptáková[a], Adriana Eštoková[b], Martina Kovalčíková[b], Nadežda Števulová[b], Mária Praščáková[a]

[a] *Slovak Academy of Sciences, Institute of Geotechnics, Department of Mineral Biotechnologies, Watsonova 45, 040 01 Košice, Slovak Republic*

[b] *Technical University of Košice, Civil Engineering Faculty, Institute of Environmental Engineering, Vysokoškolská 4, 042 00 Košice, Slovak Republic*

ABSTRACT

Sulphur cycle and its compounds in the biosphere belong to the one of the basics biological cycles. It consists of assimilation and dissimilation part. Microbial ecosystem of dissimilation part consisting from coherent sulphur-oxidizing bacteria (SOB) and sulphate-reducing bacteria (SRB) named as sulphuretum.

The main representatives of the SOB are bacteria genera *Acidithiobacillus*. It represent autochthonous bacterial culture of sulphates deposits, were it takes part on sulphates minerals oxidation and acid mine drainage (AMD) production. The most studied SRB are bacteria genera *Desulfovibrio*. It occurs especially in the anaerobic zones of soil, waters (sewage, lake, reservoir, mineral spring etc.) as well s oil and sulphate minerals deposits. This are characterized by reduce sulphates to hydrogen sulphide.

Nature of the SOB and SRB represent essence for the biologically catalyzed oxidative and reductive reactions of sulphur compounds, which occurs in the sulphuretum. Sulphuretum activity is the base for positive and negative processes in the nature and has its application also in the industry (Tab. 1).

Table 1 General positive and negative aspects of sulphuretum in industry and environment

Positive aspects	Negative aspects
sulphur, rock-oil and sulphide deposits formation	AMD production
bacterial leaching of sulphidic minerals (or sulphidic concentrates)	biocorrosion of the metallic pipes and pumping machinery
bacterial leaching of electronic wastes	biocorrosion concrete materials (mainly sewer systems)
removal of metals and sulphates from waters	biocorrosion other building materials
preparation of the biosorbents	biodeterioration of historical monument (sculptures, pictures etc.)
desulphurisation of coal	cancellation of the diesel and jet fuels
biodegradation of organic pollutants	blackening and discoloration of products
biomachining	production of smell

Paper is focused on the study any of the sulphuretum negative properties – concrete materials biocorrosion. The concrete biocorrosion has a serious economic impact worldwide, especially when the replacement or repair of municipal sewer systems is required. The general mechanism for the sulphuric acid caused corrosion of sewer systems is following: in the first step, hydrogen sulphide is produced by SRB under anaerobic conditions in sewer pipes; this one enters the sewer atmosphere by volatilization and dissolves in the condensate on the sewer crown; finally, sulphur-oxidizing bacteria *Acidithiobacillus thiooxidans* oxidize the dissolved hydrogen sulphide and other sulphur compounds to sulphuric acid, which corrodes the concrete. The primary prevention of pipeline biocorrosion under the influence of sulphuretum activity is hydrogen sulphide and sulphate removal from transported water, also hydrogen sulfide removal from the air part of the pipe. To avoid biocorrosion, it is important to know the composition of the microbiota, substrates source, and conditions for its growth; the addition of suitable additives in the production of concrete, cover the surface of the pipe by suitable protective material, the application of crown spray process etc.

Keywords: *Acidithiobacillus thiooxidans*, sulphate-reducing bacteria, sulphuretum, biocorrosion.

Acknowledgement: the work was supported by the Slovak grant agency for project No. 2/0166/11.

BIOLOGICAL REMOVAL OF INORGANIC POLLUTANTS FROM ACID MINE DRAINAGE

Eva Mačingová, Alena Luptáková, Mária Praščáková
Slovak Academy of Sciences, Institute of Geotechnics, Department of Mineral Biotechnologies, Watsonova 45, Košice 040 01, Slovak Republic

ABSTRACT
Acid mine drainage (AMD) is a serious problem related to mining and post-mining activities throughout the world. These waste waters exhibit different chemical and physical properties depending on their origin and the geochemical nature of the site of exploitation. Low pH, high concentration of sulphates and various heavy metals cause their deleterious effect on the environment. Treatment methods to address AMD are focused on neutralizing, stabilizing and removing pollutants through various physical, chemical and biological processes. Known are two basic types of treatment processes – active and passive. Table 1 demonstrates the comparison of active and passive treatment methods.

Table 1 Comparison of passive and active methods for acid mine drainage treatment

passive ⟵	Method	⟶ active
low	Costs	high
small	Need of labour	high
large	Treatment area	small
difficult	Metal recovery	easy
poor	Control	good
poor	Predictability	good

The conventional chemical techniques utilize alkaline reagent to raise pH and remove metals from AMD, however by this technique voluminous amount of metal-laden sludge is produced. Some heavy metals, such as copper and zinc, form highly insoluble sulphides and are readily removed from aqueous solution in contact with hydrogen sulphide at different pH conditions. Chemical sulphide precipitation is not used widely due to high cost, toxicity and corrosiveness of chemicals. Biogenic sulphidogenesis has the advantage in commercial operations, in that it can be established at the site of use, thereby avoiding the transport and storage of unsafe hydrogen sulphide. The treatment processes for removing sulphates may be broadly categorized as chemical treatment with mineral precipitation, membrane processes and biological technologies.

This paper reports the results of studies conducted to develop the process of biological removal of sulphates from AMD using sulphate reducing-bacteria (SRB). SRB are a heterogeneous group of anaerobic bacteria that reduce sulphates to hydrogen sulphide by dissimilatory sulphate reduction pathway (reaction 1 and 2). A nascent hydrogen sulphide for recovery of Cu and Zn from the same AMD has been used (reaction 3).

$$4H_2 + SO_4^{2-} + H^+ \xrightarrow{SRB} HS^- + 4H_2O \quad (1)$$

$$Organic\ matter + SO_4^{2-} \xrightarrow{SRB} HS^- + HCO_3^- \quad (2)$$

$$Me^{2+} + HS^- \longrightarrow MeS + H^+\ (Me^{2+}\ \text{- the cation of particular metal}) \quad (3)$$

Thus, by this way it is possible to reduce in successive steps the concentration of sulphates and metals. The experiments were performed at laboratory condition using water collected from the site of the AMD outflow at the shaft Pech from the enclosed and flooded Smolnik sulphidic deposit (Slovakia).

Keywords: acid mine drainage, sulphate-reducing bacteria, biogenic sulphide, heavy metals recovery.

Acknowledgements: the work was supported by the Slovak Research and Development Agency under the contract No. SRDA-0252-10 and Slovak grant agency for project No. 2/0166/11.

IMPACT OF OXYGEN DEMAND ON AERATION IN *Acidithiobacillus ferrooxidans* CULTURES: BIOTECHNOLOGICAL IMPLICATIONS

Martin Mandl, **Eva Pakostová, Lenka Poskerová**
Masaryk University, Faculty of Science, Department of Biochemistry, Kotlářská 2, Brno 61137, Czech Republic

ABSTRACT
The critical values of volumetric oxygen transfer coefficient $(k_La)_{crit}$ and oxygen concentration (C_{crit}) were used to define the conditions necessary for minimum aeration and to eliminate potential oxygen limitation during substrate oxidation by the model bioleaching *Acidithiobacillus ferrooxidans* culture.
The actual oxygen concentration (C) was determined using a Clark-type oxygen electrode. The oxygen uptake rate (Q) in the batch cultures was determined at 26°C in a closed 20-ml oxygen electrode chamber. C_{crit} was determined based on the oxygen uptake by bacterial cultures that were not limited by the substrate. The criterion of C_{crit} value is related to the point where a linear decrease in the oxygen concentration (corresponding to the maximum Q) is changed to a nonlinear trend, indicating the beginning of oxygen limitation. Because this deflection occurred at a very low oxygen concentration, a two-fold higher oxygen concentration was taken as C_{crit} to avoid underestimation. The Michaelis constant for oxygen (K_m) was determined in the bacterial suspension as the oxygen concentration that reduced the maximum oxygen uptake rate by half, based on the experimental data used for the C_{crit} determination. Based on the steady-state method, $(k_La)_{crit}$ was calculated using Eq. (1):

$$(k_La)_{crit} = Q / (C^* - C_{crit}) \qquad (1)$$

where C^* is the saturated dissolved oxygen concentration (250 μmol O_2 l^{-1}).

The maximum Q value, which corresponds to the culture at a phase with maximum respiration rate, was used in Eq. (1). The gassing-out technique was used to verify the results under steady-state conditions. To confirm oxygen unlimited substrate oxidation at C_{crit}, iron- and sulfur-oxidizing activities were determined in resting cell suspensions using a 5-l bioreactor. Formation of ferric iron and protons was used for determination of the oxidation rates.
Based on the data shown in Fig. 1, the C_{crit} concentrations for the oxidation of ferrous iron and elemental sulfur were 6.250 and 3.125 μmol O_2 l^{-1}, respectively. The C_{crit} values correspond to cellular oxygen demand and are

directly connected with K_m, the values of which were 1.07 ± 0.16 and 0.71 ± 0.08 μmol O_2 l^{-1} (mean ± SD, n = 3) for ferrous iron and elemental sulfur oxidation, respectively. The difference between the K_m values was significant (P < 0.05). For ferrous iron oxidation (mean ± SD, n = 3), Q = 31.3 ± 1.5 μmol O_2 l^{-1} min^{-1} and $(k_La)_{crit}$ = 7.70 ± 0.37 h^{-1}. For elemental sulfur oxidation (mean ± SD, n = 3), Q = 20.08 ± 0.20 μmol O_2 l^{-1} min^{-1} and $(k_La)_{crit}$ = 4.88 ± 0.05 h^{-1}. The gassing-out technique confirmed the above $(k_La)_{crit}$ values. The difference between the $(k_La)_{crit}$ values for iron- and sulfur-oxidizing cultures of 7.70 and 4.88 h^{-1}, respectively, was highly significant ($P < 0.01$). The lower $(k_La)_{crit}$ value for the sulfur-oxidizing culture is in agreement with its lower C_{crit} and K_m values. The iron and sulfur oxidation rates determined in the bioreactor confirmed the lack of oxygen limitation at C_{crit}.

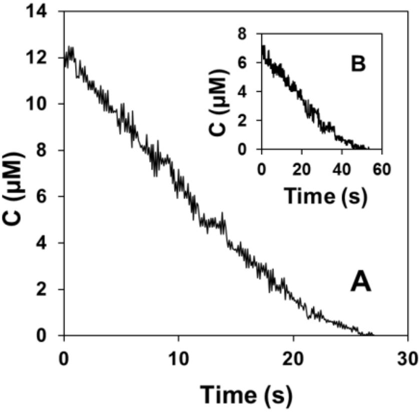

Fig. 1 Oxygen concentration time course of an *A. ferrooxidans* culture respiring either on ferrous iron (A) or elemental sulfur (B) in a closed Clark electrode chamber. Low oxygen concentration was detected with a 10-times higher sensitivity compared to the standard measurements.

The intensity of aeration has multiple consequences at the biochemical and bioleaching process control level. The comparison of the $(k_La)_{crit}$ and C_{crit} data for iron and sulfur oxidation shows that if there is no oxygen limitation in the culture with ferrous iron, no oxygen limitation appears in the culture with elemental sulfur with similar aeration conditions. The $(k_La)_{crit}$ and C_{crit} values are valid guidelines for minimum aeration criteria for pilot- and commercial-scale bioreactors employed in biohydrometallurgy.

Keywords: *Acidithiobacillus ferrooxidans*, aeration, bioleaching, oxygen limitation, volumetric oxygen transfer coefficient.

Acknowledgement
Supported by the Masaryk University Program, Project no. MUNI/A/0981/2013.

LITHIUM BIOLEACHING FROM LEPIDOLITE USING *Rhodotorula rubra*

Renáta Marcinčáková, **Jana Kaduková**, **Oksana Velgosová**, **Anna Mražíková**
Technical University in Košice, Faculty of Metallurgy, Park Komenskeho 11, 042 00 Kosice, Slovak Republic renata.marcincakova@tuke.sk

ABSTRACT
In this present work lithium recovery from lepidolite (3.79% Li_2O) by bioleaching was investigated. Lithium is of a great importance in many industrial fields and nowadays it is becoming more and more interesting as the energy source for hybrid vehicle and electromobile. Lepidolite is among the principal lithium minerals in the world. Its destruction and consequent lithium extraction is a high capital and energy intensive process. It is necessary to seek an efficient, economic technique to handle this ore. Nowadays, biohydrometallurgy, which uses microorganisms and their metabolic activities for metal recovery from primary and secondary sources, is coming into perspective.
In nature many bacteria, fungi and yeasts contribute to weathering processes and mineralization of metal containing materials. The most active leaching fungi are *Penicillium simplicissimum* and *Aspergillus niger*. They are able to produce great amounts of organic acids which play an important role in metal dissolution. On the other hand, there is a lack of studies on metal bioleaching from solid substrates using the yeast *Rhodotorula rubra*. In nature *R. rubra* may be found in silicates near lithium mining deposits. It is a slime producer and by means of macromolecules such as polysaccharides or polypeptides present in the capsule and the wall can enhance silicate weathering processes. This yeast is characterized by a high ability to accumulate metals.
It is suggested that the processes involved in metal accumulation are extracellular binding by active groups of biopolymers within the cell wall-membrane system and intracellular accumulation combined with metal ions transport through biological membrane into the cell interior. Taking these facts into account we investigated lithium dissolution from lepidolite using that microorganism. As the bioleaching medium a low nutrient medium was used since it is supposed that microbial adaptation to a low nutrient medium can enhance bioleaching process. The experiments were carried out at ambient temperature and on a rotary shaker at the operating speed of 160 rpm for 60 days.

During the bioleaching process the pH decreased from the initial pH = 5.12 up to 2.45 measured on day 27 and afterwards it remained more or less constant. The overall lithium extracted during the bioleaching using *Rhodotorula rubra* was 629 μg/l.

Key words: *Rhodotorula rubra*, lepidolite, lithium, bioleaching

Acknowledgements
This work was supported by the Slovak Grant Agency VEGA, Grant No. 1/0235/12.

METAL BIOLEACHING FROM SPENT LITHIUM-ION BATTERIES USING ACIDOPHILIC BACTERIAL STRAINS

Renáta Marcinčáková[a], Jana Kaduková[a], Anna Mražíková[a], Oksana Velgosová[a], Alena Luptáková[b], Stefano Ubaldini[c]
[a]*Technical University in Košice, Faculty of Metallurgy, Park Komenskeho 11, 042 00 Kosice, Slovak Republic, E-mail: renata.marcincakova@tuke.sk*
[b]*Institute of Geotechnics, Slovak Academy of Sciences, Kosice, Slovakia*
[c]*Institute of Environmental Geology and Geoengineering, CNR, Roma, Italy*

ABSTRACT
Lithium-ion batteries (LIBs) are ubiquitous in advanced economies, sensors, computers, electronic and medical devices. Due to their favourable properties they have gradually replaced Ni-Cd and Ni-MH (nickel-metal-hydride) batteries. Lithium batteries are and will also continue to be needed for powering all electric and hybrid vehicles. There is concern that demand for battery metals could increase, possibly to the point at which a shortage of these metals will occur. Scarcity of primary resources of metals forces us to seek new technologies on metal recovery from secondary sources and wastes. Nowadays, microbial leaching plays a crucial role in recovery of metals from various sulphide minerals and low grade ores. The major microorganisms, which play a significant role in metal bioleaching from waste, belong to the acidophilic group. These acidophilic bacterial strains help in dissolving metals from solid phase of waste into the aqueous phase.
In this present work lithium and cobalt recovery from spent lithium – ion batteries (27.5% $LiCoO_2$) by bioleaching was investigated. The assay was carried out using the consortia of acidophilic bacteria of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans.* For the metal bioleaching two different media were used. A rich nutrient medium was consisted of all minerals needed for bacterial growths, whereas a low nutrient medium contained only sulphuric acid and elemental sulphur. Initial pH of both media was adjusted to 1.5.
The percentages of lithium and cobalt dissolution in two different bioleaching media using the consortia of the bacteria are plotted in Fig. 1. As it can be seen the overall lithium and cobalt bioleaching efficiency was 80% and 67%, respectively, in the rich nutrient medium, whereas in the low nutrient environment only 35% Li and 10.5% Co were released. The experimental results revealed that the presence of minerals in the bioleaching media is one of factors which to a large extent influence lithium and cobalt dissolution from LIBs.

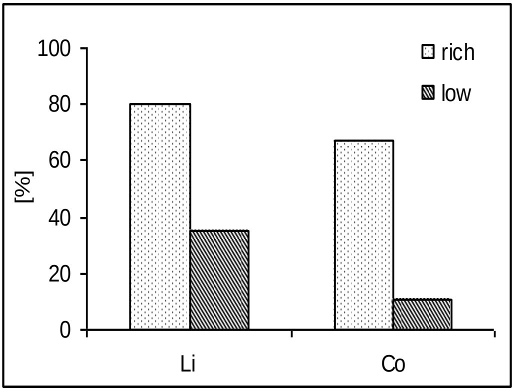

Fig. 1 Li and Co extraction [%] by the mixed culture of *A.ferrooxidans* and *A.thiooxidans* in the rich and low nutrient media..

Key words: *A. ferrooxidans*, *A. thiooxidans*, lithium, cobalt, bioleaching

Acknowledgements

This work was supported by the Slovak Grant Agency VEGA, Grant No. 1/0235/12.

INFLUENCE OF USED BACTERIAL CULTURE ON ZINC AND ALUMINIUM BIOLEACHING FROM PRINTED CIRCUIT BOARDS

Anna Mražíková[a], Renáta Marcinčáková[a]. Jana Kaduková[a], Oksana Velgosová[a], Magdaléna Bálintová[b]
[a] *Technical University in Košice, Faculty of Metallurgy, Letná 9, 042 00 Košice, Slovak Republic*
[b] *Technical University of Košice, Civil Engineering Faculty, Institute of Environmental Engineering, Vysokoškolská 4, 042 00 Košice, Slovak Republic*

ABSTRACT
Waste from printed circuit boards (PCBs) belongs to the typical waste of electric and electronic equipments and their recycling has attracted a great attention not only from the perspective of waste treatment but also of valuable metal recovery. Bioleaching processes were used to solubilize zinc and aluminium from printed circuit boards (PCBs). The aim of this study was to evaluate the influence of the acidophilic bacteria and their mixture on zinc and aluminium recovery from PCBs. The PCBs-adapted pure culture of *A.ferrooxidans,* pure culture of *A.thiooxidans* and PCBs-adapted mixed culture of *A.ferrooxidans* and *A. thiooxidans* were used as the predominant bacteria in this bioleaching process. The isolates used in the experiments were cultured and acclimated in presence of PCBs for two weeks and consequently used as bioleaching bacteria for zinc and aluminium extraction from PCBs.

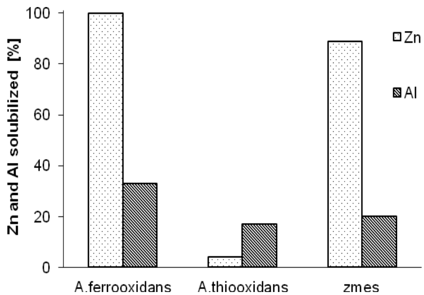

Fig. 1 Percentage of zinc and aluminium solubilized from PCBs by pure culture of *A.ferrooxidans, A.thiooxidans* and mixed culture of *A.ferrooxidans* and *A.thiooxidans*.

Percentages of zinc and aluminium released into the solution using the pure culture of *A.ferrooxidans,* the pure culture *A.thiooxidans,* and the mixed culture

of *A.ferrooxidans* and *A.thiooxidans* are shown in Fig. 1. As it can be seen 100% of Zn and 33% of Al were dissolved using the pure culture of *A.ferrooxidans*. By the use of the mixed culture of *A.ferrooxidans* and *A.thiooxidans* almost 90% Zn and 20% Al dissolved. The lowest bioleaching efficiency of both metals was reached using the pure culture of *A.thiooxidans,* when only 4% Zn and 17% Al dissolved.
The results demonstrate that Fe^{3+} ions produced by bacterial culture of *A.ferrooxidans* are main leaching agents in zinc recovery. It also shows that the aluminium solubilization depends on ferric ions and sulphuric acid, as well. Based on the results it may be concluded that the optimal bacterial culture for zinc mobilization seems to be the pure culture of *A.ferrooxidans* and mixed culture of *A.ferrooxidans* and *A.thiooxidans,* as well. The PCBs-adapted pure culture of *A.ferrooxidans* was found to be more effective, able to leach 100% Zn. As regards aluminium, there were no significant differences in percentages of aluminium solubilized using the pure culture *A.ferrooxidans, A.thiooxidans* and mixed culture of *A.ferrooxidans* and *A.thiooxidans*.

Keywords: bioleaching, electronic waste, zinc, aluminium, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*

Acknowledgement
This work was supported by the Slovak Grant Agency VEGA, Grant No. 1/0235/12.

NOVEL COMPOSITE MATERIAL FOR ARSENIC REMOVAL FROM WATERS

Lenka Oroszová[a], Miroslava Václavíková[a], George Gallios[b], Katarína Štefušová[a], Silvia Dolinská[a], Vladimír Girman[c]

[a] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, Košice 040 01, Slovak Republic E-mail: vaclavik@saske.sk*

[b]*Laboratory of Inorganic Chemical Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece*

[c]*Department of Condensed Matter Physics, Pavol Jozef Šafárik University, Park Angelinum 9, Košice 040 01, Slovak Republic*

ABSTRACT

The use of activated carbons for the removal of heavy metals in cationic form (i.e. Cd, Cu, Pb) and organic pollutants from waters is widely extended, due to their unique structure with high surface area, porous structure, high adsorption capacity and surface chemical nature. However these adsorbents are less efficient for the removal of metal species in anionic form (i.e. As, Cr, Se). Our earlier research has shown that Fe-oxide/oxyhydroxide based nanomaterials have high affinity towards arsenic and chromium oxyanions. However, the use of free nanoparticles is problematic due to their difficult manipulation and/or non-controllable behavior, and potential health and environmental effects. Thus, the incorporation of iron nanoparticles into a well organized mesoporous carbon matrix may be an effective technique for the secure deposition of nanoparticles, while retaining the sorption affinity of both iron and carbon sites towards various toxic substances in aqueous media.

This contribution deals with the adsorption of arsenic from model aqueous solutions onto three adsorbends based on activated carbon (pure activated carbon (AC), Fe-modified activated carbon (FeAC) and Fe-modified activated carbon syntetized with ultrasound power (FeAC US)). All adsorbent were fully characterised by XRD, FTIR, TEM, HR-SEM, sureface analys by N_2 adsorption etc. Experiments have been carried out in batch mode giving a description of the equilibrium adsorption capacity of the activated carbon as a function of the initial arsenic concentration, pH of the solution and contact time). The initial arsenic concentration was 10-700 mg/L and the sorbent concentrations was 2 g/L. The pH of the solutions was adjusted with suitable concentrations of sodium hydroxide and nitric acid. All experiments were performed at constant temperature 23±1°C in a rotary shaker set at 30 rpm. Determination of As concentration in solutions was performed by flame and graphite AAS, respectively.

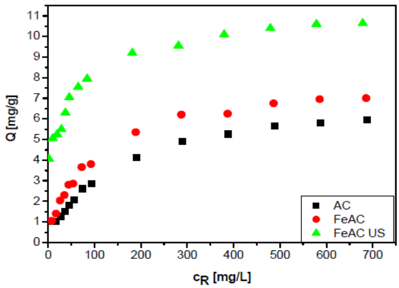

Fig. 1 Effect of arsenic initial concentrations at pH 3

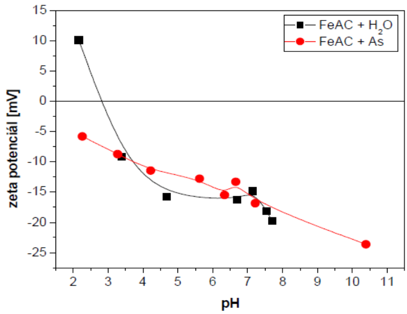

Fig. 2 Zeta potential measurement for the FeAC

Best results have been achieved for the removal of arsenic by FeAC US in comparison with pure AC and FeAC, respectively. Adsorption capacity as a function of arsenic concentration shows the typical behaviour of the Langmuir-like adsorption mechanism, where the chemisorption takes place (Fig. 1). The adsorption capacity is the highest at acidic pH conditions, when the surface charge of the adsorbent surface is positive (Fig. 2). The effect of pH can be described by the As(V) speciation analysis and by the competitive effects of the hydroxyl anions. At increasing pH>6, the increase in arsenic dissociation tends to enhance the adsorption capacity, while competition phenomena with hydroxides tend to reduce the adsorption capacity at higher pH. Best adsorption capacity occurs when the dissociation of arsenic acid is high and the concentration of OH^- ion is sufficiently low to reduce the competition with the arsenic species. It was observed that the kinetics of adsorption of As ions is relatively fast, the equilibrium was achieved within 12 hours.
It has been assumed that the composite material is promising sorbent for arsenic removal from aqueous solutions. Improved deposition of iron oxide nanoparticles into porous structure of activated carbon has been observed using power ultrasound. It has also improved sorption properties of the composite compared to the classical synthesis of FeAC.

Keywords: activated carbon, iron oxides, ultrasound, adsorption, arsenic.

Acknowledgement: The research leading to these results has received funding from the project APVV-0252-10 supported by Slovak Research and Develepment Agency. Authors are greatful to Mast Carbon International Ltd. for provisions of activated carbons.

DEVELOPMENT OF BIOSORBENTS APPLYING *Undaria pinnatifida* MACRO ALGAE

József Paulovics[a], **Ljudmilla Bokányi**[b]
[a] *EMK North Hungarian Environmental Protection Ltd., Gyártelep, Sajóbábony 3792, Hungary*
[b] *University of Miskolc, Institute of Raw Material Preparation and Environmental Processing, Egyetemváros, Miskolc 3515, Hungary*

ABSTRACT

Numerous research attempts on biosorption have been carried out by different universities and research groups around the world for the last three decades. One of the most important research teams in this field is the Technische Universität Berlin, where research on bioprocess engineering, among this research on biosorption has been performed. University of Miskolc, Institute of Raw Material Preparation and Environmental Processing have been cooperating with the research group in Berlin in the framework of DAAD program on investigation of the biosorption process.

Different research groups have been accomplished a large number of laboratory investigations and experiments with different kinds of biomass. The most investigated materials by the development of biosorbents are the micro- and macro algae. From the biomasses of the latter group, *Undaria pinnatifida* macro algae have outstanding heavy metal sorption ability.

Our investigations have been proved so far, that if more heavy metal ions occur in a solution with equimolar concentration in the same time e.g. lead (Pb2+), cadmium (Cd2+), copper (Cu2+), nickel (Ni2+) and zinc (Zn2+) which are important metals regarding the environmental impact and industrial usage as well, than *Undaria pinnatifida* macro algae will adsorb the lead in the largest amount (Fig. 1.).

This adsorption seems to be as an undisturbed sorption of lead and it is similar to the adsorption process in mono cationic system. It can be stated that *Undaria pinnatifida* has selective adsorption ability towards the lead (Fig. 1.).

Nevertheless, it is not enough if the applied biomass has a high capacity of heavy metal sorption for the development of biosorbents, but it is also necessary to be easily and cheaply accessible, easy to separate and after regeneration it shall be able to the biosorption process again.

Our biosorption research with the *Undaria pinnatifida* have been carried out at the University of Miskolc, in the Laboratory of Bioprocessing and Reaction Techniques applying an own built biosorption column with a ground water

from a heavy metal contaminated site and with a scrubber water from the wet flue-gas cleaning process of a hazardous waste incineration plant.
We have found that the applied biosorbent is able to reduce the heavy metal concentration (especially of the lead) in case of the both tested liquids (contaminated ground water and scrubber water), but to achieve an effective biosorption it is needed to adjust the pH value of the initial solution between pH = 4...6.

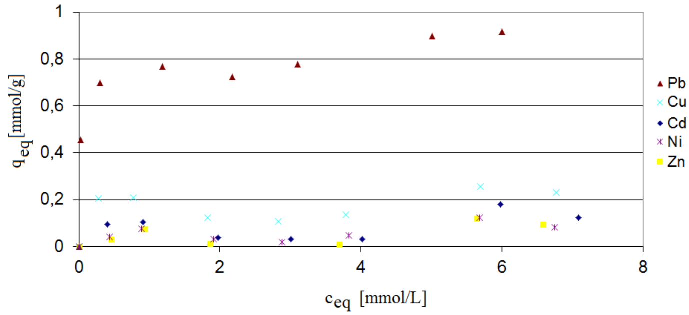

Fig. 1 Adsorption ability of *Undaria pinnatifida* in the simultaneous presence on five cations

By the selection of the raw material of the biosorbent, it can be stated that it is important to know the place of origin of the biomass - especially by macro algae - because it can be contaminated with pollutants (e.g. heavy metals) from the environment during its grown and from the treatment processes after harvesting.
The porosity of the biosorbent is also important by the designing and planning process of biosortption columns, it needs special circumspection in case of macro algae, because the dried macro algae can be swelled significantly by contacting with water.

Keywords: biosorption, heavy metals, algae, *Undaria pinnatifida*

BACTERIA FROM EXTREME ENVIRONMENT AND THEIR POTENTIAL IN METAL AND RADIONUCLIDE BIOREMEDIATION

Martin Pipíška[a], Lenka Tišáková[b], Andrej Godány[a,b], Miroslav Horník[a], Jozef Augustín[a]

[a] *Department of Ecochemistry and Radioecology, University of SS Cyril and Methodius, J. Herdu 2, Trnava, SK-917 01, Slovak Republic (pipiska@ucm.sk)*

[b] *Institute of Molecular Biology, Slovak Academy of Sciences, SK-845 51, Bratislava, Slovak Republic*

ABSTRACT

As a result of adaptation to extreme environment, many bacteria have evolved unique properties of considerable biotechnological significance. Microorganisms have been shown to survive and grow in high levels of radiation and in highly pure water. They can tolerate both high as well as low osmolarity and extreme pH and temperature values. The objective of this contribution is to summarize the results of several years of our research focused on characterization of bacteria isolated from spent nuclear fuel pools and their potential in bioremediation of water contaminated with metals and radionuclides. Although the role of microorganisms in such environment is predominantly negative (e.g. biofouling, biocorrosion processes), analyzing of bacterial communities exposed to ionizing radiation gives opportunities to find bacterial strains with interesting properties such as radioresistance and radionuclide sequestering.

Several bacterial species have been isolated from pool water of the Interim Spent Nuclear Fuel Storage (ISFS, JAVYS Inc., Jaslovské Bohunice, Slovakia) and subsequently identified by 16S rDNA molecular phylogenetic approach. Epifluorescence and light microscopy revealed that bacteria form biofilms on stainless steel coupons both under laboratory conditions and in situ on coupons immersed in pool water. Isolated bacteria taken up radionuclides (137Cs and 60Co) and other metal ions (Cd^{2+}, Mn^{2+} and Zn^{2+}) with various efficiencies by two different processes: metabolically active transport into intracellular space and passive entrapment on bacterial cell surface. Both processes are also involved in radionuclide accumulation by biofilm formed on stainless steel surfaces of construction materials in contact with pool water. With the aim to enhance uptake of Co^{2+} by bacteria recombinant DNA technology was also employed. Engineered Escherichia coli expressing the Mg/Co/Ni transporter from Micrococcus luteus isolate effectively accumulate Co^{2+} ions in cytoplasm.

Since biofilms found in the ISFS pools are directly involved in the accumulation of radionuclides we suppose that bacteria could be effective in in situ bioremediation processes.

NON-FERROUS METAL INDUSTRY WASTE DISPOSAL SITES AS A SOURCE OF POLYEXTREMOTOLERANT BACTERIA

Peter Pristas[a, b], Zuzana Stramova[a, c], Simona Kvasnova[d]

[a] *Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4-6, 04001 Kosice, Slovakia*

[b] *Institute of Biology and Ecology, Faculty of Science, Pavol Josef Safarik University, Srobarova 2, 04154 Kosice, Slovakia*

[c] *Department of Biochemistry, Faculty of Science, Pavol Josef Safarik University, Srobarova 2, 04154 Kosice, Slovakia*

[d] *Department of Biology and Ecology, Faculty of Natural Science, Matej Bel University, Tajovskeho 40, 97401 Banska Bystrica, Slovakia*

ABSTRACT

During billions years of evolution bacteria have adapted to nearly all possible, sometimes very extreme environments on the Earth. Extremotolerant bacteria can resist extreme temperatures, pH values, salinity etc. which are detrimental to the majority of life on Earth. With the advent of industrial technologies a new type of extreme environments appeared with conditions unseen before. Especially waste disposal sites from non-ferrous metal industry constitute environments very hostile for life. In our experiments microflora of two waste disposal sites in Slovakia – brown mud disposal site from aluminium production near Ziar nad Hronom and nickel sludge disposal site near Sered - was analyzed for cultivable bacteria.

Brown mud is disposed as a slurry having a solid concentration in the range of 10-30%, pH in the range of 13 and high ionic strength. The nickel sludge contains of about 80% of Fe and high concentrations of other metals e.g. 3.5% of Cr_2O_3 and 0.17% of Ni. Despite extremely harsh conditions (extreme pH values and heavy metal content in red mud disposal site from aluminium production or high heavy metal content in nickel sludge) relatively high numbers of bacteria were recovered. Isolated bacteria were characterized by a combination of classical microbiological approaches and molecular methods. At least some of isolated bacteria are probably representatives of new, up to now unrecognized bacterial species and most of them are polyextremotolerant. The most frequently halotolerant (resistant to the high level of salt concentrations) and alkalitolerant (resistant to the high pH level) bacteria belonging to the Actinobacteria class were detected (Table 1). The most of bacteria shown very high level of heavy metal resistance e.g. more than 500 μg/ml for Zn^{2+} or Cu^{2+}.

Based on our data, waste disposal sites thus on one side represents an important environmental burden but on other side they are a source of new

polyextremotolerant bacterial strains and species possibly used in many biotechnology and bioremediation applications.

Table 1 Characteristics of studied environments and occurrence of extremotolerant phenotypes in isolated bacteria.

Environment / Characteristics	Red mud disposal site near Ziar nad Hronom		Nickel sludge disposal site near Sered
	Drainage water	**Red mud**	
pH	13.1	11.6	8.1
Cultivable bacteria counts	80 cfu/ml	3500 cfu/g	32000 cfu/g
Total number of isolates	12	19	23
Gram-positive	10	16	23
Actinobacteria	7	8	21
Gram-negative	2	3	0
Number of species	6	14	7
Dominant genus	*Microbacterium*	*Bacillus*	*Arthrobacter*
Frequency of isolates growing at:			
pH=7.0	12/12	19/19	23/23
pH=10.0	12/12	17/19	21/23
3% NaCl	12/12	19/19	23/23
5% NaCl	11/12	6/19	23/23
10% NaCl	5/12	0/12	0/23
500 µg/ml Zn^{2+}	nt	17/19	nt
500 µg/ml Ni^{2+}	0/19	12/19	2/23
200 µg/ml Co^{2+}	nt	12/19	22/23
500 µg/ml Cu^{2+}	0/19	12/19	23/23

nt - not tested

Keywords: non-ferrous metal industry, waste, environment, extremotolerant bacteria, biotechnology

BIOCHAR DERIVED FROM TRITICUM AESTIVUM: STUDY OF CHARACTER AND MECHANISM OF METAL IONS REMOVAL

Lucia Remenárová[a], **Martin Pipíška**[a], **Augustín Jozef**[a], **Gerhard Soja**[b]
[a]*Department of Ecochemistry and Radioecology, University of SS. Cyril and Methodius, J. Herdu 2, Trnava, SK-917 01, Slovak Republic (remenarova@ucm.sk)*
[b]*AIT Austrian Institute of Technology GmbH, Department of Health & Environment,* Konrad-Lorenz-Straße 24, 3430 Tulln, *Austria*

ABSTRACT

Biochar prepared from *Triticum aestivum* biomass by pyrolysis at low temperatures (450°C) was characterized and investigated as biosorbent for the removal of Cd^{2+}, Zn^{2+} and Co^{2+} ions from aqueous solutions.

The character of biochar as well as sorption mechanism were studied using potentiometric titration (Fig. 1), specific surface area (SSA) determined by the BET adsorption method, SEM-EDX analysis, FTIR analysis and chemical blocking.

To obtain reliable data in broad concentration range $^{109}CdCl_2$, $^{65}ZnCl_2$ and $^{60}CoCl_2$ were used as a tracers and radioactivities were measured by NaI(Tl) gamma spectrometric assembly. Biosorption of metals was well-described by the Langmuir isotherm. The maximum sorption capacities onto biochar from single solutions at pH 6.0 calculated by Langmuir equation were (μmol/g): 193 (Cd^{2+}), 148 (Zn^{2+}) and 90 (Co^{2+}). The affinity of biochar for metal ions decreased in order: $\mathbf{Cd^{2+}>Zn^{2+}>>Co^{2+}}$.

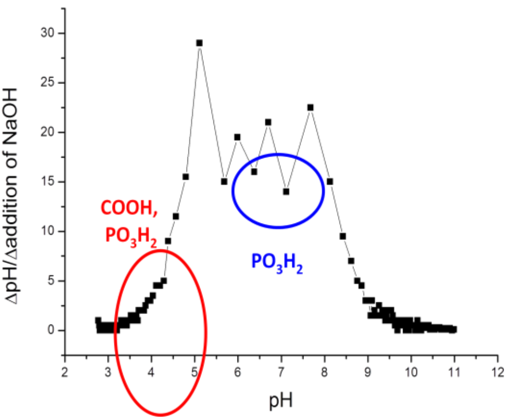

Fig. 1 Potentiometric titration of pyrolyzed biochar from straw (3.0 g/dm^3) expressed in the form of first derivation according to Gran. Predicted functional groups are color coded. Titration performed with 0.1 M NaOH at background electrolyte 0.1 M NaCl and 25°C.

Keywords: biochar, mechanism, biosorption, metal ions, radiotracer technique

FUNGAL BIOLEACHING OF ELECTRONIC WASTE

Milan Semerád[a], Slavomír Čerňanský[a], Alexandra Šimonovičová[b], Alena Kubátová[c], Alžbeta Takáčová[d]

[a] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Environmental Ecology, Mlynská dolina, Bratislava 84215, Slovak Republic*

[b] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Soil Science, Mlynská dolina, Bratislava 84215, Slovak Republic*

[c] *Charles University in Prague, Faculty of Science, Department Culture Collection of Fungi, Albertov 6, Prague 12843, Czech Republic*

[d] *ÚRUP a.s. in Bratislava, Department Laboratory of Spectral Methods, Vlčie Hrdlo, Bratislava 82001, Slovak Republic*

ABSTRACT

Electronic waste is a term used to cover almost all types of electrical and electronic equipment and their components that has or could enter the waste stream. Recycling of the electronic waste is very important not only for secondary use as raw materials such platinum, gold, silver, iron, aluminium and copper, electronic waste also contains many toxic elements such as arsenic, barium, cadmium, cobalt, chrome and lead. Nowadays, the most preferred methods for recycling of electronic waste are physicochemical methods such as pyrometallurgy and hydrometallurgy. These methods have many drawbacks including high costs, a lot of produced toxic waste and releasing of pollutants to the environment. The great alternative could be bioleaching with bacteria or fungi. These methods are cheaper and don't produce so much waste compared to conventional techniques. For leaching experiments, microscopic fungi *Penicillium pulvillorum*, *Rhizopus stolonifer* and *Trichoderma virens* were used. Distilled water was used as control leaching agent. Fungi are capable to leach metals and metalloids from electronic waste by their metabolites such as organic acids and various enzymes. Bioleaching was carried out in 250 ml Erlenmeyer flasks with 45 ml liquid Sabouraud medium, 5 ml suspension of fungal spores and 2 g of electronic waste. As control, 50 ml of distilled water with pH 6.1 and 2 g of electronic waste were used. The leaching was carried out for 14 days at constant laboratory temperature. The used electronic waste was composed from personal computer motherboards, graphics and sound cards and was powdered into the fraction under 2 mm. Fungal bioleaching was more efficient for economically significant metals such as aluminium, iron and copper if compared with the leaching with distilled water. Concentrations of toxic compounds as arsenic, cadmium and cobalt after leaching by distilled water were very similar with bioleaching with fungi. It is probably due to very good solubility of compounds containing these metal(loid)s. The concentration

of lead was higher in case of fungal bioleaching than in case of leaching with distilled water (Tab. 1). The pH value decreased during fungal bioleaching of electronic waste compared with experiment with distilled water (Fig. 1).
This work was financially supported by the grant VEGA No. 1/1155/12.

Table 1 Concentration of chemical elements in electronic waste and in solutions after leaching of electronic waste by distilled water and *Penicillium pulvillorum*, *Rhizopus stolonifer* and *Trichoderma virens*

Chemical elements	Electronic waste before leaching mg/kg	Distilled water mg/l	*Penicillium pulvilorum* mg/l	*Rhizopus stolonifer* mg/l	*Trichoderma virens* mg/l
Al	33100	0.04	3.16	4.25	2.56
As	24.12	0.06	0.07	0.06	0.07
Cd	2.00	<0.002	<0.002	<0.002	<0.002
Co	6.16	<0.02	0.03	0.04	0.03
Cr	32.20	<0.01	0.03	0.08	<0.01
Cu	42690	0.43	189.60	140.53	281.18
Fe	1208	<0.01	6.47	3.91	3.75
Pb	146.90	0.03	0.52	0.13	0.45

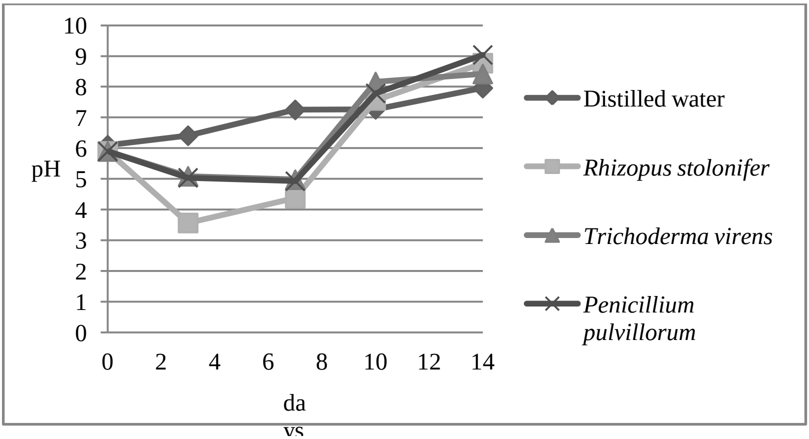

Fig. 1 Changes in pH during leaching of electronic waste by distilled water and *Penicillium pulvillorum*, *Rhizopus stolonifer* and *Trichoderma virens*

Keywords: bioleaching, fungi, electronic waste, heavy metals

EARTHWORMS AS A TOOL OF TOXICITY DETERMINATION OF SEDIMENT POLLUTED BY MINING ACTIVITY ON THE TERRITORY OF EASTERN SLOVAKIA

Oľga Šestinová, Lenka Findoráková, Silvia Dolinská, Tomislav Špaldon, Tomáš Kurbel

Slovak Academy of Sciences, Institute of Geotechnics, Department of Environment and Hygiene in Mining , Watsonova 45, Kosice 04 001, Slovak Republic

ABSTRACT

This study is devoted to Ecotoxicity tests, Terrestrial Plant Test (modification of OECD 208, Phytotoxkit microbiotest on Sinapis Alba) and chronic tests *of Earthworm (Eisenia Veneta*, modification of OECD Guidelines for the testing of chemicals 317, Bioaccumulation in Terrestrial *Oligochaetes*) on polluted sediments. Bioassays are considered a simple, quick and sensitive way of evaluating the potential environmental risk. Negative effects of contaminants on the ecosystems and humans are characterized by their environmental toxicity. Earthworms can accelerate the removal of contaminants from soil. The area of Eastern Slovakia in which the water reservoir Ružín No.I is located is classified as a loaded region. Branches of the Hornád and Hnilec Rivers drained a former mining area following extraction and treatment of the sulfide ore connected with the abandoned mines. The study materials are river sediments, which were obtained from a monitoring station - the Water reservoir the Ružín No.1 concretely, the river Hornád, Hnilec and sample from sludge bed Rudňany. The samples of sediment were used to assess of the potential phytotoxic effect. Total mortality was established at earthworms using chronic toxicity test after 7 and 28 exposure days. The total concentrations of heavy metals (Tab. 1) and FTIR (Fig.1) spectroscopy before and after Ecotoxicological tests were determined.

Table 1 The concentrations of heavy metals in the studied sediments

Sample	Concentration (mg.kg^{-1})							
	Cu	Zn	As	Pb	Cr	Ni	Cd	Hg
H	408,5	399,5	56,7	90,3	54,3	45,8	7,8	2,2
H-7	397,7	397,4	49,5	94,3	53,3	46,0	2,8	2,1
H-28	404,1	404,0	54,8	93	61,8	45,6	2,1	2,9
HO	161,2	263,0	32,8	47,1	95,9	76,5	6,5	7,7
HO-7	150,1	264,5	35,6	50,1	82,9	67,9	4,2	5,9
HO-28	144	255,6	34,5	49,2	84,9	67,6	3,8	8,4

R	1429	2246	133,5	870,06	521,7	150,1	63,7	188,5
R-7	1269	1038	124,7	406,7	359,1	126,9	58,5	0,9
R-28	1415	1756	144,4	656,7	476,7	155,4	67,7	187
C	<0,5	8,0	0,7	31,7	175,5	7,4	8,4	<0,5
C-7	<0,5	5,9	0,2	21,4	127,8	9,4	11,6	0,2
C-28	<0,5	7,1	0,4	20,1	95	8,4	9,6	0,4
Norm used for comparison								
(mg.kg^{-1})								
TV	36	140	29	85	100	35	0.8	0.3
MPC	73	620	55	530	380	44	12	10
IV	190	720	55	530	380	210	12	10

Norm No. 549/1998-2: **TV**-Target Value (Negligible Risk), **MPC**–Maximum Permissible Concentration (Max. Tolerable Risk), **IV**-Intervention Value (Serious Risk), Control soil (C), sediment of Hnilec (H) and Hornád (HO) River, sludge bed of Rudňany (R)

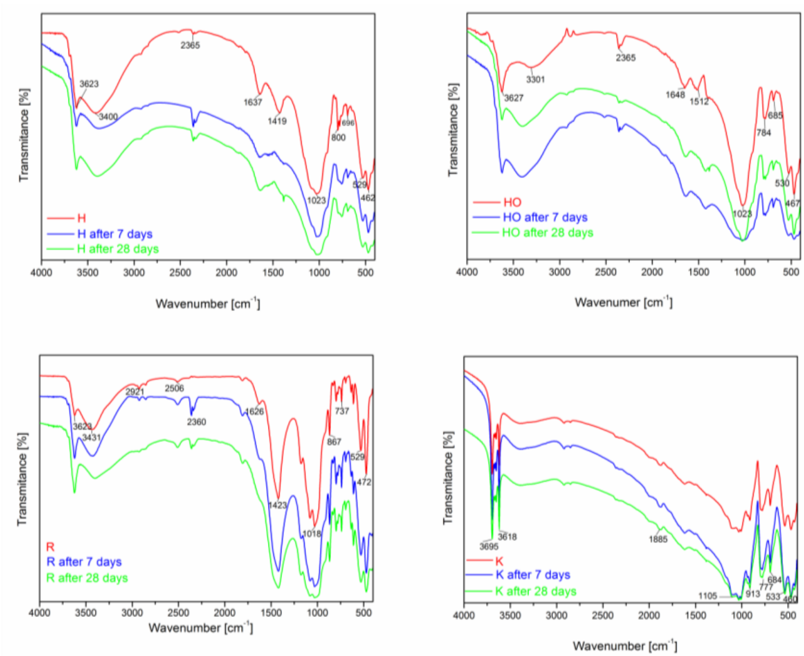

Fig. 1 The FTIR spectrum of sediments: H, HO, R and K (Control soil)

It was found that earthworms in same cases caused decline of metals concentration in contaminated sample. FTIR spectroscopy confirmed in samples of R and R 7 the presence of vibrations of C-H groups at 2926 and

2921 cm-1. It demonstrates the presence of organic matter. The absence of that vibration in sample R28 probably related to the viability and metabolic activity of earthworms. The results of our study confirmed that no mortality was observed in any of the study sediments. Based on the phytotoxicity testing, no phytotoxic effects of the metal contaminated sediments from the water reservoir Ružin No.I, Hnilec (H, H-28) and Hornád (HO, HO-28) on *Sinapis alba* seeds was observed. Potential phytotoxic effects of the metals contaminated sediments from the sludge bed Rudňany (R, R-28) on *Sinapis alba* seeds was observed. The inhibition of the germination rate (phytotoxic and mortality tests) of sludge bed Rudňany was probably the result of their high contamination by heavy metals and of their physico-chemical properties

Keywords: sediment, phytotoxkit, test of earthworm, heavy metals, FTIR

Acknowledgements: The authors greatly acknowledge the fellowship support of APVV - Slovak Research and Development Agency (No - 0252-10) and VEGA - Slovak Grant Agency for Science (grant No- 2/0187/11).

INHIBITION OF FUNGAL PELLET GROWTH BY MONTMORILLONITE

Alexandra Šimonovičová[a], Karol Jesenák[b], Slavomír Čerňanský[c]

[a] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Soil Science, Mlynská dolina, Bratislava 842 15, Slovak Republic*

[b] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Inorganic Chemistry, Mlynská dolina, Bratislava 842 15, Slovak Republic*

[c] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Environmental Ecology, Mlynská dolina, Bratislava 842 15, Slovak Republic*

ABSTRACT

Mutual occurrence of microscopic fungi and microcrystalline inorganic silicates is very often in natural systems. For example, both components are frequented part of most soils and sediments. Both components are capable to decrease the concentration of various water-soluble substances (including harmful ones) in their surrounding environments. For the mutual transport of these substances from the surrounding environment, the presence of water in its fluid phase, which significantly increases surface of interaction for both components with its surroundings and it also facilitates the implementation of more effective sorption mechanisms (in case of silicates).

Bioaccumulation properties of microscopic fungi and sorption properties of microcrystalline silicates result in consideration about their potential application for decontamination of the environment. Thereby bioaccumulation properties of microscopic fungi as well as sorption properties of microcrystalline silicates are known, overall decontamination effect in technological processes followed by implementation of bioaccumulation and sorption units may be relatively well estimated. On the contrary, decontamination effect of the mutual application of microscopic fungi and silicate sorbents cannot be estimated and it is necessary to estimate it experimentally. It can be supposed that this effect will be lower than sum of effects of both components. The main reason should be an inhibition effect of the sorbent to fungal growth together with the decreasing of the rapidity of solution transport towards the sorbent surface due to decreasing of rapidity of fluid-phase circulation in the surrounding of fungal pellets as well as directly inside the pellets. Therefore mutual use of microscopic fungi and silicate sorbents in decontamination technologies wouldn't have any significant advantages, it provides opportunity for observation of crucial problems connected to the interaction of fine-grained particulate materials with microscopic fungi in the aqueous environments. To such problems, explanation

of the inhibition effect of fine-grained sorbents on the fungal growth also belongs.

This is the first contribution to the solution of this problem and the aim of this work was to appreciate the inhibition effect of the clay material of montmorillonite on the growth of the *Aspergillus niger* fungus in the aqueous environment without addition of any contaminant. To appreciate the inhibition effect, comparison of the distribution of fungal pellets cultivated for 7 days at 12°C in standard culture medium containing glucose, peptone and distilled water with the addition of montmorillonite and the size of fungal pellets in the culture medium without the addition of montmorillonite were used. 10 ml of water suspension of fungal spores (*Aspergillus niger* strain isolated from the Šobov mining site), 70 ml culture medium and 1, 5, 10, 15, and 20 g monomineral sample of montmorillonite with the grain size lower than 5 μm were used in the experiment.

The main conclusion of the experiments is a clear confirmation of the retardation of fungal growth and significant reducing of the final size of spherical fungal pellets. Within the observed range of additions of the clay mineral, this size was in indirect relation, however, this influence may be evaluated as insignificant. More significant influence can be expected under the level of 1 g addition of montmorillonite in 80 ml of total volume of the suspension. The inhibition effect of montmorillonite is shown on Fig. 1. The detailed microscopic analysis of fungal pellets referred to the presence of clay particles in the spatial structure of the pellets. Unexplained questions include:

1. What are the main reasons of the inhibition effects?
2. How is this effect affected by shape and size of the inorganic sorbent particles?
3. How is this effect affected by physicochemical properties of the sorbent?

Answers to these questions could be provided by experiments with other types of fine-grained inorganic minerals that will be observed later.

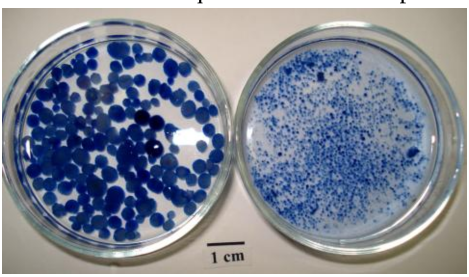

Fig. 1 Fungal pellets of the *Aspergillus niger* strain isolated from the Šobov site after a 7-day cultivation with (on the right) and without (on the left) addition of montmorillonite (1 g)

Keywords: montmorillonite, *Aspergillus niger*, fungal pellets

Acknowledgement

This work was financially supported by the grant VEGA No. 1/1155/12.

SELECTIVE RECOVERY OF ANTIMONY FROM WASTEWATERS

Dana Luminita Sobariu[a,b], Yannick-Serge Zimmermann[a], Sebastian Müller[a], Maria Gavrilescu[b], Markus Lenz[a]

[a] *University of Applied Sciences and Arts Northwestern Switzerland, Institute for Ecopreneurship, Gründenstrasse 40, 4132 Muttenz, Switzerland*

[b] *Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Department of Environmental Engineering and Management, 73 Prof. dr. docent Dimitrie Mangeron Rd., Iasi, 700050, Romania*

ABSTRACT

Antimony (Sb) is a natural element used in the production of flame retardants, lead-acid batteries, glass, plastics and alloys. Sb is classified as a critical raw material, in a recent report of the European Union next to other 13 materials, due to its high economic importance and supply risk. Currently only a small proportion of Sb is recycled (from lead-acid batteries) and it is thus important to find new secondary sources.

In mine drainage waters and smelting industry effluents, Sb can be found in high concentrations, often associated with As. Due to the chemical similarities between the trivalent and pentavalent As and Sb species a separation of these two elements is challenging. The aim of this study was to selectively recover Sb from waters contaminated with both oxyanions using a combination of bioreduction and adsorption. We explored the capability of a specialized dissimilatory As reducing organism for the reduction of pentavalent arsenic in presence of pentavalent Sb. Indeed, the microorganism could selectively reduce As to high extents (96.6% of 100μM As), while not being inhibited by equimolar concentrations of Sb. In a second step, pentavalent Sb was fully retained on a commercially available anion exchange whereas trivalent As remained in solution to high proportions. Trivalent As can be removed from solution by standard (precipitation) methods, whereas Sb(V) is won in purified form.

Keywords: antimony, arsenic, bioreduction, wastewaters, selective recovery.

HUMIC ACID SORPTION INTERACTIONS WITH BIOLOGICAL SURFACES AND HEAVY METALS

<u>Martin Urík</u>, Katarína Gardošová, Marek Bujdoš, Peter Matúš
Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Mlynska dolina 1, 842 15 Bratislava 4, Slovakia

ABSTRACT
Humic acids are complex natural organic macromolecules with highly variable chemical composition allowing them to interact with various solid surfaces. Their exceptional sorption properties also affect the mobility of various elements in the natural environment, including the potentially toxic metal(loid)s.
The mutual sorption interactions between heavy metals, humic acids and microscopic fungi (*Aspergillus niger* and *A. clavatus*) were evaluated in two and three component batch experiments. The residual content of heavy metals and humic acids in filtrates were determined by ICP-OES on Jobin Yvon 70 Plus (Longjumeau, France) and on spectrophotometer SP-300 (Optima, Japan) at 410 nm, respectively. Data were evaluated using standard kinetic and sorption models (Langmuir and Freundlich isotherm models).
Experimental data of humic acids sorption onto pelletized fungal biomass of *A. niger* and *A. clavatus* suggest the maximum sorption capacity of *A. niger* is higher under alkalic conditions, while the removal efficiency of humic acids by *A. clavatus* is higher in acidic regions of pH (Fig. 1).

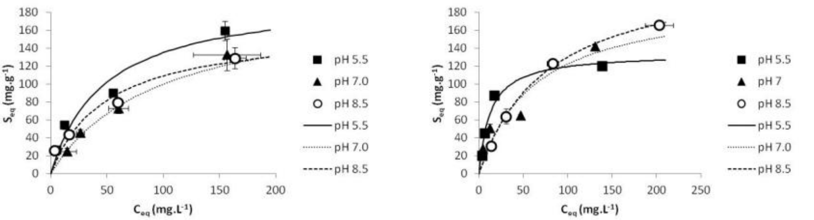

Fig. 1 Humic acids sorption by *A. clavatus* (left) and *A. niger* (right) fitted by Langmuir isotherm (25°C, 120 rpm, 20 h contact time)

The effect of mutual interactions between humic acids and mycelial pellets on immobilization of Zn(II) indicates that zinc affinity is higher towards the fungal surface rather than to humic acids, which do not supply enough active sorption sites for zinc, resulting in decrease of sorption capacity of mycelila pellets modified with humic acids when compared to unmodified biomass (Fig. 2).

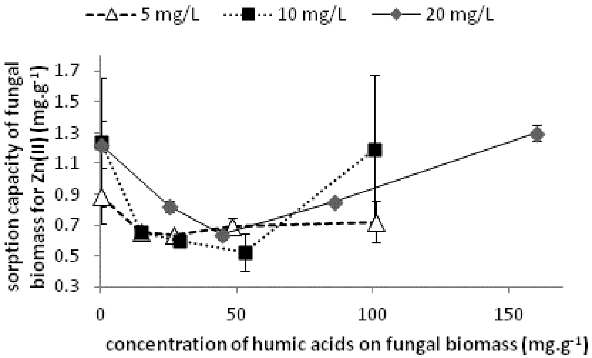

Fig. 2 Influence of humic acids concentration pre-adsorbed on mycelial pellets on Zn(II) uptake by *A. niger* strain

Keywords: filamentous fungi, toxic metal(loid)s, adsorption

Acknowledgement
The work was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences under the contract Nos. 1/0860/11 and VEGA 1/0203/14.

INHIBITION EFFECTS OF SEDIMENTS FROM THE ČERNÝ PŘÍKOP SITE ON GROWTH AND BIOLEACHING OF MICROSCOPIC FILAMENTOUS FUNGI

Hana Vojtková[a], Alexandra Šimonovičová[b], Slavomír Čerňanský[c], Radmila, Kučerová[a], Vojtech Dirner[a], Eva Pauditšová[d], Pavla Švanová[a]

[a] *VŠB-Technical University of Ostrava, Faculty of Mining and Geology, Institute of Environmental Engineering, 17. listopadu 15/2172, Ostrava – Poruba 708 33, Czech Republic*

[b] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Soil Science, Mlynská dolina, Bratislava 84215, Slovak Republic*

[c] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Environmental Ecology, Mlynská dolina, Bratislava 84215, Slovak Republic*

[d] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Landscape Ecology, Mlynská dolina, Bratislava 84215, Slovak Republic*

ABSTRACT

The Černý Příkop site is an artificial water course in the city of Ostrava (Czech Republic) built in 1952 for industrial purposes. Now, it is used as a water body for industrial and waste water. Sediments (CP1 and CP2 samples) in this water course contains high concentrations of various organic and inorganic pollutants such as PAH, PCB, NEL, Fe, Al, Pb, Co, Zn, Cr, Cd and Hg (Tabs. 1, 2).

Table 1 Contents of organic pollutants in sediments from the Černý Příkop water course (mg/kg dried weight)

Benzene	BTEX	EOX	NEL	PCE	TCE	PAH	PCB
3.58	8.72	14.4	2880	0.76	1.06	541	0.64

BTEX – volatile organic compounds such as benzene, toluene, xylenes, ethylbenzene; EOX – extractable organic halogens; NEL – non-polar extractable substances; PCE – tetrachlorethylene; TCE – trichlorethylene; PAH – acenaphtene, naphtalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, dibenzo[*a,h*]anthracene, benzo [*b*]fluoranthene, benzo[*k*]fluoranthene, benzo [*a*]pyrene, chrysene, benzo[*ghi*]perylene, indeno[*1,2,3-cd*]pyrene; PCB – congeners (28, 52, 101, 138, 153, 180)

Table 2 Contents of inorganic pollutants in two different sediments (CP1, CP2) from the Černý Příkop water course (mg/kg dried weight)

	Fe	Al	Pb	Ni	Co	Zn	Cr	Cd	Hg
CP1	40800	37200	150	48.1	36.0	760	175	3.7	2.7
CP2	40800	37200	929	48.1	-	760	-	4.6	-

BTEX – volatile organic compounds such as benzene, toluene, xylenes, ethylbenzene; EOX – extractable organic halogens; NEL – non-polar extractable substances; PCE – tetrachlorethylene; TCE – trichlorethylene; PAH – acenaphtene, naphtalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, dibenzo[*a,h*]anthracene, benzo [*b*]fluoranthene, benzo[*k*]fluoranthene, benzo [*a*]pyrene, chrysene, benzo[*ghi*]perylene, indeno[*1,2,3-cd*]pyrene; PCB – congeners (28, 52, 101, 138, 153, 180)

The toxicity of sediments expressed as the inhibition of fungal growth was tested using several fungal strains such as *Aspergillus clavatus, A. fumigatus, A. niger* and *Penicillium glabrum*. In both samples (CP1 and CP2), growth of biomass of all fungal strains was inhibited. The growth of biomass in the CP1 sample was only slightly inhibited in all fungal strains compared with control samples without the sediment. However, significant inhibition of the fungal growth was observed if the CP2 sediment was used. The most inhibited growth was recorded using *P. glabrum* followed by *A. fumigatus* and *A. niger* . The minimum inhibition effect was observed using *A. clavatus*.

Besides the growth inhibition, releasing of nickel, zinc, copper and iron from sediments into the solution mediated by microscopic filamentous fungi were observed. The *A. clavatus* strain leached the highest content of nickel into the solution using both sediment samples CP1 and CP2 representing 0.13 and 0.16 mg/l of nickel, respectively. It is interesting that *A. fumigatus* was capable to leach nickel from the sample CP2 (0.08 mg/l) but not from the sample CP1. The highest content of leached zinc was observed by using *A. clavatus* and *A. niger* (0.3 mg/l of zinc for both strains and sediments). Strains *A. fumigatus* and *P. glabrum* were able to leach zinc approximately less by half. The results from bioleaching of copper varied enough. *A. clavatus* and *P. glabrum* were capable to leach zinc from both sediment samples; contents of released zinc were lower using the sample CP2 by both strains. However, the best bioleaching agent for zinc using the sediment sample CP1 was *A. niger* (0.05 mg/l of zinc) but this strain did not leach any zinc if the sample CP2 was used. No releasing of zinc using both samples CP1 and CP2 was observed if *A. fumigatus* was applied. The amounts of released iron were higher using all fungal strains when the CP2 sample was used. Only in this case, fungi were capable to leach higher contents of the element into solution from the CP2 sample. The highest contents of iron were leached when *P. glabrum* was used for both sediments CP1 and CP2, e.i. 6.97 and 10.21 mg/l of iron, respectively.

According to results, microscopic fungi of various species are capable to leach iron, zinc, nickel and copper into solution from highly contaminated sediments. The amount of released metals significantly varied according to applied fungal strain indicated that bioleaching process is species specific.

Keywords: bioleaching, fungi, heavy metals, growth inhibition

Acknowledgements

This work was financially supported by the grant VEGA No. 1/1155/12 and the contribution is also the result of the project implementation: SPECTRA+ No. 20240120002 “Centre of Excellence for the Development of Settlement Infrastructure of Knowledge Economy” supported by the ERDF.

SELECTIVE RECOVERY OF COPPER FROM SOLUTIONS AFTER BIOLEACHING OF ELECTRONIC WASTE

Joanna Willner, Agnieszk Fornalczyk, Mariola Saternus
Silesian University of Technology, Faculty of Materials Engineering and Metallurgy, Institute of Metals Technology, Krasińskiego 8, Katowice 40-019, Poland

ABSTRACT
Over the past decades, there is an increasing interest in biological methods and possibilities of their applications in metals recovery. Among many types of waste (e.g. fly ashes, tannery sludge, jewellery waste and spent lithium-ion or Ni-Cd batteries) much attention is paid to the growing group of electronic waste, which are examples of complex material, containing a mixture of various metals, their alloys and also plastics and ceramics. As a result of bioleaching of electronic waste, a multi-component solution is obtained, containing various metal ions, of which copper is predominant component. Due to the presence of additional metal cations, especially iron cations direct recovery of copper (i.e. by electrolysis) is much more hindered. In this case, an effective method that allows the separation of desired metal (Cu) from their mixture may be solvent extraction. In practice solvent extraction is applied in hydrometallurgical production of copper from low grade ores within operations consisting of (bio)leaching – solvent extraction – electrowinning (BL–SX–EW).

There are very few publications which refer to recovery of metals from solutions after bioleaching of e-waste. The purpose of the study was to identify the selectivity and efficiency of LIX 860N-IC (5-nonilsalicylaldoxime) relatively to copper from polymetallic solution after bioleaching of electronic waste. Composition of this solution was shown in Table 1 and next steps of the experiment are illustrated in Figure 1.

Table 1 Concentration of metals in solution after bioleaching PCBs

Metal	Cu	Fe	Sn	Pb	Ni
Concentration, (g/L)	4.525	3.437	0.061	0.0005	0.0695

Due to the possibility of extractant reaction with cations present in the solution (mainly Fe) and process of solvent extraction performed in a non-specific manner, preliminary test of copper extraction from the solution after bioleaching, using shaking time of 5 and 10 min., pH 2,4 and phase ratio O:A =1, were carried out. It was found that (data not shown) within 5 minutes 99.6% of Cu and 14% of Fe was extracted from aqueous to the organic phase.

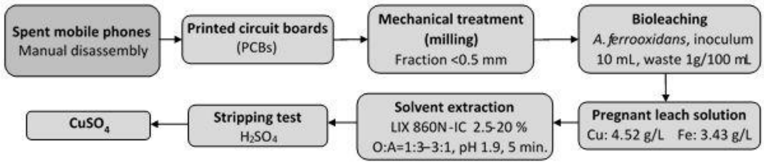

Fig. 1 Schematic diagram of experimental stages during solvent extraction of copper from solution after bioleaching of PCBs

The amount of iron in the organic phase was greater when the mixing time of phases lasted for 10 minutes. Together with copper, 24.9% of Fe was co-extracted to organic phase. This effect combined with the lack of efficiency of copper extraction, confirmed the need to shorten time of mixing and reducing the pH of aqueous solution.

Further research of copper extraction was carried out at different concentrations of LIX 860N-IC (2.5-20%), different phase ratios O:A from 1:3 to 3:1, at pH 1.9 and contact time 5 min.

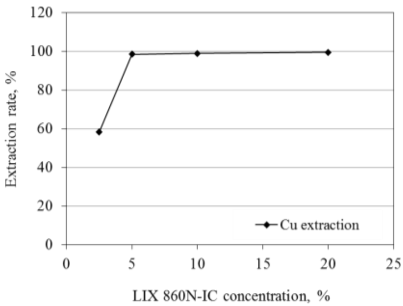

Fig. 2 Effect of LIX 860N-IC concentration on copper extraction rate, (pH=1.9, O:A=1, contact time 5 min)

Copper rate extraction (Fig.2) increased from 58.0% to 98.3% as the concentration of LIX 860N-IC increased from 2.5% to 5.0%. Simultaneously co-extraction of Fe was observed when concentration of extractive reagent raised (from 1.9% to 5.2%). However effect of co-extraction of Fe was significantly reduced at pH=1.9 comparing to pH 2.4. Similar observations of elimination (or reduction) of the iron effect co-extraction at lower pH value was observed and reported in literature. It was found that the extraction rate of copper increases with the LIX 860N-IC concentration. Best results of Cu extraction (98.3%) were achieved with extractant concentration of 5% and pH 1.9 and phase ratio O:A = 1:1. Higher pH value of aqueous phase (pH=2.4) favours the simultaneously greater effect of Fe co-extraction.

Keywords: solvent extraction, copper, solution after bioleaching, electronic waste, PCBs

POSSIBILITIES OF USING CYANOGENIC MICROORGANISMS IN THE RECOVERY OF PRECIOUS METALS FROM WASTE MATERIALS

Joanna Willner, Agnieszka Fornalczyk, Mariola Saternus
Silesian University of Technology, Faculty of Materials Engineering and Metallurgy, Institute of Metals Technology, Krasińskiego 8, 40-019 Katowice, Poland

ABSTRACT
Platinum Group Metals - PGMs (platinum, palladium, rhodium and iridium, ruthenium and osmium) are widely applied in many industrial areas such as: electronics sector, production of liquid crystal displays, jewellery or as catalysts in chemical industry. The sector of automotive industry where catalytic converters for cars are manufactured is still leading one demand for PGMs (especially Pt, Pd and Rh). Today almost half of the produced platinum, the majority of palladium and 80% of rhodium is used for the production of auto catalytic converters. A more new car on the market, causes increase the number of used and inefficient ones. Large quantities of used catalytic converters give possibility to recover the considerable amount of platinum.
To recover precious metals from spent catalysts many hydro- and pyrometallurgical methods are used. But none of these methods is an universal method that can be used to recover all type of spent catalysts. These recovery methods have also some disadvantages: pyrometallurgical methods require special equipment, reaching the desired temperature, and they are not only expensive but also highly energy consuming; application of hydrometallurgical methods requires to solve the problem of harmful waste solutions generated during the process.
Right now many actions are taken up, leading to the scientific description of new strategies of waste materials recycling, focused on the recovery of precious metals. At the same time all ecological standards are maintained. New methods for the metal recovery from waste materials, which are of interest among scientists are biological methods, especially biohydrometallurgical technologies, may become an economic alternative, fulfilling the above criteria.
In the presence of cyanide, most metals (except lanthanides and actinides) form well-defined cyanides complexes, which show very good water solubility and high chemical stability. For many years, this knowledge has had practical application - cyanide is used commercially to the complexation and recovery of gold (and other precious metals) from ores, concentrates or secondary raw materials.

Among the diverse flora used in biotechnology, there are microorganisms able to form hydrocyanic acid (HCN) e.g. bacteria *Chromobacterium violaceum, Pseudomonas fluorescens, Pseudomonas aeruginosa*, or fungi - *Marasmius oreades, Clitocybe Sp., Polysporus Sp*. Although cyanide formation by microorganisms has been known for many years the knowledge about the possibility of HCN formation by various species of microorganisms and their potential use in biotechnological processes is still incomplete.
Previous laboratory studies focused mainly on the ability to create cyanide complexes of Au and Ag in the presence of different strains of cyanogenic bacteria while information about the creation of the platinum complexes is significantly depleted (Table 1).

Table 1 Cyanogenic bacteria used in bioleaching of precious metals from waste

Genus of microorganisms	Leached material	Level of leached metal
C. violaceum	electronic strap	Au -14.9%
C. violaceum, P. fluorescens, P. plecoglossicida	electronic scrap, jewellery waste, automobile catalytic converters	Ag – 5% Au – 65.5% Pt – 0,2%
C. violaceum	printed circuit boards	Au- 10,8% Cu – 11.4%
Mixture: *C. violaceum* and *P. aeruginosa*	electronic waste	Cu – 83%, Au -73%, Zn – 49%, Fe – 13% Ag - 8%

There are unrecognized issues in the area of bacterial cyanide leaching of platinum group metals (platinum, palladium and rhodium). There is a lack of complete characterization of cyanide complexes formation mechanisms, description of physicochemical parameters that influence the kinetics of metals extraction and metabolic activity of microorganisms in terms of obtaining the highest degree of platinum metals transition from solid into solution.
The article presents currently used pyro-and hydrometallurgical platinum group metals recovery methods. However, particular attention is dedicated to the new trends in biohydrometallurgy, focused on the possibility of using cyanogenic microorganisms potential in biological leaching of these metals. A review of the achievements and results of laboratory tests for the extraction of precious metals from waste materials by bacteria leaching has been presented. The areas of research and suggestions for further laboratory work towards the use of cyanogenic microorganisms for PGMs recovery from solid waste that require further explanation and solutions have been indicated.

Keywords: platinum group metals, spent auto catalytic converters, biohydrometallurgy, cyanogenic microorganism.

Technical University in Kosice
Faculty of Metallurgy

The Faculty of Metallurgy is an integral part of Technical University of Košice. The main aim of the Faculty is to prepare new specialists in metallurgy and related fields. Scientific research is oriented on metallurgy and material technologies.

Technical University in Košice was founded in June 1952 with its three Faculties: Faculty of Mining, Faculty of Metallurgy and Faculty of Heavy Machinery.

Currently Faculty of Metallurgy has the following departments:
- Department of Ceramics
- Department of Chemistry
- Department of Ferrous Metallurgy and Foundry
- Department of Furnaces and Thermal Technology
- Department of Integrated Management
- Department of Materials Science
- Department of Metal Forming
- Department of Non-Ferrous Metals and Waste Treatment

http://www.tuke.sk/tuke/faculties-1/hf/top-info?set_language=en&cl=en

Department of Materials Science

Department of Materials Science

The Department of Materials Science as Department of Metallurgy was established in 1952 as one of two departments of Faculty of Metallurgy in Kosice and consisted of seven members. Over the next sixty years the departments' name was changed seven times.
The Department of Materials Science with an academic staff of over 17 plays a central and major role in research and teaching in materials science.

Department consists of 3 divisions:
- Division of structural engineering of materials,
- Division of mechanical properties and corrosion of materials,

- Centre of nanomaterials and nanotechnologies.

The department has well-equipped laboratories focused on metallography and physics of solid substance with light and electron microscopes, heat treatment and chemical-heat treatment of materials with various furnaces, mechanical properties of materials with testing equipments, corrosion of materials with equipments to measurement of chemical and electrochemical corrosion parameters and laboratory of nano and biotechnology.

Study

Materials science emphasizes the study of the structure of materials and of processing - structure – property relations in materials. To understand how the useful properties of materials can be modified, it is necessary to understand the fundamental relationships between structure and properties and how the structure can be changed and controlled by the various chemical, thermal, mechanical, or other treatments to which a material is subjected during manufacture and in use.

The emphasis in undergraduate teaching is always on the underlying principles which are applicable to the whole range of engineering materials; ceramics, metals, polymers, composites, solid state device materials. The materials engineer must understand thermodynamics, heat and kinetics, material structure, electronic and mechanical properties of materials, biomaterials, nanomaterials and materials processing. Lectures are also complemented by a variety of laboratory experiences.

Research

At the Department of Materials Science, research is done in all areas of materials science, including advanced materials characterization, iron and steel production, mechanical and thermal forming of aluminium, materials processing, corrosion/biocorrosion and surface chemistry, nano-structured materials, and metal recovery and recycling.

The Department of Materials Science has also several large ongoing projects, including in cooperation with U.S. Steel Košice and Železiarne Podbrezová.

Slovak Academy of Sciences
Institute of Geotechnics

The institute of Geotechnics SAS has a dominant position in Slovak Republic within the basic and applied research in the area of rock disintegration, mineral processing, mechanochemistry, mineral biotechnologies and environmental protection.

Activities of the Institute of Geotechnics SAS

Nanosciences and nanotechnologies

Development of concepts for synthesis and applications of nanocrystallic compounds prepared from precursors based on mineral, synthetic and waste materials. Potential applications presume the use of nanomaterials in chemical technologies (water treatment), electrotechnical industry (semiconductors and photovoltaic/solar cells), medicine (cancer therapy), and for specialized purposes.

Environmental protection

Investigation of effects of present and historical influence of mining, metallurgical and other industry on basic factors in living and working environment. Exploration of solid phase of aerosols and atmospheric deposition of selected contaminants from industrial, municipal and transport domains. Development and application of methods for analysis, detection, monitoring and distribution of contaminants in the environment and examination of possibilities to eliminate the implying environmental hazards.

Mineral biotechnologies

Research of biogeochemical processes in superior parts of geosphere related to weathering of the earth crust, mineral transformation, element cycle and formation of mineral deposits. Application of bacterial oxidation-reduction processes in reuse of the earth resources and wastes and in environmental protection. Application of biogenic minerals and nanomaterials in environmental technologies (water and soil treatment of heavy metals and persistent organic pollutants). Use of methods of conventional and molecular biology in assessment of biodiversity of observed ecosystems. Implementation of sophisticated analytical instruments of high resolution and accuracy in monitoring the environmental components in order to improve the life quality and health protection of population.

Geotechnics

Monitoring and optimization of rock cutting processes in the interaction of tool-rock. Development of inverse and non-standard methods for determination of rock mass condition and process effectiveness. Application of the means of artificial intelligence, fuzzy methods and frequency analysis in rock cutting. Elaboration of physical interpretation of selected rock characteristics. Application of the patterns of fracture mechanics into rock mechanics in rock cutting processes.

Scientific activities shall results in the designs of advanced technological approaches in evaluation of raw material base of Slovak Republic and recovery of localities, that were denoted as the most hazardous ones within the audit of the old pollutions (by Ministry of Environment SR) where their research and management represent the key tasks of environmental policy of advanced countries. Issues of mining pollutions and localities with organic pollutions represent the substantial part of the research scope in accordance with National Implementation Plan for Stockholm Convention on Persistent Organic Pollutants focused on health and environment protection against the persistent organic pollutants.

Scientific departments of the Institute of Geotechnics SAS

1. Department of destructional and constructional geotechnics,
2. Department of mineral biotechnology,
3. Department of physical and physiochemicasl mineral processing methods,
4. Department of mechanochemistry,
5. Department of environmental and hygiene in mining.

Department of Mineral Biotechnologies

Research course was founded by Prof. Dr. h. c. Ing. Frantisek Spaldon, DrSc. in 1985.

In 1988 was founded autochthonous microorganisms internal bank.

In 1992 was established detached department, where headed by assoc. prof., Ing. Maria Kusnierova, PhD. are working specialists in different research areas of mineral biotechnologies with specific orientation on the problems of own raw materials basis and environment. From 2006 is department working under leading MVDr. Daniel Kupka, PhD.

Study of fundamental knowledge and possibility of their application is aimed at research areas:

- **Explanation of biological - chemical oxidation processes and sulfide transformation for** the development of environmental technologies, their processing with utilization of autochthonous and physiological adapted bacteria Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans. It was developed the technology of biological - chemical treatment and Au obtaining from refracted Au-As-Sb-Fe ores at Pezinok, or methods of non-extraction application of microorganisms in the processes of selective bioflotation.
- **Determination on factors affecting metabolic functions and interactions of bacteria with mineral surface.** These factors are enable to rectify bioleaching processes rate and efficiency in Fe extraction, which is main ineligible component of non-metallic raw materials (kaolin, quartz sands, feldspars, zeolites, and fly-ashes).
- **Study of sulfate reducing-bacteria metabolism and their applic**ation in the processes of heavy metals and sulfates elimination from acid mine drainage, study of mineral raw materials and their wastes treatment and processing, study of nanosorbents biological-chemical preparation on sulfide base.
- **Study of biodiversity of autochthonous acidophilus bacteria** Acicdithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxians in the areas of abandoned and flooded mines as well old mine loadings after mining activities of sulfide ores in Slovakia.
- **Study of organic pollutant biodegradation**, mainly oil hydrocarbons in soils and waters. In the co-operation with the company Environcentrum Ltd. was developed kinetics monitoring of biodegradation processes in laboratory as well as "in situ" in surroundings of ecological disaster or decontaminating station.
- **Study of autochthonous microflora influence** at the exogenous process behavior in energetic wastes stock-piles - fly-ashes and at the coal-masse transformation from the point of its adsorptive characteristics.
- **Biological-chemical oxidation study of metallurgical wastes with Fe content** in order to prepare Fe nanodispersions industrial utilizable like a sorbents and pigments.

Department of mineral biotechnologies get equipments as well as:

- Real-time RT-PCR (Q-PCR) system ROCHE Light Cycler 2.0
- Gel-documentation system Major Science UV TransLuminator
- Aseptic box for the work with DNA and RNA
- CO_2 analyzer in gases – Stable Systems
- Paramagnetic analyzer of oxygen (0-100 %) Sable Systems

- Ion chromatograph for the simultaneous anions and cations analysis DIONEX LCS-5000
- Liquid chromatograph UHPLC DIONEX RSLC-U-3000 with MS spectrometer Bruker micrOTOF-Q II
- Gas chromatograph Agilent 7890A with MS detector Agilent 5975C (GC-MS)
- ICP-MS Agilent 7700 with 2-260 amu detection range
- Epifluorescence microscope Leica DM 6000B
- Bioreactors with automatic control of parameters and data collection in the cultivation process of the microorganisms (aerobic / anaerobic)
- Respirometric apparatus
- Equipment for the deionized and ultrapure analytical water preparation Elga-PureLab-Option + Elga PureLab-Ultra (analytic)
- Multisizer 4 Beckman Coulter Counter

SPONSORS

ITES Vranov, s.r.o.

kvalita overená rokmi ...
Môžete sa na nás spoľahnúť.
Už 20 rokov.

ITES Vranov, s.r.o.
Čemernianska 137
093 03 Vranov nad Topľou
Slovenská republika

Ponuka:
- laboratórne sklo
- sklenené priemyselné aparatúry a diely
- laboratórne pomôcky
- laboratórne plasty
- papier
- alobal pre laboratórne účely
- prístroje a zariadenia pre kontrolu odpadových a povrchových vôd
- laboratórne a prenosné prístroje a zariadenia
- súčasti a náhradné diely laboratórnych zariadení
- laboratórny nábytok
- laboratórne chemikálie

VWR International GmbH
Graumanngasse 7
A-1150 Vienna
Tel.: (02) 321 010 33; Fax: +43 1 97002-276
Email: info@sk.vwr.com

Reprezentačná kancelária :

VWR International s.r.o.
Prievozská 6 SK-821 09 Bratislava
Tel.: (02) 326 038 31 Fax: (02) 326 038 34 Email: info@sk.vwr.com

---

Wireless Network Equipment

RF elements. s.r.o.
Jasenovská 2528
066 01 Humenné, Slovakia
Email: cv@rfelements.com
Phone: + 421 (57) 3210848

1rst International Scientific Conference on Biotechnology and Metals

2nd International Scientific Conference on Biotechnology and Metals

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Proceedings:	Book of Abstracts of the 3rd International Scientific Conference on Biotechnology and Metals
Editors:	Jana Kaduková, Alena Luptáková, Oksana Velgosová
Publishers:	Faculty of Metallurgy, Technical University in Košice & Institute of Geotechnics, Slovak Academy of Sciences
Printed:	EQUILIBRIA, Poštová 13, 040 01 Košice
Year:	2014
Edition:	First edition
Print run:	55
Pages:	96
ISBN:	978-80-553-1787-8