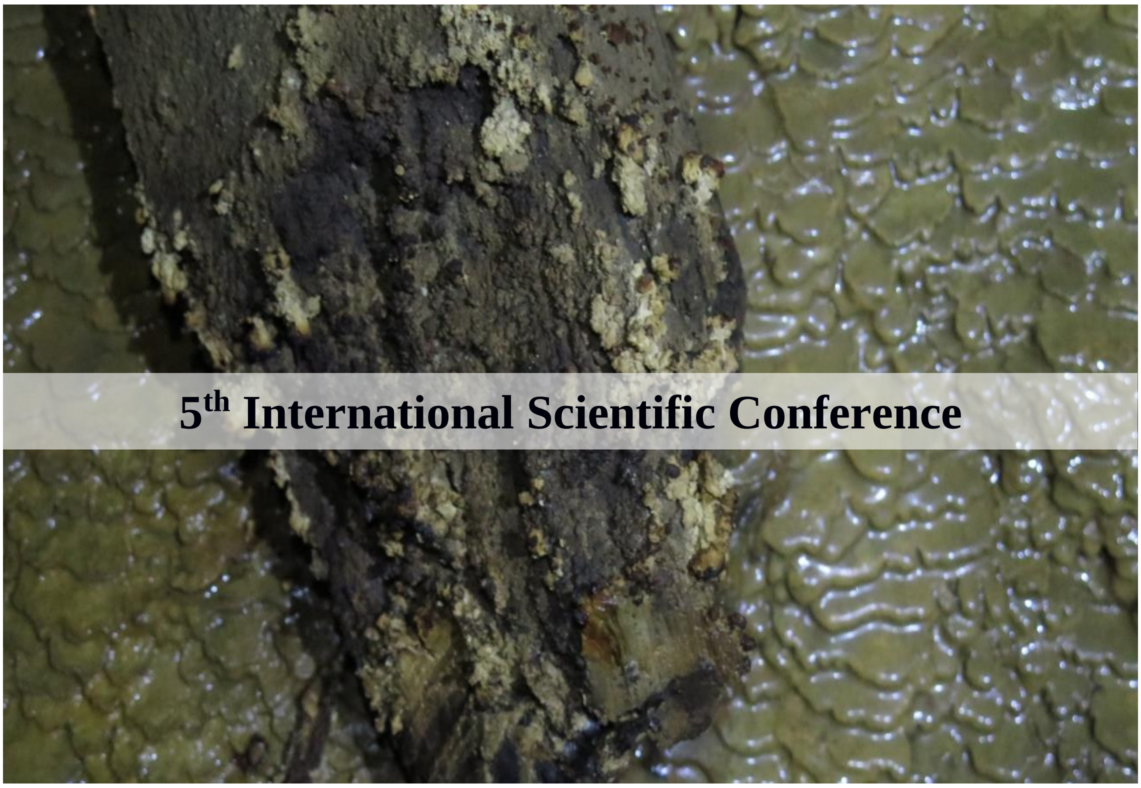
Biotechnology & Metals

Košice 2018

Faculty of Science, Pavol Jozef Šafárik University in Košice
Institute of Geotechnics of Slovak Academy of Sciences
Slovak Mining Society at the Institute of Geotechnics SAS



5th International Scientific Conference

Editors:
Jana Kisková, Jana Sedláková-Kaduková, Alena Luptáková

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Preface

Dear colleagues and friends,

we are very pleased that we can meet here in Košice at Biotechnology and Metals conference for the fifth time.

Not just we but also biotechnological research passed long journey for those 10 past years. From young biotechnologies it grew to teenage age and now we do need to explain to our colleagues what biotechnologies are and why there are interesting or beneficial for the society. On the other hand, many of the good ideas are still at the laboratory scale and there is quite a job to move them forward real technological applications to widen advanced technologies based on biological systems.

The scope of the conference concentrates on a wide and dynamic spectrum of biotechnological methods applicable in the environmental protection or raw material processing including bioremediation, wastewater treatment, biodegradation or waste management. All these disciplines gain their fundamental knowledge from the understanding of complex interactions among microbial communities or synergies among microorganisms and plants. However, there are still numerous “white sites” on the map of our knowledge on microbial diversity, population and structure. Thus, there are many exciting fields that still can be investigated. Our aim is to bring jointly the multidisciplinary skills of researchers, engineers and hopefully in the future also industrial partners to take part in the development of innovative technologies in order to utilize a vast potential of microorganisms and plants for the restoration and conservation of the environment and for the sustainable utilization of resources.

We are very grateful for your precious contributions showing how biotechnology can have a significant benefit in a wide range of environmental biotechnology research. We hope that the discussion during the conference will result in creation of new net of cooperation and will engender novel research questions leading to subsequent inventive and exciting research.

We wish you to enjoy your time at the conference and in Košice, as well. You are cordially welcome to come again for next, 6th year of Biotechnology and Metals conference held in Košice in two years.

Jana Sedláková
Alena Luptáková

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CONTENT

CU(II) AND ZN(II) REMOVAL BY VARIOUS ADSORBENTS BY BATCH AND COLUMN MODE

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Abstract

This study presents the possibilities of utilization of natural raw materials and also modified materials as potential adsorbents of Cu(II) and Zn(II) ions. Natural raw materials; siderite (S), kaolin (K), perlite (P), quartz sand (QS), glauconite sand (GS), zeolite (Z) and dolomite (D) were studied. To enhance their adsorption capacity, the surfaces of the most effective materials were coated by MnO_2 particles (samples denoted as SM, KM and GM). The changes of their textural properties after the modification were studied by the low temperature nitrogen adsorption measurement. The most expressive increase of the value of S_{BET} value was observed for the sample SM, from 6 to 36 $m^2\ g^{-1}$. The nonhomogenous distribution of MnO_2 particles of needle shape on the siderite surface was observed by SEM. The results from the batch adsorption experiments of Cu(II) and Zn(II) ions removal were processed by the Langmuir adsorption isotherm. The calculated value of maximum adsorption capacity increased after the modification from 12.9 to 19.8 mg g^{-1} for siderite and from 10.8 to 39.8 mg g^{-1} for kaolin sample. In the introductory experiments in dynamic regime, the columns filled with mixtures of quartz sand-modified siderite (QS-SM) and quartz sand-modified kaolin (QS-KM) were percolated with binnary Cu(II)/Zn(II) model solution. During the first hour, the effectivity of both mixtures in columns reached between 80- 100 % for both metal ions, except of Cu(II) in case of QS-KM, where removal of 100% was achieved an copper removal remains almost constant up to the end of the experiment (12h).

Keywords: adsorption, copper, zinc, siderite, kaolin, quartz sand

1 Introduction

The continual entry of copper into the environment, owing to long-term anthropogenic activities, such as mining, smelting, metal processing, and the application of copper salts as fungicides, has seriously impacted soil and water safety and increased the exposure risk to humans [1]. With the increasing discharge of industrial wastewater, copper has been listed as one of the most widespread heavy metal contaminants [2]. Zinc is considered as an essential element for life and acts as a micronutrient when present in trace amounts. The WHO recommended the maximum acceptable concentration of zinc and copper in drinking water as 5 mg L^{-1} and 3 mg L^{-1} , respectively [3]. Heavy metal ions which occur in industrial wastewater, Zn(II) ions stand out since they are generated by industries such as mineral extraction, metal plating and battery producing [4-6]. Zinc occurs in the list of primary contaminant elements proposed by the U.S. Environmental Protecting Agency (EPA), since it has caused serious poisoning events. The main indications of zinc poisoning are desiccating muscles, imbalance of electrolytes, stomach ache, vertigo and disharmony [7].

Frequently used methods for toxic elements removal include chemical precipitation [8], coprecipitation/sorption with hydrous metal oxides such as manganese, filtration, and adsorption [9]. Of these methods, adsorption is a highly effective [10] and attractive process because it is simple and cost efficient [11]. Several types of adsorbents have been applied for the removal of toxic elements from wastewater. High surface area of manganese oxides are effective sorbents for the removal of toxic metals from aqueous solutions because of their affinity for several heavy metals [12,13]. Moreover, nano-MnO_2 has a large number of reactive hydroxyl groups on the surface, which results in large adsorption capacities for heavy metal ions [14].

Removal of heavy metals from industrial wastewater is of primary importance because they are not only causing contamination of water bodies and are also toxic to many life forms. The copper and zinc ions have been chose for the adsorption studies with regard to their wide use in industry and potential pollution impact. For enhancement of studied parameters as well as their utilization in dynamic adsorption conditions the chemical modification – precipitation of MnO_2 on the surfaces of selected materials/samples was used. Adsorption of Cu(II) and Zn(II) ions from the aqueous solutions on natural materials and also modified materials was investigated and compared in this study. In more detailed were characterized siderite (S) and kaolin (K) samples.

2 Materials and methods

2.1 Characterization of samples

Samples of various non-metallic raw materials were tested in the batch adsorption experiments. All natural samples used in this study were obtained from the Slovakia deposits especially from the eastern region. In the first series the siderite, perlite, quartz sand and glaukonite sand with the fraction of particle size under 630μm and in the second series samples of zeolite, kaolin and dolomite with the fraction of particle size of 100 μm were tested. To enhance their adsorption capacity, as well as simplify their utilization in the dynamic conditions of the adsorption processes, the coating of surfaces by MnO_2 of selected samples with best adsorption capacity was used, according to the method of Sivasankar et al. [15]. The samples were denoted as siderite modified (SM), kaolin modified (KM) and glauconite modified (GM).

2.2 Adsorbents characterization

The adsorbents morphology was observed by the field-emission scanning electron microscope (FE-SEM) of type TESCAN MIRA 3 (Oxford Instruments) equipped with EDX detector. The adsorption and desorption isotherms were measured with the Quantachrome NOVA 1200 apparatus (Quantachrome Instruments, USA) by the method of physical adsorption of nitrogen at 77 K. To obtain the value of specific surface area S_{BET} of the studied materials the experimental data were processed by the Brunauer, Emmett, Teller (BET) isotherm in the range of relative pressure 0.05–0.2 p/p_0. The micropore volume V_{micro} and the external surface area S_t were calculated from the *t*-plot method using the Harkins-Jura standard isotherm. The value of total pore volume V_a was estimated from the maximum adsorption at relative pressure close to the saturation pressure. The pore size distribution was obtained from the Barret–Joyner–Halenda (BJH) method from the desorption branch of the isotherm [16].

2.3 Adsorption experiments

The adsorption of Cu and Zn ions was studied by the batch and column method. Analytical grade metal salts were used without the further purification. Stock solution of Cu and Zn ions at concentrations 1000 mg Cu(II)/Zn(II) L^{-1} were prepared by dissolving the appropriate amount of metal salts of $CuSO_4$. $5H_2O$ and $ZnSO_4$. $7H_2O$ in de-ionized water. The stock solutions were diluted to obtain standard model solutions containing 10 – 300 mg Cu(II)/Zn(II)L^{-1}. The sorption measurements were made by batch technique at the ambient temperature using the rotary shaker (30 rpm). Through the study, the pH was varied from 2 to 9. The adsorbents dose was 1 g L^{-1}. The diluted NaOH and HCl were used to adjust the pH of the solutions. The suspensions were shaken for 24 hours to reach equilibrium and then consequently filtered using the filter paper. The supernatant solutions were analyzed by the atomic absorption spectroscopy (AAS, Varian 240 RS/2400). The linearized Langmuir model was used for the analyses of the adsorption isotherms [17]. In preliminary experiments, the efficiency of selected samples in the mixtures was tested in dynamic mode. In this study glass columns were used for the experiments. The filtration column was 110 mm high with an inner diameter of 12 mm. Two types of column were filled with appropriate amounts of mixture of quartz sand/modified siderite and quartz sand/modified kaolin. Then 50 ml of binnary solution of Cu(II)/Zn(II) ions of concentration 100 mg.g^{-1} for each was passed through the column at a constant flow rate of 6 rpm. The filtrate was sampling each hour for pH and Cu(II) and Zn(II) concentration measurement. The pH of the suspension was measured and adjusted to required value.

3 Results and discussions

3.1 Adsorbents characterization

The qualitative phase analyses of natural samples obtained from the XRD measurement confirmed the presence of siderite, quartz, muscovite, chlorite and gyps in S sample and kaolinite, quartz, feldspar, muscovite, rutile, plagioclase and smectite in K sample. In spite of the suppression of synthetic MnO_2 reflections by diffraction lines of mineral phases, its presence in SM and KM was confirmed by the phase analyses (not shown here). According to that fine MnO_2 particles were assumed what was also proved by SEM, Fig. 1.

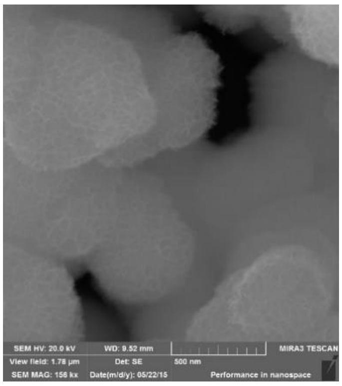

Fig. 1 SEM micrograph of siderite sample modified b MnO_2 particles

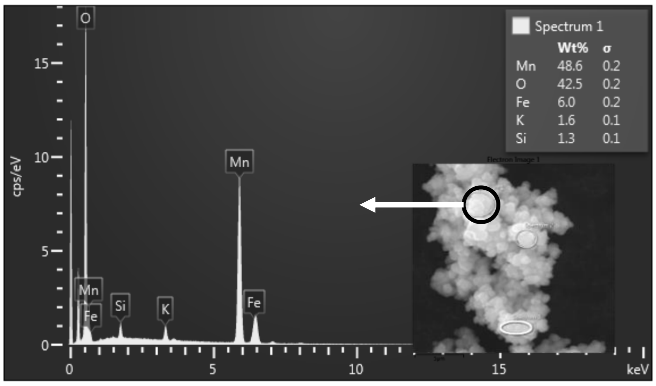

Fig. 2 EDX analysis of siderite sample modified by MnO_2 particles

The manganese oxide particles of needle shape and non-homogenous distributed on the siderite surface were observed. The EDX analysis confirmed the presence of basic structural elements in natural and modified sample, Fig. 2. The S and K samples contain the silicate mineral phases confirmed by XRD analyses. The influence of modification on the change of surface properties was in both cases observed by the broadening of hysteresis loop. By the non-homogenous coating of surfaces by MnO_2 particles, new interparticle spaces created with the size corresponded with range of mesopores, what is also in agreement with the results from SEM analyses. The value of specific surface area of S and K increased after their modification. Higher increase was obtained for S. The total pore volume of K, for that case of isotherm type, could not be determined from the value of total volume of adsorbed nitrogen. All surface parameters are summarized in Table 1.

Table 1 Surface parameters of studied materials

Sample	S_{BET} [m^2 g^{-1}]	V_a [cm^3 g^{-1}]	V_{micro} [cm^3 g^{-1}]	S_t [m^2 g^{-1}]
S	6.89	0.0143	0.001	4.19
SM	36.10	0.0634	0.002	30.95
K	10.76	-	0.00003	10.29
KM	16.09	0.0755	0.00005	15.40

3.2 Adsorption experiments

The adsorption of Cu(II) and Zn(II) ions onto various natural and modified samples as a function of pH was studied, and the results are shown in (Fig.3-5). The results showed the increase of the amount of adsorbed copper ions amount with the increasing pH from 4.5 to 6.0, Fig.3. After the value of pH 5,8 Cu(II) ions precipitated out from the solution. Fig.4 shows the increase of the adsorption of Zn(II) until pH 5,5 and then the decrease of the adsorption for all samples at pH 6, at which at pH 6.8 zinc ionc precipitated from the solution. From this series of samples the most effective sorbents in the whole pH range were samples S and GS. The S sample achieved the maximum adsorption capacity 9.1 mg.g^{-1} Cu(II) and 11 mg g^{-1} Zn(II) at pH 5.5. For the sample GS 8.2 mg.g^{-1} Cu(II) and 13.1 mg.g^{-1} Zn(II) at pH 5.5 was calculated.

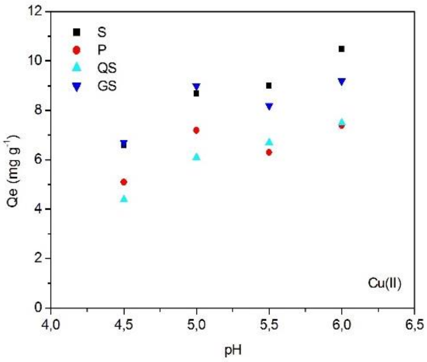

Fig. 3 Dependence of Cu(II) adsorption on the pH of the solution

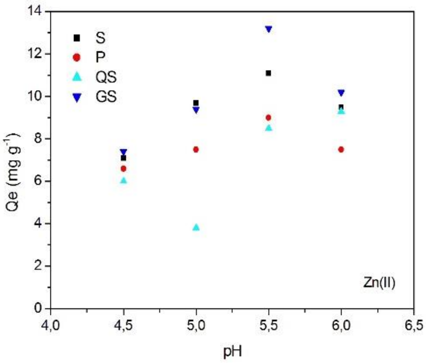

Fig. 4 Dependence of Zn(II) adsorption on the pH of the solution

The adsorption of Cu(II) and Zn(II) ions onto adsorbents from the second serie as a function of pH shows Fig.5. The K sample shows the highest affinity to Cu(II) ions at pH 5, for Zn(II) ions at the same pH value the effectivity of sorbents was comparable. The K sample obtained the maximum adsorption capacity 23.2 mg.g^{-1} Cu(II) and 12.1 mg.g^{-1} Zn(II) at pH 5.

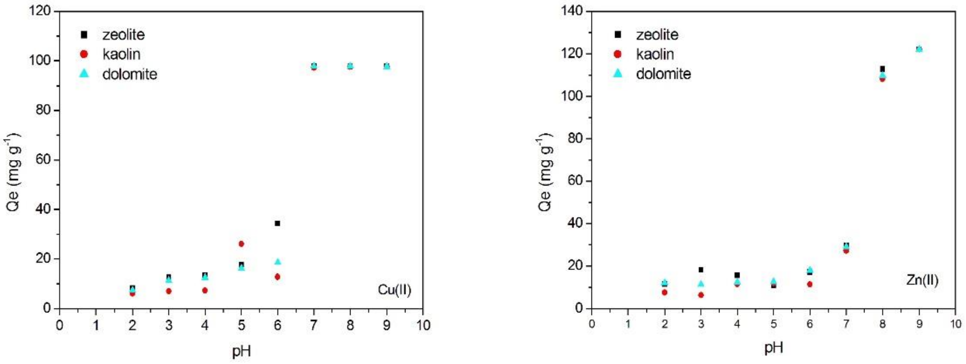

Fig. 5 Dependence of Cu(II) (left) and Zn(II) (right) adsorption on the pH of the solution

Whereas the value of specific surface area to a certain extent relates with the adsorption properties it was expected, that the modified samples by MnO_2 particles will be better sorbent for selected metal ions. The modification of selected samples enhanced their sorption capacity. The influence of the modification and also the influence of initial ion concentration on sorption properties of the samples show Fig.6 and Fig.7. Fig.6 also illustrates that the maximum adsorption of Cu(II) ions appears at pH 5 at the concentration of 200 mg.l^{-1} for all samples. Cu(II) ions demonstrated higher affinity onto modified samples compare to zinc ions. The adsorption of copper ions on SM, GM and KM samples increased with the increasing of initial ion concentration up to 250 mg.l^{-1} , then the equilibrium was established in case of KM sample. In the case of zinc ions with the increasing initial ion concentration increased the adsorption up to 110 mg.g^{-1} of Zn(II). Adsorption effect of modified samples to copper and zinc ions decreased in this order KM > SM > GM. The values of maximum adsorption capacity calculated from the linearized Langmuir model applied to the experimental data increased for selected natural adsorbents after the modification what corresponds with the obtained adsorption isotherms (not shown here). The highest value was calculated for KM, Table 2.

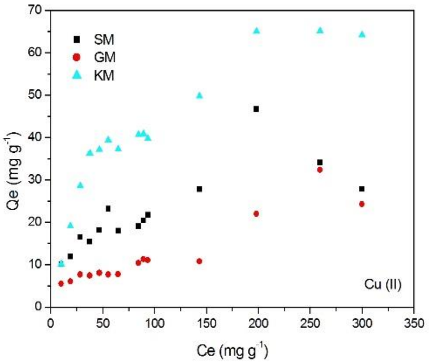

Fig. 6 Adsorption isotherms of Cu(II) adsorption onto studied materials

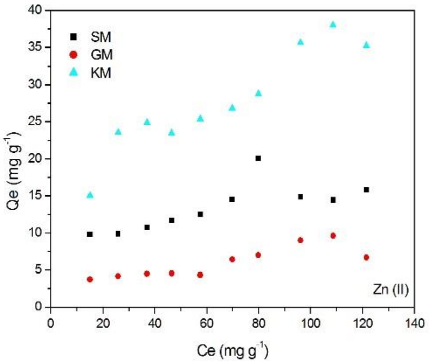

Fig. 7 Adsorption isotherms of Zn(II) adsorption onto studied materials

Table 2 Langmuir parameters for Cu(II) adsorption onto studied materials

Sample	Q_0 [mg g^{-1}]	b [L mg^{-1}]	R^2
SM	47.8	0.441	0.9754
KM	65.6	3.803	0.9976

Q_0 – maximum adsorption capacity, b – Langmuir constant related with sorption energy, R^2 -correlation coefficient

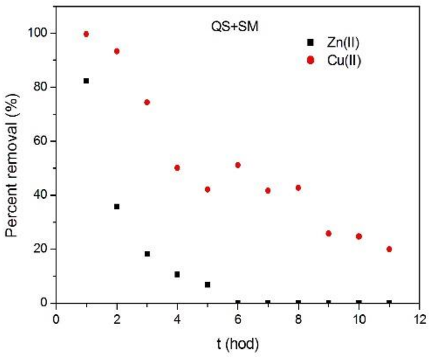

Fig .8 Effect of time on Cu(II)/Zn(II) removal in column with modified siderite

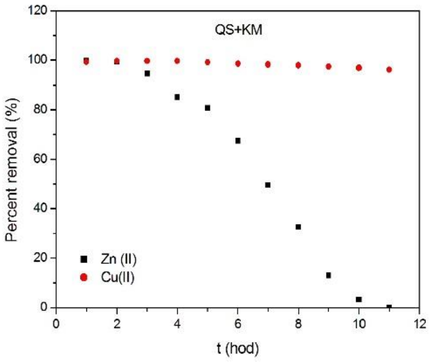

Fig. 9 Effect of time on Cu(II)/Zn(II) removal in column with modified kaolin

Effect of time on the adsorption of Cu(II) and Zn(II) ions on mixture samples of quartz sand/modified siderite (QS/ SM) and quartz sand/modified kaolin (QS/KM) in column study is illustrated in Fig.8 and 9. In case of QS/ SM column, maximum Cu(II) adsorption was almost 100% in a contact time of 1h. A maximum Zn(II) removal of 82% was observed also in 1h for this sample. After this time adsorption of both ions decreased rapidly, Fig.8. A similar trend is observed for Zn(II) ions to QS/KM sample in column, whereas Cu(II) ions reached 100% removal and with and increase in contact time remains almost constant up to the end of the experiment (12h), Fig.9. This results corresponds with the results of batch adsorption experiments, where Cu(II) ions had the highest affinity to KM sample.

4 Conclusions

The results obtained from this study confirmed that many of non-metallic raw materials located in Slovakia can be utilize as suitable adsorbents of Cu(II) and Zn(II) ions at lower concentration range. From the results of batch adsorption study with natural samples., siderite, kaolin and glaukonite sand with the highest adsorption capacity were modified by manganese oxide. By the chemical precipitation of MnO_2 particles on the surfaces of natural samples their value of specific surface area increased which pointed on the enhanced adsorption properties. In the batch experiments all modified materials showed better affinity towards Cu(II) compare to Zn(II) ions. The highest value of maximum sorption capacity was calculated for KM, which efficiency in the Cu(II) removal from the solutions of lower concentrations reach almost 100 %. In the introductory dynamic conditions (column study) effect of S and K sample modification was compared in a mixture with quartz sand. A maximum removal of 100% was observed for Cu(II) ions in column filled with QS and modified kaolin and with the increase in contact time becomes almost constant up to the end of the experiment (12h). These sorbents can be utilized for the wastewater treatment where the concentration of metal ions are up to 100 mg g^{-1} .

Acknowledgement

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PRELIMINARY EXPERIMENTS ON THE BIOLEACHING OF INDIUM FROM WASTE LCD PANELS

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Abstract

The present study deals with the preliminary research of In recovery from discarded LCD panels by bio-hydrometallurgical way. For the experiments, discarded TVs were dismantled and LCD panels were pre-treated in order to enhance the solubilisation. Bioleaching was carried out using *Acidithiobacillus ferrooxidans* and a mixed culture of *A. ferrooxidans and Acidithiobacillus albertensis.* The experiments were carried out in Wise Cube shaker in 250ml flasks with 1:10 S/L ratio, the temperature and the rotation speed were permanent during the 7days of retention time, 25°C and 150rpm respectively. Samples were taken from the liquid phase after 1, 4 and 7 days for pH measurements, and ICP analyses of the end solution was carried out. The precipitate, formed during the leaching process, was separated and also directed to chemical analysis. It was revealed that in case of mixed cultures, the retention time was not sufficient for In dissolution, however it was also predicted by the changes in the observed pH values. Based on the chemical analyses of the liquid phase and the precipitate it was revealed that when *A. ferrooxidans* strain was used alone, the indium recovery was 75% in the liquid phase and further 4.5% was obtained in the precipitate.

Keywords: LCD panels, *A. Ferrooxidans*, *A. albertensis*, bioleaching

1 Introduction

The European Commission launched the European Raw Materials Initiative in 2008 with the aim to establish Critical Raw Materials (CRMs) sustainable supply in the EU. Indium is one of the identified CRMs and regarding our former research work and studies available in literature, it has good technical recycling potential trough waste LCD panels as secondary resource. In the framework of the CriticEl project the Institute of Raw Material Preparation and Environmental Processing investigated the recovery of indium from the ITO (indium-tin-oxide) layer of waste LCD panels by chemical solubilisation. Based on our laboratory scale experiments, -after a proper pre-treatment method- when the solid/liquid ratio was 1:1 (g/mL), 1M HCl and 1M H_2SO_4 solution are both appropriate for the leaching process, but 1M HCl solution with 1h residence time and leaching at 55°C was suggested as most appropriate, because the dissolution of other elements, which could be disturbing during the solution treatment process, is lower, while the indium recovery reached the value as high as 99% [1, 6].

In the field of bioleaching of In from LCD panels, there are only one available paper, a recently released work of Jowkar et al., in which *Acidithiobacillus thiooxidans* was used for the bioleaching of In from waste LCD screens of laptops. In this study the particle size of LCD was below 37μm and the bacterium was adopted to the waste material by serial sub-culturing up to 4% (w/v). The maximum extraction for In was 100 %, and 10% for Sr [2]. There are research results, when mixed bacteria cultures are used for metal extraction, however in these studies, the material resources are usually raw materials or mine tailings, just as in a paper published by Nguyen et al. when mixed cultures of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* were used to remove heavy metals from Philippinian mine tailings. The results showed that mixed cultures were more efficient than each bacterium alone for the dissolution of Cu and As [3]. Although, there can be cases, depending on the feedstock, when mixed cultures do not work well together. When the leaching characteristics of mixed culture of *A. ferrooxidans* and *A. albertensis* was investigated on pyrite, chalcopyrite and sphalerite by Xia et al., it was revealed that the mixed culture consisting of both strains could accelerate the leaching rate of chalcopyrite and sphalerite, but not better than the pure culture on pyrite. In case of pyrite, Fe recovery was 80% when *A. ferrooxidans* was used, but only 0.59% when leaching was carried out by the mixed culture, besides the recovery was 2.64% using *A. albertensis* alone [4].

2 Materials and methods

For the bioleaching experiments, the pre-treatment of the waste LCD panels was carried out by the same method, as in case of our above mentioned chemical solubilisation research. The first step was to remove the LCD panel from the device.Then the liquid crystal and plastic polarisation and protective films were removed as well. Removing of protective foils was done by a 300s long thermal treatment at 225-227 °C. After the comminution of the LCD below 10-20 mm, the next step was to remove the liquid crystal by ultrasonic treatment in distilled water with surfactant. Finally the waste glass substrate with ITO layer was crushed below 5mm for the solubilisation using a hammer crusher [5].

The chemical composition of a so-produced waste sample is rather complex. It contains high amount of Al, B, Ba, Ca, Fe, Sr, as well as several critical elements such as Ce, Co, In, La, Mg, Nd, Y and Yb, although the amounts of these elements are quite low.In and some other metals concentration of LCD sample is shown in Table 1.

Table 1 Metals content of LCD sample

Element	*Weight (mg/kg)*
In	128
Sn	142
Sr	6460
Cu	77,7
Mn	36,2

The bioleaching experiments were carried out in Wise Cube shaker in 250mL flasks with 1:10 S/L ratio, after 5 day of inoculation in case of *A. ferrooxidans* Karitas in 9K medium. Mineral solution contained $(NH_4)_2$ SO_4,KCl, K_2HPO_4, $MgSO_4.7H_2O$, and $Ca(NO_3)_2.4H_2O$ was autoclaved and $FeSO_4.7H_2O$ solution was filter sterilized before inoculation. *A. albertensis* bacteria were isolated from gold mine Hodruša-Hámre, Slovakia using thiosulphate media which consists of a mineral medium with KH_2PO_4, K_2HPO_4, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$, NH_4Cl, Na_2CO_3 and 10% $Na_2S_2O_3$ in 90ml:10ml ratio. Since we do not have any detailed information about the growing characteristics of the *A. albertensis* bacterium, in case of it, the inoculation time was chosen to be two weeks in sterilized thiosulphate medium. The mixed culture of *A. ferrooxidans* Karitas and *A. albertensis* was prepared in 1:1 ratio, just before the leaching experiments. The temperature and the rotation speed were permanent during the 7days of retention time, 25ºC and 150rpm respectively, pH measurements were carried out after 1, 4 and 7 days.

Precipitates formed during bioleaching were separated from the liquid phase via filtration, and after washing and drying of the solid phase it was separated from the LCD glass. It was also directed to chemical analyses after dissolution in HCl.

3 Results and discussion

The aim of our preliminary research was to investigate the possibility of indium recovery from pre-treated waste LCD panels by *A. ferrooxidans* Karitas and a mixed culture of *A. ferrooxidans* Karitas and *A. albertensis* without preliminary adaptation to the material source.

A.albertensis are acidophilic, mesophilic, obligatory chemoautotrophic, aerobic, Gram-negative rods. Its closest relative is *A. thiooxidans*, the main energy source is oxidation of S^0 and/or S^{2-}, $S_2O_3^{2-}$ and $S_4O_6^{2-}$ reduction. The optimum temperature for their growth is between 25-30ºC and the optimum pH is 3.5-4, but they grow down to pH 2, therefore the simultaneous use with the highly acidophilic *A. ferrooxidans* is feasible. *A. ferrooxidans* can oxidize ferrous ions, and elemental sulphur, thiosulphates and sulphides. The mechanisms of bioleaching by *A. ferrooxidans* are usually discussed in terms of direct bacterial attack on sulphide minerals and indirect oxidative dissolution of minerals by Fe^{3+}.

Fig 1 shows the measured pH values after 1, 4 and 7 days. It can be seen, that while the iron oxidizing bacterium was applied alone, the pH decreased constantly, but when the mixed culture was used, the pH first rose up above pH 3, than started to decrease. In this case it is sure, that the applied retention time was not sufficient for the bacterium to solubilize metals.

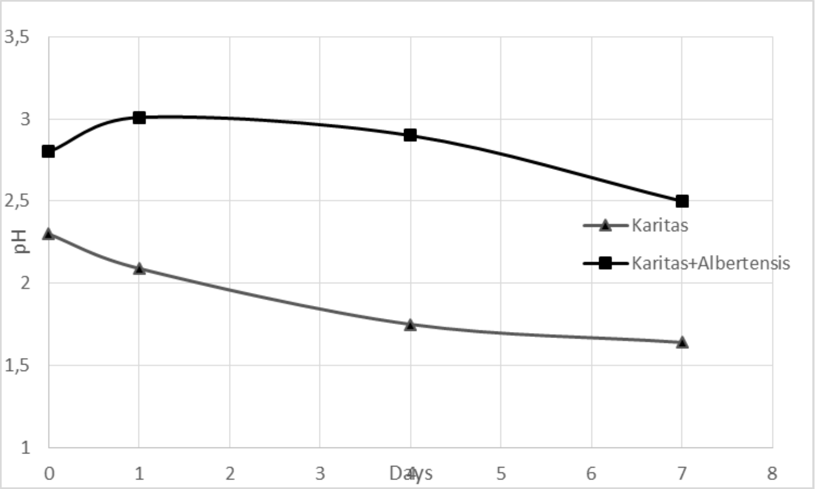

Fig 1 Changes in pH values during leaching experiments

During bioleaching experiments a precipitate forming was observed, Fig 2 shows the photos of the flasks, which were taken on the 4th day of experiment.

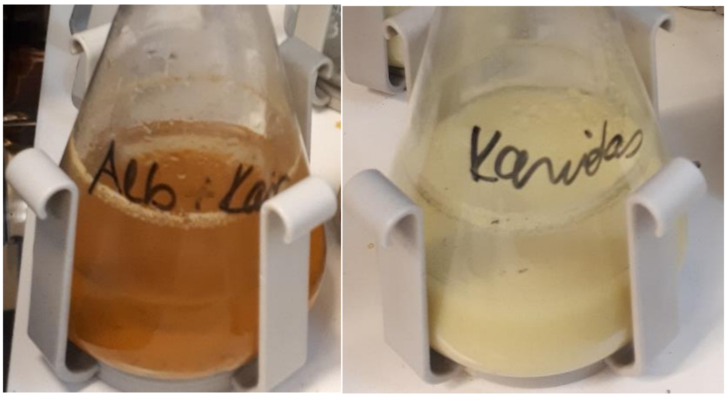

Fig 2 Precipitate in reactor vessels during bioleaching (day 4)

Table 2 shows the concentration values of the bioleaching solutions at the end of the experimental time. It can be seen, that hazardous elements, such as As and Sr presented in the solution, however with quite small amount and the *A. ferrooxidans* bacterium was a little more prosperous. In case of Cu, the mixed bacterium culture was more efficient than the *A. ferrooxidans* Karitas alone, as it was expected, but in case of indium, while the *A. ferrooxidans* Karitas worked well, the leaching rate of the mixed culture with *A. albertensis* was very poor. Such a phenomenon was observable regarding the results of Mn and Sn, but compared to the case of indium, the differences between the mixed and pure bacterium solutions, were not so pronounced.

Table 2 Metals content of the liquid phase after 7 days of retention time

	As(mg/L)	Cu (mg/L)	In (mg/L)	Mn(mg/L)	Sn (mg/L)	Sr (mg/L)
Karitas	0.011	10.018	9.668	6.737	0.088	1.019
Karitas+albertensis	0.009	14.958	0.249	3.484	0.038	0.560

Three-fold amount of precipitate was formed in case of the pure culture experiment, compared to the mixed one. All the above mentioned metals were presented in the precipitates, when mixed culture was used, in case of Cu and In the concentrations were quite high, 934.8 mg/kg and 677.8mg/kg. The further treatment of this precipitate has to be also solved, because of its complex composition and hazardous waste content: As content was 14,3mg/kg; Sr was 149,2mg/kg when mixed culture was used. Based on the chemical analyses of the liquid phase and the precipitate it was revealed that when *A. ferrooxidans* strain was used alone, the indium recovery was 75% in the liquid phase and further 4.5% was obtained in the precipitate.

4 Conclusions

Preliminary bioleaching experiments of the mechanically pretreated LCD panels were carried out in order to investigate the leaching possibility of indium by *A. ferrooxidans* and a mixed culture of *A. ferrooxidans* and *A. albertensis.*

It was found that pH during 7 days bioleaching decreased constantly in case of *A. ferrooxidans*, but when the mixed culture was used, the pH first rose up above pH 3, than decreased.

Based on the chemical analyses of the liquid phase and the precipitate, formed during leaching process, it was revealed that while *Acidithiobacillus ferrooxidans* Karitas strain was used alone, the indium recovery was 75% in the liquid phase and further 4.5% was obtained in the precipitate. The biosolubilisation of other materials was also higher in case of pure culture. Copper although was the exception*:* mixed culture was more effective.

When the mixed culture was used, only poor recovery of In in liquid phase was reached, 92.26% of In remained in the solid phase.

Based on these preliminary results further experiments should be carried out to reveal the phenomena and find the technological and micro biological optimal conditions.

Acknowledgement

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THE STUDY OF COPPER REMOVAL FROM WATERS BY WOODEN SAWDUST

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Abstract

The heavy metal remediation from wastewater is very important due to their persistency in aquatic environment. Conventional treatment technologies as precipitation for the removal of these toxic heavy metals are not economical and further generate huge quantity of toxic chemical sludge. The using of wooden sawdust is emerging as a potential alternative to the existing conventional technologies for the removal of metal ions from aqueous solutions.

The aim of this article is a study of the removal of Cu(II) from water by unconventional waste products including the wooden sawdust of poplar, cherry, spruce and hornbeam. Data obtained by neutron activation analysis revealed that ion exchange is one of the mechanisms underlying metal removal by the selected sawdust from the model solutions. The FT-IR spectra confirmed the presence of functional groups that have potential for heavy metal binding.

Keywords: heavy metals, water treatment, adsorption, wood sawdust.

1 Introduction

The heavy metal ions are introduced to the aquatic environment by various industrial activities as mining, tanneries, batteries, paper industries, fertilizer or pesticides etc. The heavy metals are toxic and accumulate in living bodies where causing serious diseases and disorders [1-3]. The major toxic metal ions hazardous to humans are Cr, Se, V, Cu, Co, Ni, Cd, Hg, As, Pb, and Zn that are toxic already at low concentrations [4].

The most commonly methods used for removal of metal ions from waters are chemical precipitation, ion exchangers, chemical oxidation/reduction, reverse osmosis, electro dialysis, ultra-filtration etc. [4, 5]. However these conventional techniques have limitations such as less efficiency at low concentrations or production of secondary sludge and further the disposal is a costly affair [6]. Another suitable technology is adsorption of heavy metals by activated carbon for treating of contaminated waters [7]. However the high cost of activated carbon and its loss during the regeneration restricts its application.

In last two decades is booming the use a low cost organic materials on the heavy metal ions adsorption. It started with the utilization of biomass and agricultural waste materials for removal of heavy metals as peat, rice husk, leaves or seeds [2, 8-10]. The major advantages of natural sorbents in comparison to conventional treatment methods include: low cost, high efficiency, minimization of chemical or biological sludge, no additional nutrient requirement, and regeneration of biosorbents and possibility of metal recovery [11].

Wooden materials or wastes are cheap sorbent materials [12, 13]. The benefits of application of wooden by-products or wastes for wastewater treatment are determined by their high removal selectivity, good adsorption capacity and possibility of regeneration. The use of original and untreated wooden materials or wastes such as straw, tree bark, peanut skins, wood sawdust, moss and peat has been reported for pollutants removal in several studies [14-16]. Wood sawdust, a low-costs adsorbent, is perspective for removing metal ions, some types of acid and basic dyes as well as other unwanted compounds from wastewaters [17]. The efficiency of the adsorption processes strongly depends on the composition of the wastewater showed formation of complex compounds of metal cations with wood sawdust functional groups [18]. Lignin, cellulose and hemicellulose, carbohydrates, and phenolic compounds, which contain carboxyl, hydroxyl, sulphate, phosphate, and amino groups, are the main metal binding [19, 20]. The application of wood sawdust for removal of pollutants brings many benefits for the protection of environment and timber industry. Contaminated water could be treated, and a new market would be opened for the sawdust [14].

The paper deals with a Cu(II) ions removal from model solutions by poplar, spruce, cherry and hornbeam wooden sawdust under static and dynamic conditions. The selected wooden sawdust were analysed by infrared spectrometry for characterization of functional groups, which can be responsible for metal binding. Efficiency of

Cu(II) ions removal was analysed by colorimetric method and changes of pH values were also measured. Neutron activation analysis was used to determine the elemental composition of wooden sawdust (raw and Cu-loaded).

2 Materials and methods

The wooden sawdust of poplar, spruce, cherry, and hornbeam species of locally available wood was sieved, and only the fractions with a particle size under 2.0 mm were used for the experiments. The IR measurements of the wooden sawdust were performed on a Bruker Alpha Platinum-ATR spectrometer (BRUKER OPTICS, Ettingen, Germany). A total of 24 scans were performed on each sample in the range of 4,000 to 400 cm^{-1}.

The model solution with initial concentrations of Cu(II) ions 10 mg.L^{-1} was prepared by dissolving of $CuSO_4.5H_2O$ in deionised water. Concentrations of the Cu(II) ions were determined using the colorimetric method with a Colorimeter DR890, (HACH LANGE, Germany) and the appropriate reagent. The input pH value of model solution was measured by pH meter inoLab pH 730 (WTW, Germany).

The batch adsorption experiments were carried out on static and dynamic conditions.. In both experiments, 1 g of dry wooden sawdust were mixed with 100 mL of model solutions containing 10 mg.L^{-1} of Cu(II) ions. In static conditions, the sorbent-sorbate interaction time was 1 day. To determine the contact time required for equilibrium sorption in dynamic condition the samples were analysed in different time intervals 5, 10, 15, 30, 45, 60 and 120 min, respectively. After the adsorption experiments, wooden sawdust were removed by filtration through a laboratory filter paper. Residual concentrations of Cu(II) ions were determined by colorimetric method and pH changes was also measured. In both cases, the percentage efficiency of ion removal η removal was calculated using the following equation (Eq. 1):

$$\eta = \frac{(c_0 - c_e)}{c_0} \cdot 100\%, \qquad (1)$$

where c_0 is the initial concentration of appropriate ions (mg.L^{-1}) and c_e equilibrium concentration of ions (mg.L^{-1}). All adsorption experiments were carried out in triplicate under the batch conditions and results are given as arithmetic mean values.

A neutron activation analysis (NAA) of raw and Cu-loaded wooden sawdust was performed at the pulsed fast reactor IBR-2 of the Frank Laboratory of Neutron Physics, JINR, Dubna, Russia. For the analysis the used wooden sawdust were dried at 40 °C to a constant weight. Samples of approximately 0.3 g were packed in polyethylene foil bags for short-term irradiation or in aluminium cups for long-term irradiation. A total of 6 elements (Na, Mg, Cl, K, Ca, and Ba) were analysed. More details concerning the irradiation time and gamma spectra processing can be found in [21]. The difference between the certified and measured content of elements of the certified material varied between 1 and 10%.

3 Results and discussion

3.1 Infrared spectra of wooden sawdust

The functional groups present in natural materials as alcoholic, carbonyl, phenolic, amide and amino, sulfhydryl groups etc. have affinity for heavy metal ions to form metal complexes or chelates. The mechanism of biosorption process includes chemisorption, complexation, adsorption on surface, diffusion through pores and ion exchange [22]. The functional groups of poplar, spruce, cherry, and hornbeam were determined using FTIR spectroscopy (Fig. 1). The metal adsorption capacity of wooden sawdust is influenced with the presence of surface structures of –OH (3,650-3,000 cm^{-1} and 1,700-1,600 cm^{-1}), -COOH (1,750-1350 cm^{-1} and 1,250-1,000 cm^{-1}), and $–NH_x$ (3,337 cm^{-1}) functional groups that are present in organic materials [23]. According to the literature [24], the structure of wooden sawdust is primarily formed by cellulose, hemicellulose, and lignin. The detailed FT-IR spectra of wooden sawdust characterisation and the band assignments are available in the literature [23-28].

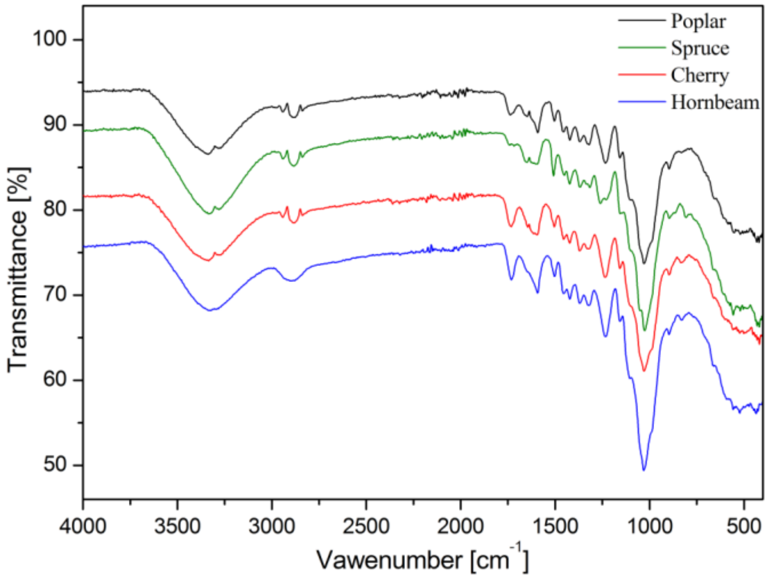

Fig. 1 Infrared spectra of selected wooden sawdust

3.2 Static adsorption study

Results of sorption experiments under static conditions for solutions with Cu(II) ions concentrations 10 mg.L-1 are shown in Table 1. The data suggest that used wooden sawdust has a very good efficiency of Cu(II) ions removal. The most efficient sorption was for poplar sawdust with efficiency about 86 %. It is comparable to the results of research Šćiban et al. [15]. They found that 2 g of poplar sawdust on 100 mL of contaminated water is efficient for removal of most of the Cu(II) ions.

The adsorption experiments were accompanied by decreasing of pH values in solutions. The pH of the solutions is an important controlling parameter in the adsorption process, since pH affects the surface charge of the adsorbent, the degree of ionization and speciation of sorbate during adsorption [29]. Authors [14] found that at lower pH values, the positively charged metal ion M^+ species may compete with H^+ and be absorbed by the ion-exchange mechanism. At an increased or neutral pH, metal ions M^+ may be absorbed by the hydrogen bonding mechanism along with ion-exchange mechanism.

Table 1 Results of static adsorption experiments with selected wooden sawdust (c_0(Cu(II))= 10 mg.L^{-1})

Sorbent	$Cu(II)$ **Initial** pH**= 5.8**		
	c_e **[mg.L^{-1}]**	η **[%]**	pH
Poplar	1.42	85.8	5.2
Spruce	1.90	81.0	4.9
Cherry	2.30	77.0	4.9
Hornbeam	2.72	72.8	5.2

3.3 Dynamic adsorption study

The poplar wooden sawdust exhibited under static conditions the best adsorption properties. From this reason these sawdust was used for detailed study under dynamic adsorption mode. The efficiency of Cu(II) ions removal and changes of pH values over time are shown in Table. 2. Using the poplar sawdust for absorption experiment indicates the rapid progress of sorption. After 5 min of sorbent-sorbate interaction, approximately 88 % of Cu(II) ions were removed from the model solution. The residual time of experiment can be considered as a relative settled with slower changes of removal efficiency. From this results we could suppose that the removal of Cu(II) ions might occur a two-stage process. At the beginning of the sorbent-sorbate interaction, the intensive increase in pH is accompanied with ion-exchange. In the second stage, the adsorption of metals ions takes place at stabilised pH values. The highest efficiency of Cu(II) removal from model solution (98 %) was observed after 30 minutes.

Changes of pH values in solutions were also observed. A significant change of pH value was revealed at the begging of dynamic experiment. Authors [30] observed that intensive pH changes are caused by the high initial concentration of dissolved heavy metals as a result of intensive ion exchange. The change of pH value was recorded after 5 minutes of the adsorption (from 5.8 to 6.1). During the increase of pH, Cu(II) ions were absorbed by mechanism of ion exchange by hydrogen bonding, while with the decreasing metal ion concentrations the ion exchange is not so dominant and does not affect the pH [31]. After completion of the ion exchange, the pH began to decrease gradually to the approximately input values.

Table 2 Results of metal sorption efficiencies η and changes of pH values over the experimental time at dynamic conditions

Time	**[min]**	0	5	10	15	30	45	60	120
c_e	**$[mg.L^{-1}]$**	10.0	1.22	0.88	0.72	0.24	0.68	0.50	1.12
η	**[%]**	0.0	87.8	91.2	92.8	97.6	93.2	95	88.8
pH		5.8	6.1	5.9	5.8	5.7	5.7	5.7	5.7

3.4 Neutron activation analysis of wooden sawdust

The NAA data obtained for raw wooden sawdust and Cu(II)-loaded wooden sawdust are presented in Table 3. The absorption experiments of Cu(II) ions was influenced with the content of the other elements in the sorbents. The amount of potassium in the Cu-loaded wooden sawdust after the 24-hour adsorption experiments decreased under the detection limit. A significant decrease (10 times) in the calcium content of Cu-loaded wooden sawdust was also observed for spruce sawdust. Based on the obtained NAA results, it can be concluded that ion exchange is one of the mechanisms underlying copper removal by the wooden sawdust. A change of the sodium, magnesium, chlorine, and barium content in the sawdust was also observed; the most pronounced chlorine decrease was noted for spruce and hornbeam, significant magnesium decrease was observed for spruce and cherry.

Table 3. Chemical composition of natural wooden sawdust and Cu-loaded wooden sawdust

Element	**Average content of elements in adsorbents $[mg.kg^{-1}]$**					
	Na	**Mg**	**Cl**	**K**	**Ca**	**Ba**
Poplar	12.5 ± 0.9	264 ± 18.5	12.9 ± 1.42	1790.0 ± 286	1810.0 ± 181	32.1 ± 1.6
Poplar_Cu(II)	12.3 ± 0.9	174 ± 12.2	10.4 ± 1.14	**< 151.0***	**1520.0 ± 152**	24.5 ± 1.23
Spruce	5.8 ± 0.4	497 ± 34.8	51.9 ± 5.71	287.0 ± 45.9	5210.0 ± 521	5.4 ± 0.54
Spruce_Cu(II)	17.9 ± 1.3	37.4 ± 2.62	11.6 ± 1.28	**< 63.0***	**495.0 ± 49.5**	4.0 ± 0.4
Cherry	21.2 ± 1.5	175 ± 12.3	27.8 ± 2.5	606.0 ± 97	1040.0 ± 104	6.5 ± 0.52
Cherry_Cu(II)	15 ± 1.1	8.7 ± 0.61	29.6 ± 2.66	**< 232.0***	**697.0± 69.7**	3.7 ± 0.29
Hornbeam	48.6 ± 3.4	376 ± 26.3	35.0 ±3.85	1570.0 ± 267	4350.0 ± 435	30.2 ± 1.51
Hornbeam_Cu(II)	113 ± 7.9	220 ± 15.4	14.1 ± 1.55	**< 214.0***	**2660.0 ± 266**	26.4 ± 1.32

* Values under detection limits

4 Conclusions

The wooden materials as sawdust are promising by-product that they are used in the removal of metal ions mainly in their natural form or after some physical or chemical modification.

The FT-IR analysis of the wooden sawdust confirmed the presence of the functional groups that they are able to bind heavy metal ions as was also confirmed by static adsorption experiments with efficiencies of Cu(II) ions removal approximately 80 %.

At dynamic conditions the sorption processes reached approximately 88 % efficiency already after 5 min. The highest removal efficiency of Cu(II) (98 %) was observed after 30 min. Significant changes of pH were recorded after 5 min of adsorption as a consequence of ion exchange between metal ions in model solutions and chemical elements in poplar wood sawdust.

The neutron activation analysis revealed a decrease of concentration of the certain elements (K and Ca) after the adsorption, indicating that ion-exchange is one of the mechanisms underlying the interaction of metal with the natural sorbents.

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ADSORPTION PROPERTIES OF MODIFIED BENTONITE FROM THE SLOVAK DEPOSIT

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Abstract

This article examined the impact of natrification agents and methods on the adsorption properties of bentonite. The natural bentonite with the most amount of calcium cations in the interlayer space was first modified in the slurry, which contained the activating agent - Na_2CO_3 and distilled water. The second modification of natural bentonite was carried out in suspension through NaOH as activating agent. The shift of peak of the basal plane peak towards the higher values of 2 Theta for modified bentonites was due to the cation exchange of Ca^{2+} with Na^+ cations with smaller atomic radius. The diffraction pattern of Na_2CO_3/bentonite, especially NaOH/bentonite indicates overlapping peaks, resp. decrease in crystallinity probably due to the unequal natrification of the structure of the montmorillonite.

Keywords: bentonite, natrification, sorption

1 Introduction

Bentonite is one of the most popular clay rock with exceptional adsorption properties. The main clay mineral presents in bentonite is montmorillonite which belongs to smectite mineral group. The properties of bentonite result from the crystal structure of this group. The particles of montmorillonite have negative charges on their faces due to isomorphic substitutions in its structure. This negative charge is compensated by the presence of the cations in the interlayer space, which are not fixed and have the character of so called "exchangeable cations"(i.e. Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}) [1]. It is possible to use bentonite as a sorbent either without any treatment or in its modified form. The chemical modification of bentonite is feasible due to the presence of water molecules at the surface and exchangeable cations in the interlayer space of montmorillonite structure. The basic chemical treatment includes preparation of the monoionic form of bentonite which can be received by the ionic exchange of cations of the alkali metals or alkaline earth metals. Example of such modification is saturation with monovalent cations of sodium. This process is called natrification. Different natrification agents may be used for this purpose such as NaCl, NaF, Na_2CO_3, NaOH. Natrification salt Na_2CO_3 is used almost exclusively thanks to its financial accessibility [2, 3]. Most likely, cations of sodium have dispergative effect on the montmorillonite structure which means they prevent aggregation of particles and thereby increase the specific surface area. On the other hand, polyvalent cations such as calcium have coagulative effect, they support clustering of particles [4]. The paper deals with the characterization and use of activated bentonites as adsorbents for the removal of heavy metals from aqueous solutions.

2 Material and methods

For the experiments, natural bentonite from the deposit of Stará Kremnička – Jelšový Potok with the chemical formula $[Si_{7.95}Al_{0.05}][Al_{3.03}Fe_{0.22}\ Mg_{0.75}]\ O_{20}(OH)_4\ (Ca_{0.42}Mg_{0.04}Na_{0.01}K_{0.01})$ was used [5]. This bentonite was pre-treated by the sedimentation method, while almost monomineral fraction of montmorillonite with the size of particles below 20μm was received [5, 6]. Bentonite with montmorillonite as a main clay mineral was first activated by Na_2CO_3 by "semidry" process. The slurry contained Na_2CO_3 as activating agent (4 mass %), destilled water and natural bentonite with the content of montmorillonite higher than 80%. After 24 hours stabilization, natrified bentonite was dried at 60 °C. Second sample was prepared using NaOH as activating agent by wet method of natrification. Natural bentonite (also with the content of montmorillonite higher than 80%) was mixed with 3.5 M solution of NaOH. Stabilization takes place 72 hours and final sample was dried at 60 °C.

X-Ray diffraction data of studied samples were obtained by a diffractometer Bruker D8 Advance (40 kV, 40 mA) working with CuKα radiation. The JCPDS (Joint Committee for Powder Diffraction Data - International Centre for Diffraction Data) database was used to interpret the diffraction peaks of the investigated samples. The important parameter to evaluate the sorption properties of the as-received and activated bentonites is the CEC (Cation Exchange Capacity). It is defined as the quantity of cations reversible adsorbed by clay particles, expressed in milliequivalents per 100g of dry clays [7]. The methylene blue stain test makes it possible to quantify the cation exchange capacity of the samples by measuring the quantity of methylene blue cations necessary to cover the total (external and internal) surface of the particles [8]. Laboratory tests were performed as combination of two applicable standards: AFNOR (French Association for Standardization) and ASTM (American Society for Testing and Materials). All the measured samples and methylene blue powder were first dried at 105 °C. 2g of each sample and methylene blue solution with the concentration of 10g/l were used to determine CEC. The methylene blue solution was titrated stepwise first in 5 and then in 1 ml doses to a stirred bentonite suspension [9]. The end point of titration of the methylene blue adsorption was determined when light blue halo appeared around the solid particles. In order to compare the sorption properties of the studied materials, sorption of cadmium, lead, zinc cations from synthetic solutions $Cd(NO_3)_2.4H_2O$, $Pb(NO_3)_2$ and $Zn(NO_3)_2$. $6H_2O$ were performed at the ambient temperature. The initial concentration of cadmium, lead and zinc was in the range of 10 to 700mg/l. The concentration of adsorbent was 1g/l. The sorption was done in polyethylene tubes on the rotary shaker. Shaking took 24 hours. The concentration of the metals was determined by the method of atomic adsorption spectroscopy (Varian 240 RS/2400).

3 Results and discussion

Preparation of bentonite monoionic forms (natrification) is a common method of the chemical activation of bentonite. Activation takes place by cation exchange method inside the interlayer space of montmorillonite structure due to presence expandability of montmorillonite structure and exchangeable cations (Fig. 1).

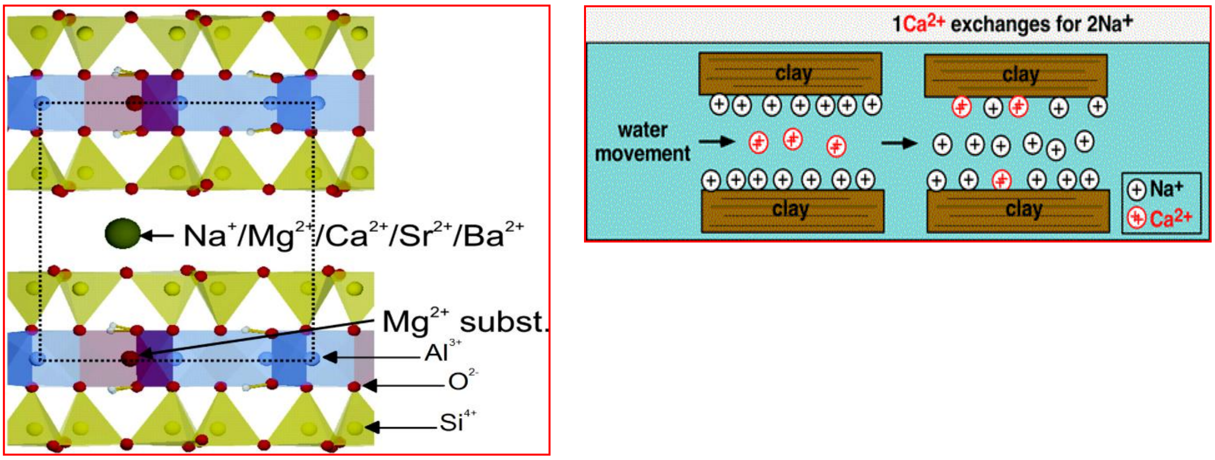

Fig. 1. Expandability of structure and presence of exchangeable cations in the interlayer area of montmorillonite

The X – ray diffraction analysis of natural bentonite confirmed the dominant presence of montmorillonite with Ca^{2+} cations at the exchangeable positions (Fig. 2). The effect of the natrification was observed by comparing basal plane diffraction before and after activation of the montmorillonite structure. The shift of peak of the basal plane peak towards the higher values of 2 Theta for modified bentonites was due to the cation exchange of Ca^{2+} with Na^{+} cations with smaller atomic radius. The diffraction pattern of Na_2CO_3/bentonite, especially NaOH/bentonite indicates overlapping peaks, resp. decrease in crystallinity probably due to the unequal natrification of the structure of the bentonite.

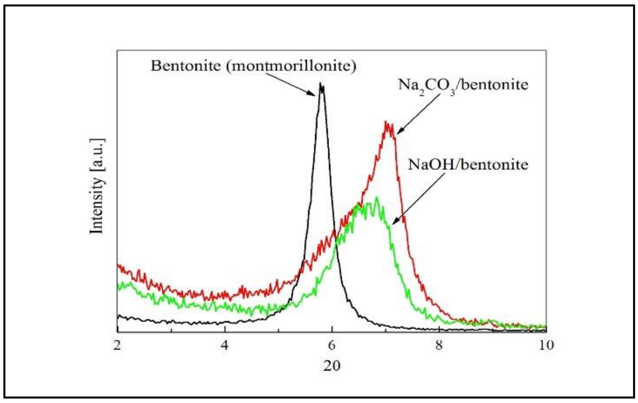

Fig. 2 X-ray diffraction pattern of the natural and modified bentonite

Natrification – decrease of interlayer space: 1.51 nm (bentonite) → 1.24 nm (Na_2CO_3/bentonite) and 1.31 nm (NaOH/bentonite).

From the final consumptions of methylene blue solution values of CEC were calculated according to the formula [9, 10]:

$$CEC = \frac{V \frac{C}{M_r}}{m} 100 \qquad (1)$$

where V is the total volume of methylene blue solution titrated (ml), C is the concentration of methylene blue solution (g/l), M_r is the molecular mass of methylene blue and m is the sample mass (g). The data of CEC for all measured samples are shown in Table 1.

Table 1 Values of CVC for natural and modified bentonites

Sample	**CVC [meq/100g]**
Natural bent.	84.4
Na_2CO_3/bent.	114.1
NaOH/bent.	115.9

The changes in the structural properties of the chemically modified samples of bentonite show the possibilities of their use for the sorption purposes. The Fig. 3-5 show the isotherms of $Cd^{2+,}$ Pb^{2+} and Zn^{2+} sorption on the modified samples, while the sorption data were fitted with the help of linear shape of Langmuir isotherm [11]:

$$\frac{C_e}{q_e} = \frac{1}{Q_m b} + \frac{C_e}{Q_m}, \qquad (2)$$

where C_e is the equilibrium concentration of the metal ions in the solution, q_e is the amount of the adsorbed metal related to the weight unit of the adsorbent (mg/g), Q_m represents the maximal adsorption capacity (mg/g) and b is the sorption equilibrium constant (L/mg). The values of Langmuir parameters for the sorption of Cd^{2+}, Pb^{2+} and Zn^{2+} cations are given in Tables 2-4.

Table 2 Langmuir parameters for sorption of Cd^{2+}

Sample	**Q_m [mg/g]**	**b [L/mg]**	**R^2**
Na_2CO_3/bent.	72.99	0.0265	0.9967
NaOH/bent.	81.97	0.0184	0.9884

Table 3. Langmuir parameters for sorption of Pb^{2+}

Sample	**Q_m [mg/g]**	**b [L/mg]**	**R^2**
Na_2CO_3/bent.	208.33	0.0489	0.9902
NaOH/bent.	249.8	0.0498	0.9905

Table 4. Langmuir parameters for sorption of Zn^{2+}

Sample	**Q_m [mg/g]**	**b [L/mg]**	**R^2**
Na_2CO_3/bent.	76.34	0.0181	0.9928
NaOH/bent.	83.33	0.0132	0.9902

The natrification of bentonite has improved its adsorption properties. Bentonite modified by NaOH reached higher maximum adsorption capacities for each of tested heavy metals.

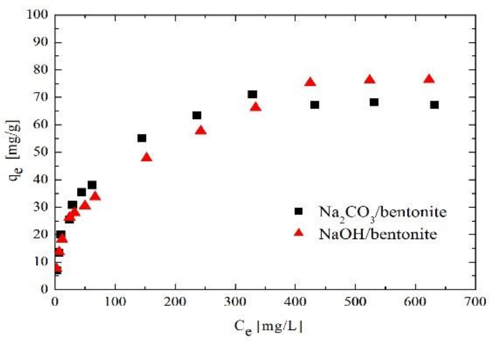

Fig. 3 The adsorption isotherms of Cd^{2+} sorption

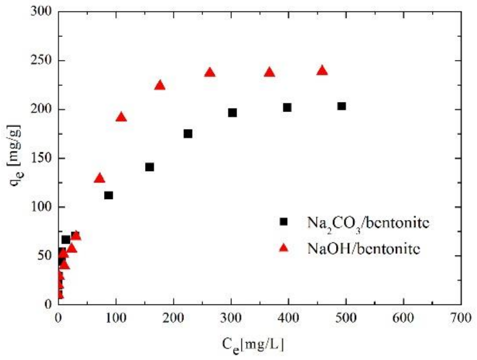

Fig. 4 The adsorption isotherms of Pb^{2+} sorption

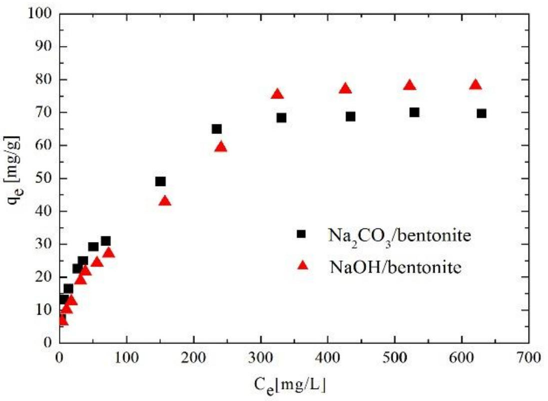

Fig. 5 The adsorption isotherms of Zn^{2+} sorption

4 Conclusions

The qualitative phase analysis of studied sample confirmed the presence of montmorillonite. Effect of natrification can be observed by the comparison of basal plane diffractions d_{001} before and after modification. Shift of NaB peak to the right on x axis points on the cation exchange from interlayer space for cations with the smaller atomic radius. Chemical activation of bentonite like natrification belongs to cost saving and rapid method to increase of ion exchange – adsorption properties of bentonite. The comparison of adsorption properties: bentonite modified by NaOH reached higher maximum adsorption capacities for each of tested heavy metals.

Acknowledgements

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BACTERIAL LEACHING OF POLYMETALLIC ORES FROM ZLATÉ HORY LOCALITY

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Abstract

This work deals with the use of *Acidithiobacillus ferrooxidans* to bioleach polymetallic ores (Cu, al, Zn, and Fe) from locality Zlaté hory. As for geology, Zlaté Hory is a highly varied locality with frequent, quartz, muscovite, albinite as well as deposits of sulphide ores (pyrite, chalcopyrite). Leaching lasted for 5 weeks in a bioreactor that ensured optimal conditions for bioleaching. The results imply that the experiments were successful, leaching 54% of Fe, 47% of Zn and 4% of Cu.

Keywords*:* bacterial leaching, pollymetallic ores, pyrite, chalcopyrite, Acidithiobacillus ferrooxidans, bioreactor

1 Introduction

1.1 Description of Zlaté Hory locality

Zlaté Hory is a geologically diverse locality. The most frequent rocks are metaquartzites, amphibolites, quartz and sulphidic ores. The existing raw material and mineral resource policy of the Czech Republic does not consider reopening of mining in the locality. However, based on the statistical data provided by the Czech Ministry of the Environment in 2016, Zlaté Hory is still registered as a source of Au, Ag, Cu, Pb and Zn. Metal extraction in the locality is impeded by legal requirements and economic limitations of the current era. [1] From the point of view of the Czech mineral wealth, the good news is that the resources are available, and it is the development of mining technologies and the development of the world mineral market that will be the key for future mining. [2]

The origin of the studied sediment is guaranteed by the state enterprise Diamo. This company is responsible for treating the mine water in the locality and the mine water character is subject to regular monitoring. The locality is protected from mineralized water, which represents a potential threat for groundwater. The maximum karst permeability is about 100 m below ground level. Therefore, we find there a system of channels finished with wells, from which water is pumped into a seepage pit. The second source of water is a water treatment plant for bottom headings and acid mine drainage. The mine wastewater contains diverse minerals, which leads to the acid character from pH 2.3 to 2.8. The water is accumulated for final clarification, where it is neutralized by lime hydrate. Water with iron and copper content is conducted into the channels mentioned above, from which it is pumped. Thanks to lime hydrate in the settling tank, the components coagulate under the formation of thick sludge. The neutralized thick sludge is pumped into a secondary settling basin, which helps to make the process more efficient. The primary sludge is pumped into a settling lagoon for sludge sedimentation and once a year the sediment is pumped from the lagoon to the sludge disposal site. Technologically, the sludge disposal site is equipped with pipes to collect the water seeping through the sludge to be further used in the process. [1]

1.2 Bioleaching

The process of bioleaching may be defined as the dissolution of minerals caused by direct or indirect action of diverse microorganisms. In the course of such processes, the natural flora of microorganisms consists of a mixture of acidophilic autotrophic bacteria. [2] Their role is to produce chemical agents vital for leaching. [3] It is a simple and effective method to recover metals from mineral raw materials, where conventional methods cannot be applied. The fundamental mechanism is the direct or indirect oxidation of sulphide ore. Through oxidation, metal releases into solution, from where it can be recovered using conventional chemical-physical methods. In this case, we aim to recover metals from sulphide minerals through the activity of bacteria of *Acidithiobacillus* genus, which convert insoluble metal sulphides into soluble sulphates. With regard to the character of the sample, we selected the most suitable species to leach sulphide ores, i.e. *Acidithiobacillus ferrooxidans*. [4,5,6]

1.3 Bacteria *Acidithiobacillus*

Acidithiobacillus is considered a biotechnologically and ecologically significant group of bacteria that is the most active in the dissolution of sulphides. It is Gram-negative, acidophilic, rod-like bacteria that thrive in acidic media of a low pH between 1.8 and 2.5. They gain energy from the oxidation of sulphur and reduced sulphur compounds, or via the oxidation of bivalent iron and hydrogen. Considering their metabolic properties, they are suitable to process mineral and electronic waste, and for the desulphurisation of gas and coal. A negative aspect of the metabolic activities of the bacteria is biocorrosion due to the production of sulphuric acid. As for their physiological and morphological characteristics, it shows that *Acidithiobacillus* genus contains at least seven species, e.g. *Acidithiobacillus ferrooxidans, Acidithiobacillus ferridurans, Acidithiobacillus ferrivorans* and *Acidithiobacillus ferriphilus*, *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus* and *Acidithiobacillus albertensis*. [7,8]

Acidithiobacillus is a group of microorganisms important for the dissolution of copper, zinc, iron and arsenic from diverse ores, sediments and waste [9]. Their genes determine the transport routes of nutrients, including K, P and Fe, which are vital for the intercellular balance as well as elimination of toxic elements, such as Hg, Pb, As, Cr, Cd and Ag. [10]

2 Materials and Methods

The samples come from Zlatý Chlum. Subsequently they were processed in the laboratory of the Department of Environmental Engineering at VŠB-TU Ostrava using a vibrating attrition mill. The samples were ground to the grain size fraction -0.040 mm. The sediment is dark brown and is highly viscous. The grain size is below 0.5 mm. The dark brown colour is accompanied by a visible shine due to a high water content. Mineralogical tests and metal content tests were carried out when preparing the sediment for the experiments. The sediment is analogues to sand abrasives. Thanks to the modifications of sediment pH by lime hydrate before disposal, the sediment pH was 7.8.

2.1 Mineralogical analysis

The mineralogical composition analysis of the samples was carried out. An X-ray diffraction analysis was done in the laboratories of the Institute of Geological Engineering at VŠB-TU Ostrava. The measurements were carried out on a modernized, fully automated diffractometer URD-6 (Rich. Seifert-FPM, SRN). The following phases were identified on the samples in question: quartz (68.14%), chlorite (5.02%), muscovite (13.96), albite (6.51%), pyrite (5.47%), sphalerite (2.05%). (See Table 1).

Table 1 Results of X-ray diffraction

Name of mineral	Content (%)
Quartz	68.14
Sphalerite	2.05
Chlorite	5.02
Chalcopyrite	0.52
Albite	6.51
Pyrite	5.47
Siderite	0.55

The sediment element analysis was carried out using XRF Delta professional. The analysis shows that the most abundant element is Ca, which may be most likely explained by the sediment treatment in Diamo. The most abundant metals are Fe, Zn and Cu in the concentrations 6.04 % 5.79 % and 3.95 % respectively.

Table 2 Results of XRF analysis

Element	Content (%)
Cu	3.95
Si	2.59
Ca	12.36
Mn	1.76
Fe	6.04
Al	1.51
Zn	5.79

1.1. Methodology of bacterial leaching

For the bioleaching process, we prepared 9K medium according to Silverman and Lundgren, in which *Acidithiobacillus ferrooxidans* was cultivated.

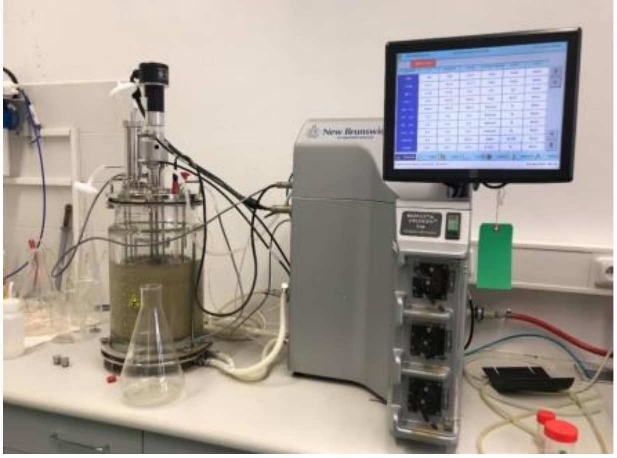

Fig. 1 Bioreactor Bioflo 310

The leaching process took place in New Brunswick BIOFLO®&CELLINGEN® 310 (Figure 1). Having sterilized the bioreactor, 500 g of sample from Zlaté Hory at 100% grain size under 0.040 mm and 10 litres of 9K medium free of $FeSO_4$ were inserted inside the bioreactor. After one-hour stirring and homogenization of the suspension, a bacterial culture of *Acidithiobacillus ferrooxidans* in the amount of 880 ml was introduced into reactor. A pre-cultivated culture of *Acidithiobacillus ferrooxidans* from the Institute of Microbiology in Brno was applied.

The bioreactor New Brunswick BIOFLO®&CELLINGEN® 310 is a glass vessel of 14 l. To make the bioleaching process most effective and yielding, it is vital to ensure optimal conditions for the growth of bacteria. The bioreactor Bioflo 310 is able to maintain constant pH of the environment, temperature, degree of aeration, concentration of CO_2 and O_2, and rate of agitation.

Conditions:

- Dissolved O_2:100/l
- Temperature: 30°C
- pH: 1.8
- Agitation: 150 RPM
- Leaching time: 5 weeks

The samples were leached for 5 weeks. In the course of leaching, samples were drawn after the 1st, 2nd, 3rd, 4th and 5th week. All the samples were washed in 0.1.mol HCl and later in distilled water to stabilize pH. The drawn samples were analysed using a portable spectrometer Dynamic XRF DELTA PROFESSIONAL by BAS Rudice s.r.o. In the course of experiment, the colour of the leached sample changed. At the start, the colour was dark brown and it became lighter during the experiment, which was likely caused by the highly acidic environment.

3 Results and discussion

The results showed that the application of *ATF* bacteria disrupted the strong sulphidic bonds and led to the release of metals into the solution. We observed the following metals of interest: Fe, Zn and Cu.

3.1 Leaching of Fe

The feed percentage of Fe in the sample was 6.04 %. During the first week of bacterial leaching, no leaching bacterial activity was observed. It appears that the bacteria were adapting to the environment and they replicated and grew slowly. As a result, only 2% of Fe passed into the solution (Table 3). In the second week of bioleaching, there was a prominent increase in Fe release amounting to 6.62 %. Bacteria vitality was verified in a test and only a slight increase in the bacterial culture was observed. Therefore, a new bacterial culture was added into the sample.

In the following weeks (third to fifth) the metal recovery grew gradually. In the third week the recovery of Fe was 30.05 %, in the fourth it was 37.25 % and 54.12 % in the fifth.

Table 3: Leaching of Fe

Leaching time	Metal concentration (%)	Recovery of metal (%)
Input	6.04	
1. week	5.91	2.15
2. week	5.64	6.62
3. week	4.01	33.60
4. week	3.79	37.25
5. week	3.26	54.12

3.2 Leaching of Zn

Zinc was 5.79 % at the input. Similarly to iron, hardly any leaching occurred in the first week. The decrease was only 0.35 %. In the second week, the bacterial culture must have adapted as the Zn decrease was already 15.71 %. The drops in Zn concentration were more prominent in the remaining weeks of leaching - see Table 4. The overall zinc recovery after 5-week leaching was 47.32 %. It is well established that zinc in the form of ZnS leaches better in the presence of other minerals, especially FeS_2. This was also confirmed by the tests reported herein, and the experiments showed that zinc may be successfully leached in a bioreactor using *Acidithiobacillus ferrooxidans*.

Table 4: Leaching of Zn

Leaching time	Metal concentration (%)	Recovery of metal (%)
Input	5.79	
1. week	5.74	0.35
2. week	4.88	15.71
3. week	4.15	28.32
4. week	3.90	32.64
5. week	3.05	47.32

3.3 Leaching of Cu

The input percentage of Cu in the sample was 3.95 %. In the course of leaching, the copper concentration hardly changed. The bacterial culture was revived after two weeks as the copper recovery stayed low. After 5-week leaching, the copper content decreased to 3.79 %, which corresponds to the overall copper recovery of 4.05 %. (Table 5)

Table 5: Leaching of Cu

Leaching time	Metal concentration (%)	Recovery of metal (%)
Input	3.95	
1. week	3.94	0.25
2. week	3.92	0.75
3. week	3.90	1.26
4. week	3.81	3.54
5. week	3.79	4.05

4 Conclusion

The samples were drawn from the locality Zlaté Hory, in the Czech Republic. Bacterial leaching was carried out under pilot-plant conditions in a bioreactor New Brunswick BIOFLO®&CELLINGEN® 310 using the bacterial strain of *Acidithiobacillus ferrooxidans*. This strain was selected for its fitness for the sample in question. The drawn samples were ground below 0.040 mm grain size, mixed with Silverman 9K medium and leached with the bacteria in the bioreactor.

The experiment lasted for 5 weeks (35 days) under optimal conditions set for the bioreactor. With regard to the mineralogical composition of the sample, we observed the following metals: Fe, Zn and Cu. The most striking was the course of iron leaching, during which only 2% leached in the first week, followed by a rapid increase to 54% in the weeks after. With regard to the lower metal recovery in the first two weeks of leaching, fresh bacterial culture was added into the bioreactor to revive the leached sample. This step showed as successful in leaching Fe and Zn. The course of zinc leaching was analogous to the leaching of iron. The metal recovery was higher in the third week. The Zn recovery was 47%. The lowest metal recovery was obtained in leaching copper, i.e. only 4% Cu. Therefore, the used method is not suitable for leaching copper and it is necessary to choose a different bacterial strain, or a different processing method. Nevertheless, bacterial leaching of sediments from mine water treatment has its advantages and it may be successfully applied as an alternative processing method and recovery of certain metals (Fe and Zn). The disadvantage is the long duration of the leaching process.

Acknowledgement

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ANALYSIS OF AUTHOTROPHIC SULPHUR OXIDISING BACTERIA FROM SLOVAK GOLD MINE HODRUŠA-HÁMRE

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Abstract

Sulphur-oxidising autotrophic bacterial communities in deep biosphere from weathered ore samples collected from active gold mine Hodruša-Hámre, Slovakia were analysed using cultivation approach followed by DNA extraction, PCR amplification and 16S rRNA gene analyses. Indirect measurement of pH changes in cultivation media evidenced the presence of acidophilic bacteria with active production of acids, decrease of pH was observed at the beginning of isolation and later pH in range of 1.5 – 2 was maintained in both, sulphuric acid and thiosulphate, media. The presence of homogenous population of gram-negative rods was proved by Gram staining. Molecular analyses have revealed that the population of sulphur-oxidising bacteria in gold mine is dominated by a single species of *Aciditiobacillus* genus, particularly *A. albertensis*, suggesting the low level of autotrophic bacteria diversity in deep deposits. For the first time this species was isolated from weathered rocks of a gold mine subsurface environment.

Keywords: autotrophic bacteria, *Acidithiobacillus albertensis*, gold mine

1 Introduction

In the past years, our perspective on the Earth's biosphere has expanded from just the terrestrial and oceanic realms to include deep subterranean and subseafloor environments [1]. The deep terrestrial subsurface environments such as those exemplified by deep mines represent an emerging area for exploring microbial populations with bewildering arrays of metabolic capabilities [2]. Majority of these environments are characterised as extreme because of their hostile life conditions such as extreme temperature, pH, pressure, low oxygen content, no light and toxic metals presence. The existence of microorganisms in such environment is of increasing scientific and practical interest because subsurface microorganisms with novel metabolic properties may be of potential value to industry for applications in bioremediation and biotechnology [3]. Microbes in the deep terrestrial subsurface environment are considered to play a key role in deposition and weathering the minerals being a part of geochemical processes and are the only life forms that have been encountered in the deeper regions of the Earth´s crust [4].

Microbial communities existing in deep subsurface soil especially in gold mines have been studied very rarely and therefore they remain largely uncharacterized. The Banská Hodruša Au + Ag, Pb, Zn, Cu deposit at the Rozália mine (48°27´N, 18°51´E) is the last operating ore mine in Slovakia and it offers a unique opportunity for direct exploration of the mining-impacted deep subsurface environment. It occurs in the central zone of the large Middle Miocene Banská Štiavnica stratovolcano (15.0 – 10.7 mil.years) within the Banská Štiavnica – Hodruša ore district, which belongs to the largest ore districts in the Carpathian arc, famous for Ag-Au mining since the Middle Ages. The subhorizontal vein system occurs in 400-650 depth, it is about 1,2 km long, hosted by andesite, near to the flat roof of the pre-mineralization subvolcanic granodiorite pluton. The deposit consists of two parts, separated by a thick sill of quartz-diorite porphyry. The eastern part is currently mined, and the western part has already been depleted [5, 6].

Gold–sulphide ore from epithermal vein deposit Banská Hodruša consists of gold, electrum, galena, sphalerite, chalcopyrite, and pyrite. Minor amounts of tellurides are present in the form of hessite, petzite and other rare Au sulphides and tellurides. Gangue minerals are represented by abundant quartz and adularia, clay minerals and carbonates. The ore has excellent metallurgical properties, content of gold is in range of 5 – 600 g/t, with average 20 – 30 g/t and maximum achievable gold recovery by flotation is 96–98 % [7].

Microbial diversity present in Hodruša-Hámre mine have not been reported yet. In the present study, our objective was to elucidate the existence and composition of sulphur-oxidising autotrophic bacterial population in the weathered ore samples of Hodruša-Hámre gold mine by culture-based molecular methods.

2 Materials and Methods

2.1 Sample collection

The samples (500 g each) were collected along the junction of the drift wall and the floor in vein Blanka. Yellow mats were covering the rock surface around the sample colleting site. The area was not disturbed by any type of activities including human trafficking from the end of 2000. The weathered ore was collected 10 cm below the surface with sterile spatula. The temperature at the time of sampling was 19°C, it was measured using VWR EU 620-1259 thermometer. The samples were transported to the laboratory in sterile zip lock sacks on ice and in refrigerator.

2.2 Isolation of autotrophic bacteria

6 g of homogenized soil sample was resuspended into 100 ml of sulphuric acid with pH 1.5 (medium 1) and 100 ml of thiosulphate medium (medium 2). The composition of thiosulphate medium was 90 ml of mineral medium and 10 ml of 10% $Na_2S_2O_3$, mineral medium was composed of 1.2 g KH_2PO_4, 0.2 g K_2HPO_4, 0.75 g $MgCl_2.6H_2O$, 0.15 g $CaCl_2.2H_2O$, 0.5 g NH_4Cl, 0.5 g Na_2CO_3 into 1000 ml of deionised water. Few drops of HCl were added into sterilised mineral media to dissolve precipitates. Cultures were cultivated statically at 25°C and pH values of media were measured once a week for 237 days. Fresh media (20 ml) were added into culture flask after 29 days of cultivation.

2.3 DNA isolation, 16S rRNA gene amplification, RFLP analysis and sequence analysis

Genomic DNA was extracted from bacterial cultures as described by Chen et al. [8].

Amplification of 16S rDNA fragments was performed with fD1 (5’-AGAGTTTGATCCTGGCTCAG-3’) and rP2 (5’-ACGGCTACCTTGTTACGACTT-3’) universal bacterial primers according to Vandžurová et al [9]. Products were compared by performing restriction fragment length polymorphism (RFLP) analysis with restriction endonucleases AluI, HpaII, HaeIII, HhaI, EcoRI and PstI (Thermo Scientific, USA) according of the manufacturer’s instruction. The restriction fragments were separated by electrophoresis in 1% agarose gel.

The 16S rDNA amplicon of the culture from thiosulphate medium was cloned into the pTZ57R/T vector using an InsTAclone PCR Cloning Kit (Thermo Fisher Scientific, USA) following manufacturer’s instructions. Recombinant plasmids were isolated with GenElute^{TM} Plasmid Miniprep Kit (Sigma-Aldrich, USA), checked on 1% agarose gel and purified using Wizard$^{®}$ SV Gel and PCR Clean-Up kit (Promega, USA). Sequencing was performed using Sanger dideoxy sequencing method using plasmid specific primers by GATC Biotech sequencing facility (GATC Biotech AG, Germany).

Sequences from both primers were assembled in CAP3 Sequence Assembly Program (http://doua.prabi.fr/software/cap3) [10] and analysed using MEGA7 software [11]. The sequences were taxonomically classified using BLASTN analysis against a database of 16S rDNA sequences of the type strains of bacteria and archaea (http://blast.ncbi.nlm.nih.gov/Blast) [12].

3 Results and discussion

The presence and activity of sulphur-oxidizing bacteria in both media was indirectly observed by changes of the solution pH values (Fig. 1). In sulphuric acid (medium 1) slight increase of pH was observed, the pH value increased from 1.5 up to 2.6 at the 35th day followed by the decrease to 1.6 later on. After that time significant changes in pH were not observed till the end of monitoring period after 237 days. In thiosulphate medium (medium 2) with initial pH 4 significant decrease of pH was observed to 2.1 after 17th days of cultivation followed by the decrease to 1.54 after 94 days. This pH value was kept till the end of the observation period. Values of pH of 5.8 – 6.4 were measured in control experiments without bacterial activity so it is visible that bacterial activity is responsible for the pH decrease.

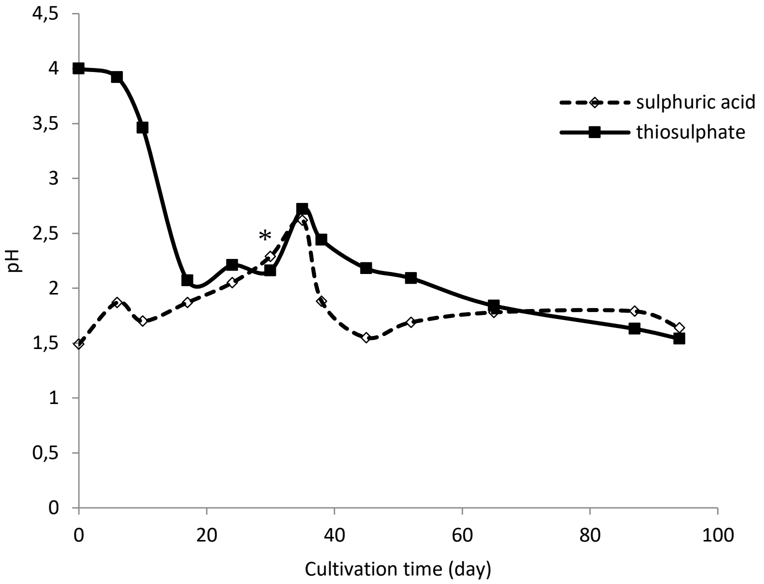

Fig. 1 Changes of pH during cultivation of bacteria in two cultivation media (*20 ml of fresh media were added into flasks after 29 days of cultivation)

Changes of pH in both cultivation media suggested acidifying activity of bacteria indirectly confirming presence of the bacteria. As no carbon source was added to the cultivation media bacterial activity observed have to be accounted for the presence of autotrophic bacteria.

Under microscopic observation a morphologically homogenous population of gram-negative rods was observed just in thiosulphate medium (Fig. 2). Bacterial DNA was however isolated from both media. RFLP analysis of the amplified 16S rRNA gene revealed genetic homogeneity of analysed bacterial population. Moreover, the identical RFLP banding pattern for both, H_2SO_4 and thiosulphate media, was observed (data not shown) indicating high genetic relatedness of autotrophic sulphur-oxidising bacteria grown in both media. The amplification and sequence analysis of 16S rRNA gene was used for molecular identification of bacteria grown in thiosulphate medium

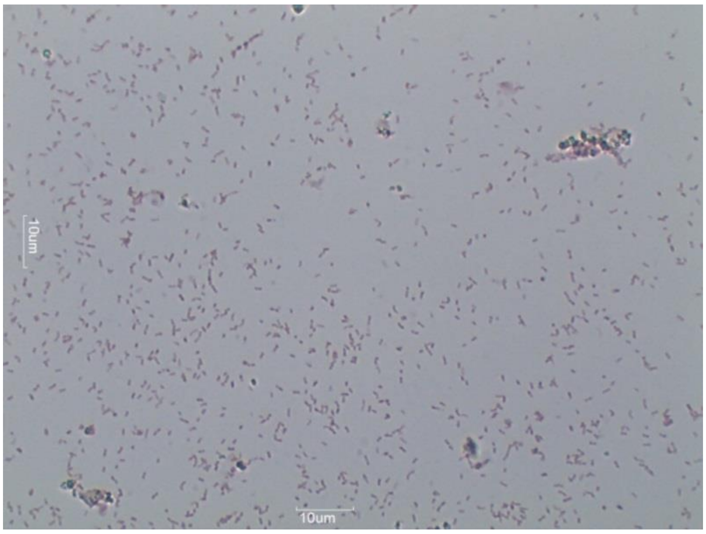

Fig. 2 Photograph of *A. albertensis* isolated from gold mine (magnification 640 x).

Analysis of obtained 16S rRNA sequence against the database of prokaryotic_16S_ribosomal_RNA available at NCBI site (http://www.ncbi.nlm.nih.gov/blast) shown that bacterium grown in thiosulphate medium is highly similar to the *Acidithiobacillus albertensis* DSM 14366/ATCC 35403 strain with similarity as high as 99.7%. Similarity to other well established species of *Acidithiobacillus* genus were lower e.g. *A. thiooxidans* (98.7%), *A. ferrooxidans* (97.7%) or *A. ferridurans* (97.6%), respectively. Based on these analyses the autotrophic sulphur-oxidising bacterium cultivated from weathered ore samples from active gold mine Hodruša-Hámre could be identified as *Acidithiobacillus albertensis*. It is the first time when this bacterium was isolated from gold mine subsurface environment. In previous studies these species were isolated from acidic soil adjacent to a sulphur stockpile [13] and from acid-mine drainage reach of sulphur [14]. *A. albertensis* are acidophilic, mesophilic, obligatory chemoautotrophic, aerobic, gram-negative rods with size 0.3 – 0.5 x 0.7 – 2 μm. They do not produce spores. The main difference between *A. albertensis* and its closest relative *A. thiooxidans* is lophotrichous locations of flagella responsible for their movement. They produce glycocalyx important in bacterial attachment to ore surface [15]. The main energy source is oxidation of S^0 and/or S^{2-}, $S_2O_3^{2-}$ and $S_4O_6^{2-}$ reduction [14]. They are able to fix CO_2 but not N_2. Temperature range for their growth is in the range of 10 – 40°C with optimum between 25 – 30°C. Optimum pH is 3.5 – 4, but they grow down to pH 2. They are forming yellowish colonies on solid sulphur-containing media.

Although *A. albertensis* species was for the first time described by Bryant et al. in 1983 [13], it is not very well known species. Up to now it was just found that this bacterium is able to bioleach metals. The genome of this bacterium was published just recently, the study revealed that the species is very similar to *A. thiooxidans* [15] so probably its biotechnological utilisation might be oriented more to sulphur dissolution or as a part of bioleaching consortium with iron-oxidising bacteria to metal dissolution.

4 Conclusions

The subsurface biosphere in gold deposits was very rarely studied, however, according to the newest findings it is obvious that bacteria actively contribute and regulate biogeochemical cycles in this environment. The diversity of culturable microorganisms involved in sulphur oxidation was investigated in the weathered rocks of gold mine Hodruša Hámre, Slovakia. Just the single species of *Acidithiobacillus albertensis* was isolated from the samples suggesting the low level of biodiversity of autotrophic bacteria in deep subsurface deposits. In this study we first time reported autotrophic bacterial diversity and isolation of *A. albertensis* from solid sample of weathered rocks in deep gold mine. Although *A. albertensis* is not a new species, still there is very little known about these bacteria and their potential in bioleaching of metals from ores or metal-bearing waste. Further studies of functional bacteria and potential new functional species need to be carried out to explore their specific contributions to gold biogeochemical cycling.

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THE FATE OF ANTIBIOTIC RESISTANCE DURING WASTEWATER TREATMENT

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Abstract

Antibiotics are widely used for the treatment and prevention of bacterial diseases. A serious problem of their intensive use is the development of antibiotic resistant bacteria and resistance genes. Bacterial community including resistant strains can be significantly reduced during wastewater cleaning process but on the other side, many studies documented that wastewater treatment plants can be reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. In our work, we investigated if wastewater treatment plant acts as a barrier for antibiotic resistant bacteria in the wastewater stream. The samples of inlet and outlet water were taken in the wastewater treatment plant for the city Košice in Kokšov-Bakša. After cultivation on R2A medium at 37 °C and laboratory temperature, bacterial isolates were identified by matrix assisted laser desorption/ionization time-of-flight mass spectrometry and their sensibility to ampicillin, kanamycin, tetracycline and chloramphenicol was examined. *Escherichia coli* was the most common bacterial species in inflow (46%), followed by *Aeromonas* spp. (17%) and *Klebsiella* spp. (11%). In outflow, *Aeromonas* spp. (38%) and *Acinetobacter spp.* (28%) showed the highest abundance. While microbial community was significantly changed during water treatment process, the occurrence of antibiotic resistance was not affected. In inlet water sample, 90% of isolates were resistant to ampicillin, 26% to kanamycin, 31% to tetracycline and 26% to chloramphenicol. In outlet, statistically important reduction was found only within isolates resistant to tetracycline. Based on these results, we can assume that the bacterial community in the wastewater treatment plant may represent a potential source of antibiotic resistant bacteria and genes, which could be further disseminated in an aquatic environment.

Keywords: bacteria, wastewater treatment plant, antibiotics, antibiotic resistance

1 Introduction

Antibiotics are widely used for improving human, animal and plant health and for preventing and treating infections caused by pathogenic bacteria. However, antibiotics are not completely degraded in the organism. Metabolic rates for the unchanged active compounds range from 10 to 90% and it is estimated that up to 70% of the used antibiotics are excreted unchanged into wastewater. So, their intensive use results into their release into the environment. A serious problem of their release is the development of antibiotic resistant bacteria and resistance genes [1]. Some bacteria (e.g. *Pseudomonas aeruginosa*) are naturally resistant to antibiotics, but secondary resistance can develop during contact of a microorganism with an antibiotics at their sub-inhibitory concentration. Secondary resistance may be mediated between bacteria by extra-chromosomal genetic material (e.g. plasmids) exchange by horizontal gene transfer [2, 3].

The main role of wastewater treatment plants (WWTP) is the elimination of pollutants including pathogenic microorganisms. Their efficiency varies depending on the purification process [4]. Some studies documented that the number of bacteria including resistant strains are reduced during the wastewater cleaning process up to 99% [5, 6]. On the other site, wastewater treatment plants are characterized by a high microbial density of biofilms and activated sludge and the presence of antibiotics at sub-inhibitory concentrations. These conditions may facilitate genetic exchange among bacteria and therefore WWTP can be important reservoirs of antibiotic resistant bacteria and antibiotic resistance genes persisting in the final effluent and spreading into the environment [7, 8].

In the present study, we investigated the prevalence of antibiotic resistant bacteria in the wastewater treatment process in Kokšov-Bakša WWTP near Košice (Slovakia).

2 Material and methods

WWTP in the village Kokšov-Bakša treats sewage from the city Košice and is located on the right bank of the Hornád River. Wastewater samples were taken in January 2018 at the water inlet and outlet into sterile 10 ml tubes. The samples were immediately transferred to the laboratory for further processing.

Water samples were serial diluted from 10^{-1} up to 10^{-10} in sterile phosphate-buffered saline and aliquots of 100 µl from each dilution were inoculated onto R2A medium and parallel incubated overnight at 37°C and laboratory temperature. One hundred isolates from each sample site were subcultured on R2A again and identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).

Bacterial samples were prepared according to manufacturer's instructions and analysed by Microflex LT MALDI-TOF MS system (Bruker Daltonics GmbH, Germany) with FlexControl software 3.0. Protein profiles were analysed by Biotyper software 3.0 against the reference library with default setting. Dendrograms, generated using Biotyper software, were used to infer the relationships between isolates.

For antibiotic susceptibility testing, isolates were cultivated overnight on the Mueller-Hinton agar supplemented with ampicillin (8 µl.ml^{-1}), kanamycin (16 µl.ml^{-1}), tetracycline (4 µl.ml^{-1}) or chloramphenicol (8 or 16 µl.ml^{-1}) at 37°C and laboratory temperature. The concentration of each antibiotic was applied according to European Committee on Antimicrobial Testing [9] and Clinical and Laboratory Standards Institute [10] guidelines as official standards for susceptibility testing of antimicrobials.

One-way ANOVA was used to analyze the changes in bacterial count between inflow and outflow. Changes in bacterial composition and occurrence of resistant strains were analyzed using Chi-square test.

3 Results and discussion

The wastewater treatment process significantly reduced a total number of bacteria in outflow (1180 CFU/ml at 37 °C and 8150 CFU/ml at laboratory temperature) compared to inflow (220000 CFU/ml and 1380000 CFU/ml, $p \leq 0.05$). From water inlet samples 90 bacterial isolates were identified at species or genus level. Nine isolates were not identified and were classified as unknown samples. Bacteria belonged to the phyla *Proteobecteria*, *Actinobacteria* and *Firmicutes*. *Escherichia coli* showed the highest relative abundance (46%), followed by members of *Aeromonas* (17%) and *Klebsiella* (11%) genera. In water outlet sample, 92 bacterial isolates were identified at species or genus level. Four isolates were classified as unknown samples. All bacteria belonged to the phylum *Proteobacteria*. The relative abundance of genera *Aeromonas* and *Acinetobacter* significantly increased (38% and 28%). While *E. coli* dominated the bacterial community in inflow, its abundance significantly decreased in outflow sample (5%) (Fig. 1).

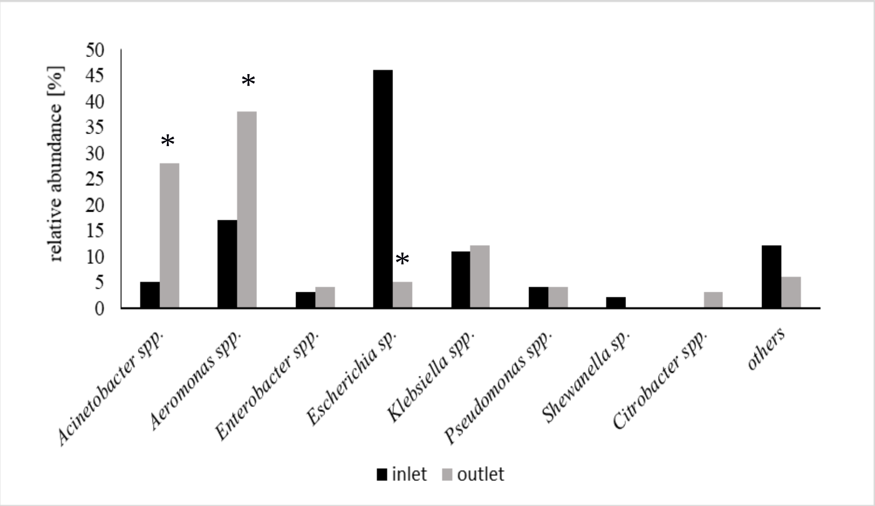

Fig. 1. Relative bacterial abundance at the genus level.
* significant changes in the prevalence of bacterial taxa between inflow and outflow water samples (Chi-test, < 0,05)

Among bacteria from water inlet, 90% of isolates were resistant to ampicillin, 26% to kanamycin, 31% to tetracycline and 26% to chloramphenicol. In outlet sample, the number of isolates resistant to each antibiotics decreased, but statistically important reduction was found only for isolates resistant to tetracycline (Fig. 2). This reduction could be caused by significantly reduction of *Enterobacteria* counts during wastewater treatment process as these bacteria showed the highest occurrence of tetracycline resistance.

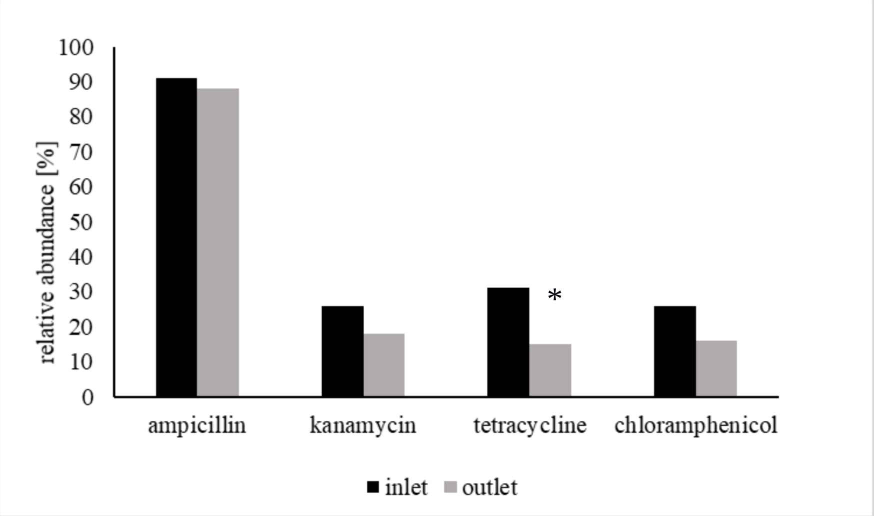

Fig. 2. Relative abundance of antibiotic resistant bacterial isolates.

* significant changes in the prevalence of antibiotic-resistant bacterial isolates between inflow and outflow water samples (Chi-test, < 0,05)

Our findings, similar to several other studies [5, 6, 8], confirmed a significant decrease in total of bacterial biomass during wastewater cleaning process, e.g. numbers of E.coli one of the important pathogenic bacteria decreased from 46% to 5%.

Several studies have demonstrated a high incidence of antibiotic resistance in wastewater all over the world [2, 3, 11]. Our results showed that relative abundance of antibiotic resistant bacteria remained unchanged after wastewater processing (except tetracycline resistant bacteria), which is consistent with the research of Xu et al. [12]. On the other hand, several studies recorded increasing of resistant bacterial strains [8, 13, 14]. However, these studies demonstrated a significantly higher prevalence of resistant bacteria from WWTPs receiving wastewaters from clinical environments than influents only treating municipal wastewater [15].

4 Conclusions

While the total bacterial load was significantly reduced during the wastewater treatment process, the relative abundance of antibiotic-resistant bacteria was not significantly changed, except isolates resistant to tetracycline. Our results showed that the WWTP for Košice may represent a potential source of resistant bacteria and genes which are further spread in the aquatic environment of Hornád River. Therefore, it is very important to develop progressive methods that would effectively eliminate the spread of bacterial resistance against clinically relevant antibiotics.

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MICROBIOLOGICAL EVOLUTION OF NEW TELLURIUM-CONTAINING COMPOUNDS BASED ON 1,2,4-TRIAZOL MOIETY

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Abstract

The introduction of Tellurium into the molecule of organic compounds is a promising branch of pharmaceutical chemistry, because of growing investigation and therapeutic usage of these compounds. Organotellurium structures provide new impetus for the development of enzyme inhibitors and redox modulators, some of which may be of interest in the field of antibiotics and anticancer drug design [1,2]. Low-molecular-weight of organotellurium compounds shows the pharmacologic interest catalyze the reduction of hydroperoxides or peroxynitrite with various cellular reducing equivalents [3]. There are organotellurium compounds with peroxidase [4,5], anti-tumor [6], antibacterial activity [7]. That is why the development of new organic Te-containing heterocycles production via energy-save technologies is an actual task because of their lower toxicity in comparing with a toxicity of inorganic analogues.

Keywords: Tellurium, green chemical technologies, 1,2,4-triazole, antibacterial activity, fungal activity.

1 Introduction

In our previous studies we reported the highly regioselective method of synthesises of bioactive Te-containig [1,3]thiazolo[3,2-b][1,2,4]triazol-7-ium and [1,2,4]triazolo[5,1-b][1,3]thiazin-4-ium salts via electrophilic heterocyclization [8-10]. Chemical properties of above-mentioned salts were also investigated [11]. The reactions were provided in harmless solvents such as water and ethanol according to “clean industry” objectives [12-15]. It was found that above-mentioned compounds affect fungal and antibacterial activity. The calculated toxicity of obtained Te-containing 1,2,4-triazoles has shown that organic compounds are much less toxic as inorganic analogues [16]. In continuation of above research in present paper we report the synthesis of new Te-containing triazoles and their microbiological evolution. In current work, we also would like to summarize the investigation of bioactivity of known and new-produced compounds and to emphasize the laws of bioactivity changing depending on the structure of the sample.

2 Material and methods

2.1 Chemical reagents and apparatus

Chemical reagents used in this study were purchased from Aldrich. Melting points were determined using a Stuart Melting Point 30. 1H and 13C NMR spectra were recorded in deuterated dimethyl sulfoxide with TMS as internal standard using a Mercury-400 spectrometer at 400 MHz. Elemental analyses were carried out on a Elementar Vario MICRO for C, H, and N.

2.2 Biological activity

Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Penicillin (10 μg/mL), cefatoxim (10 μg/mL), nystatin (10 μg/mL) and klotrymazole (5 μg/mL) were used as standard drugs. The antimicrobial activity of chemical substances in relation to collection strains of Staphylococcus aureus 25923 and Pseudomonas aeruginosa 27853 as well as clinical isolates of Salmonella enteritidis, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus epidermidis and Candida albicans has been investigated.

As a screening test, the Kirby-Bauer method was used in accordance with generally accepted requirements. For substances with pronounced bactericidal activity, the method of serial (2-fold) micro-dilutions in liquid nutrient media was additionally provided, in which the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. These experiments were carried out twice.

2.3 General procedure for the synthesis of compounds 1-8

The synthesizes of compounds **1-6** were carried out via described procedures [8-10]. The experimental data for received compounds **1-6** are the same as described [8-10].

The experimental data on newly synthesized compounds are shown below:

General procedure for the synthesis of compounds 7,8

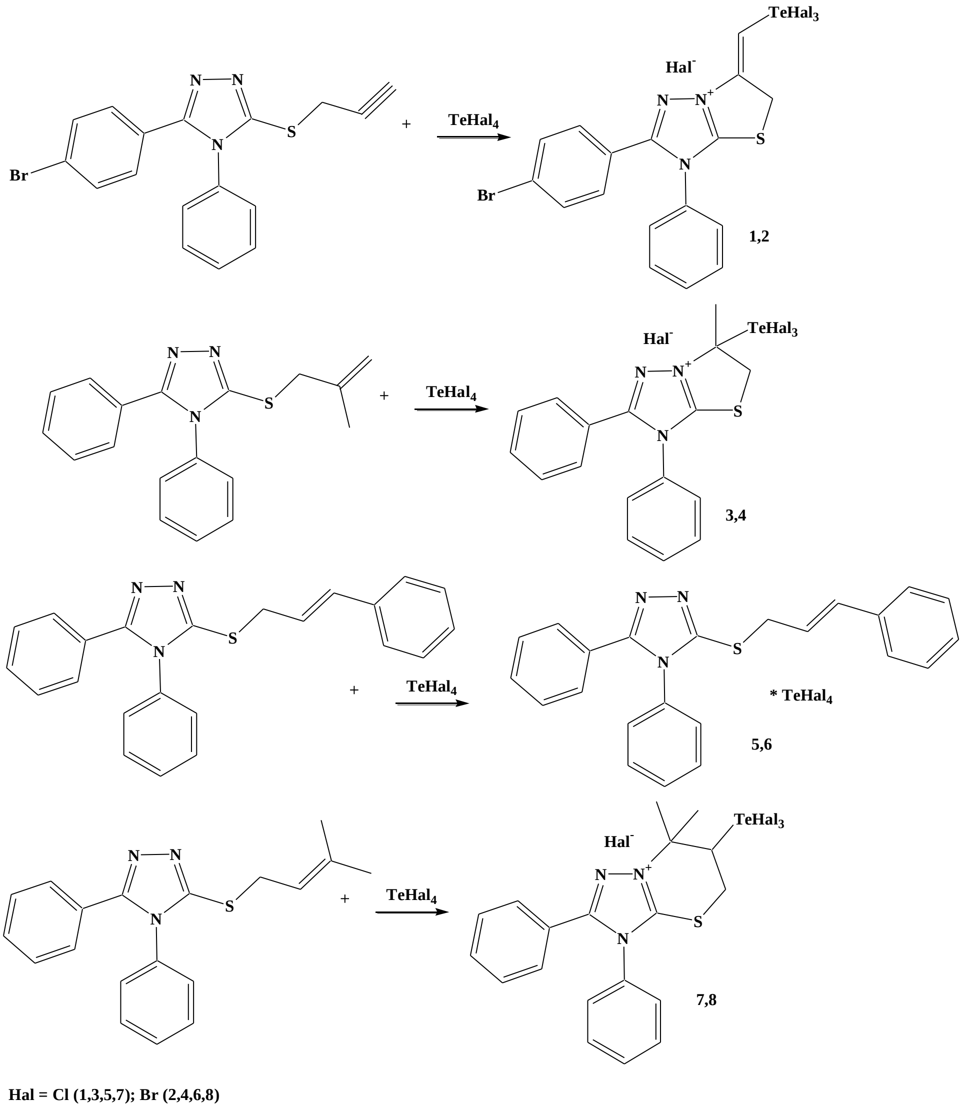
To 10 mmoles of the starting unsaturated thioethers of 1,2,4-triazole were dissolved in alcohol-water medium (4:1), the equimolar amount of Tellurium tetrahalides was dropped to solution of starting thioether. The reaction mixture was stirred at room temperature for 24 hours, the target precipitate was filtered and washed with water.

7,7-dimethyl-6-(trichloro-λ^4-telluranyl)-2,3-diphenyl-3,5,6,7-tetrahydro-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-8- ium chloride (7) Yield 72%. Mp 160-161°C. NMR ^{1}H, δ, (DMSO-*d*$_6$): 1.84 (3H, s, CH_3), 1.90 (3H, s, CH_3), 4.05-4.46 (2H, m, CH_2), 5.12-5.27 (1H, m, CH), 7.21-7.75 (10H, m, 2Ph). NMR ^{13}C, δ, (DMSO-*d*$_6$): 27.1, 31.5, 67.6, 68.9, 123.2, 126.0, 128.2, 128.6, 129.2, 129.8, 130.4, 131.5, 155.2, 156.3. Calculated for C19H19Cl4N3STe$_4$: C 38.62; H 3.24; N 7.11. Found: C38.59; H 3.28; N 7.08.

7,7-dimethyl-6-(tribromo-λ^4-telluranyl)-2,3-diphenyl-3,5,6,7-tetrahydro-[1,2,4]triazolo[5,1-*b*][1,3]thiazine-8- ium bromide (8) Yield 68%. Mp 158-159°C. NMR ^{1}H, δ, (DMSO-*d*$_6$): 1.57 (3H, s, CH_3), 1.66 (3H, s, CH_3), 3.77 (2H, d, *J* = 7.6 Hz, CH_2), 5.30 (1H, t, *J* = 6.7 Hz, CH), 7.21-7.65 (10H, m, 2Ph). NMR ^{13}C, δ, (DMSO-*d*$_6$): 25.8, 31.0, 119.0, 128.2, 128.77, 129.0, 129.2, 129.8, 130.2, 130.3, 130.4, 134.5, 152.1, 154.8. Calculated for C19H19Br4N3STe$_4$: C 29.69; H 2.49; N 5.47. Found: C 29.71; H 2.46; N 5.45.

3 Results and discussion

The compounds 1-8 were synthesized according to the procedure by the method of electrophilic heterocyclization. This method is inexpensive, convenient and meets all the requirements of green chemistry, because of the usage of harmless solvents, throughout the synthesis there are no pollutants, the process is carried out at room temperature conditions with the use of a small amount of equipment and electricity.



According to the potential promising of the resulting compounds, the predicted low toxicity and effective pharmacological action, the investigation of bioactivity was conducted in relation to the selected bacterial cultures (Table 1). The concentration of compounds 1-8 is 100 μg/ml. The antibiotic disks of penicillin (III), cefatoxime (IV), nystatin (V), clotrimazole (VI) were used as standard solutions of comparison.

Table 1. Bioactivity of compounds 1-8.

Bacteria Compound	***E. coli***	***Klebsiella pneumoniae***	***Staph. Aureus***	***Pseudomonas aeruginosa***	***Shigella dysenteriae***	***Salmonella enteritidis***	***Enterococcus Faecalis***	***Enterobacter cloacae***	***Candida albicans***	***Saccharomyces cerevisiae***
1	++	0	0	-	+++	-	-	-	++	-
2	+++	++	++	-	-	+++	-	-	0	-
3	+++	0	++	0	++	0	0	0	0	0
4	++	+++	+++	0	+++	0	+++	+++	0	+++
5	++++	++	++	0	++	++	+++	+++	0	++
6	++++	++++	++++	0	0	++++	++++	+++	+++	0
7	-	++++	-	-	-	++++	-	-	-	-
8	-	++++	-	-	-	++++	-	-	-	-
III	++	0	+++	+	0	0	+++	0	0	0
IV	+++	0	++++	++	0	0	+++	0	0	0
V	0	0	0	0	0	0	0	0	+++	0
VI	0	0	0	0	0	0	0	0	+++	0

The difference in the obtained results shows that there are certain patterns between the chemical structure of investigated compounds and the detected bioactivity.

Biological activity against gram-negative bacteria:

In the case of E.coli bacterium, the compounds **5,6** (which structure differs from other by donor-acceptor bond) showed the highest effect. As for *Klebsiella pneumoniae*, the high activity also was found for compounds **7,8**. From the whole range they are distinguished the presence of two methyl groups in the 7-position of the thiazine cycle, which also probably influence on the increasing of bioactivity. Unfortunately, there is no active compound against Pseudomonas aeruginosa. When investigating the bioactivity of the compounds in relation to the bacterium Shigella dysenteriae, the triazolothiazolium salts **1,4** were the most active. In the case of Salmonella enteritidis, the compounds 6-8 were the most effective and *Enterobacter cloacae* – **4-6**.

Biological activity against gram-posive bacteria:

It was found that the compound 6 showed the highest activity against Staphylococcus aureus and the compounds **4-6** – against *Enterococcus faecalis*.

Biological activity against fungi bacteria:

The compound 1 showed the moderate biological activity against the Candida albicans bacteria and the compound 6 showed he same activity as an activity of known drugs – nystatin, clotrimazole. In the case of bacterium Saccharomyces cerevisiae the compounds **4,5** the most active the most active.

For the selected compounds the quantitative data on antimicrobial activity were measured (Table 2).

Table 2. Quantitative data on antimicrobial activity of compounds 7,8

Bacteria	Compound			
	7		**8**	
	MIC (mcg/ml)	MBC (mcg/ml)	MIC (mcg/ml)	MBC (mcg/ml)
K. pneumonia	31,25	62,5	31,25	125
S. enteritidis	62,5	125	-	-

To determine the most promising compounds for further targeted synthesis and preclinical studies, we have systematized the physical characteristics and yields of reactions for compounds **1-8** (Table 3).

Table 3. Physical data of compounds 1-8.

Compound	Molecular weight	Mp,℃	Yield, %
1	639.5	188-189	61
2	818	179-181	48
3	563	255	45
4	741	267	59
5	591	160-161	72
6	769	158-159	68
7	640	255	45
8	818	267	39

According to biological activity data it was found that the compound **6** is the most active, it showed both antibacterial and fungal activity. The yield of reaction is quite high – 68 %, which is also appropriate for further synthesis and investigation. The compounds **7** and **8** have demonstrated extremely high biological activity against *Klebsiella pneumoniae* and *Salmonella enteritidis*, which wasn't found in any case of standard solutions of drugs. The compounds 7,8 have high melting point, they are stable; that is why it's reasonable to find out ways for increasing of production reaction yield. The compound **4** demonstrated high activity against gram-negative bacteria *Shigella dysenteriae, Enterobacter cloacae*; gram-posive bacterium *Enterococcus faecalis* and fungi bacterium *Saccharomyces cerevisiae*. The further study and synthesis of compound **4** is very promising because of high stability and yield.

4 Conclusions

Organotellurium compounds based on 1,2,4-triazole were synthesized with using green approaches. The biological activity of obtained compounds was investigated. Certain patterns between the chemical structure, physical data of investigated compounds and the detected bioactivity were found. The most promising compounds for further preclinical investigation have been determined.

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SUITABLE ELECTRON DONOR FOR BIOLOGICAL SULPHATE REDUCTION

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Abstract

The bioprecipitation of metals with the bacterially produced sulphide by sulphate-reducing bacteria represents the positive effect of the sulphate-reducing bacteria existence in the environment. It finds the industrial exploitation in the area of the removal metals and sulphate from industrial waste waters mainly the acid mine drainage. The basis of the bioprecipitation first stage is the hydrogen sulphide bacterial production by the cultivation of sulphate-reducing bacteria. In the laboratory conditions the sodium lactate is the energetic substrate for the growth of bacteria. Its price is not economic for the application in the practice and is needed investigate the alternative substitutes. The aim of this work was the cultivation of SRB using the selected energetic substrates - calcium lactate and ethanol. Experimental studies confirm that in the regard to the amount of reduced sulphates the calcium lactate and ethanol are the suitable alternative substrates for the bacterial sulphate-reduction.

Keywords: biological sulphate reduction, sulphate-reducing bacteria, electron donor, calcium lactate

1 Introduction

Acid mine drainage (AMD) is a serious problem related to mining and post-mining activities throughout the world. This drainage exhibits very 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 [1]. Treatment methods to address AMD are focused on neutralizing, stabilizing and removing pollutants through various physical, chemical and biological processes [2]. Nowadays attention is pay to methods for the selective recovery of metals from AMD. These methods have the potential to recover metals in a suitable form for commercial use. One of the best available technologies for the removal of metals from AMD is precipitation as metal sulphides. Furthermore, due to the different pH values of metal sulphide precipitation, selective recovery of valuable metals (Cu, Ni, Co, Zn etc.) from AMD can be achieved by hydrogen sulphide. However, chemical sulphide precipitation has not been generally used probably due to the high cost of reagents. A good way is the preparation of hydrogen sulphide by the biological sulphates reduction using sulphate-reducing bacteria (SRB). SRB reduce sulphates present in AMD to hydrogen sulphide which consequently precipitates the metals from AMD [3, 4].

SRB may be one of the oldest forms of life on Earth. They can be found back billions of years in the geologic rock record to the Early Archean (3900 to 2900 million years ago) [5]. Ancient SRB left their first mark on their environment in pyrite minerals (FeS_2) as old as 3400 million years [6]. Today, these bacteria are widespread in marine and terrestrial aquatic environments. Their ability to adapt to extreme physical and chemical conditions enables them to play an important role in global geochemical cycles [1, 7] predominantly in the sulphur cycle that constitutes one of the best examples of the impact exerted by living organisms on geochemical cycles. SRB is a wide term that is applied to diverse collection of obligate anaerobic bacteria that use sulphates as an electron acceptor in the anaerobic oxidation of inorganic (H_2+CO_2) or organic substrates (lactate, acetate etc.). They can produce a considerable amount of hydrogen sulphide, which reacts easily in aqueous solution with the available metal ions to form insoluble products, most commonly FeS_2, leading to the production and transformation of natural mineral deposits [7]. Sulphates are reduced by SRB according to the generalized reactions (1 and 2):

$$4\ H_2 + SO_4^{2-} \xrightarrow{SRB} H_2S + 2\ H_2O + 2\ OH^- \qquad \text{Inorganic substrate} \quad (1)$$

$$\text{Organic matter} + SO_4^{2-} \xrightarrow{SRB} HS^- + HCO_3^- + H_2O \qquad \text{Organic substrates} \quad (2).$$

SRB can use a large range of substrates as an electron donors and carbon sources, which oxidize incompletely (to acetate) or completely (to CO_2). These substrates are usually organic compounds including sewage sludge, leaf mulch, wood chips, animal manure, vegetal compost, sawdust, mushroom compost, whey, and other agricultural waste. Various types of synthetic organic compounds have also been used, especially small molecular weight compounds, such as lactate, acetate, propionate, pyruvate, ethanol and other alcohols [8].

Methods of SRB applications for AMD treatment involve a few principal stages. The first stage is the cultivation of SRB i.e. the bacterial sulphate reduction. In the laboratory conditions the sodium lactate is the energetic substrate for the growth of bacteria. Its price is not economic for the application in the practice and is needed investigate the alternative substitutes. Therefore the aim of this work was the cultivation of SRB using the selected energetic substrates - calcium lactate and ethanol.

2 Material and methods

2.1 Microorganisms

The cultures of SRB (genera *Desulfovibrio*) isolated from the potable mineral water (Gajdovka spring, Slovakia) were used for the experiments. The SRB were selected from the mixed cultures by the modified dilution method using the selective nutrient medium DSM-63 in the solid and liquid form [4, 5].

2.2 Cultivation of sulphate-reducing bacteria

Cultivation conditions were following: temperature 30°C, anaerobic conditions, 10 % inoculum of SRB, 400 ml of the selective nutrient medium DSM-63 (the energetic substrate for the growth of SRB was sodium lactate (Lac-Na)) or its modifications [4, 9]. The basis of this modification was the Lac-Na substitution by the adequate amount of chosen substrates: calcium lactate (Lac-Ca) and ethanol (Eta). The necessary amount of substrates was assigned by the chemical oxygen demand (COD) determination by photo- colorimetric method using Hach Lange Colorimeter DR/890 at 610nm [9, 10]. Sodium lactate, calcium lactate and ethanol were used in the form of chemicals with the analytical grade. At the sterile conditions the abiotic controls (without inoculum of SRB) were prepared just for the chosen substrates. In process of the experiments the presence of SRB was monitored by the microscopic observation (after the gram stained of the microscopical preparations, by oil immersion, the magnification – 1000X) using the light microscope Nikon Eclypse 400.

2.3 Analytical procedures

Nefelometric method was used to measure the sulphate concentration using a Spectromom 195 instrument. The absorbance of the sample was measured at a wavelength of 490 nm. A glass pH electrode combined with the reference Ag/AgCl electrode was used to measure pH. The acid solution of $CuSO_4$ was used for the monitoring of the hydrogen sulphide presence.

3 Results and discussion

Attributes of the positive SRB growth are the black precipitates occurrence, the hydrogen sulphide formation and the SRB cells presence. These evidences were observed in all studied substrates except the abiotic controls (Fig. 1). Black precipitates represent iron sulphides (salts of iron are present in the nutrient medium [5]. Their creation can be described according reaction (2) and following reaction (3):

$$Fe^{2+} + H_2S \longrightarrow FeS + 2H^+ \quad (Fe^{2+} \text{ is standard compound of nutrient medium DSM-63}) \qquad (3).$$

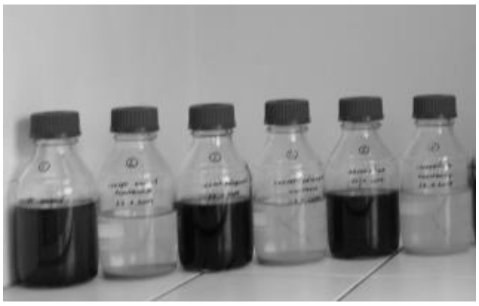


Fig. 1 The comparison of the black precipitates formation during SRB cultivation using chosen substrates and abiotic controls

(1) Lac-Na – sodium lactate; (2) Lac-Na (AC) – abiotic control of sodium lactate; (3) Lac-Ca – calcium lactate; (4) Lac-Ca (AC) – abiotic control of calcium lactate; (5) Eta – ethanol; (6) Eta (AC) – abiotic control of ethanol

Figures 2 – 5 describe the changes of the sulphate concentration and pH values in the course of SRB cultivation using selected substrates and corresponding abiotic controls. In all cases the Lac-Na was used as reference growth substrate. The decrease of the sulphates concentration was observed in all studied substrates except all abiotic controls.

One of the characteristics of all known SRB is their sensitivity to even mild acidity. Most of them are inhibited at pH<5.5 [1]. Therefore the value of pH is very important for the regular course of the bacterial sulphate reduction (i.e. in fact the bacterial sulphide-genesis). In the case of the Lac-Ca pH value changes during SRB cultivation showed the similar profile in the compare with the classical substrate Lac-Na (Fig. 3). After the initial decreasing of the pH from 7.7 to 6.7 followed minor increasing of the pH values to 7.0. Then it remained without the strong changes and the average pH values were about 7.1 that is suitable for the bacterial sulphate reduction (Fig. 2). The lactate oxidation by the SRB was realized without problems and lactate was converted to the acetate at the simultaneously bicarbonate alkalinity production according to the reaction (4).

$$2\ CH_3CHOHCOO^- + SO_4^{2-} \xrightarrow{SRB} 2\ CH_3COO^- + 2\ HCO_3^- + H_2S \qquad (4)$$

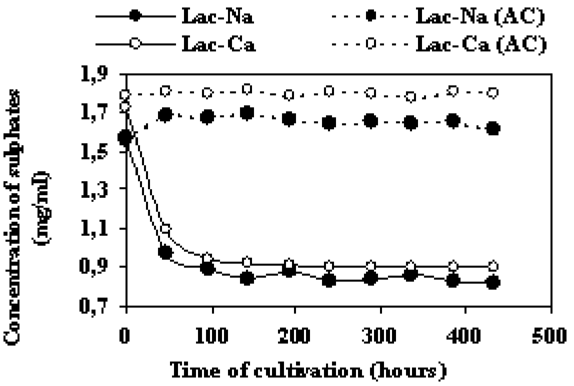


Fig. 2 Sulphate concentration changes during the SRB cultivation
Substrates: Lac-Na – sodium lactate; Lac-Na (AC) – abiotic control of sodium lactate; Lac-Ca – calcium lactate; Lac-Ca (AC) – abiotic control of calcium lactate

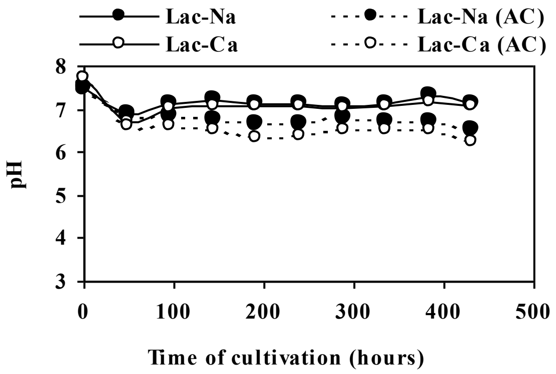
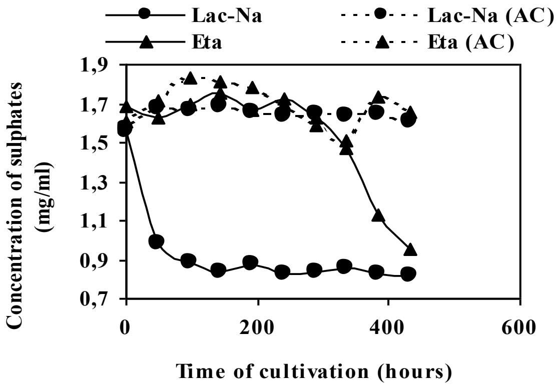


Fig. 3 pH values changes during the SRB cultivation
Substrates: Lac-Na – sodium lactate; Lac-Na (AC) – abiotic control of sodium lactate; Lac-Ca – calcium lactate; Lac-Ca (AC) – abiotic control of calcium lactate

At the using Eta the pH values after 48 hours decreased to 6.8. Until 300 hours it was almost identically (Fig. 5) and after was observed gradually decreasing up to pH 6.0. It related with the major sulphates decreasing (Fig. 4). The profile curves on the Fig. 4 and 5 can be explained to the next considerations. According to Liamleam and Annachhatre [8] the ethanol oxidation by SRB illustrated reactions (5) and (6). Acetate is a major degradation intermediate and its oxidation (reactions 6) produces the bicarbonate alkalinity, which neutralizes solution.

$$2\ CH_3CH_2OH + SO_4^{2-} \xrightarrow{SRB} 2\ CH_3COO^- + HS^- + H^+ + 2\ H_2O \qquad (5)$$

$$CH_3COO^- + SO_4^{2-} \xrightarrow{SRB} 2\ HCO_3^- + HS^- \qquad (6)$$



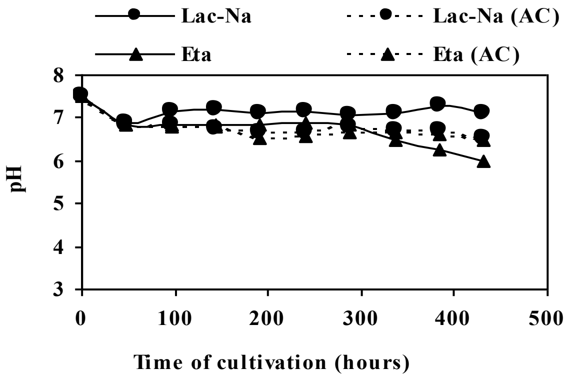


Fig. 4 Sulphate concentration changes during the SRB cultivation
Substrates: Lac-Na – sodium lactate; Lac-Na (AC) – abiotic control of sodium lactate; Eta – ethanol; Eta (AC) – abiotic control of ethanol

Fig. 5 pH values changes during the SRB cultivation
Substrates: Lac-Na – sodium lactate; Lac-Na (AC) – abiotic control of sodium lactate; Eta – ethanol; Eta (AC) – abiotic control of ethanol

Figure 6 documented that chosen substrates are suitable alternative substitutes. In the regard to the amount of reduced sulphates the calcium lactate and ethanol are suitable alternative substrates for the bacterial sulphate-reduction.

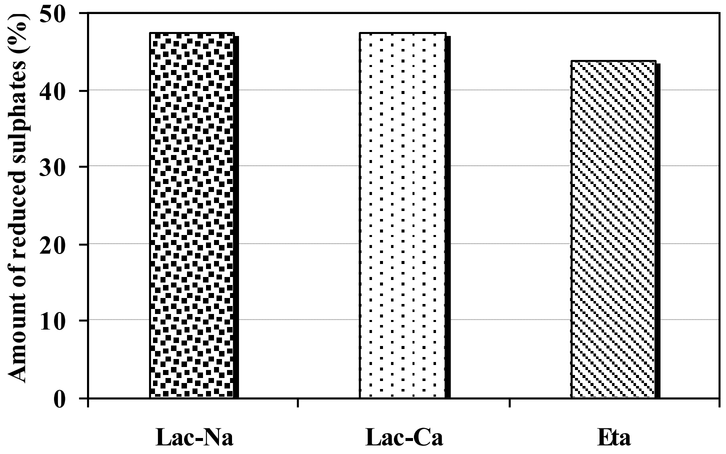


Fig. 6 Comparison of reduced sulphates amount by SRB using substrates: Lac-Na – sodium lactate, Lac-Ca – calcium lactate, Eta – ethanol.

4 Conclusions

The first stage of SRB applications for AMD treatment is the cultivation of SRB i.e. the bacterial sulphate reduction. For the economically effective utilization is needed to investigate the alternative substitutes of sodium lactate that presents the standard growth substrate for SRB. The main aim of this work was the cultivation of SRB using the classical selective nutrient medium DSM-63 (the energetic substrate for the growth of SRB was sodium lactate (Lac-Na)) or its modifications. The basis of modification was the Lac-Na substitution by the adequate amount of chosen substrates: calcium lactate (Lac-Ca) and ethanol (Eta). Experimental studies confirm that in the regard to the amount of reduced sulphates the calcium lactate and ethanol are the suitable alternative substrates for the bacterial sulphate-reduction.

Acknowledgements

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EXTRACELLULAR PRECIPITATION FOR CRITICAL RAW MATERIALS RECOVERY

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Abstract

Europe is confronted with an increasing supply risk of critical raw materials (CRMs). CRMs can be defined as materials of which the risks of supply shortage and their impacts on the economy are higher compared to most of other raw materials. The European Commission has created the three lists of critical raw materials for the EU (in 2011, 2014 and 2017). To tackle the supply risk challenge, innovation is required with respect to sustainable primary mining, substitution of critical metals, and urban mining. In these three categories, bio-hydrometallurgy can play a crucial role. The natural biogeochemical cycles of elements under the microorganisms influence are the base of the bio-hydrometallurgical methods. Indeed, microbe–metal interactions have been successfully applied on full scale to win materials from primary sources, but are not sufficiently explored for metal recovery or recycling. Bio-hydrometallurgy is the advanced technology of the mineral raw recovery and processing as well as the environmental protection. This paper focuses on one of the basic processes of bio-hydrometallurgy – the extracellular precipitation of metals with biogenic compounds.

Keywords: bio-hydrometallurgy, critical raw materials, heavy metals, extracellular precipitation.

1 Introduction

The consumption of resources (raw materials, energy, water and land) has continually accelerating trend. Europe is facing an increasing risk of supplying critical raw materials. Major parts of the economy depend on the supply of raw materials [1]. Given the continued strategic importance of raw materials for the EU manufacturing industry1, the Commission is implementing a wide range of actions under the EU Raw Materials Initiative to help ensure their secure, sustainable and affordable supply. The list of CRMs for the EU is a central element of this Initiative. In 2011 a list of 14 CRMs was published by EU in the communication on raw materials [2]. This first list was established as a priority action of the EU 'raw materials initiative' of 2008. The Commission is committed to updating the list at least every 3 years to reflect production, market and technological developments. Therefore in 2014 EU prepared second list of CRMs. This list represents of a first revised list of 20 CRMs was published in the communication on the list of critical raw materials 2014 [3]. Third list of CRMs was prepared in 2017. A third list of 27 CRMs was published in the communication on the list of critical raw materials 2017 [4].

The third assessment of critical raw materials was conducted based on a refined methodology developed by the Commission, while ensuring comparability with the previous methodological approaches (2011 and 2014). Economic importance and supply risk remain the two main parameters used to determine the criticality of a raw material. Main improvements in the revised methodology relate to trade (import reliance and export restrictions in calculating supply risk), substitution as a factor correcting both economic importance and supply risk, and detailed allocation of raw materials end-uses based on industrial applications to define economic importance. Raw materials, even if not classed as critical, are important for the European economy as they are at the beginning of manufacturing value chains. Their availability may quickly change in line with trade flows or trade policy developments underlining the general need of diversification of supply and the increase of recycling rates of all raw materials. The 27 raw materials listed below are critical for the EU because risks of supply shortage and their impacts on the economy are higher than those of most of the other raw materials (Table 1). Additional elements were found without immediate supply risk but above average economic importance, thus a change in supply chain may result in one of these materials being reclassified as 'critical' in the future (Table 1).

Table 1 List of by today critical raw materials at EU level and materials with above average economic importance [2, 4]

Critical raw materials			Raw materials with high relative economic importance	
Antimony	Germanium	Phosphorus		
Baryte	Hafnium	Scandium		
Beryllium	Helium	Silicon metal		
Bismuth	Indium	Tantalum	Aluminum	Molybdenum
Borate	Magnesium	Tungsten	Bauxite	Nickel
Cobalt	Natural graphite	Vanadium	Chromium	Rhenium
Coking coal	Natural rubber	Platinum Group Metals	Iron	Tellurium
Fluorspar	Niobium	Heavy Rare Earth Elements	Magnesite	Vanadium
Gallium	Phosphate rock	Light Rare Earth Elements	Manganese	Zinc

The crucial dependency on raw materials is acknowledged by the recent launch of the European Innovation Partnerships (EIPs) on raw materials. The main goal of the EIPs is to support the effective utilization of state's own natural resources, thus eliminating the import dependence, based on the research and development of the progressive technologies of exploitation and processing of natural resources, considering the environmental protection. It is necessary to develop the methods for the better valorisation of primary and secondary sources of metals by using environmental technologies.

Biotechnologies can already contribute to meet the EIPs main objectives, and which research and development priorities should be followed to tackle this grand challenge in the future. Although scarcity is a multidimensional problem which cannot be solved by technological solutions alone, biotechnologies can nevertheless play a crucial future role in particular on the extraction, processing and recycling of critical raw materials, allowing for economic utilization of limited/depleted reserves and especially of waste streams that can be considered as new and unexplored resources.

2 Bio-hydrometallurgical methods for metal recovery

Diverse processes can be used for biotechnology based metal recovery. Bio-hydrometallurgy is the interdisciplinary scientific field of mining and mineral processing connecting knowledge of microbial activities (bio-) in aquatic environment (-hydro-) for metal recovery (-metallurgy) from primary as well as secondary metal-bearing materials [5, 6]. Basic processes of bio-hydrometallurgy are biomining by autotrophic and heterotrophic microorganisms, extracellular precipitation of metals with biogenic compounds, intracellular biosynthesis of solid metals, bio-electrochemical recovery of metals, microbial sorption and methylation [7]. The extracellular precipitation of metals belongs to the most significant process for recovery metals/metalloids from primary and secondary sources.

2.1 Extracellular precipitation of critical raw materials with biogenic compounds

Metal can be removed from solution even without contact with the microorganisms. Some microorganisms in the effort to avoid metal entrance to the cell release different compounds into the environment which participate in the formation of insoluble compounds (e.g. organometallic complexes, metal sulphides or oxides) [8]. This process is called extracellular precipitation which can or do not have to depend on metabolisms [9], it often is slow, irreversible and temperature-dependent process [10]. Organisms often within the defence against toxic metals produce various compounds facilitating their precipitation on the cell surface or in the solution (this mechanism depends on the metabolism). Thus, they can be regarded as cheap producers of precipitating agents [7]. Extracellular formed phases are of particular interest for recovery, because no downstream processing (e.g. ultrasonic rapture, treatment with surfactants) is needed to separate materials from the cells [11]. However, regarding direct functional application, materials precipitated in the surrounding medium are often of poor crystallinity, broad particle size distribution, poor mineral specificity and/or show inclusion of impurities. Extracellular precipitation involves several mechanisms such as the physical-chemical interactions with cell surface, direct metal reduction, rf precipitating agents into solution and release of oxidation agents.

Physical-chemical interactions with cell surface:

– precipitation of metals do not depend on the metabolism and are probably results of the chemical interactions between metal and cell surface. This mechanism is probably responsible for Cd removal by bacteria *Arthrobacter* and *Pseudomonas* [12] and algae *Alcaligenes denitrificans* and *Alcaligenes eutrophus* [10].

Direct metal reduction:

– by dissimilatory metal reducing bacteria such as *Shewanella spp.*, *Geobacter, Thermoanaerobacter* etc., which can remove metals from the environment by their reduction to lower oxidation stage. This process is used in the recovery of metal such as Cr, U, Tc, Fe, Co, Mn Se etc. Bacteria use organic compounds (e.g. aromatic carbohydrates) as a source of electrons for the reduction and this way they can simultaneously remove organic pollutants as well as metals [13];

– by eukaryotic organisms e.g. algae *Chlorella kessleri* [14] and *Saccharomyces cerevisiae* [15]. Probably, reducing enzymes are responsible for the process of metal precipitation in the metallic form.

Release of precipitating agents into solution:

– by sulphate-reducing bacteria (SRB), which are to able realize the conversion of sulphates (SO_4^{2-}) to hydrogen sulphide (H_2S) under anaerobic conditions [16]. The typical species of SRB is *Desulfovibrio desulfuricans*, which may reduce sulphates autotrophically with hydrogen gas, as shown in reaction (1) as well as heterotrophically with an organic electron donor like lactate, as shown by reaction (2) [17]:

$$4\ H_2 + SO_4^{2-} \xrightarrow{\textbf{SRB}} H_2S + 2\ H_2O + 2\ OH^- \qquad (1)$$

$$2\ C_3H_5O_3Na + MgSO_4 \xrightarrow{\textbf{SRB}} 2\ CH_3COONa + CO_2 + MgCO_3 + H_2S + H_2O \qquad (2)$$

SRB can produce a considerable amount of hydrogen sulphide, which reacts easily in aqueous solution with the cations of heavy metals, forming metal sulphides that have low solubility. Reaction (3) summarizes this type of reaction:

$$Me^{2+} + H_2S \longrightarrow MeS + 2\ H^+ \qquad \text{(Me2+ is the cation of a particular metal)} \qquad (3)$$

SRB play a crucial role in metal mobility in the environment by precipitating metals through their metabolic end product, sulphide [18]. In industry, hydroxide is still the most widely used precipitation chemical for metal removal. However, sulphide precipitation offers some advantages when material recovery and reuse is aimed at: - metal sulphide precipitates can be formed in more diluted aqueous solutions, concentrating the metal to be recovered in a solid phase; - the pH dependent solubility allows selective precipitation of a specific metal in the presence of others; - the generated sulphide sludge has lower volumes and show superior thickening characteristics [19, 20]. Bacterially produced (biogenic) sulphide has additional decisive advantages, because it can be produced on site from wastes using an electron donor that can be a waste product itself [21, 22]. Selective precipitation has been demonstrated on a limited number of critical (Co) [23] and economically important metals (Fe, Zn, Ni and Mn [24, 20], whereas for some metals, sulphide precipitation and element recovery has even reached full scale application (for instance recovery of Zn in the Netherlands [16]). Notably, selective precipitation and metal recovery can be well combined with bioremediation needs, for instance in the treatment of acid mine drainage. Here, metal recoveries of >97% have been reported for Co, Cu, Mn and Zn [19, 20]. In addition to precipitation of critical metal sulphides, several elements of interest have been precipitated as result of microbial activity in the extracellular space, including Co (as Co_3O_4 [25]), Te [26], Se and uraninite (although they can be at times formed also in the periplasm or intracellular) [27, 28];

– by production of organometallic complexes. In this mechanism cell releases into its environment polymeric compounds forming organometallic complexes with metal ions [29]. Biopolymers are produced by several types of bacteria. They often have capsular structure linked to external cell membrane or they are completely separated from the cell wall. Primarily they are formed by polysaccharides and proteins but the presence of nucleic acids and lipids was also found [30]. Formation of complexes takes place on the cell surface after the interaction between metal and active groups. Complexes can be formed with monovalent ligands or also chelates can be produced. Production of complexes is considered to be the only mechanism responsible for the accumulation of Ca, Mg, Cd, Zn, Cu and Hg by bacteria *Pseudomonas syringae* [31];

– by production of hydrogenphosphates – in bacteria *Citrobacter sp.* over-production of enzyme phosphatase was observed. This enzyme is related to the release of hydrogenphosphates HPO_4^{2-} into solution [32]. The suitable organic phosphate is the source of these ions. This mechanism is responsible for the precipitation of Cd by bacteria *Citrobacter sp.* in the form of hydrogenphosphate on the cell surface [33] as well as U precipitation in the form of insoluble $NH_4UO_2PO_4$ [34]. This mechanism can be used for the recovery of metals together with organic pollutants which serve as the source of phosphate. Recovery of uranium from nuclear waste was recorded after the U precipitation with inorganic phosphate released during mineralisation of three-butyl-phosphate (TBP) by TBP-degrading microorganisms [13].

Release of oxidation agents:

– metal precipitation is also possible by the mechanism when cell synthesises oxidation agents such as molecules O_2 or H_2O_2, which diffuse to its outer environment [35].

3 Conclusions

Bio-hydrometallurgical processes will proceed to play a important role for the supply with critical raw materials, considering they propose eco-friendly alternatives to classical pyro- or hydrometallurgical processes. Highly selective metal/metalloids microorganisms interactions suggest the ability to unite bioremediation with resource recovery for many elements. For the future, it is important to research the application of bio-hydrometaluric methods for the processing of real multi-metal containing wastewaters using mixed bacterial cultures and organic wastewaters that serve as the source of substrates. The experimental conditions of the laboratory should be as realistic as possible. Basic research must go hand in hand with application-oriented studies that require the real robustness of biotechnology in real life. Only then will the industries be convinced that bio-hydrometallurgy is important and they will want to use of the full biotechnological potential for resource recovery.

Acknowledgements

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THE INFLUENCE OF *A. NIGER* PRE-CULTIVATION ON THE EFFICIENCY OF Li DISSOLUTION FROM Li-ACCUMULATOR

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Abstract

Very fast development of mobile electric products leads to an increasing demand for lithium, the main material in rechargeable batteries. The irreplaceable industrial value of lithium increases the global demand for this light metal. However with increasing use of the batteries there is also a growing amount of spent Li-containing accumulators and batteries which can serve as valuable secondary source of Li. The aim of our work was to understand the effect of cultivation on utilisation of *A. niger* on lithium bioleaching from Li-accumulators. The inhibition effect of Co^{2+} ions on fungi spore germination at the beginning of bioleaching period was observed. To avoid the inhibition, A. niger biomass was pre-cultivated prior the start of bioleaching experiments. The increase of bioleaching efficiency from 5 to 11% was observed. To increase the efficiency more, further experiments are necessary.

Keywords: lithium, *Aspergillus niger*, bioleaching, batteries

1 Introduction

Lithium, known as “the new energy metal promoting the world forward”, is widely used in lithium-ion batteries, aircraft alloys, aviation, atomic energy, medicine and other industries due to its unique electrochemical reactivity as well other properties [1]. According to increasing price of crude oil and natural gas and pressure to higher environmental protection, lithium becomes more significant alternative source of electrical energy for electromobiles and hybrid cars. Regarding to market development it is estimated that lithium ion batteries demand could increase to 66% of global lithium production by 2025 [2]. Great amounts of lithium ion batteries (LIBs) have been manufactured to meet the global demand for power source resulting in very fast growing amount of spent Li batteries, annually 200 – 500 million tons of Li-containing waste is produced [3]. Li content in such waste exceed the natural occurrence of Li in ores or brines several times what makes a valuable secondary Li resource from spent Li accumulators and batteries, nowadays. Advantage of spent Li batteries processing are not only in Li recovery because they contain several valuable metals such as Co, Li, Mn, Ni and their compounds. There are several technologies suggested for the treatment mostly based on hydrometallurgical and pyrometallurgical processes, however, majority of them is on semi-pilot level. Eco-friendly and cost effective alternative to these processes represent biohydrometallurgical methods based on the ability of microorganisms to transform insoluble solid compounds into soluble ones which can be recovered. Various bacteria or microscopic fungi are used in published studies. In our work we focused on utilisation of microscopic fungi, particularly *Aspergillus niger*, because compared to bacteria fungi have more ability to tolerate toxic materials, have a shorter lag phase and a faster leaching rate. They also excrete metabolites such as organic acids which enable metal leaching by replacing metal ions from the waste with hydrogen ions or by forming soluble metal complexes and chelates [4]. The potential of heterotrophic fungi *Aspergillus niger* has already been investigated for several waste materials such as flying ash [5], spent catalysts [6] and/or electronic waste [7]. *Aspergillus niger* is a haploid filamentous fungi found in mesophilic environments such as decaying vegetation [8].

The aim of the present work was to study possibility to use the *A. niger* to dissolve Li and Co from cathode powder of Li accumulator, to optimise the process of bioleaching and to find the most important parameters influencing the bioleaching process.

2 Materials and Methods

2.1 Characteristics of spent lithium-ion battery (LIBs)

Spent Li-ion batteries used in laptops of different manufacturers were collected for this study. The LIBs were manually cut up into different portions; anode and cathode were uncurled manually, separated and dried for 24h at

60°C. Black material covering the cathode was separated from the copper foil, analysed and used for the bioleaching experiments. Based on the AAS results the content of Li and Co was 5% and 58%, respectively.

2.2 Fungi cultivation

Heterotrophic microorganism of *Aspergillus niger* was obtained from Department of Soil Science, Faculty of Natural Sciences in Bratislava and maintained at 4 °C on a solid Sabourad Dextrose Agar slant. Stock cultures were subcultured every month.

2.3 Bioleaching experiment

The experiments were carried out in 250 ml Erlenmeyer flasks containing 200 ml of liquid bioleaching media composed of glucose – 5 g/l and $(NH_4)_2SO_4$ – 0.5 g/l with the initial pH value of 5.1. To the bioleaching media 2 g cathode powder and 2 ml of 8-day old spores or conidia were added. The flasks were sealed with removable cotton and the experiment was carried out at 21 °C. Prior to leaching the medium was sterilized by autoclaving for 20 min at 120 °C before spores added. Each experiment was conducted in duplicate. For elemental analysis the samples were collected by disposable sterilized pipettes and filtered through the 0.45 μm-pore-size membrane filter. At the end of the experiments the biomass was easily removed and washed with distilled water. The biomass and residue samples were air-dried for 24 h. Thereafter, the biomass was digested by the hydrochloric acid method to determine lithium accumulated in the biomass. Li concentration was measured by Atomic Absorption Spectrophotometer (Perkin Elmer 3100) at 670 nm. The initial sample and final leaching residues were also mounted with silver paste on aluminium stubs, then coated with 300 – 400 A Au/Pd in a sputtering unit and finally examined in a JEOL scanning electron microscope.

3 Results and discussion

Bioleaching efficiency of Li from Li accumulator using *A. niger* was very low just about 5%. Similar low efficiency was observed also by Kolenčík [9] during bioleaching of Li from accumulators obtained from electronic waste. Although microscopic fungi *A. niger* is known for its high tolerance to heavy metal ions presence in the environment it was found by Gadd [10] that spore germination is very sensitive to presence of Co^{2+} ions. As we assume that Co was dissolved together with Li from the beginning of the process that could lead to inhibition of *A. niger* biomass growth. Sporulating hyphae of *A. niger* shown in Fig. 1A can be the response of microscopic fungi on unfavourable environmental conditions.

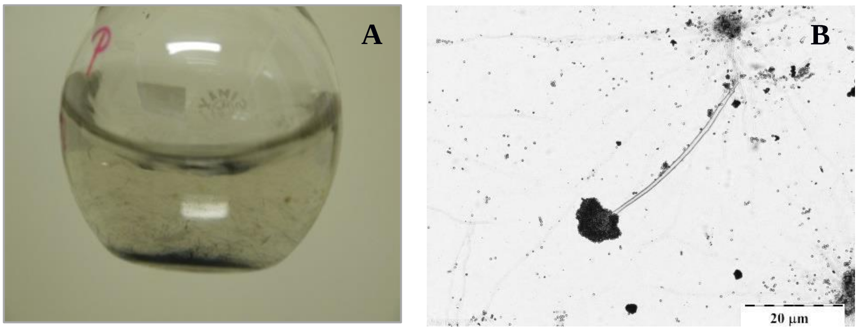

Fig. 1 Hyphae in leaching solution (A) and sporulating hyphae of *A. niger* (B) observed during Li bioleaching from Li accumulators.

Regarding to high sensitivity of spore germination in the presence of Co^{2+} ions pre-cultivation of *A. niger* in nutrition media before addition cathode powder could positively affect the bioleaching process. Therefore, *A. niger* biomass was firstly pre-cultivated in nutrition media. Along with increase of biomass, significant decrease of medium pH up to 3.5 was observed. In that time cathode powder was added into bioleaching solution. The decrease of pH was related to production of organic acid into the solution. Bioleaching efficiency using non-cultivated and pre-cultivated biomass of *A. niger* for Li dissolution from Li accumulators is shown in Fig. 2.

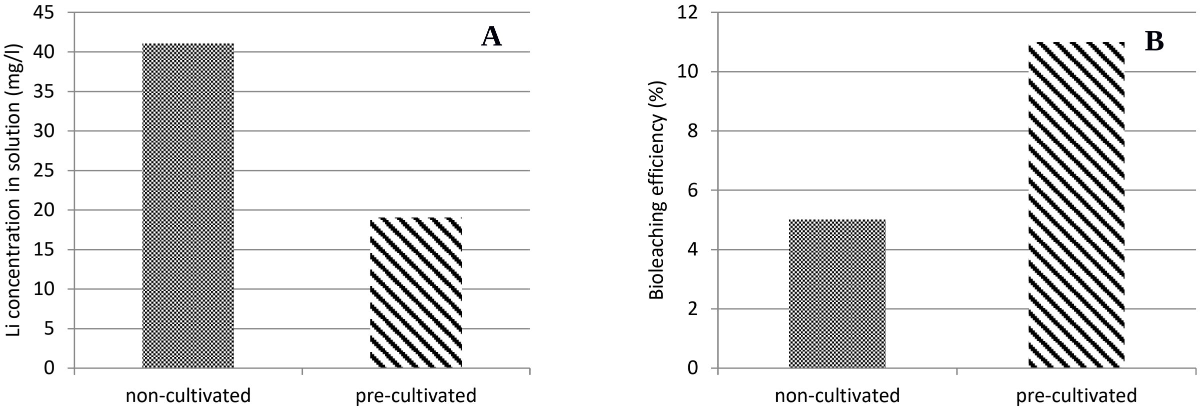

Fig. 2 Differences in Li$^+$ ions concentration in solution (A) and bioleaching efficiency (B) using non-cultivated and pre-cultivated *A. niger* biomass.

Although higher concentration of Li in solution was observed using non-cultivated biomass higher bioleaching efficiency was found in system using pre-cultivated biomass. As in solution with pre-cultivated *A. niger*, biomass was already present dissolved Li$^+$ ions were immediately accumulated inside the biomass resulting in low Li$^+$ ions concentration in solution. However, after analysis of biomass, Li bioleaching efficiency of 11% was found. It is visible that pre-cultivation has significant effect on Li bioleaching with *A. niger*, though future study is necessary to optimise bioleaching conditions to reach even higher bioleaching efficiency.

4 Conclusions

As energy and environmental issues are becoming increasingly prominent, lithium has been a great concern because it is one of the important materials in green energy. But with its increasing use also amount of Li-containing waste is growing. According to our results it is visible that utilisation of biological methods has strong potential to become a basis for the development of alternative eco-friendly technologies. However, there is a necessity to understand conditions required by organisms which can strongly affect the recovery efficiency. Even though *A. niger* is known as a fungus with very high tolerance to heavy metals ions, the high amount of Co might significantly decrease the process efficiency. To overcome this problem pre-cultivation of *A. niger* biomass was added into experimental procedure. After this step the bioleaching efficiency increased more than twice. However, to reach the commercially interesting efficiency further study is necessary.

Acknowledgement

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BIODEGRADATION OF PHENOL UNDER LOW CONCENTRATION FROM MODEL SOLUTIONS BY SULPHATE-REDUCING BACTERIA

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Abstract

In view of the wide present of phenol in different wastewaters and its toxicity, even at low concentration, it is necessary to remove phenol before discharge of wastewaters into waterbodies. Therefore, it is important to employ appropriate methods of wastewater treatment, in order to reduce impact on the environment. Several treatment technologies have been developed, including physical, chemical or biological methods. Biological treatment of phenolic effluents is limited by toxicity of phenol to microorganisms. Low concentrations of phenol can cause inhibition of the microbial growth. The limits of the biological processes are related to the adaptation of biomass to phenol and to the variability of the wastewaters composition. The aim of the present study is to investigate possible adaptation of SRB isolated from natural source to phenol and possible biodegradation of phenol under low concentration.

Keywords: Adaptation, biodegradation, cultivation, phenol, sulphate-reducing bacteria

1 Introduction

Contamination by phenol is a serious concern because of its toxicity to humans, plants and animals [1]. Wastewaters containing phenol are generated from various industrial activities, including cruide oil refineries, pulp-paper manufacturing plants, steel plants, coal conversion processes, manufacturing units of phenolic resins, pesticides, chemicals [2]. Concentration of phenol, in industrial wastewaters may achieves value up to 10 000 mg/l [3]. Number of physical, chemical, physico-chemical methods (adsorption, ion exchange, chemical oxidation, etc.) have been developed for the removal of phenol from wastewaters [4,5,6]. These processes are effective, on the other hand, there are high energy consuming and expensive methods with secondary pollution. Biological treatment methods of wastewater containing phenol are generally more cost-effective and eco-friendly than chemical or physico-chemical treatment processes [2,4]. Various microorganisms are able to degrade phenolic compounds. Their perspective proportion depends mainly on the specific substrate concentration and the potentiality of growth [7]. Anaerobic phenol-degrading bacteria have been isolated under denitrifying (*Thauera aromatic strain K172),* iron-reducing (*Geobacter metallireducens*), methanogenic and sulphate-reducing (*Desulfobacterium phenolicum, Desulfotomaculum, Desulfovibrio*) conditions [8].

Anaerobic sulphate reduction, as part of carbon and sulphur cycle in nature, is an important anaerobic process. The organisms responsible for this process are known as sulphate-reducing bacteria (SRB). In general, SRB were found in fresh waters, marine, sediments and oil or hydrocarbons pollution areas. SRB are facultative anaerobic microorganisms that occur in oxygen-free environments and utilize sulphates as a terminal electron acceptor to produce hydrogen sulphide as one of the metabolic end products. To obtain energy for their growth and maintenance, SRB utilize a wide range of organic carbon substrates, including alcohols, fatty acids, lactate, aromatic or aliphatic hydrocarbons, as the electron donors. In this process, organic compounds are oxidized in two ways, either completely to water and carbon dioxide, or incompletely to acetate and water as end products. At present, about 125 organic compounds used by SRB are known and this number continues to grow [9]. Anaerobic biodegradation of phenol by SRB has been study extensively. Boopathy (1997) isolated sulphate-reducing bacteria from pig feces that could degrade phenol into acetate [5]. Häggblom and Young (1995) studied anaerobic degradation of halogenated phenols by sulphate-reducing consortia. The sulfidogenic consortium utilized phenol, 4-bromophenol, and 4-iodophenol in addition to 4-chlorophenol [10]. Fang et al. (2004) studied anaerobic degradation of phenol from synthetic wastewaters containing 1260 mg/l of phenol, using UASB reactor. The sludge contained five groups of microorganisms *(Desulfotomaculum, Clostridium, Syntrophus, Methanogens* and *Proteobacteria).* In the process, 98% of phenol was removed. *Desulfotomaculum* and *Clostridium* were likely to be responsible for the conversion of phenol to benzoate, which was further degraded by *Syntrophus* to acetate and H_2/CO_2. *Methanogens* lastly converted acetate and H_2/CO_2 to methane. The role of *Proteobacteria* was unclear [2]. Mort and Dean-Ross (1994) found sulphate reduction abilities in bacteria obtain from fresh water river sediments composed of SRB, when only 10 mg/l of phenol was introduced as carbon source without any prior acclimatization [11].

The aim of the present study is to investigate possible adaptation of SRB, isolated from natural source, to phenol and biodegradation of phenol at low concentration.

2 Material and methods

2.1 Isolation and cultivation of SRB

SRB utilized in experiments were obtained from potable mineral water (Gajdovka spring, located in Kosice, Slovakia). The SRB were grown and maintained in Postgate's medium C. At isolation and cultivation phase, lactate was used as the carbon and energy source and ferrous sulphate heptahydrate was used as a sulphate source. The medium was prepared in 1200 ml glass bottle with 1000 ml volume of medium and bubbled with nitrogen gas, for adherence to anaerobic conditions. The pH was finally set at 7.5. 20 ml of bacterial inoculum (natural mineral water Gajdovka) was added into the each of four 250 ml flask containing 180 ml of medium. The bottles were incubated at 30°C, in dark, without shaking, for 2 weeks. Periodically, each two weeks in next 2 months, 20 ml of bacterial culture was transferred into fresh cultivation medium under the same conditions.

2.2 Study of phenol biodegradation

Batch experiments were designed to examine the adaptation and biodegradation of phenol, at low concentration by SRB. The Postgate's medium C was modified for these experiments. Instead of lactate, phenol with concentration 10 mg/l, was used as sole energy and carbon source. Ferrous sulphate heptahydrate was used as sulphate source.

Two biotic sets, including one biotic set with previously cultivated SRB culture, and one set with mineral natural water Gajdovka, containing SRB, and one abiotic control set were carried out simultaneously. In the biotic experiments, 20 ml of the cultivated SRB was inoculated into the 180 ml of modified medium, containing phenol with concentration 10 mg/l. The second biotic set was prepared with samples of natural mineral water Gajdovka. 500 ml of natural mineral water was filtered onto 0.2 μm nucleopore polycarbonate filter under vacuum pressure. Then the filter was rinsed at 200 ml of modified medium containing phenol solution with concentration 10 mg/l. Control abiotic set was prepared under same conditions, with 200 ml of modified medium and addition of phenol solution with final concentration 10 mg/l, without SRB.

All experimental samples were prepared under strictly anaerobic conditions, pH was modified to final pH value 7.5, with NaOH. Bottles were incubated at 30°C, in dark, without shaking, for 21 days. After 21 day, bacterial culture was transfer into fresh modified medium with 10 mg/l of phenol under same conditions. In all, 3 transfers were carried out into fresh modified medium.

2.3 Analytical methods

Samples for analyses were collected each 7 days for determination of sulphate and phenol concentration. 2 ml of samples were centrifuged at 10 000 rpm for 10 minutes and filtered with 0.22 μm HPTFE filter before analyses. Sulphates were determinated by ion chromatography using Dionex ICS 5000 instrument, equipped with an IonPac AS11-HC anion column and suppressed conductivity detector. Phenol was determined by high performance liquid chromatography (HPLC). The HPLC analyses were performed using Dionex UltiMate 3000 system with diode array detector, equipped with Acclaim C18, 150 mm x 2.1 mm column.

3 Results and discussion

3.1 Sulphate reduction

In biodegradation experiments by SRB, sulphate is utilized as final electron acceptor. The decline of sulphate throughout the experiment reflects the performance of the system. The experimental results showed, that sulphate reduction was observed in all biotic samples. The sulphate reduction with phenol as carbon source, for first cycle, is shown in Fig.1. In samples with previously cultivated SRB, concentration of sulphates decreased from 1650 mg/l to 1547 mg/l (F1-1), from 1583 mg/l to 1549 mg/l (F1-2), and for non-cultivated bacteria (F1-G) from 1591 mg/l to 1512 mg/l. The low decrease of sulphate concentration was observed in abiotic control, from 1646 mg/l to 1614 mg/l (F1-K), during 132 days.

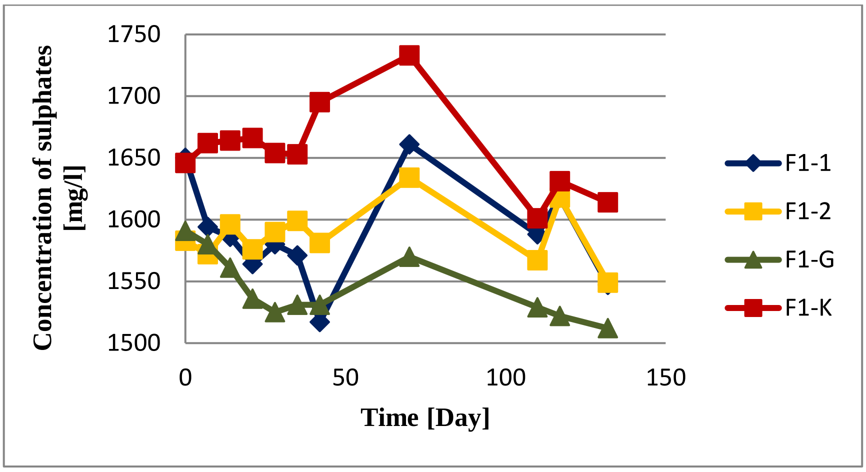

Fig. 1 Cycle 1. Reduction of sulphates during 132 days of cultivation, in addition of phenol to cultivation medium. F1-1: biotic sample with previously cultivated SRB with phenol, F1-2: biotic sample with previously cultivated SRB with phenol, parallel to F1-1, F1-G: biotic sample with phenol and pure natural mineral water Gajdovka with SRB, F1-K: abiotic control.

In cycle 2, the higher decrease of sulphates was observed at 111 day. In samples with previously cultivated SRB, (F2-1) and (F2-2), concentration of sulphates declined from initial 1652 mg/l to 1589 mg/l, and from 1679 mg/l to 1575 mg/l, respectively. In biotic sample with Gajdovka, (F2-G), sulphate decline from 1649 mg/l to 1529 mg/l at 111 day.

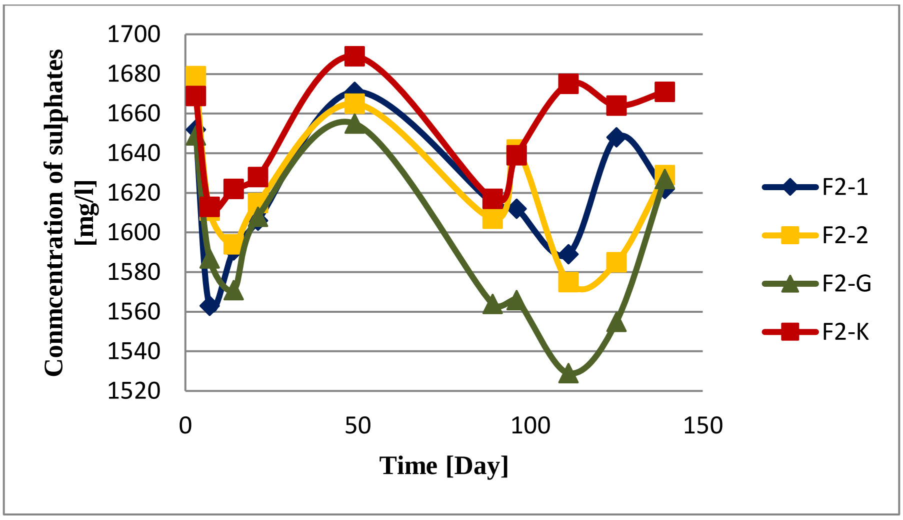

Fig. 2 Cycle 2. Reduction of sulphates during 139 days. F2-1: biotic sample with previously cultivated SRB with phenol, F2-2: biotic sample with previously cultivated SRB with phenol, F2-G: biotic sample with phenol and pure natural mineral water Gajdovka with SRB, F2-K: abiotic control.

3.2 Phenol biodegradation

At the biodegradation experiments, phenol was utilized as final electron donor and sole source of carbon and energy for SRB. Results of phenol biodegradation are shown in Fig. 3. During first cycle, the best results of SRB cultivation on phenol were obtained after 35 days, where phenol concentration decreased from initial concentration 10 mg/l to 0.6 mg/l for mix culture sample without prior cultivated bacterial culture (F1-G). The fast decline can be caused by other microorganism present in mineral water Gajdovka. After 132 days, the same result was obtained in this sample.

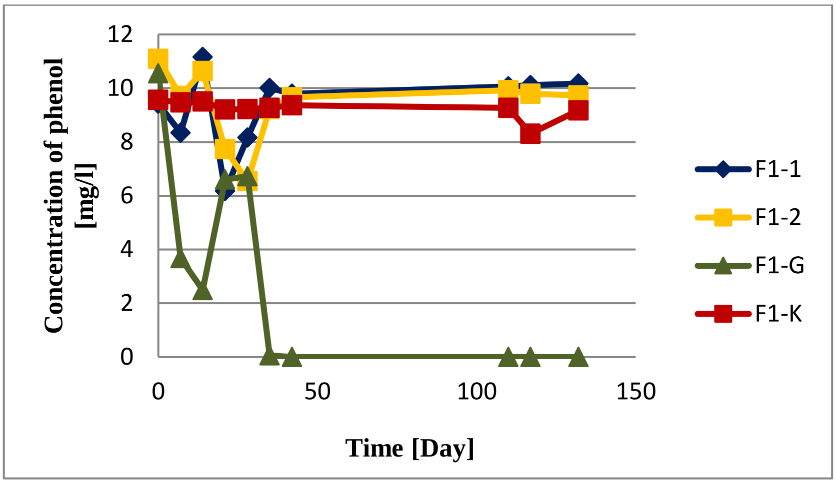

Fig. 3 Cycle 1. Biodegradation of phenol. F1-1: biotic sample with previously cultivated SRB with phenol, F1-2: biotic sample with previously cultivated SRB with phenol, F1-G: biotic sample with phenol and pure natural mineral water Gajdovka with SRB, F1-K: abiotic control.

The degradation of phenol, during second cycle, is shown in Figure 4. After 139 days of adaptation-biodegradation study, phenol concentration decreased from initial concentration 10 mg/l to 0 mg/l, for sample with prior cultivated bacterial culture (F2-1). Slow progress was obtained in sample with mix culture without prior cultivated bacterial culture (F2-G), from 9.27 mg/l to 7.72 mg/l. Other samples were without significant changes.

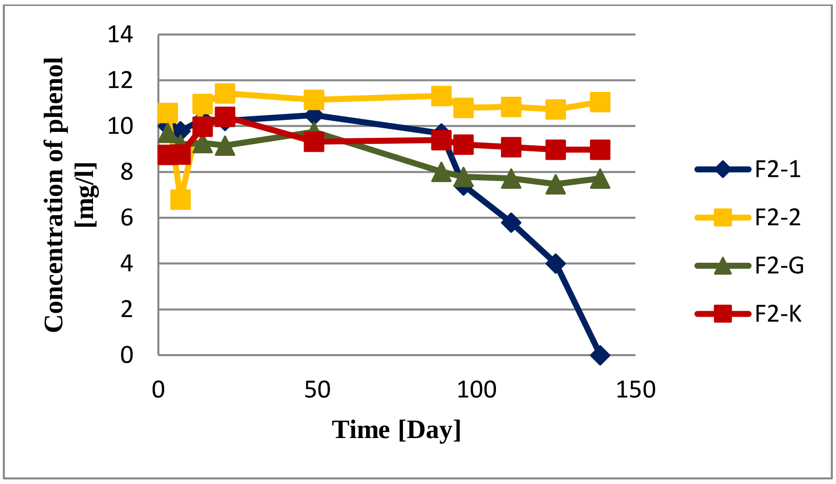

Fig. 4 Cycle 2. Biodegradation of phenol during 139 days of cultivation, in addition of phenol to system. F2-1: biotic sample with previously cultivated SRB with phenol, F2-2: biotic sample with previously cultivated SRB with phenol, F2-G: biotic sample with phenol and pure natural mineral water Gajdovka with SRB, F2-K: abiotic control

4 Conclusions

In the present study, the possible adaptation and biodegradation of phenol under sulphate-reducing conditions was studied. Experimental results demonstrate that, SRB are able to metabolized phenol at low concentration, during the anaerobic sulphate reduction. Indigenous microbial culture has been shown as capable to be adapt and grow on phenol, as the sole source of carbon and has the potential for biodegradation of phenol. Because of bacterial culture was isolated from natural source (mineral water Gajdovka) without any organic contamination, it is necessary to adapt SRB to organic substrate. For this reason, initial concentration of phenol was very low, only 10 mg/l. During first cycle, the best results of cultivation of SRB on phenol, were obtained after 35 days, where phenol concentration declined from initial concentration 10 mg/l to 0.6 mg/l, for mix culture sample without prior cultivation. This fast decrease can be caused by other microorganism present in mineral water Gajdovka. After 132 days, the same result was obtained in this sample. At second cycle, after 139 days, phenol concentration decreased from initial concentration 10 mg/l to 0 mg/l, for sample with prior cultivated bacterial culture. Slow progress was obtained in sample with mix culture without prior cultivated bacterial culture, from 9.27 mg/l to 7.72 mg/l. Other samples were without significant changes. SRB from mineral water Gajdovka have been not at the contact with organic compounds, before. If they are supposed to be able to degrade phenol with higher concentration, adaptation mechanisms responsible for degradation of phenol, have to be created. For this reason, adaptation is challenging and long process, however, results showed, that it is possible to slowly adapt SRB from pure natural sources without present of any organic contaminants. Results obtained provide bases for further biodegradation study working with higher phenol concentration and understanding processes performed by SRB.

Acknowledgements

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POSSIBILITIES OF CRITICAL METALS RECOVERY FROM WASTE MATERIALS USING BIOHYDROMETALLURGICAL METHODS

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Abstract

The outdated technologies and end-of-life products causing creation of constantly growing stream of waste, which are treated as a potential source of metals, especially critical ones. The sources of critical metals, which can be recovered by use of microorganisms, are defined. Microbial technologies become more and more promising for the selective recovery of metal ions due to low cost, technical feasibility for large scale applications and no generation of hazardous wastes. Such methods as bioprecipitation, biosorption, bioreduction and bioaccumulation have been examined for the removal of metal ions from the leachates and aqueous wastewater and in the article shortly characterized. The exemplary of microbe-metal interactions for immobilization of some of critical metals were given. The focus concentrated mainly on PGM and REE metals; the available adsorbents for such metals were presented.

Keywords: biohydrometallurgy, bioleaching, waste treatment.

1 Introduction

Critical raw materials are defined as materials of which the risk of supply shortage and their impacts on the economy are higher compared to most of other raw materials. To this group belongs also metals such as: antimony, beryllium, cobalt, gallium, germanium, indium, magnesium, niobium, platinum group metals (PGM), rare earth metals (REE), tantalum, tungsten [1]. The above-mentioned metals, especially PGM and REE metals, play a key role in some industrial sectors. For example new green low-carbon technologies, which development is currently constantly increasing, is strongly dependable on metals such as indium, dysprosium, gallium, neodymium or niobium [2]. Another sector of industry crucially affected by metals (REE, cobalt, lithium) demand is market of hybrid and electric vehicles. Table 1 shows the factors of criticality, which are related to supply and demand of metals.

Table 1. Factors of criticality related to supply and demand of metals [3]

Supply	Demand
factors used for evaluating supply risk	factors used for evaluating vulnerability
- Country concentration - Country risks - Depletion time - By-product dependency - Proved substitute - Import dependence - Price volatility	- Value of products affected - Spread of utilization - Strategic importance - Value of the utilized material - Proved substitute - Demand share - Import dependence

The outdated technologies and end-of-life products causing creation of constantly growing stream of waste, which are treated as a potential source of metals. These metals can be recovered by hydro- or pyrometallurgical methods. However, currently biohydrometallurgy becomes more and more attractive due to economic factors. Bioleaching is not so expensive method and requires considerably lower investment inputs taking into account the traditional refining and melting methods. If bioleaching method is used the following advantages are observed: no emission of sulfur dioxide, low energy consumption or possibilities of applying for not easily accessible sources containing metals. Fig. 1 presents the schematic diagram presenting the sources of critical metals, which can be recovered applying microorganisms in biomining, bioleaching and bioelectrochemical Systems (BES). The main problems with metals recovery are: low concentration of critical metals, low pH, co-existing metals and salts [3,4].

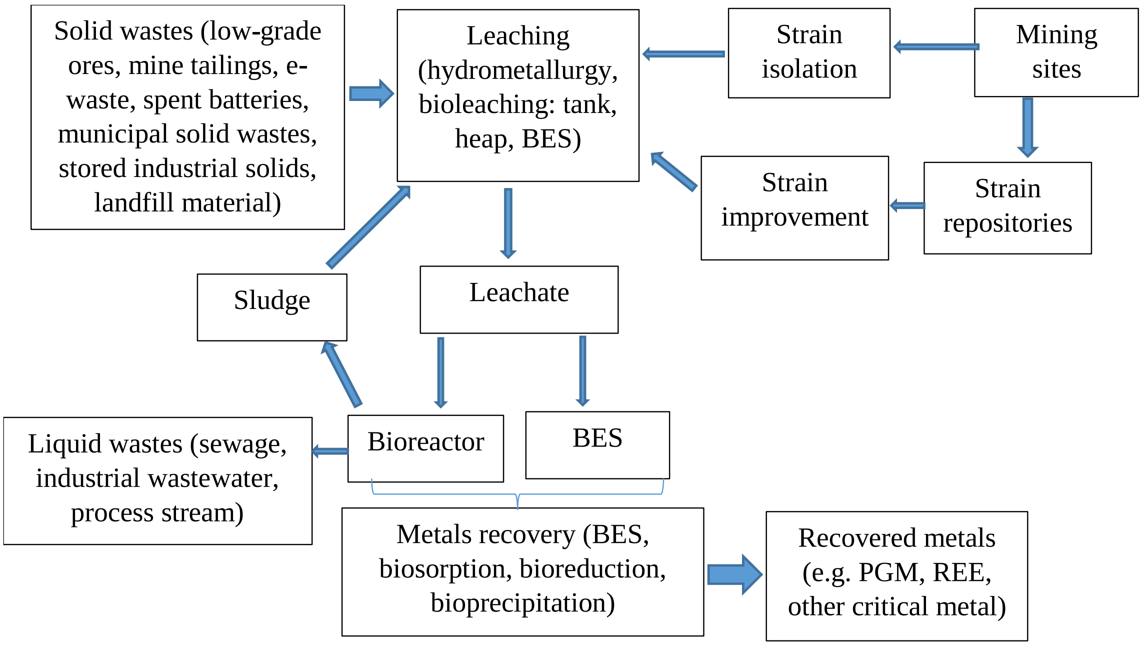

Fig. 1 Schematic diagram presenting the sources of critical metals, which can be recovered applying microorganisms in biomining, bioleaching and bioelectrochemical Systems (BES) [5].

2 Microbe-metal interactions

Microbial technologies become more and more promising for the selective recovery of metal ions due to low cost, technical feasibility for large scale applications and no generation of hazardous wastes. Such methods as bioprecipitation, biosorption, bioreduction and bioaccumulation have been examined for the removal of metal ions from the leachates and aqueous wastewater [6]. Fig. 2 presents the process of microbe-metal interactions showing various mechanism of metals solubilization and immobilization used for biorecovery of metals. To be more precisely, two possible areas for the bioprocessing of metals from waste can be divided to recover critical metals: bioleaching and biosorption along with bioreduction. As consequence the leachate stream or solid precipitate is enriched in metals [7-12]. Table 2 shows short characteristics of above-mentioned methods of bioprocessing.

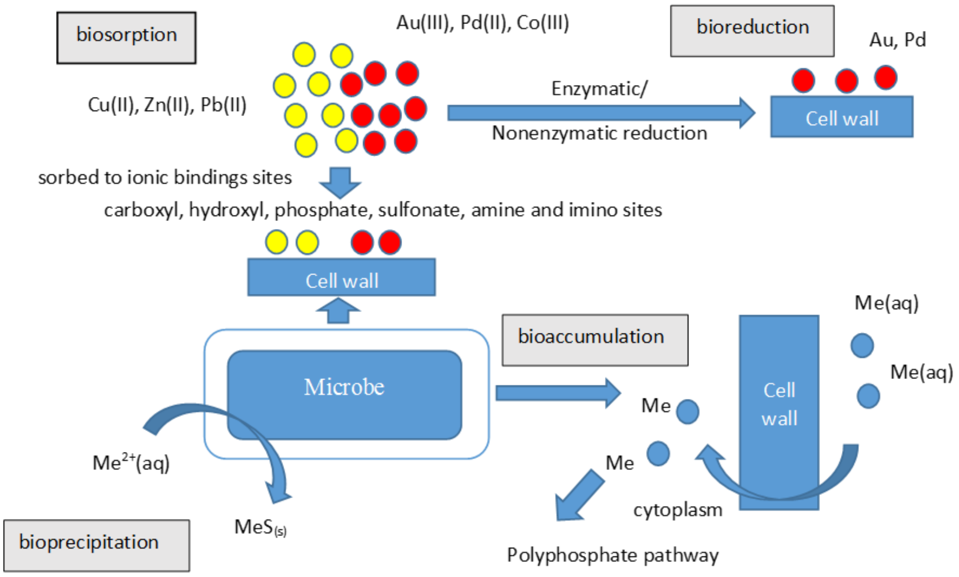

Fig. 2. Mechanism of biorecovery metals from the leachates or aqueous solutions [5,7].

Table 2. Characteristics of bioprocessing methods [7,12-19]

Method	Short characteristics
Bioprecipitation	Biogenic sulfide precipitation has industrial application; sulfate reducing bacteria (SRB) are special group of microorganisms (eubacteria and archaea) - they utilize oxidized sulfur compounds as electron acceptors while oxidizing organic compounds for energy and produce sulfides; the biogenic sulfide production can be conducted by two mechanisms: assimilatory sulfate reduction and dissimilatory sulfate reduction; biogenic sulfide, which are produced during sulfate reduction can precipitate and remove heavy metals from wastewater; metal sulfide precipitation can be divided into two stages: biological hydrogen sulfide production by SRB and metal sulfide precipitation by the biologically produced H_2S; metal sulfide precipitation is successfully applied to remove metals from industrial wastewater, synthetic bioleachate, Cu-metallurgical slags, Zn leach residues bioleachate and also various other sources; an advantage of bioprecipitation: the possibility of selective recovery of metals from multi metallic leachate; there is still need of more research prior to any practical applications.
Biosorption	It is one of technologies developed for the removal of heavy metals from wastewater; bacteria and fungi can be used as sorbents; they can sorb metals on their cell wall either by their property or for a defense mechanism; also some plants would be able to sorb metals by active processes (at the expense of energy) or passive processes (electrostatic attachment to the cell wall); different biosorbents have been investigated to metal recovery from industrial waste streams or mine waste disposal sites; still the laboratory trials are required.
Bioreduction	It is a process that convert the soluble metal species (mainly precious metals: Au, Ag, Pd) to insoluble species; it catalyzed different enzymatic or non-enzymatic processes; it can be applied to precious metal; but also to toxic metal species: Cr(VI), Se(IV), Se(VI), Co(III); this metals can be biologically reduced to nontoxic or less toxic Cr(III) and Se(0) and Co(II); bioreduction is a biorecovery possibilities for recovery of precious metals from the leachate, still research are necessary to implement it at large-scale applications.
Bioaccumulation	It differs from biosorption - both living and dead biomass can be used as biosorbents but only active biomass can be used for bioaccumulation; only living organisms can transport metals to the cell and accumulate the metals inside the cell; whereas biosorption is passive bioaccumulation is an active process (needs energy and respiration); it is dependent on structural properties, physiological and genetic properties of the bacteria and speciation and bioavailability of the heavy metals; there is lack of publication referring bioaccumulation of precious metals from real industrial wastes.

3 Possibilities of critical metals recovery

Table 3 shows the exemplary of microbe-metal interactions for immobilization of some of critical metals. The focus concentrated mainly on PGM and REE metals; however in the last years also other critical metals like gallium or indium start to attract researcher. Naturally occurring microbes can sorb PGMs (Pt, Pd), REEs and heavy metals (such as Fe, Ni, Zn, Pb, Cu, Ag, Cd, Au, Hg) with binding capacities in the range of 10^{-5}–10^{-3} mol metal/ g microbe [2, 20-22]).

3.1 Bioleaching of precious metals and rare earth metals from electronic waste

For leaching process of precious metals such as gold, silver, palladium and platinum from electronic waste the cyanide forming bacteria were utilized. The bacterial cyanide generation is considered to replace the cyanide chemicals to leach gold under alkaline conditions due to: metal recovery is much easier, reduction of transportation charges of chemicals, environmental safety issues (cyanide is produced from glycine for short periods at the late exponential or early stationary phase of its growth) [23]. Different kind of microorganisms were tested to recovery gold and other precious metals from electronic waste like *P. fluorescens*, *Pseudomonas plecoglossicida* and *C. violaceum*, which was found to be the most effective cyanogen for mobilizing gold from the used printed circuit boards. In case of silver and platinum the effect of recovery is not so impressive due to their resistance and toxic effects on the microbes [24].

When considering bioleaching of REEs from electronic waste it occurs that there is almost no literature available. The process of recovery REEs from red mud was studied using *Penicillium tricolor* showing that at 10% (w/v) pulp density and under two-step bioleaching process gives the maximum extraction yields [25]. As consequence similar research can be done to recover REEs from electronic waste.

Table 3. Microbe–metal interactions for immobilization of critical metals [5]

Metal	Organism/Mixed Culture/ Composites	Metal Source	Biochemical process
Ag	*Aeromonas sp. SH10, Bacillus cereus, Bacillus subtilis, B. megaterium, B. licheniformis, Brevibacterium casei, Corynebacterium sp. SH09, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Lactobacillus fermentum, Proteus mirabilis, Pseudomonas putida, P. stutzeri, Serratia nematodiphila*	$AgNO_3$	Bioreduction
Au	*B. megatherium, B. subtilis, Desulfovibrio desulfuricans, D. vulgaris, Escherichia coli, Plectonema boryanum, Pseudomonas aeruginosa, P. fluorescens, Rhodopseudoomonas capsulata, Shewanella algae*	$HAuCl_4$	Bioreduction
Pd	*Anabaena, Bacteroides vulgatus, Bacillus sphaericus, Calothrix, Clostridium pasterianum, Cupriavidus metallidurans, C. nector, Citrobacter braakii, Delsulfovibrio desulfuricans, D. fructosivorans, D. vulgaris, Paracoccus denitrificans, Pseudmonas putida, Plectonema boryanum*	Na_2PdCl_4	Bioreduction
Pt	*D. sulfuricans, Shewanella algae, Pseudomonas sp.*	$PtCl_6^{2-}$	Bioreduction
Ru	Bacterial biomass/chitosan	Ru-acetate	Biosorption
Dy	*Penidiella* sp. T9	$DyCl_3$	Bioaccumulation
	P. aeruginosa	$DyCl_3$	Biosorption
La	*Pseudomonas aeruginosa, Bacillus subtilis, Myxococcus xanthus, Myxococcus smegmatis, E. coli, Pseudomonas sp.*	$La(NO_3)_3$	Biosorption
Nd	*P. aeruginosa*	$NdCl_3$	Biosorption
Sc	*Saccharomyces cerevisiae, Rhizopus arrhizus, Aspergillus terreus*	$ScCl_3$	Biosorption
Eu	*P. aeruginosa, Myxococcus xanthus*	$EuCl_3$	Biosorption
Yb	*M. smegmatis*	$YbCl_3$	Biosorption

3.2 Biosorption of precious metals from waste

Table 4 presents the commonly applied adsorbents used for precious metal recovery. The biosorption capacities is different, varied from 0.003 to 40 mmol/g. Therefore, further research is necessary to select a most probable biomass from a lot of microorganisms. The biosorption is more efficient in acid conditions. The biomass can be used in its natural state or modified. Immobilized biomass particles in packed or fluidized bed reactors is working more effective then freely suspended microbial biomass due to their small particle size, low mechanical strength and difficult biomass/effluent separation [23].

3.3 Biosorption of rare earth metals from waste

Table 5 shows the adsorbents that can be used for recovery rare earth metals. Biomass of several fungal species (*Saccharomyces cerevisiae*, *Rhizopus arrhizus, A. niger*) has been tested for biosorption of rare earths metals from leach solutions; as a result it was stated that the presence of glutamate, sulfate, phosphate, carbonate, chloride, and EDTA (ethylenediaminetetraacetate) in solution with metal ratios up to 15, influence the biosorption of lanthanum by fungal biomass [23,27]. It was also investigated that scandium biosorption by different microorganisms could be decreased between 56 and 94% in the presence of aluminum, iron, and titanium [27].

Recovery of REEs can be achieved by bacterial enzymes. *D. desulfuricans* was stated as the ideal adsorbent for Tc^{7+} reduction [28]. Comparison of Tc^{7+} reduction rates by *Escherichia coli* and *D. desulfuricans* showed greater potential of the last one, and this was attributed to the high activity of the soluble, more accessible, periplasmic hydrogenases in the SRB (sulfate reducing bacteria) [23]. Lanthanum was accumulated at the surface of *Pseudomonas aeruginosa* inducing crystalline precipitation. It was also indicated preferential adsorption for europium ions by *P. aeruginosa* biomass in case of La, Eu and Y. Additionally, biosorption of lanthanum by *Mycobacterium smegmatis* was greatly decreased by the presence of Th or U ions in solution at pH 1.0 [28].

Table 4. Adsorbents applied for precious metals biosorption [26]

Adsorbents		Metal	ph	Biosorption, mmol/g
Algae	*Fucus vesiculosus*	Au^{3+}	7.0	0.35
	Dealginated Seaweed Waste		3.0	0.4
	Sargassum fluitans		2.0	0.0032
	Chlorella vulgaris		6.0-7.0	0.5
	Sargassum natanss		2.5	2.1
	Ascophyllum nodosum		2.5	0.15
Fungi	*C. cladosporioides Strain 1*	Au^{3+}	4.0	0.4
	C. cladosporioides Strain 2		4.0	0.5
	Fomitopsis carnea		1-13	0.48
	Saccharomyces cerevisiae		5.0	0.026
	Cladosporium cladosporioides		4.0	0.5
	Cladosporium cladosporioides		4.0	0.18
	Aspergillus niger		2.5	1.0
	Rhizopus arrhizus		2.5	0.8
	C. cladosporioides Strain 1	Ag^{+}	4.0	0.6
	C. cladosporioides Strain 2		4.0	0.12
	Aspergillus niger		5-7	0.9
	Neurospora crassa		5-7	0.6
	Fusarium oxysporium		5-7	0.5
Bacteria	*Streptomyces erythraeus*	Au^{3+}	4.0	0.03
	Spirulina platensis		4.0	0.026
	Desulfovibrio desulfuricans	Pt^{4+}	2.0	0.32
	Desulfovibrio fructosivorans		2.0	0.17
	Desulfovibrio vulgaris		2.0	0.17
	Desulfovibrio desulfuricans	Pd^{2+}	2.0	1.2
	Desulfovibrio fructosivorans		2.0	1.0

Table 5. Adsorbents applied for rare earth metals biosorption [23]

Adsorbents		Metal	ph	Biosorption, mmol/g
Fungi	*Saccharomyces cerevisiae*	Sc^{3+}	0.6	134
	Rhizopus arrhizus	La^{3+}	3.5	350
	R. arrhizus	Sc^{3+}	0.6	366
	A. niger	Sc^{3+}	0.6	51
	Aspergillus terreus	Sc^{3+}	0.6	153
Bacteria	*P. aeruginosa*	La^{3+}	5.0	1000
		Eu^{3+}	5.0	290
		Yb^{3+}	5.0	326
		Gd^{3+}	5.0	322
		La^{3+}	4.0	144
		Pr^{3+}	5.0	940
		Nd^{3+}	5.0	1100
		Eu^{3+}	5.0	830
		Dy^{3+}	5.0	1000
	Pseudomonas putida	Eu^{3+}	6.4	330
	Bacillus cereus	La^{3+}	4.0	33
	B. subtilis	La^{3+}	4.0	114
		Gd^{3+}	5.0	350
	Shewanella putrefaciens	Pm^{3+}	4.0	2
	M. smegmatis	Yb^{3+}	1.5	103
		La^{3+}	1.5	57
		Eu^{3+}	1.5	101
		Gd^{3+}	5.0	110
	Escherichia coli	La^{3+}	4.0	70

4 Conclusions

Currently, biohydrometallurgical treatment of waste becomes more and more popularly. It decreases the demand for natural sources (ores of metals), energy and places of waste deposits. Wide game of waste (waste after flotation process, slimes, slags, sludge and slurry from electroplating, fly ashes, bauxite residues obtaining after bauxite leaching process, jewellery and electronic scrap, spent petrochemical and auto catalysts, spent Ni-Cd and Li-ion batteries) [30] are treated by microorganisms; however the process is complicated due to complexity of the material containing basic metals, precious metals, rare earth metals as well as dangerous substances. The more and more attention is paid to rare earth metals, which together with precious metals are identified as the most critical raw materials.

In the biological leaching of waste the natural abilities of microorganism are applied to transform metals (present in solid matrix of waste) to the dissolved form. Bioleaching process is carried out in the acidic environment, thus the acidophilic microorganisms play the key role. The most often are used consortium of chemolitotrophic bacteria (liking acidic environment) such as: *A. ferrooxidans*, *A. thiooxidans*, *Leptospirillum ferrooxidans* and heterotrophs e.g. *Sulfolobus*. Apart from this, fungi such as: *Penicillium* and *Aspergillus niger* are the example of some eukaryotic microorganism applied in bioleaching to recover metals from industrial waste [31].

The recovery of precious metals from waste water has been investigated applying living or dead biomass (especially bacteria, fungi and algae). Further research should be carried out to find out modification of a biomass of high uptake capacity and good biosorption characteristics. The research concerning on rare earth metals are not so popularly as for gold and other precious metals, however because of scarcity of these metals become more and more interesting. Special attention should be focused on biosorption of Ag, Pd, Pt, Sr, Ce, La, Th, Am, Co, Ni and Pu metals from multi-elemental solutions. It is also suggested to use of hybrid processes consisting of biological and chemical approach to improve the kinetics and overall performance of metal extraction [30].

Considering recovery of critical metals by biometallurgy the most focus is put on precious metals and rare earth metasl, the other metals are more or less negligible. However, in the last years also indium is considered as a metal possible to recover from used LCD pannels by bioleching methods [32].

However, the biometallurgy is considered as promising and attractive alternative to conventional methods of recovery, still microbiological methods of metals recovery from waste are in majority of cases in experimental stage. Therefore, they require further advanced research work.

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PRACTICAL APPLICATIONS OF BACTERIA ISOLATED FROM EXTREME ENVIRONMENTS: CASE STUDY – SLOVAKIA

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Abstract

Microorganisms are ubiquitous in all known ecosystems on the Earth forming a vital part of nature. Microorganisms that inhabit extreme environments are classified as extremophiles. The fascinating abilities of these organisms to adapt and survive in environments where other living forms are not able to live for longer time make them special candidates not just for research but also for technological applications. For research they represent an interesting reservoir of the hidden genetic variations. In technology they can have broad applications in both environmental (such as bioremediation) and industrial biotechnology (as a source of new enzymes or various bioactive compounds). The aim of our work was to study microbial populations living under Slovak "extreme" conditions including high metallic pollution, high and low pH, elevated salinity, high radiation etc. We found several microorganisms that could be probably classified as novel species or microorganisms with interesting bioremediation potential such as Zn removal by novel species of the genus *Streptomyces* found in brown mud disposal site in Žiar nad Hronom.

Keywords: extreme environments, extremophiles, applied microbiology, biotechnology

1 Introduction

Through to the extensive molecular method utilization in microbiology our knowledge on the life limits on the Earth has dramatically extended. Today we know that microorganisms inhabit environments where we would not imagine any life. New discoveries significantly extended also our knowledge about Earth´s biosphere, opened new horizons in life´s history study and increased the probability of life out the Earth.

Organisms which can be found in the most extreme environments – named extremophiles are able to survive under the conditions which are according to humans' standards harsh, causing death for other organisms. Extremophiles have physiological characteristics which differ them from "common" organisms, e.g. ability to tolerate or even accumulate high metal concentration, ability to live in acidic environment or under high temperature, pressure etc. Basic characteristic of extremophiles is that they occur in the areas where such conditions have been maintained for long time [1]. Extremophiles teach us where the limits of the life are.

Extreme environmental conditions involve physical as well as chemical parameters. Extreme environment is defined taxonomically as an environment with low biodiversity lacking the whole taxonomic groups. Extreme environment has natural or anthropogenic origin [2]. Extremophiles were found in all three domains of life. They are able to live under extremely difficult conditions but often they are not able to live under "normal" conditions. Names of particular groups of extremophiles are derived from environments they inhabit, such as thermophiles or psychrophiles, alkalophiles or acidophiles, barophiles etc. [3]. Exceptional abilities of these organisms to survive are interesting to understand how the life functions but also due to the fact that these unique bacteria produce special compounds or are able to process various compounds in a very specific ways creating the platform for the development of new interesting biotechnologies.

The aim of the article was to study extremophilic microorganisms isolated in Slovak extreme environments and understand the possibilities of their practical applications. We worked in the anthropogenically as well as naturally extreme environments around the Slovakia. Although these environments are not the most extreme in comparison with globally existing extremes, they still are enough extreme to complicate life of organisms.

2 Metal polluted environments

Regarding Slovakia being a country with long mining history, there are large dumps in our country remaining our history but they are full of metals. Metals are also naturally occurring in our soils so it is not very difficult to find various environments where metal content extend the permissible limits.

2. 1 Nickel smelter near Sereď

Production of Nickel smelter near Sereď finished after 30 years because of environmental and ecological reasons. Although the production ended in 1993 there is still remaining pollution in the form of landfill threatening surrounding villages due to wind and water erosion. In 1994 the smelter with landfill became private property. As there was no recultivation performed environmental burden persists until now. It represents serious problem because surrounding field are used in agriculture. The body of the landfill is formed by the anthropogenic sediment – leaching residuum. It is very fine material with grain size under 0.01 mm forming a black dust resulting in specific soil type with high heavy metal content. The Ni concentration is 198 – 3151 mg/kg (limit is 500 mg/kg) and Cr is 1443 – 24300 mg/kg (limit is 1000 mg/kg). There is no vegetation able to cover the landfill even after 30 years what would decrease the extent of wind erosion and pollution of the country around the landfill. We used both cultivation and non-cultivation approaches to determine the bacterial community of waste disposal site. By cultivation analysis, 136 isolates belonging to the *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* phyla were identified which accounted for 44, 38, 17 and 1%, respectively. Preliminary results suggest that at least 2 of the isolates obtained are representatives of new bacterial species. According to phylogenetic analysis they are the most related to species *Sphingobacterium daejeonense* and *Arthrobacter enclensiss.* Based on Shannon Diversity Index, the degree of bacterial diversity in the studied area is lower compared to the diversity of bacterial communities in uncontaminated areas.

These bacteria were further studied and we found that one of the isolates *Arthrobacter sulfureus* can be successfully used in metal bioremediation study as it was able to remove 95% of zinc from water solution. Several isolates expressed resistance against heavy metals as well as antibiotics [4] and the correlation between tetracycline and zinc resistance was confirmed in the tested isolates.

2.2 Elisabeth shaft

Slovinky mining area (north part of Slovak Ore Mountains territory, Spišská Nová Ves district, Slovakia) is considered the largest source of copper ores (chalcopyrite, cuprite, malachite, delafossite) but it is also rich in iron ores (siderite, pyrite, chalcopyrite, delafossite). The most frequent secondary and tertiary minerals are iron oxides such as goethite or crystalline hydrous ferric oxide. Mining in Slovinky was stopped in 1999 but due to the sludge bed accessibility the area still represents a high risk threatening all environmental components. Elisabeth's shaft was built in 1900 and today it works as mining drainage water system. Due to the presence of carbonates is mine drainage flowing in the shaft neutral.

Direct microscopic observations identified iron–oxidizing proteobacteria of the genera *Gallionella* and *Leptothrix*. *Ferribacterium* spp. and *Pseudomonas* spp. were detected via cultivation based methods. We identified members of 204 bacterial genera that belonged to 25 phyla. *Proteobacteria* (69.55%), followed by *Chloroflexi* (10.31%) and *Actinobacteria* (4.24%) dominated the bacterial community. Genera *Pseudomonas* (14.15%), followed by iron–oxidizing proteobacteria *Dechloromonas* (11%) and *Methyloversatilis* (8.53%) were most abundant within bacterial community. Also members of sulfur bacteria were detected represented by families *Desulfobacteraceae* (0.25%), *Desulfovibrionaceae* (0.16%) or *Desulfobulbaceae* (0.11%). The most important finding is obvious shift from neutral mine drainage to acid mine drainage even though carbonate minerals around. The decrease of pH can be probably attributed to the presence of bacteria obtaining their energy by oxidization of minerals [5].

2.3 Hodruša-Hámre

Mine Rozalia near Hodruša Hámre deals with gold mining which is mostly embedded in quartz. Content of the gold is in range of 5 – 600 g/t, in average it is 20 – 30 g/t [6]. Using cultivation based analyses we isolated several heterotrophic bacteria, nowadays we continue with their identification and characterisation of the physiological and biochemical abilities with focus on the abilities suitable for their biotechnological use. One of our aims is to find bacteria able to dissolve gold, so we intend to study the ability of these bacteria to produce cyanogenic acid or organic acids as well as their ability to reduce gold into gold nanoparticles. Among already identified bacteria blue-pigment producing *Vogessella indigofera* able to work as a very sensitive biomonitoring species in early warning systems especially focused on chromium presence in water was identified. High antibiotic resistance was found in isolated bacteria but to explain it is the aim of our further studies.

Except of heterotrophic bacteria we used cultivation in three different media to isolate autotrophic bacteria from deep subsurface samples as the existence of microorganisms in such environment is of increasing scientific and practical interest because subsurface microorganisms with novel metabolic properties may be of potential value to industry for applications in bioremediation and biotechnology. Molecular analyses have revealed that the population of sulphur-oxidising bacteria in gold mine is dominated by a single species of *Aciditiobacillus* genus, particularly *A. albertensis,* suggesting the low level of autotrophic bacteria diversity in deep deposits. For the first time this species was isolated from weathered rocks of a gold mine subsurface environment. In the present time abilities of this bacterium to bioleach In and Sn from LCD screens in cooperation with our partners are studied.

3 Strong alkaline environment – landfill near Žiar nad Hronom

Alkaline environment of the highly alkaline waste brown mud landfill near Žiar nad Hronom, Slovakia also belongs among extreme environments. The brown mud was produced during the alumina production by Bayer process within 1957 – 1967. It is formed by an insoluble mixture of several compounds originally present in the bauxite, and of compounds formed or introduced during the treatment. Brown mud is disposed as slurry having a solid concentration in the range of 10-30%, pH ranges over 9 - 13 and high ionic strength. The high alkali and heavy metal content of bauxite mud deposits accompanied by poor structure and water holding capacity make it extremely hostile environment for the life.

Up to date, there are very limited data on variability of bauxite residues autochtonic bacteria. We succeeded to isolate two species of Streptomyces [7, 8]. One of them seems to be very interesting because of its very high zinc resistance because it is able to tolerate up to 150 mM (almost 10 g/l) while yet known *Streptomyces zinciresistens* tolerates up to 35 mM. Very important is also very high ability of this bacterium to accumulate zinc significantly exceeding known species of *Streptomyces*. Within bioaccumulation experiments this bacterium was able to sequester more than 99% of zinc, its bioaccumulation capacity was 288 mg/g – just to compare biosorption capacity found for *S. zinciresitens* was 165 mg/g (for non-living cells) and in living cells was up to date published capacity just 65 mg/g. Its ability to survive in high metal ions and low nutrient environment with very high bioaccumulation capacity makes it especially good candidate for bioremediation [9].

4 High salinity – travertine lakes in Vyhňa

Vyhňa village in the west part of Štiavnické vrchy Mountain in Central Slovakia is known for its travertine lakes. Travertine is a form of limestone that is formed through the loss of carbon dioxide gas from calcium bicarbonate mineral water sources, especially hot springs, leading to calcium carbonate deposition. Low concentration of calcium bicarbonate would not result in its precipitation but it is assumed that present bacteria are responsible for travertine formation which would not be possible without bacteria in some environments. From the point of view of living organisms this environment is on the limits of extreme environments. Total salinity reaches the levels slightly exceeding the limits for drinking water but near the forming travertines it is several times higher. This area is rich in various bacterial species, more than 95% of bacterial isolates were identified as gamma-proteobacteria. We succeeded to find novel, until now not described, species of bacteria which should be placed in the genus *Oceanobacillus*. This species represents just second species isolated form freshwater environment because majority of *Oceanobacillus* species were found in seas. Study of microorganisms from these environments contributes to understanding of biogenic mineral formation, as it is in the case of travertines [10].

5 High radiation – Jaslovské Bohunice

Primarily, spent nuclear fuel pools were established only for temporal spent fuel storage for at most 20 years. It has been assumed that it is sufficient period to which final disposal of spent nuclear fuel would be determined. However, nowadays it is more convenient in many nuclear power plants in Europe to enhance pool capacity and to store spent fuel during the whole nuclear power plant lifetime, which is about 40 years. Deionized water in storage pools serves as a coolant and, at the same time, as a protection against radiation. The measured radiation in the pools was 4.10^5 Bq/m^3 for beta and gamma radiation and 125 Bq/dm^3 for deuterium radiation [11]. Occurrence of living organisms is here limited not just by radiation but also but oligotrophic environments. Despite this fact there was biofilm formation observed here. Bacteria *Pseudomonas oryzihabitans* and probably novel, species of the genus *Methylobacterium* are major biofilm constituents. Importance of this study lies mostly in the determination of the safety of this way of spent fuel storage in nuclear power plants, however novel extremophilic species can have very interesting abilities and can be used in the production of important bioactive compounds as well as in bioremediation processes [12].

6 High mountains environment – the High Tatras

Environment in high mountains is characterised by several extreme parameters – cold, low nutrient content and high UV radiation. We focused on the area of High Tatras in North Slovakia. Our samples originated from Lucne sedlo from height 2185 meters above sea level. We searched for streptomycetes which are widely used in production of antibiotics and other bioactive compounds. All isolates look similarly so they might represent one or several relative species. Production of several biotechnologically interesting enzymes was detected in these isolates (e.g. cold active lipase and proteinase).

7 High temperature – hot spring in Piešťany

Water boiling point is 100°C. People, majority of plants and animals are able to survive up to 40°C. Some insects can live under 50°C and some plants and fungi up to 60°C. Above this temperature we believe life is not possible. However, today we know organisms with optimum growing temperature in range of 60 – 110°C, the highest temperature living organisms were found is 122°C [13]. Slovakia is a country rich in mineral and thermal sources. In Piešťany is one of our hottest springs with water temperature of 67°C. We found there thermophilic bacteria *Meiothermus ruber*, bacteria with ability to produce thermostabile enzymes, used for example in DNA analyses or bioremediation processes [14].

8 Low temperature, darkness - caves near Košice

Photosynthesis is the basis of energetic metabolism for 99% of the living organisms, just remaining 1% uses thermal of chemical sources to obtain their energy. Caves represent extreme environments because of the lack of light, cold as well as low nutrient content. Slovakia, especially the Košice region is known for great number of caves and in the cooperation with the Department of zoology we take part in cave biomass characterisation. Caves are home of several unusual animals which contains various microorganisms in their body. We looked on the microbiome of *Eukoenenia spelaea (Palpigradi)* and *Arrhopalites aggtelekiensis (Collembola)*. Our results confirmed the assumptions that predatory species *Eukoenenia spelaea* due to unfriendly conditions changed into plant food in the cave ecosystem. We found very high amount of cellulase-positive bacteria in its intestine. As by-product of this research we obtained many psychrophilic isolates producing interesting enzymes – cellulases, proteases etc.

9 Conclusions

Microorganisms that can be found in extreme environments offer an excellent source of new genes and their potential applications in biotechnology are almost unlimited. They have evolved a number of strategies through various mechanisms to cope up with the effect of the prevailing adverse factors including modification in their cell structure and biochemical activities. Extremophiles from several extreme environments were isolated in the study. Bacterial diversity in these places was according to intensity of extreme parameters of physico-chemical conditions significantly reduced in comparison with normal environmental conditions. Several new bacterial species were isolated which can be potentially exploited in bioremediation studies because of their specific abilities to e.g. dissolve metals (*Acidithiobacillus albertensis*), bioaccumulate Zn (*Arthrobacter sulfureus*, *Streptomyces* K11), produce bioactive compounds or antibiotics (streptomycetes from High Tatras), utilise methanol and C_2 to C_4 compounds (*Methylobacterium* sp.) or act in travertine formation (*Oceanobacillus*). However, majority of these bacteria needs further study to understand their potential in biotechnological applications.

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SELECTIVE PRECIPITATION OF METALS AFTER BIOLEACHING

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Abstract

Biohydrometallurgy is generally regarded as a green technology for the recycling of metal-bearing waste, including various types of electronic waste. It offers several possibilities not only to dissolve metals but also to recover them from leachates and form fully biological processes producing just limited amount of waste. In the present work we dealt with a study of metal recovery from leachates obtained after bioleaching of printed circuit boards. We developed biological-chemical method based on selective precipitation process to recover Fe, Cu, Al, Zn and Ni. Iron in the form of $Fe(OH)_3$ and copper in the form of CuS were precipitated from leachate with very high purity. No impurities were detected in precipitates. Al and Zn were also successfully removed from leachate; however, they contained several metallic impurities from original solution. Purity of all precipitates was studied using EDX analysis. During the process, all metals were removed from solutions with very high efficiency exceeding 98%. To recover Ni further research is necessary.

Keywords: bioleaching, selective bioprecipitation, printed circuit boards, sulphate-reducing bacteria.

1 Introduction

In the present time world faces the increasing amount of waste, only in 2010 the total waste production in Europe was 2.5 billion tons, on the other hand there is just limited amount of primary sources suitable for metal recovery to fulfil growing needs of human population [1, 2]. For these reasons there is increasing pressure to develop new technologies which would be cost effective, energy and environmentally friendly but also able to adapt to changing quality of initial materials. Among various possible technologies there is a growing interest on biotechnological application to recover metals not only from low grade ores but also from waste materials [3].

Bioleaching is the way of valuable metals recovery from primary and secondary raw materials using microorganisms. Generally, two mechanisms can be responsible for the process – direct mechanism when metals are directly leached by metabolic products of bacteria. These products are not known but it is assumed that enzymes are mainly responsible for meal dissolution. Main bioleaching mechanisms, however, is indirect, where bacteria are responsible only for iron oxidation from Fe^{2+} ions to Fe^{3+} ions and metals are leached chemically by Fe^{3+} ions. There is a growing interest in application of bioleaching on metal recovery from various sources including metal-bearing waste such as printed circuit boards (PCB). PCB represents an attractive source of metals because they contain high amount of various metals. They represent a specific part of the electronic waste. They consist of ceramics, plastics and approximately 40% are metals [4]. There are several strategies how to recover metal from them and there are various publications dealing with the bioleaching part [5-7], however, there is still a question of metal recovery from leachates produced during bioleaching process. These leachates have strong acidic character and are rich of various dissolved metals. Metals from solutions can be recovered by several methods based on conventional processes, such as adsorption, cementation, electrolysis, ionic exchange, precipitation, liquid extraction or membrane separation or biological methods involving biosorption, bioaccumulation or bioprecipitation [7-8]. However, interesting possibility offers combination of chemical and biological process in one technology as it is in the case of selective precipitation. Selective precipitation uses chemical precipitation by NaOH in combination with hydrogen sulphide microbiologically produced by sulphate reducing bacteria [9]. This method enables consequent recovery of metals in the forms of their hydroxides or sulphides.

The aim of the article was to apply biological process, particularly bioprecipitation with biogenic H_2S produced by sulphate-reducing bacteria to recover Fe, Cu, Al, Zn and Ni from leachate obtained after PCB bioleaching by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* consortium. Biological precipitation was combined with chemical precipitation using NaOH resulting in selective precipitation of metals with the aim to obtain pure precipitates of selected metals suitable for consequent use.

2 Materials and Methods

2.1 Bacteria isolation and cultivation

A mixed culture of sulphate-reducing bacteria (with predominant genus *Desulfovibrio*) was isolated from mineral water collected at Gajdovka spring (Košice, Slovakia) using medium Postgate C [10]. It is water with pH 7.5, H2S odour and with natural content of SRB. Bacteria were grown for 10 days at 30 °C in glass reaction flasks in anaerobic conditions that had been generated by introducing an inert gas (N2) and chemically with sodium thioglycollate.

2.2 Selective recovery of metals

200 ml of leachate obtained after PCB bioleaching by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* consortium was consequently used for Cu, Al, Zn and Ni precipitation. pH value of the leachate was 2.03. Metal concentration is shown in Table 1.

Table 1 Metal concentration in the leachate

Metal	Fe	Cu	Al	Zn	Ni
Metal concentration [mg.l^{-1}]	580.5	574.15	219.5	92.2	239.4

Precipitation was conducted by hydrogen sulphide produced by sulphate-reducing bacteria dominated by genus *Desulfovibrio*. Experiments were conducted in reagent bottles under anaerobic conditions. The inoculum of bacteria 10% (v/v) was applied. Sodium lactate was used as a carbon source. To increase pH of the leachate 0.2M NaOH was used. Samples were regularly withdrawn within the process duration, they were filtered through paper filters. Solid residues were dried at 50°C, homogenised and analysed by EDX analysis (Link 850). Metal concentrations in supernatans were analysed using atomic absorption spectrometry (Perkin Elmer 3100).

3 Results and discussion

Metals concentrations in leachates are listed in the Table 1. High iron concentration in initial leachate decreased precipitation yield of other metals. Iron has precipitated as the first metal at about pH 2 and all other metals were partially co-precipitated. For this reason oxidation of Fe^{2+} ions to Fe^{3+} ions was conducted. Fe^{3+} ions were consequently precipitated in the form of $Fe(OH)_3$ by titration with 0.2M NaOH (Fig. 1a).

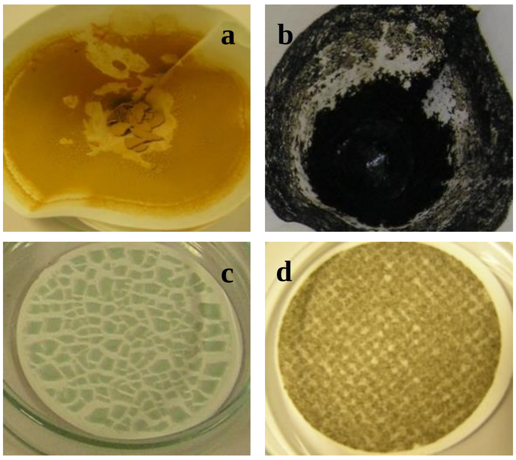

Fig. 1 Precipitates formed during the selective biological-chemical precipitation – (a) $Fe(OH)_3$, (b) CuS, (c) $Al(OH)_3$ and (d) ZnS.

The increase of pH from 2 to 3.5 was observed. After this step iron concentration in leachate decreased by 98.4%. Presence of $Fe(OH)_3$ was later confirmed by EDX analysis. In the next step hydrogen sulphide produced by sulphate-reducing bacteria was added into leachate resulting in biological precipitation of CuS (Fig. 1b), its presence was again confirmed by EDX analysis of solid residuum. In this step 98.9% of copper was removed from solution. Later pH of the solution was adjusted to 6. Formation of white precipitates, later identified as $Al(OH)_3$, was observed (Fig. 1c). Subsequent concentration of Al was below the determination limit confirming that almost all Al was removed from the solution. Again biogenic H_2S was added into solution resulting in ZnS precipitation (Fig. 1d). On contrary to $Fe(OH)_3$ and CuS precipitates, precipitates of $Al(OH)_3$ and ZnS contained trace amounts of metallic impurities, such as Cu, Na or Si, so further optimisation of the process to obtain pure compounds is necessary. The scheme of the process is shown in Figure 2. According to the literature Ni would be precipitated by H_2S at pH above 7, however, in the present experiments volume of leachates decreased so significantly that it was not possible to recovery Ni, as well.

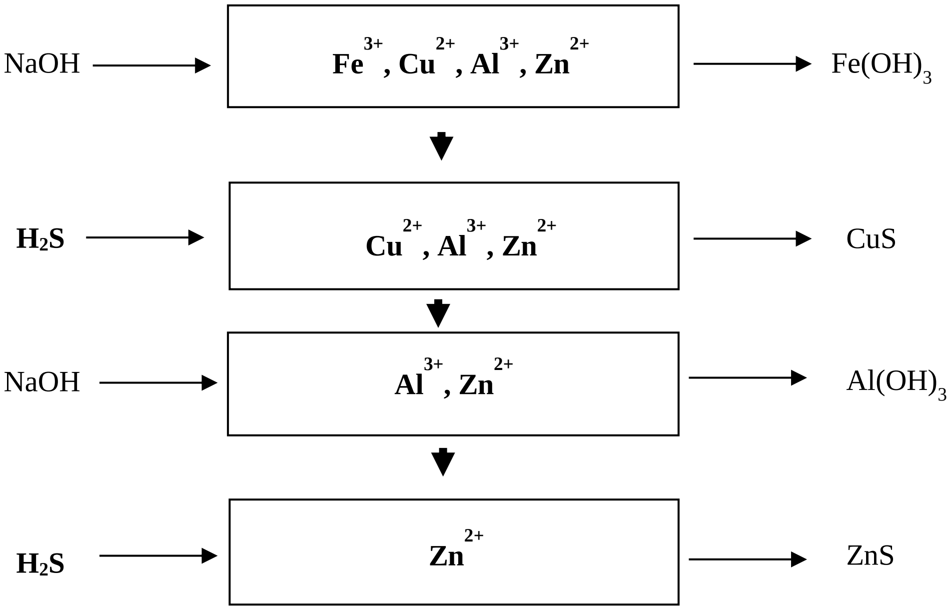

Fig. 2 Scheme of the selective biological-chemical process.

4 Conclusions

Nowadays, large amounts of waste electrical and electronic equipment (WEEE) are being produced with the rapid economic growth and shortened lifespan of electrical and electronic products. It was reported that WEEE is the fastest growing wastes, indicating the urgency for their treatment or recycling with suitable methods. Biohydrometallurgy offers simple, eco-friendly and energy-saving process to recover metals from WEEE, including PCB as important secondary source of various metals. Cu, Al, Zn and Ni were recovered from solutions obtained after PCB bioleaching by mixture of acidithiobacilli showing the possibility of biohydrometallurgy not only dissolve metals but also recover them after bioleaching. The combined biological-chemical precipitation method was developed for metal recovery after bioleaching as pure precipitates. According to obtained results selective precipitation may represent a powerful tool for metal recovery from solutions after bioleaching using environmentally friendly combination of biological-chemical precipitation, however future study is necessary to optimise the final part of the precipitation so all of the formed precipitates would have high purity and would be suitable for consequent utilisation in the commercial processes.

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INDUSTRIALIZED SOILS TOXICITY ASSESSMENT USING EARTHWORMS

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Abstract

This study was conducted to investigate of heavy metals bioaccumulation in industrialized soils from the Košice, city in eastern Slovakia. In present research, we used ecotoxicity tests with *Dendrobaena veneta* to infer about potential toxic risks to the agricultural and permanent grass vegetation of soils around the plant U. S. Steel Košice were performed. The combination of chemical measurements with the calculation of the BAFs - bioaccumulation factor and r - Person matrix correlations can be a useful tool in risk assessment. The total Fe, Cu, Zn, Pb, Cd, Cr and As concentrations and eco-toxicological tests of industrialized soils from the Košice area were determined of 12 sampling sites in 2016-2017 years. An influence of the sampling sites distance from the largest steel producer U. S. Steel Košice on the total concentrations of heavy metals was recorded for iron, cadmium, chromium and arsenic. It was found that earthworms (*D. veneta*) in some cases caused decrease of metals concentration in contaminated soils, the largest metal concentration differences were recorded in the samples PGV (4) U.S.Steel-plant-main gate. The results of the BAFs were lower than 1 for more studied metals: Fe, Cu, Pb, Cr and As and the higher values BAFs for Zn and Cd. The results obtained show the relatively highest positive correlation between mortalities of earthworms and concentrations with Fe (r= 0.25 to 0.85) and the highest negative correlation between mortalities of earthworms and concentrations with Cu (r= -0.17 to -0.82).

Keywords: industrialized soils, *Dendrobaena veneta*, heavy metals, potential toxic risk, bioaccumulation factor

1 Introduction

Urban environmental quality is vital to be investigated as the majority of people live in city. However, continuous urbanization and industrialization in urban areas pose a great threat to human. Soils are susceptible to anthropogenic contamination with metallic trace elements and it can present some risks to ecosystems and human health. Increasing human activities affect urban ecosystem, including soils. In industrialized areas soils are highly modified and intensively managed. The pollution is related to metallic aerosols from industry as well as combustion of fossil fuels and the impact of traffic. The Košice area, in addition to typical urban contamination sources such as road traffic, municipal sphere, small industrial sources and construction is long-term environmental loaded by the iron and steel works- the largest source of contamination in Slovakia [1].

Earthworms were already used in several studies about heavy metals environmental contamination and usually referred as being a good biomonitor organism for soil pollutants. Their biological activities can also influence the accumulation and/or biodegradation of inorganic contaminants namely heavy metals [2-3]. Many authors have shown that bioassays provide a general indication of metal toxicity in soils [4-7]. These tests can be successfully used to evaluate potential toxicity and to assess the environmental dangers of heavy metals. The aim of this study was evaluation of the anthropogenic impact in urban soils from the Košice area, using bioassays on earthworms, with the BAFs - bioaccumulation factor and r - Person matrix correlations.

2 Materials and methods

2.1 Material and method for soils testing

The Košice city has about 239 000 inhabitants and is located the Košice basin in the river valley Hornád in the eastern part of Slovakia (Europe). In the years 2016-2017, 12 soil samples were collected in the Košice suburban, around U. S. Steel Košice (the largest steel producer in Central Europe), which is located about 10km south-westwards from the centre of city. Annual raw steel production capability is 4.5 million metric tons. The technology of steel production has a significant impact on the urban environment. Also, the agricultural and industrial activities contribute to contamination of the soil in this region. The seven sampling sites were localised on area of the Košice (around U. S. Steel Košice); PGV-permanent grass vegetation soils: (1-Perín, 2- U. S. Steel-slag heap, 3-Gomboš (Veľká Ida), 4-U. S. Steel-plant (main gate), 5-Pereš-south, 6-U. S. Steel-north-west and 7-U. S. Steel- north-eastern. The five samples of A-agricultural soils: 1-Perín, 2- U. S. Steel-slag heap, 3-Gomboš (Veľká Ida), 4-U .S. Steel-plant west and 5- Pereš-south was collected.

The soils were sampled at a depth of 20cm to 40cm into plastic bags. Soil samples were homogenized dried at room temperature (25°C); sieved through 2-mm sieve opening. For analysis of soil were used the mixed samples of 5kg weight. The concentrations of the heavy metals (Fe, Cu, Zn, Pb, Cd, Cr and As) in studied soils were measured through the use of SPECTRO XEPOS X-ray fluorescence spectrometer model XEPO 3 (range of elements: Na(11)-U(92), scattering targets: Mo, Co, Al2O3, Pd, HOPG-crystal, X-ray lamp (type VF50): Pd with Be window, resolution: 145keV on line Kα Mn). All the analysed samples were conducted in triplicate and the data were based on soil dry weight. The used control soil (CRM) contains: 85% quartz, 10% kaolin and 5% peat.

2.2 Material and method for soils testing of D. veneta

Earthworms are often used as terrestrial model organisms for ecotoxicity testing, because of their importance for the structure and function of soil ecosystems. The experimental design was based on OECD guideline 317/2010) for the testing of chemicals relating to environmental fate, tests of mortality [8]. The reaction to the earthworm (*Dendrobaena veneta*) was used for chronic tests in the soils. The earthworms were purchased from a local supplier. Prior to the start of the experiment, the earthworms were allowed to acclimatize for one week in the experimental conditions. The adult worms were used in the tests. Three replicates were performed for each test (of the soil 100 g dry weight) with ten earthworms added to each boxes. Then distilled water was added for purpose to obtain 30% moisture of soil. The boxes with soils were kept for 7 days at laboratory temperature. The earthworms were lyophilized (at temperature -50°C and pressure 50Pa). The concentrations of heavy metals after 7 days bioassays in the *D. veneta* were determined after mineralization with a mixture of acids in the system (MWS33, Germany) by the atomic absorption spectrometry (AAS- Variant, Australia). The results were evaluated as the percentage inhibition of mortality and compared to the control soil.

2.2.1 Bioaccumulation of heavy metals in D. veneta tissues (BAF and r-Person matrix correlations)

Bioaccumulation factor (BAF) are used in the risk assessment domain to estimate trophic transfer of contaminants such as metals from soil and can be helpful to the prediction of risks associated with this transfer. BAF can be derived from laboratory studies through the determination of steady-state concentrations or kinetic estimation methods. In this study, bioaccumulation tests were performed in the collected industrialized soils to determine uptake of Fe, Cu, Zn, Pb, Cd, Cr and As by *Dendrobaena veneta*. Bioaccumulation factor for earthworms (BAF) were calculated according to (OECD, 2010): BAF= (metal) earthworm /(metal)soil. The statistical dependence between mortality of earthworms (%) and total metal concentrations were evaluated by Pearson matrix correlation.

3 Results and Discussions

Table 1 summarizes the results of physico-chemical parameters of the soils (**PGV** and **A**) from the Košice area (around U. S. Steel Košice). Soil samples (**PGV** and **A**) presented a slightly alkaline pH/KCL and also pH/H2O (6.34 - 7.83), except for the **PGV** and **A**-soils (5 Pereš-south) were slightly acidic pH/KCL - pH/H2O (5.43 - 6.68). Organic matter (OM) content of the **PGV**-soils ranged from 4.8 to 10.7 %. Organic matter of the **A**-soils was determined in the slightly low varying from 4.11 to 4.94 %. Soil types were by silty-clay texture. The quality of the soil was established with reference to law (220/2004, No.2, Slovak Republic) from various areas to keep representativeness [9]. The used control soil contained: 85% quartz, 10% kaolin and 5% peat.

Metal concentration (Fe, Cu, Zn, Pb, Cd, Cr and As) in the soils of the around U.S.STEEL Košice are shown in Table 2, indicating the significant contamination with cadmium, chromium and arsenic, and according to law No. 220/2004-2, Slovak Republic about the quality of the soil. The highest concentrations of studied metals were measured at sites located south of the ironworks in their vicinity (4 U.S.Steel-plant main gate for **PGV**: Fe 10.5mg/kg, Zn 1084mg/kg, Pb 379mg/kg, Cd 21,4mg/kg, Cr 278mg/kg and As 94mg/kg. For the **A**-soils were determined the highest values of metals in the areas: 4 U.S.Steel-plant west mainly for As 34.8mg/kg and 3 Gomboš for Cr 160mg/kg. The mainly sources of metals contamination in soils are represented by neighbouring the iron and steel works. The lowest average concentration of heavy metals in the **PGV** and **A** soils were found in localities not very effected by the metallurgical industry, as are 5 Pereš-south (**PGV** and **A-soils**) and 1 Perín (**PGV** and **A**-soils).

Table 1 Soil physico-chemical parameters considered in this study

Soil		**pH** (KCl)	**pH** (H_2O)	**Eh** (mV)	**OM** (%)
PGV	**1** Perín	6.88	6.81	462	10.27
	2 U.S.Steel-slag heap	7.18	7.13	472	7.09
	3 Gomboš	7.45	7.55	443	6.60
	4 U.S.Steel-plant- main gate	7.83	7.72	391	5.39
	5 Pereš-south	5.67	6.10	534	4.80
	6 U.S.Steel-north-west	6.34	6.89	476	6.50
	7 U.S.Steel- north-eastern	7.22	7.38	369	7.74
A	**1** Perín	6.20	7.20	490	4.65
	2 U.S.Steel-slag heap	6.99	7.74	441	4.34
	3 Gomboš	7.23	7.95	522	4.94
	4 U.S.Steel-plant west	7.47	7.80	422	4.11
	5 Pereš-south	5.43	6.68	457	4.36

Oxidation–reduction potential (**Eh**), Organic Matter (**OM**) - dry weight (STN EN 12879),
PGV-permanent grass vegetation soils, **A**-agricultural soils

Table 2 Metal concentration (average ± standard deviation) in soils of around U.S.STEEL Košice, CRM

Soil	**Fe** (%)	**Cu** (mg/kg)	**Zn** (mg/kg)	**Pb** (mg/kg)	**Cd** (mg/kg)	**Cr** (mg/kg)	**As** (mg/kg)
Permanent grass vegetation soils (PGV) dw							
1	3.3±2.1	27.1±3.9	88.7±4.3	28.9±0.9	2.0±1.1	91.7±4.1	10.6±1.8
2	3.4±2.7	27.2±2.7	157±5.5	50.1±2.3	1.6±0.8	85.8±3.4	13.4±2.9
3	2.5±1.8	22.5±2.8	65.9±6.9	29.9±1.5	1.9±1.2	68.8±2.8	12.5±2.1
4	10.5±3.8	64.9±6.1	1084±11	379±14.3	21.4±2.9	278±14.2	94±12.8
5	2.5±1.5	24.7±2.2	64.4±5.7	30.5±1.4	3.3±1.4	106±8.7	9.9±2.3
6	2.9±1.7	30.8±4.3	86.5±4.6	36.8±2.2	1.2±0.1	248±10.5	10.8±1.8
7	3.6±2.9	28.0±3.0	95.5±6.2	46.3±2.9	2.7±0.8	264±11.6	12.7±1.1
Agricultural soils (A) dw							
1	2.5±2.0	30.5±2.6	60.2±4.7	26.3±1.2	2.1±0.7	61.6±3.1	7.3±0.9
2	3.5±2.6	28.4±3.2	83.8±6.3	30.4±1.7	9.1±2.3	55.3±2.9	15.9±4.5
3	2.6±1.4	54.0±5.8	67.9±5.1	32.9±2.3	1.8±0.6	160±9.7	10.8±3.1
4	3.6±1.9	32.3±4.6	121±9.4	69.3±5.8	4.4±1.2	69.1±4.6	34.8±8.8
5	2.7±1.8	29.9±2.7	62.4±3.9	29.5±2.7	6.9±2.1	83.3±6.2	9±5.6
CRM	2.4±0.9	83.6±1.5	439±12.1	77.2±3.3	2.7±1.3	84.0±5.3	24±3.4
Law used for comparison (mg/kg dw)							
Limit	-	70	200	115	1	90	30

CRM (certified reference material); **dw** (dry weight); **Low No. 220/2004-2**;

The results of the toxicity tests are shown in Table 3. The earthworms mortality was a little influenced by soils after 7 exposure days. The largest metal concentration differences were recorded in the samples **PGV**: (4) U.S.Steel-plant-main gate, (As=67.3mg/kg, Cr=258.5mg/kg, Cd=19.5mg/kg, Pb=368,4mg/kg, Zn=1022mg/kg and Fe=9.1mg/kg); and in the samples **A**: (4) U.S.Steel-plant west As=30.3mg/kg**,** (3) Gomboš Cr=152.1mg/kg, after 7 days earthworms exposure. It was found that earthworms (*D. veneta*) in some cases caused decrease of metals concentration in contaminated soils.

Table 3. Metal concentration after 7-day bioassay (average ± standard deviation) in soils of around U.S.STEEL Košice and CRM

Soil	**Fe** (%)	**Cu** (mg/kg)	**Zn** (mg/kg)	**Pb** (mg/kg)	**Cd** (mg/kg)	**Cr** (mg/kg)	**As** (mg/kg)
Permanent grass vegetation soils (PGV) dw							
1/7d.	3.1±1.7	15.3±3.5	49.5±4.2	27.9±1.1	1.9±0.8	90.3±3.9	7.8±0.6
2/7d.	2.9±1.8	22.6±2.2	106±3.8	48.7±2.2	1.5±0.7	87.8±2.9	9.1±1.9
3/7d.	2.0±1.9	16.4±1.9	61.2±2.9	28.1±0.6	1.9±0.6	66.9±2.4	8.7±1.3
4/7d.	9.1±2.9	55.7±7.9	1022±13	368±12.6	19.5±1.7	258±11.6	67.3±7.8
5/7d.	1.9±1.1	19.9±2.1	48.7±5.1	29.9±1.5	3.1±1.1	106±7.1	8.4±1.7
6/7d.	2.3±0.8	23.1±3.4	74.6±6.3	35.4±1.9	1.1±0.1	248±10.8	9.8±2.3
7/7d.	2.8±1.5	26.2±2.7	92.0±8.4	46.2±3.0	2.8±0.9	264±12.7	10.6±2.8
Agricultural soils (A) dw							
1/7d.	2.3±0.5	18.9±1.6	55.8±3.6	25.3±0.6	1.9±0.9	60.1±2.8	6.1±0.9
2/7d.	3.1±1.3	20.5±1.4	79.8±4.1	29.1±1.2	8.7±2.2	52.9±3.0	11.8±1.4
3/7d.	2.5±0.5	50.3±4.3	67.1±2.3	32.0±1.5	1.5±1.3	152±11.5	8.4±2.1
4/7d.	3.8±1.4	31.1±2.6	110±9.5	64.3±3.6	4.1±2.6	64.3±4.5	30.3±2.8
5/7d.	2.3±1.2	25.8±1.9	62.0±4.1	24.7±1.3	5.9±3.7	82.8±6.3	7.5±0.7
CRM	2.1±2.0	80.5±6.3	421±11.7	76.1±9.3	84.0±8.8	2.6±0.7	21.5±1.6
C. worm	0.05±0.8	4.2±1.3	49.8±2.4	1.7±1.9	0.5±0.4	1.0±0.1	0.7±1.4

C. worm-Control worm

3.1 Correlation between mortality of D. veneta and heavy metal concentrations (BAF and r-Person matrix correlations)

The results of the BAFs were lower than 1 for more studied metals: Fe, Cu, Pb, Cd, Cr and As and the higher values BAFs for Zn and Cd are presented in Fig.1. As it was already referred by some authors, Zn is an essential metal and their levels are usually efficiently regulated in some organisms by an in crease in elimination rate or a decrease in uptake from environmental media [10-11]. According to the authors [2] related that Zn is probably the most likely metal that often limits earthworm populations. The exposure time in this work was 7 days, being 7 days probably not enough to reach the equilibrium between the matrix and the earthworms resulting in only lower values for BAFs.

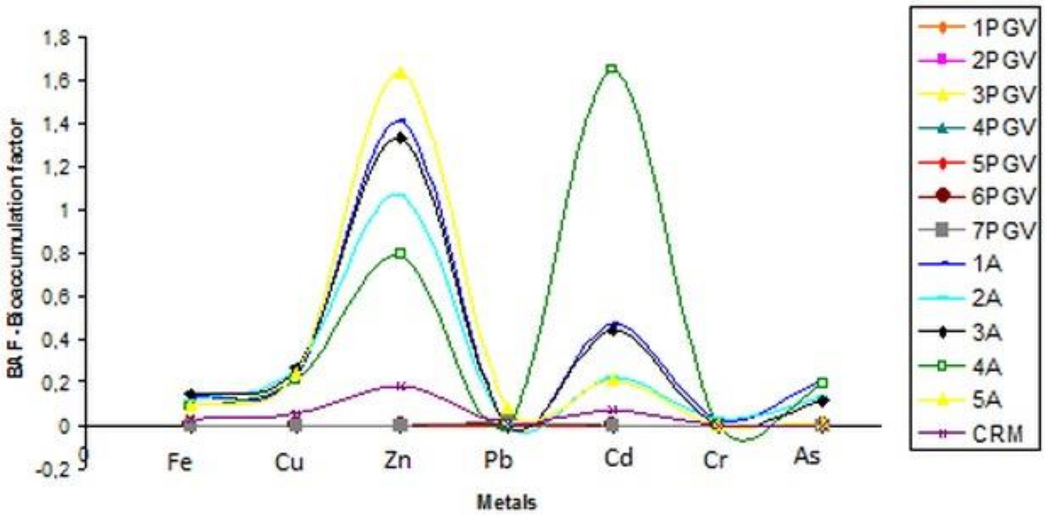

Fig. 1 Bioaccumulation factor for studied metals in earthworm tissues and soils

The correlation between mortality of earthworms (%) and metal concentrations were calculated using the Pearson matrix correlation shown in Figure 2. The results obtained show the relatively highest positive correlation between mortalities of earthworms and metal concentrations with Fe (r= 0.25 to 0.85) and the highest negative correlation with Cu (r= -0.17 to -0.82). The relatively lower values of the correlation coefficient were calculated for these metals with Zn (r= -0.37 to 0.48), As (r= 0.09 to 0.54); Pb (r= -0.22 to -0.78) and the lowest values were detected in case of Cd (r= -0.13 to 0.32) and Cr (r= -0.21 to 0.28). The correlation (positive and negative) found between the studied metals may show nearly similar levels and sources of contamination in the study area [12]. It is evident that samples with the high concentrations of metals don't influence significantly the mortality of earthworms.

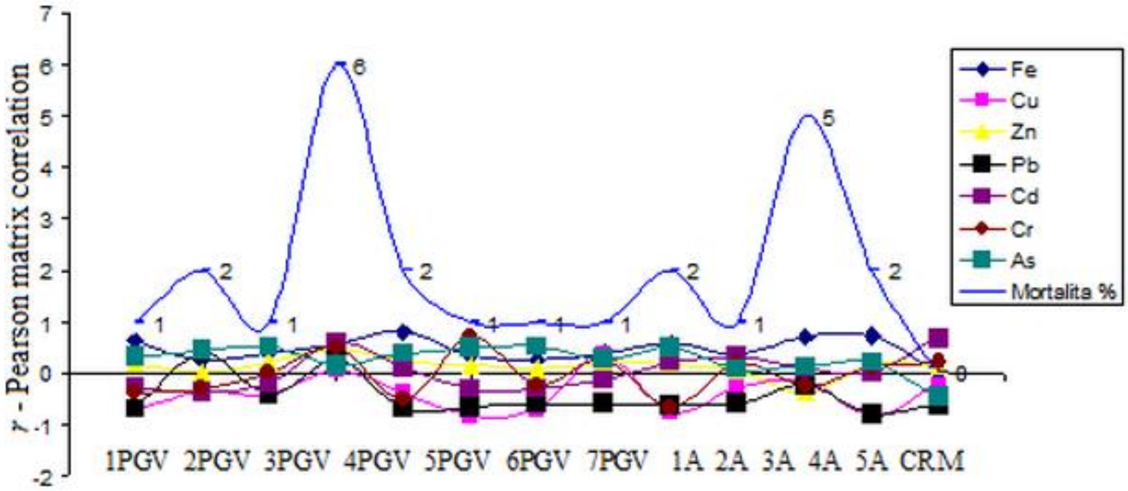

Fig. 2 Effect of metals on mortality *D. veneta* after 7 days of exposure of tests and correlation between mortality of earthworms and concentration of metals for studied soils

4 Conclusions

Local industry located in the region influences the quality of the environment, including soils. The Košice area, in addition to typical urban contamination sources is long-term environmental loaded by the iron and steel works (the largest steel producer U. S. Steel Košice in Central Europe). The total Fe, Cu, Zn, Pb, Cd, Cr and As concentrations and ecotoxicological tests of industrialized soils from the Košice area were determined of 12 sampling sites in 2016-2017 years. Metal concentration in the soils of the around U.S.STEEL Košice indicating the significant contamination with Fe, Cd, Cr, As, the highest concentrations of metals were measured at sites located south of the ironworks in their vicinity (PGV 4 U.S.Steel-plant main gate and A 4 U.S.Steel-plant west). The largest metal concentration differences were recorded in the samples (PGV soils of the U.S.Steel-plant-main gate (As, Cr, Cd, Pb, Zn,Fe), and in the samples (A soils of the U.S.Steel-plant west (As) and Gomboš (Cr) after 7 days bioassays (*D. veneta*). The results obtained show the relatively highest positive correlation between mortalities of earthworms and metal concentrations with Fe (r= 0.25 to 0.85), and the highest negative correlation with Cu (r= -0.17 to -0.82). The results of the BAFs were lower than 1 for Fe, Cu, Pb, Cd, Cr and As and the higher values BAFs for Zn and Cd were determined.

BAFs index and Person matrix correlations are useful tools to characterized potential risks posed by contaminated soils. The obtained results may provide a baseline data for soil health and information about ecosystem in urban regions subjected to emission of industrial contamination sources.

Acknowledgements

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ENVIRONMENTAL HEAVY METAL CONTAMINATION AND ANTIBIOTIC RESISTANCE SPREADING

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Abstract

Nature contaminated by heavy metals and antibiotics as a consequence of anthropogenic activity is of great concern nowadays. It was proved that environment could act as a reservoir of antibiotic resistance genes allowing them to spread among bacterial species via horizontal gene transfer resulting in antibiotic resistance also in pathogen microorganisms. Heavy metals could play an important role in this process because of their potential to create selective pressure with subsequent selection of antibiotic resistant bacteria through linkage between antibiotic resistant genes and heavy metal resistance genes. This study was conducted to investigate the antibiotic tolerance profile of bacteria isolated from contaminated environment of mine and dump; the environment where we did not expect the presence of antibiotics.

Keywords: antibiotic resistance, heavy metals, bacteria, mine, dump

1 Introduction

Antibiotics (ATBs) are essential substances with the irreplaceable role in the treatment of bacterial infections in humans and animals [1] [2] [3]. The clinical use of antibiotics may also be associated with the increasing number of antibiotic-resistant bacteria. Moreover, anthropogenic activities in agriculture (e. g. application of antibiotic compounds, manures, biosolids, irrigation with municipal wastewater etc.) could transmit residual concentrations of previously used antibiotic substances, antibiotic resistance genes and antibiotic resistant bacteria to agricultural soils [4] [5] [6]. Soil, mineral and organic particles have the ability to adsorb antibiotics from water and to induce their long-lasting stability [7]. Resistant bacteria living in such contaminated areas could be induced by low concentrations of these compounds. When dealing with antibiotic presence in their environment, their resistance genes could be duplicated and through the plasmids become available to wide range of neighbouring bacteria [8]. The acquisition of antibiotic resistance determinants by the mean of horizontal gene transfer plays an important role in the development and spread of antibiotic resistance among pathogenic species of bacteria [9]. The environment acts both as a bioreactor containing chemical stressing factors and as a reservoir of genetic resistance traits which could be transferred to clinically relevant pathogens as a consequence [10].

A statement that antibiotic resistance originates only from the overuse or misuse of antibiotics, is no longer acceptable. Despite the efforts that have been made to reduce and control antibiotic applications, antibiotic resistance still continues to increase. Antibiotic resistance occurs not only by selective pressure created by antibiotics, but also due to the contaminants, like e. g. heavy metals, that contribute to the dissemination of genetic elements by mechanisms of cross-resistance and co-resistance [11] [12] [13]. Moreover, genetic determinants of antibiotic and heavy metals resistance could be co-located on the same mobile genetic element (e. g. plasmid) and could also be linked together what can result in their co-transport in the process called conjugation. Target organisms of this transport might be not only bacteria belonging to the same bacterial genera, but also interspecific conjugation is possible [14] [15].

Tight relationship between heavy metals and antibiotic resistance expansion in the environment was noted in the highly contaminated areas for the first time. The occurence of bacterial antibiotic resistance in the presence of insufficiently treated wastewater and biosolids, outflows or industrial contamination has often been indicative of their exposure to higher concentrations of heavy metals [16] [17].

The aim of our work was thus determination of number of bacteria per gram of soil/dump, monitoring of antibiotic tolerance and establishment of MIC (minimal inhibitory concentration) of antibiotics in the bacteria isolated from contaminated environment of old dump and mine in Hodruša - Hámre, where the selection pressure of heavy metals could also play important role in formation and development of antibiotic resistance.

2 Materials and methods

Two soil samples from gold mine (labelled RB1 and RB2) and one sample from the dump located near the mine (labelled H1) were collected from Hodruša – Hámre, Slovakia (GPS N 48°45.658´ E 18°85.166´). In these sites, high concentrations of heavy metals have been accumulated over the years.

RB1 and RB2 sampling sites were located right in the mine; RB1 site was cca. 6 weeks and RB2 site was only 3 days after the blasting and initiation of mining activity. H1 sampling site was the place of dump residue from the treatment located near the mine (GPS N 48°27.274´ E 18°46.804´).

Approx. 50 g of each sample were collected from the top layer (5–10 cm) into the sterile ziplock bag. After the collection, the samples were kept on ice and transported to the laboratory, where the material was processed until 24 hours.

1 g of thoroughly homogenized sample was suspended in 10 ml PBS-T and intensively shaken for 30 minutes. Then, 50µl of appropriate serial 10-fold dilutions of soil suspensions were plated onto the TSA (Tryptic Soy Agar), NA2 (Nutrient Agar no. 2), 100x diluted NA2 agar and R2A (Reasoner´s 2A agar) plates, which were cultivated at 25°C for 48 hours to determine CFU (colony forming units) per gram of the sample. Based on the variable phenotype of bacterial colonies, 40 isolates from each type of cultivation media from one sampling site were selected (i. e. 160 isolates per one sampling site) for MALDI – TOF MS (Matrix – Assisted Laser Desorption / Ionisation Time of Flight Mass Spectrometry) identification. After the MALDI – TOF MS identification, representative isolates from each clade of phylogenetic tree were tested for their antibiotic tolerance. Antibiotic tolerance testing of bacterial isolates was performed on MH (Mueller-Hinton) agar using dilution method with the addition of antibiotics - ampicillin (AMP), chloramphenicol (CHLOR), tetracycline (TET) and kanamycine (KAN). Antibiotics were added to the medium in an appropriate amount to the achievement of final concentrations of antibiotics in the medium. Concentrations of antibiotics were as follows: AMP - 10, 20, 50, 100 µg/ml; CHLOR – 1, 2, 5, 10, 20 µg/ml; TET – 2, 5, 10, 20, 50 µg/ml and KAN – 5, 10, 20, 50, 100, 200 µg/ml. The plates were incubated in the dark at 25°C for 48 hours and then the growth of bacterial isolates was evaluated.

3 Results and discussion

Bacteria (along with fungi), predominant types of microorganisms found in the soil, have an essential role in maintaining soil functions. In our experiment we proved, that the presence of contaminants in the environment could reduce the multiplicity of these microorganisms.

After the 48 hours incubation we determined the number of bacterial CFU per gram of metal contaminated soil of mine and dump. From the Tab. 1 is apparent, that the numbers of CFU in samples collected from mine and dump are approximately the same. Our counts are thereabouts in accordance with the findings of Berg et al. [18] and Williams with Hakam [19], who set the CFU value of contaminated soil on 1.5 x 10^6 CFU/g and 2.4 x 10^7 to 1.2 x 10^8 CFU/g of soil respectively. But in comparison with the limits of CFU in non-contaminated soil determined by Popelářová et al. [20] – 6.65 x 10^{10} to 6.88 x 10^{10}, our counts differs – they are by five orders of magnitude lower, what could be an evidence of harmful effect of heavy metals creating an inappropriate conditions for soil bacterial populations growth.

Tab. 1 Counts of CFU per gram of the sample

sampling site	medium	CFU/g of sample
RB1	TSA	**1.50 x 10^5**
	NA2	**3.58 x 10^5**
	R2A	**1.38 x 10^5**
	100x diluted NA2	**2.24 x 10^5**
RB2	TSA	**3.02 x 10^5**
	NA2	**1.50 x 10^5**
	R2A	**3.21 x 10^5**
	100x diluted NA2	**3.16 x 10^5**
H1	TSA	**3.18 x 10^5**
	NA2	**1.18 x 10^5**
	R2A	**4.57 x 10^5**
	100x diluted NA2	**3.69 x 10^5**

Based on the variable phenotype of bacterial colonies, 40 isolates from each type of cultivation media from one sampling site were selected and identified using MALDI – TOF MS identification. After the identification, representative isolates from each clade of phylogenetic tree were tested for their antibiotic tolerance.

As we can see on the pictures (Fig. 1), the MIC of TET was determined in the case of all three sampling sites. The highest value of MIC for this antibiotic was 50 µg/ml observed in the H1 location followed by 10 µg/ml for RB1 and 5 µg/ml for RB2 site. MIC values established for localities RB1 and RB2 were approximately the same in comparison with dump, where this value was several times higher pointing to the probable man-made impact and environmental factors. On the contrary, Safari Sinegani and Younessi [21] observed the highest TET resistance just in the isolates obtained from the mine. In their research, TET resistance was the highest among all the antibiotics tested. 44.33 % of their bacteria were resistant to 30 µg of this antibiotic.

While Safari Sinegani and Younessi [21] noticed TET resistance as the highest one, in our experiment, KAN and CHLOR tolerance were observed as the highest. The same values of MIC for KAN were set only in samples obtained from the mine – 200 µg/ml. Malik and Aleem [22], on the contrary, tested resistance of bacterial isolates obtained from wastewater irrigated soil and groundwater irrigated soil. In the case of KAN (30 µg/disc) they detected 29% of resistant isolates from wastewater irrigated soil and 27% from groundwater irrigated soil respectively. Different situation occurred in the case of CHLOR – in the concentration of 30 µg/disc, they observed very high percentage of resistant bacteria (46% for bacteria from wastewater irrigated soil vs. 69% for bacteria from groundwater irrigated soil), while our team, in the highest CHLOR concentration tested - 20 µg/ml – observed merely from 2 – 19% of tolerable bacteria.

Only in the case of AMP we perceived high counts of bacteria capable of growing even on the highest concentration of antibiotic tested. Similarly Safari Sinegani and Younes [21] noticed very high frequency of AMP resistant isolates, even though they experimental concentration of this antibiotic was only 10 µg/ml.

As we can see from the results, we proved the presence of antibiotic resistance in the places where it was not expected. The influence of heavy metals could be considerable what might be seen from higher numbers of antibiotic resistant isolates obtained from the mine. This phenomenon could occur due to the presence of higher heavy metals concentrations in the mine than in the dump.

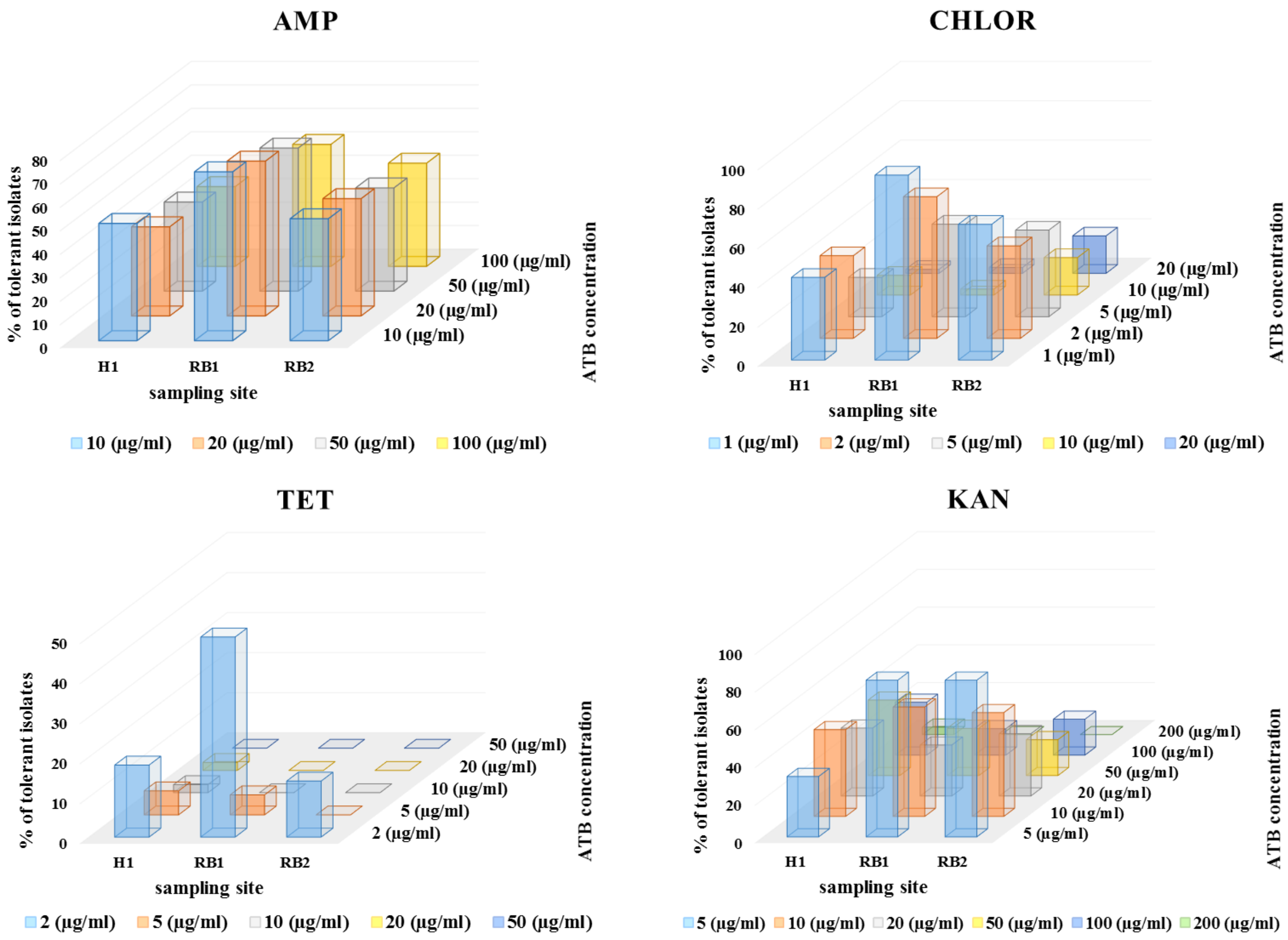

Fig. 1 Minimal inhibitory concentrations of ampicillin (AMP), chloramphenicol (CHLOR), tetracycline (TET) and kanamycine (KAN) influencing bacterial growth

4 Conclusions

In our experiment we showed the presence of antibiotic tolerance of bacteria which could arise from selective pressure created just by heavy metals. There are concerns about the probability of metal contaminated soils acting as favourable sites for antibiotic resistant bacteria and thereby pool of antibiotic resistant genes in both natural and clinical settings.

Acknowledgements

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INFLUENCE OF TEMPERATURE ON INDIUM AND TIN EXTRACTION FROM LCD GLASS DURING CHEMICAL AND BIOLOGICAL LEACHING

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Abstract

Article presents results of the indium and tin extraction from the LCD glass. Biological and chemical leaching were used for the ground LCD material. Experiments were carried out in temperature 20°C and 30°C in two separated leaching tests: i) bioleaching during 32 days in 9K medium and mixed bacteria *Acidothiobacillus ferrooxidans* and *Acidothiobacillus thiooxidans* ii) chemical leaching during 24 hours with $Fe_2(SO_4)_3 \cdot 5H_2O$ solution and Fe^{3+} concentration 9 g/dm^3. The temperature enhanced dissolution of indium and tin both in the chemical and biological leaching. However higher concentrations of these metals were achieved under biological leaching conditions at the temperature of 30°C: respectively 11,1 mg/dm^3 for In and 6,1 mg/dm^3 for Sn.

Keywords: indium recovery, LCD panels, bioleaching, leaching, recovery

1 Introduction

Indium is considered as one of the critical raw materials for the EU. It is expected that the demand for special metals as well as other rare raw materials will increase by three times up to 2030. This situation is conditioned by the growing demand for critical metals, such as lithium, indium, tantalum or germanium, needed for the production of photovoltaic modules. In this situation, the importance of recycling increases more and more. Indium as metal is used in electronics in the form of alloys and low-melting solder. However the main indium raw materials are indium oxide and In-Sn oxide (ITO: 90% In_2O_3, 10% SnO_2, the contents of other elements are less than 20 ppm), which ultrathin, transparent coatings are used in the production of liquid crystal displays (LCD). The amount of indium in LCD screens varies in different models of devices and is approximately 102 mg/kg. This value can be even higher (1400 mg In/kg) when the polymer film attached to the LCD screen is previously removed [1]. The recovery process of indium and tin is under investigation of many researches which tested different dissolution, extraction and purification methods such as leaching process, solvent extraction, selective precipitation or vacuum-chlorinated separation method [2-5]. The process of metals extraction from the solid into liquid phase is a complex process, determined by many factors among which the temperature is one of the main factor influences on kinetic of metals dissolution. This article presents results of indium and tin extraction from LCD panels during leaching and bioleaching process in different temperature range: 20°C and 30°C. These temperatures were chosen as optimal and slightly below the optimal conditions for bacteria *Acidothiobacillus ferrooxidans* and *Acidothiobacillus thiooxidans* growth. The solution of salts $Fe_2(SO_4)_3$ with the Fe^{3+} ions content of 9 g/dm^3 was used in the chemical leaching. In hydrometallurgical processes, iron (III) salt solutions are effective leachants due to their high oxidation-reduction potential (ORP = +0.77 V), rapid reaction and ease regeneration.

2 Materials and methods

LCD panels from spent mobile phones were used in experiments. The waste was ground in a cutting mill to a particle size of 0.2-0.5 mm. The content of indium and tin in the material was determined by Microwave Plasma Atomic Emission Spectroscopy (MPAES) (Agilent MP-AES 4200). Indium and tin content was 0.0427% and 0.007% respectively. Experiments of indium and tin leaching at 20°C and 30°C were carried out in two tests: i) in a leaching medium with the use of acidophilic bacteria ii) using solution of $Fe_2(SO_4)_3 \cdot 5H_2O$ as leaching medium. The ratio of solid phase of waste to volume of leaching solution was 1:5.

Regular measurements of indium and tin concentration and the oxidation-reduction potential (ORP) were taken. The content of indium and tin in the samples was determined by the Microwave Plasma Atomic Emission Spectroscopy (MPAES) (Agilent MP-AES 4200). Cells of *A. ferrooxidans* and *A. thiooxidans* bacteria were used in the experiments. Bacteria were separated from ferruginous mineral water Głębokie i Łomnica (Nowy Sącz, Poland). 9K medium was used as leaching solution. The Silverman – Lundgren (9K) medium was composed of (g/dm^3) $(NH_4)_2SO_4$-2.0; KCl-0.1; $MgSO_4 \cdot 7H_2O$-0.25; KH_2PO_4-0.5; $FeSO_4 \cdot 7H_2O$-44.2, S0-10, Fe (II) 9,0 g/dm^3, pH 1,8.

Experiments were carried out in Erlenmeyer flasks at 30 °C in a thermostat in two series lasting 32 days. The control tests under sterile conditions were conducted in paralel.

Leaching with $Fe_2(SO_4)_3$ was carried out for 24 hours under dynamic conditions using a laboratory shaker (120 rpm). The salt solutions were prepared so as to concentration of Fe (III) 9g/dm^3, pH 1,7. The control tests were carried out in solutions without addition of iron (III) salt. Measurement of ORP, indium and tin concentration were conducted during the first 4 hours and the last one after 24 h.

3 Results and discussion

Fig. 1 presents changes in ORP recorded during chemical and bacterial leaching of LCD panels waste. In biological leaching, after the initial adaptive bacteria phase, increase of ORP from 400-450 mV (10-14 days) to about 600 mV on the 32nd day of the experiment was observed. Increase of ORP was accompanied by acidification of the leaching solution and decrease of pH to 1,4 (data not shown). Rate of ORP growth for bioleaching systems conducted at 30C was higher compared to the 20°C. It results directly from the intensification of Fe (II) to Fe (III) oxidation process by *A. ferrooxidans* in more favorable temperature conditions. At the same time, in the biological control samples ORP values were maintained at the level of 350-400 mV for 20°C and 30°C. The solutions of the $Fe_2(SO_4)_3$ containing 9 g/dm^3 of Fe^{3+} were characterized by a high initial ORP - 650mV. During the leaching process there was a slight decrease of this parameter to a comparable value of 600mV, obtained in the biological process. In the chemical control, the ORP was maintained at around 450 mV.

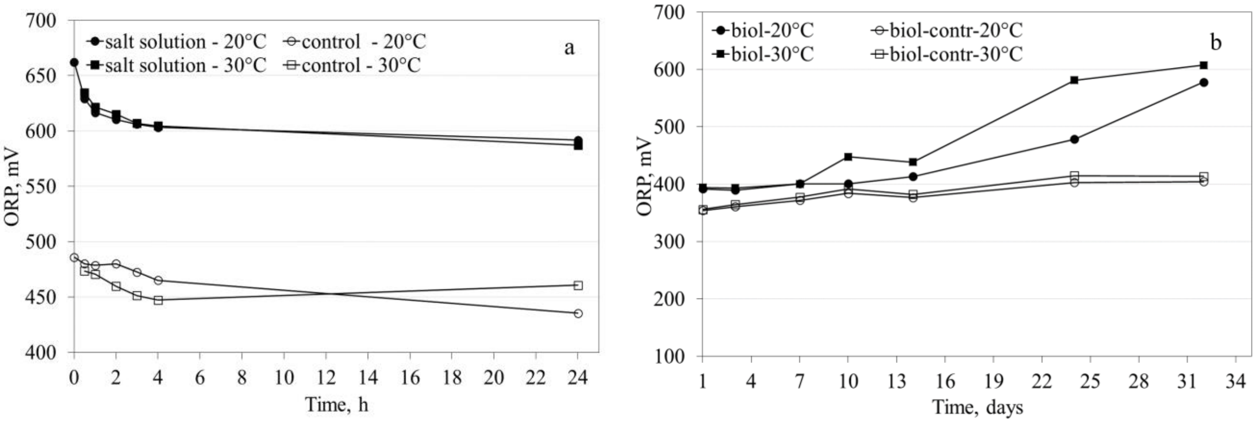

Fig.1 ORP changes during a) leaching with $Fe_2(SO_4)_3$; b) bioleaching with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*

Fig. 2. presents changes in indium and tin concentrations in solutions involving bacteria during 32 days bioleaching and application of iron salt (III) within 4 hours in leaching experiment. Temperature increase influences the dissolution kinetics both indium and tin in the conducted biological and chemical tests. Better results of In and Sn transfer into solutions were achieved at 30°C. In the bioleaching process a gradual increase in In concentration occurred in bacterial and control samples - range from 0,4 mg/dm^3 to 2,7 mg/dm^3 on 24th day. Visible increase indium concentration in the solution (up to 11,1 mg/dm^3) occurred from 24th day of bioleaching at 30°C at the ORP value of 575 mV (Fig. 1). A similar course of changes in In concentration during bioleaching was observed in the work [6]. At the same time, indium concentration at the level of 4,1 mg/dm^3 for the bioleaching system at the 20°C was achieved. In the environment of iron (III) salt with the ORP value in the range of 650-600 mV, within 4 h and at 30°C the indium concentrations at the level of 0,6 mg/ m^3 and 0,3 mg/dm^3 at the temperature of 20°C were obtained. In the case of tin for chemical systems with Fe^{3+}, during the first 0,5 h the tin was transfered into the solution at the level of 4 mg/dm^3. Over the following hours of leaching, Sn concentration increased slightly to 5,0 mg/dm^3 at 30°C. In tests without Fe^{3+}, no tin was detected in the solutions. For biological samples, tin concentration at the level of 6,0 mg/dm^3 was observed.

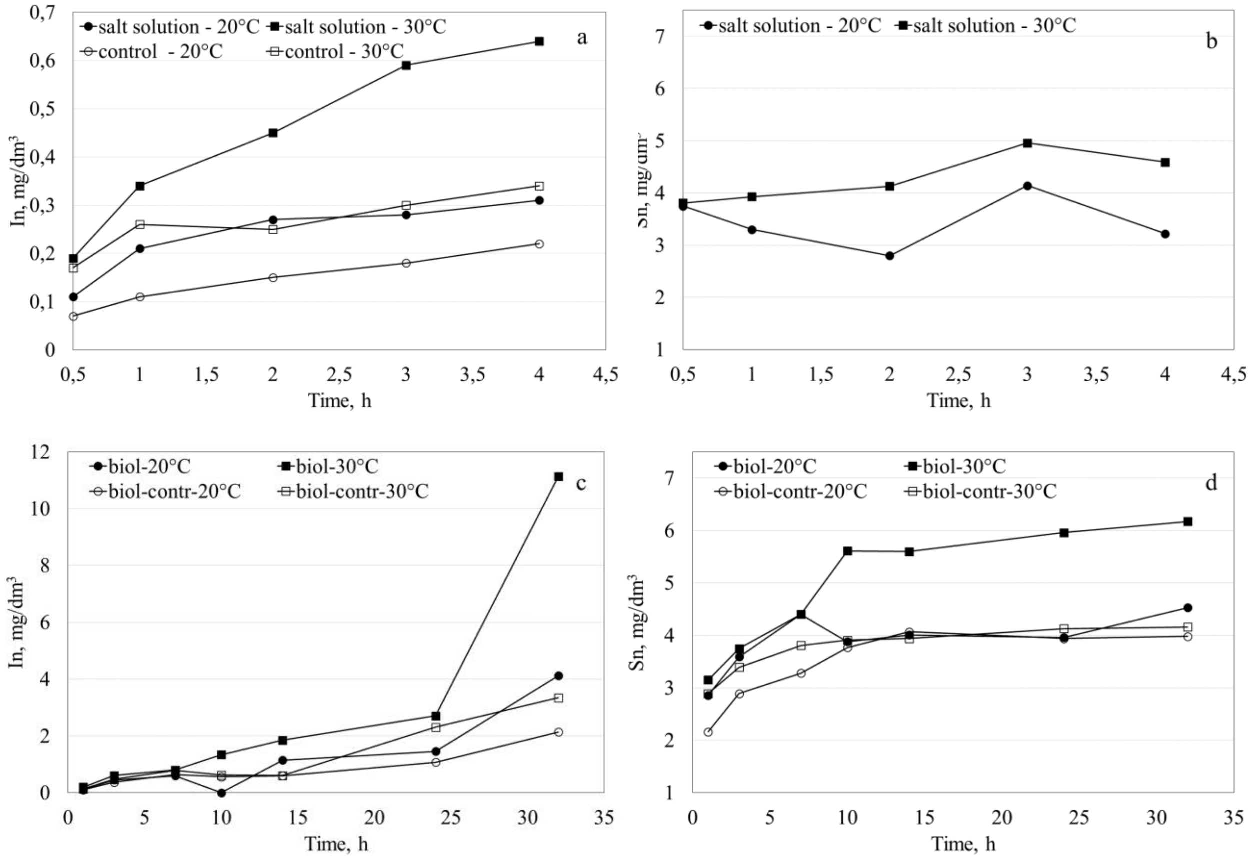

Fig. 2. Change in metals concentration: a) indium during leaching with $Fe_2(SO_4)_3$, b) tin during leaching with $Fe_2(SO_4)_3$, c) indium during bioleaching d) tin during bioleaching.

Fig. 3 compares the values of indium and tin concentrations achieved after 24 h and 32 days in the chemical and biological leaching process, respectively, at 20°C and 30°C. The degree of indium and tin transfer from solid phase into solutions increases with temperature increasing, both in biological and chemical solutions with $Fe_2(SO_4)_3$. In leaching tests with the iron (III) salt, the indium concentration was 1,1 mg/dm^3 at 20°C and 1.5 mg/dm3 at 30°C, while the indium concentration in the biological leaching process reached 4,1 mg/dm^3 at 20°C. and 11,1 mg/dm^3 for the sample at 30°C. The temperature influences microorganisms activity, the rate of bioleaching and accelerates chemical leaching. Providing favorable temperature conditions for the growth and microorganisms activity promotes the efficiency of indium and tin dissolution. In addition to the temperature, the presence of Fe^{3+} ions intensifies metals extraction, which is evident in the process of tin dissolution in systems with $Fe_2(SO_4)_3$. At 30°C, within 24 hours, tin concentration at the level of 5,7 mg/dm^3 was achieved, while in systems without iron (III) salt addition, Sn was not detected. Comparable tin concentration (6,1 g/dm^3) was obtained in biological solutions within 32 days. The concentration of Sn at 20°C in the biological medium was 4,3 mg/dm^3.

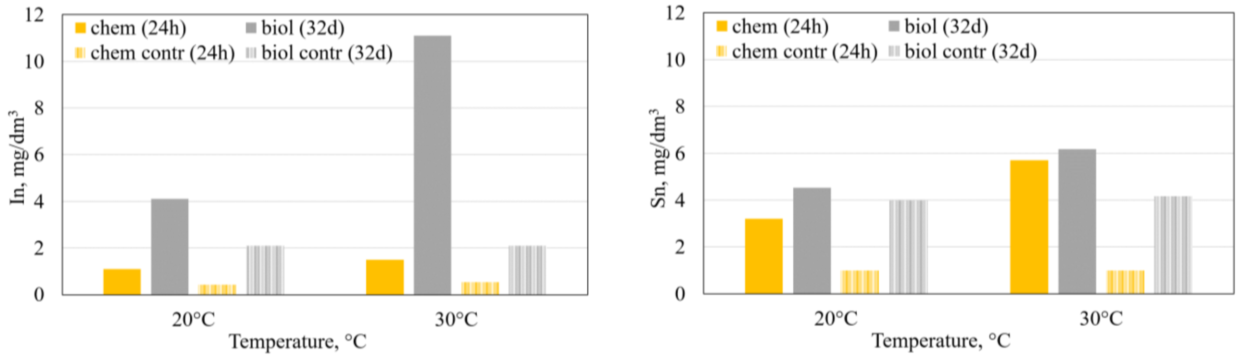

Fig. 3 Comparision of metals concentration depending on the temperature a) indium concentration in the process of bioleaching and chemical leaching b) concentration of tin in the process of bioleaching and chemical leaching

4 Conclusion

Based on the results of indium and tin bioleaching studies with the *A. ferrooxidans* and *A. thiooxidans* bacteria at 20°C and 30°C and leaching process with $Fe_2(SO_4)_3$, it was determined that temperature is a factor intensifying both indium and tin dissolution. Better results of indium and tin extraction were obtained at 30°C temperature than at 20°C. During indium bioleaching at 30°C, almost three times higher concentration of this metal in solution was obtained, relative to the temperature 20°C – 11,1 mg/dm^3. When $Fe_2(SO_4)_3$ salt was used, the indium concentration in solutions at 30C did not exceed 1,5 mg/dm^3. In addition to the temperature, presence of Fe^{3+} ions in the solutions is the factor influencing the rate of tin dissolution. No tin was detected in chemical systems without addition of $Fe_2(SO_4)_3$, while in the solution with Fe^{3+} (9g/dm^3), tin concentration at 30°C was 5,8 mg/dm3. This result is comparable with the result of tin extraction in the bacterial environment – 6,1 mg / dm^3.

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SORPTION PROPERTIES OF MAGNETICALLY SEPARATED CARBON-RICH COAL FLY-ASH

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Abstract

The paper deals with the study on the sorption properties of physically treated coal fly-ash towards cationic dye (methylene blue). Firstly, carbon rich coal fly-ash ($C^d = 21.54$ %) was magnetically separated at two inductions of magnetic field, i.e. 0.1 T and 0.4 T, respectively. Thus, three products, two magnetic and one "non-magnetic" different in carbon content and volume magnetic susceptibility were obtained. Secondly, the basic sample and selected products of separation was tested in wide pH range as a sorbent of methylene blue. pH adsorption study showed that adsorption capacity is similar for all studied samples. Additionally, the adsorption capacity increased with pH (to alkaline region). Thus, the best sorption process was observed at pH 10.7. Since the composition of physical separation products has no influence to sorption properties, basic sample was used for determination of maximum sorption capacity using Langmuir model ($Q_m = 83.33$ mg/g).

Keywords: coal fly-ash, magnetic separation, adsorption, methylene blue

1 Introduction

Utilization of fly-ashes is given by physical, chemical and mineral properties. Thus, the fly-ash properties depend on the composition and the carbonization grade of coal, and also on coal combustion technology. All types either from fusion, granular and fluidized-bed boilers usually contain silicates, alumosilicates, unburned carbon and iron. As to iron some its part is bonded to magnetite which is strong magnetic and it can be easily separated using a magnetic field of low intensity. Presence of magnetite as well as other iron-bearing components and their magnetic separation was examined by many researchers with the aim to iron removal and in such way to obtain enriched products of (alumo)silicates and/or unburned carbon.

Hredzák et al. [5] studied possibilities of iron concentrate preparation from hard coal fly-ash from the Heating plant of Košice (TEKO) using wet magnetic separation. So, the magnetic product at a mass yield of 4.09 %, Fe-content of 41.61 % and Fe-recovery of 35.04 % was won using Jones-type separator at the induction 0.09 T and additive of potato starch from the fly-ash feed with 4.85 % Fe.

Magnetic fraction with high content of iron (50.86%) was obtained from hard coal fly-ashes (heating plant Energetika VSŽ, a.s., Košice, Slovakia) by wet low-intensity magnetic separation process (Michalíková et al., 2005).

Blaha and Kaľavský [2] studied magnetic separation of hard coal fly-ash obtained from (i) electrofilters and (ii) settling pit. In the first case (i) magnetic product with iron content 44.53 % at mass yield 3.3 % was obtained. In the second one (ii), the iron content in magnetic product attained 51.54 % at mass yield 1.5 %.

Fly ash from three Bulgarian power plants was separated by wet low-intensity magnetic separation [16]. The separation was performed at different intensities of magnetic field, different flows and several averages of matrix elements. The conditions of magnetic separation were as follows: range of magnetic field induction – $0.01 < B < 0.02$ T; field gradient – $3 < \mathrm{grad}B < 30$ $T.m^{-1}$. Concentration of iron in magnetic products was 37.5 %, 16.8 % and 26 %, respectively.

Fly-ashes can be also used as the cheap adsorbents of toxic substances from water. The most popular studies are devoted on sorption experiments towards heavy metals. Thus, this was studied by many researches and results confirm that the fly-ashes can be effective and cheap adsorbents [1, 4, 8, 12, 17, 18, 21].

Moreover, the fly-ash was tested as adsorbent of organic compounds. Many times, organic dyes as model cationic or anionic compounds are used (e.g. methylene blue - MB as cationic dye). Rafatullah et al. [15] published a review, where low-cost adsorbents together with fly-ash were tested as adsorbents of methylene blue. Unburned carbon separated from fly-ash was used for MB removal in the work of Wang et al. [20]. It was found that adsorption capacity increased with the temperature. Adsorption capacity determined at 50 °C increased twice in comparison to 30 °C. Wang et al. [20] described physico-chemical properties of different fly-ashes as well as sorption properties towards MB and humic acids from water solution. Maximum sorption capacity for MB was 12.7 mg/g. Kumar et al. [9] attained maximum sorption capacity of fly-ash towards MB 5.7 mg/g. Jain et al. [6] studied the effect of various parameters (such as initial concentration, contact time, pH) on MB sorption properties. Maximum MB removal was reached at pH 7. The adsorption was described by Freundlich and Langmuir models. Maximum adsorption capacity value calculated by Langmuir isotherm was 0.434 mg/g. Additionally; Janoš et al. [7] tested the fly-ash from subbituminous coal as a sorbent of several synthetic dyes from water. They found that sorption capacities were in the range $10^{-1} – 10^{-3}$ mmol/g and it was not significant difference between basic or acidic dyes.

The main goal of this work was to show the influence of magnetic separation on the sorption properties of obtained products. Moreover, after initial adsorption experiments (influence of pH on sorption capacity), the best adsorbent was used for determination of maximum sorption capacity (Q_m).

2 Material and methods

The experiments were realized by coal fly-ash from the Heating plant (TEKO, Košice, Slovakia). Chemical composition of basic sample – TEKO determined by an X-ray fluorescence (XRF) spectrometer Spectro Xepos model XEPO3 (Spectro Analytical Instruments, Germany) was as follows: SiO_2 – 39.37 %, Al_2O_3 – 18.51 %, Fe_2O_3 – 7.76 %, CaO – 2.23 %, K_2O – 1.86 %, Na_2O – 1.35 % and MgO – 0.98 %.

Magnetic separation of TEKO sample was performed at dry conditions with two magnets (0.1 T and 0.4 T, respectively). Thus, three product of magnetic separation was received: 1. strongly magnetic (B=0.1T), 2. weakly magnetic (B=0.4T) and 3. “non-magnetic” product.

CHNS analysis was performed using the elementary analyzer Vario MACRO cube (Elementar Analysensysteme GmbH, Germany) equipped with a thermal conductivity detector. The combustion tube was set to 1150ºC and the reduction tube to 850ºC. Sulfanilamide (C = 41.81%, N = 16.26%, H = 4.65%, S = 18.62%) was used as a CHNS standard. Textural properties of ash sample were determined from the adsorption and desorption isotherms measured with the NOVA 1200e Surface Area & Pore Size Analyzer (Quantachrome Instruments, USA) by the method of physical adsorption of nitrogen at –196 °C. First, the sample was degassed at 105 °C in a vacuum oven under a pressure lower than 2 Pa for 18 hours. The measured data were processed by the BET (Brunnauer–Emmett–Teller) isotherm in the range of relative pressure 0.05–0.35 to obtain the value of specific surface area (S_{BET}). The value of total pore volume (V_{tot}) was estimated from the maximum adsorption at relative pressure close to saturation pressure. The pore size distribution was obtained from the adsorption and desorption isotherm using the Barrett-Joyner-Halenda method. Volume magnetic susceptibility (κ) was measured by Kappabridge KLY-2 apparatus (Geophysics, Brno, Czech Republic), at the following condition: the magnetic field intensity of 300 $A.m^{-1}$, the field homogeneity of 0.2 % and the frequency of 920 Hz.

The sorption properties were studied under the batch-type conditions. The sorbent concentration was 2 g/L and initial MB concentration was 10 - 500 ppm. The experiments were performed at room temperature in a rotary shaker set at 30 rpm and the equilibrium time 24 hours. The measurement of MB concentrations was performed using a UV-VIS spectrophotometer (Helios Gamma, Thermo Electron Corporation, United Kingdom). The maximum wavelength for MB was found to be 663 nm. After the adsorption, the respective concentrations were calculated using the calibration curves within the range of 0 - 20 mg/L. The pH was adjusted by the addition of 2 M NaOH or HNO_3. The sorption experiments were evaluated by Langmuir model [10], i.e. an isotherm for monolayer adsorption on homogenous surface.

The Langmuir isotherm is defined according to equation:

$$q_e = Q_m \frac{bC_e}{1 + bC_e} \qquad (1)$$

where q_e is an equilibrium adsorption capacity (mg/g), Q_m is the maximum adsorption capacity (mg/g), C_e is the equilibrium metal concentration, b is a Langmuir constant characterizing the affinity between the adsorbed molecule and the adsorbent (L/mg). Q_m and b, were determined from the experimental values using the linearized form of previous equation.

3 Results and discussion

3.1 Sample characterization

Results of two-stage magnetic separations of fly ash are summarized in Table 1. At higher induction of the field, the yield was very high (73.83 %) but volume magnetic susceptibility (κ) was comparable to basic sample – TEKO. It is because M2-TEKO contained inorganic paramagnetic substances as well as different aggregates with various magnetic properties. On the other hand, strong magnetic product with highest volume magnetic susceptibility was received in the first step by low-intensity magnetic separation (M1-TEKO).

Table 1 Results of two-stage the magnetic separation (TEKO fly-ash) and CHNS analysis magnetically separated products

Sample	Yield [%]	C [%]	H [%]	N [%]	S [%]	κ x 10^{-6} [SI unit]
M1–TEKO, magnetic product (B=0.1 T)	5.11	17.04	0.038	0.20	0.254	65897
M2-TEKO, magnetic product (B=0.4 T)	73.83	21.57	0.044	0.28	0.294	10679
N-TEKO, “non-magnetic” product	21.06	23.20	0.041	0.28	0.293	5387
TEKO, basic sample – feed to separation	100.00	21.68	0.043	0.28	0.292	11143

However, the goal was to obtain the sample with high content of organic phase (sample N-TEKO). All products and basic sample were analyzed by elemental CHNS procedure (Table 1). Finally, an increasing of carbon content from 21.54 % to 23.2 % was achieved in the “non-magnetic” product (N-TEKO).

The results obtained from textural analysis are presented on Fig. 1 and listed in Table 2. The fly-ash sample (TEKO) showed only a slight increase of adsorbed gas volume in dependence on increasing relative pressure. The value of total volume of adsorbed gas is low what points at the weak porosity of the sample and also relates with the low value of specific surface area. The expressive increase of adsorbed gas volume at the relative pressure $p/p_0 = 0.9$ is connected with the presence of macropores. The narrow hysteresis loop between the adsorption and desorption branch of the isotherm relates with the presence of mesopores (however the total porosity is inexpressive what corresponds with the low values of adsorbed gas volume and low value of specific surface area). The curves of pore size distribution obtained from the adsorption and desorption isotherm are both wide (almost equal running can be observed so the effect of capillary condensation in the present mesopores is not marked so much, probably from the reason of low mesopore content in the studied sample). Therefore the application of t-plot method to differentiate the volume of adsorbed gas in micropores is not necessary and value of external surface area can be considered as equal with the value of specific surface area.

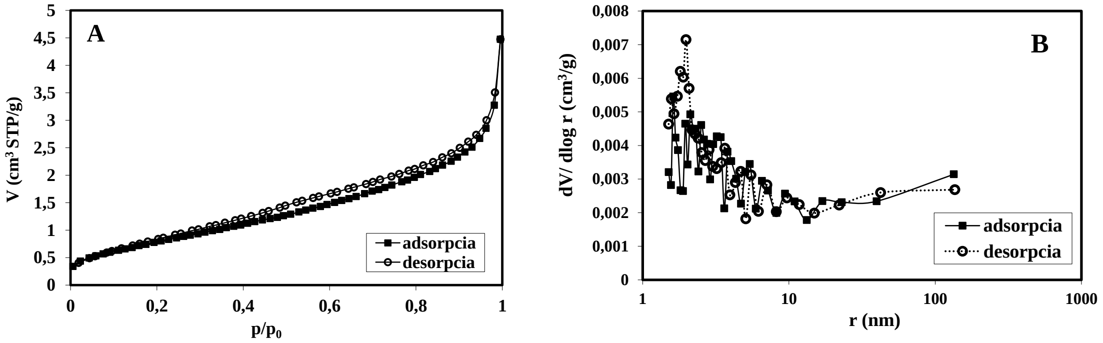

Fig. 1 Adsorption and desorption isotherms of TEKO sample (A) and pore size distribution curves derived from adsorption and desorption isotherms (STP means - standard temperature and pressure, t= 0 °C, p=101.325 kPa).

Table 2 Textural parameters of TEKO sample (the values of specific surface area (S_{BET}), C_{BET} constant, total pore volume ($V_{tot.}$)).

Sample	S_{BET} [m^2 kg^{-1}]	C_{BET}	$V_{tot.}$ [cm^3 g^{-1}]
TEKO, basic sample	3000	33.659	0.007

In general, surface properties of fly-ashes are dependent on coal properties and combustion technology. Specific surface area of TEKO sample (3000 m^2 kg^{-1}) is comparable with literature sources. For example, Mohapatra and Kanungo [14] studied surface properties of 3 different fly-ashes. Surface area was 1754 m^2 kg^{-1}, 4400 m^2 kg^{-1}, 12186 m^2 kg^{-1}. Surface area of 9 fly ashes analysed in work of Cabrera and Hopkins [3] was in the range of 480 - 8900 m^2 kg^{-1}. Lebedev et al. [11] showed different value of specific surface area depending on CaO concentration. S_A value 1100 m^2 kg^{-1} was measured for fly-ash with 38 % of CaO content and S_A = 5900 m^2 kg^{-1} was measured for fly-ash with 2.57 % of CaO.

3.2 Adsorption of methylene blue

Basic sample (TEKO) as well as the magnetically separated products (M2-TEKO, N-TEKO) was tested as adsorbents of MB from water solution. As a fist, effect of pH was monitored with the goal to select best sorbent of MB.

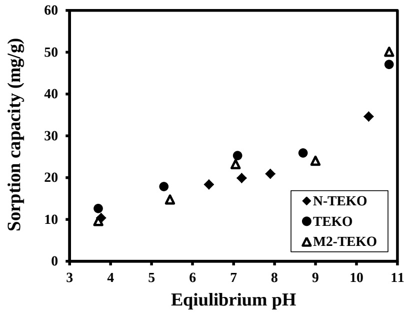

Fig. 2 Effect of pH on sorption capacity of magnetically separated fly-ashes (Initial concentration of MB = 200 mg/L)

Results showed (Fig. 2) that sorption capacity for all studied samples is similar in whole pH range. Since the composition of physical separation products has no influence to sorption properties, basic sample of fly-ash (TEKO) was chosen for determination of maximum sorption capacity (Q_m). The experiments were carried out at pH = 10.7. Langmuir adsorption isotherms are presented in Figure 3. Langmuir constants were calculated from linearized Langmuir isotherm (equation 2, Fig. 3A). Maximum sorption capacity was 83.33 mg/g and constant b = 0.0186. Correlation coefficient - $R^2 = 0.934$. This value of Q_m is higher than values published previously (0.437-16.6 mg/g) for removal of MB by fly-ashes (see Wang et al., 2008; Wang et al., 2005; Kumar et al., 2005; Jain et al., 2016).

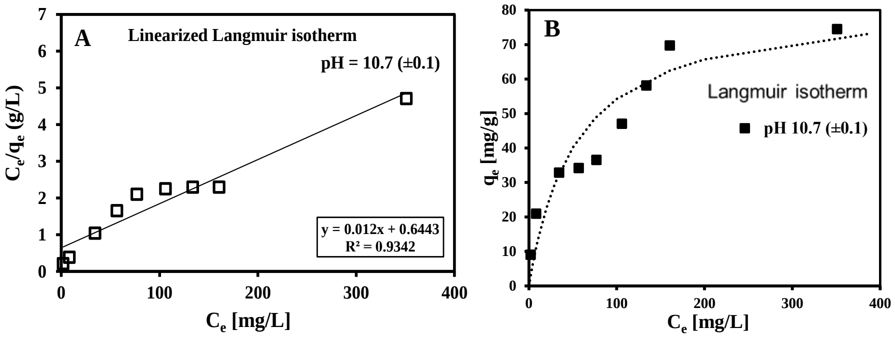

Fig. 3 Langmuir adsorption isotherm for MB (B) and its linear plots (A). Experimental values from batch type experiment are marked with filled circles. Dotted line presents Langmuir data fit.

4 Conclusion

Coal fly-ash from heating plant with high carbon content (21.54 %) was magnetically separated. Three products were obtained, where "non-magnetic" sample (N-TEKO) showed the highest carbon content (23.20 %) and lowest magnetic susceptibility value. pH adsorption test showed that adsorption capacity is similar for all studied samples (TEKO, N-TEKO, M2-TEKO) and the best results were obtained at alkaline conditions. Therefore, basic sample of fly-ash was used for determination of maximum sorption capacity. It was determined by Langmuir model and achieved 83.33 mg/g at pH = 10.7.

Acknowledgements

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UTILISATION OF FERROFLUD FOR SYNTHESIS OF EFFECTIVE MAGNETIC ADSORBENTS

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Abstract

Permanent decreasing of water quality requires the development of novel, effective and cost available adsorbents. Herein, a simple procedure for preparation of magnetic adsorbent from agricultural waste biomass and ferrofluid has been introduced. Specifically, ferrofluid mixed with wheat straw was directly pyrolysed by microwave irradiation (900 Watt, 30 min). Studied samples were characterized by X-ray powder diffraction, Mössbauer spectroscopy, textural analysis and tested as adsorbents for As(V) oxyanion. Results showed that microwave pyrolysis produces char with high adsorption capacity towards the As(V) (Q_m = 25.6 mg/g at pH 4).

Keywords*:* magnetic bio-char, microwave pyrolysis, wheat straw, ferrofluid, sorption

1 Introduction

Pollutants such as heavy metals (e.g., cations of Cd, Pb, Hg, and oxyanions of As, Cr, Mo), organic compounds (organic dyes, drugs, pesticides, PAHs, etc.) and other substances in water sources, even in relatively low concentration, have been identified as highly toxic and harmful to the environment and to human health. In recent years, considerable attention has been given to the removal of toxic metals from aqueous solutions using adsorbents derived from low-cost materials, such as agricultural waste, by-products, coal, as well as a mixture of biomass and coal [15]. The bio-char can be used as a feedstock for production of activated carbon either by chemical or physical activation in connection with second pyrolytic step (that means two-stage pyrolysis) [2, 18]. Many researchers deals with preparation of magnetic biochar from various types of biomass with conventional or microwave heating [10, 16, 17, 19]. Modified natural carbon materials with magnetic properties can be easily separated from water by applying an external magnetic field. Magnetic filtration is emerging as a water treatment technology which can provide rapid, efficient contaminant removal magnetic biochar (magnetic sorbent) from aqueous waste. In the present work: 1. natural magnetic carbon was prepared via one step microwave pyrolytic procedure 2. magnetic carbon was tested as the sorbent material.

2 Materials and method

2.1 Microwave preparation of magnetic carbon biochar

Magnetic biochars were prepared from agricultural waste biomass (wheat straw - WS) and ferrofluid (FF) as follows: wheat (*Triticum aestivum*) straw were crushed and sieved to granulometric fraction under 1 mm. Afterwards; ferrofluid was mixed with wheat straw (40 g of dry biomass with 200 ml of ferrofluid). The ratio between the biomass and liquid ferrofluid (1:5) was chosen depending up to absorption capacity of biomass sample to create magnetic biomass paste. After homogenizing, the magnetic paste was dried overnight at 80ºC and briquettes were made and then used either for microwave pyrolysis.

Microwave pyrolysis was performed in microwave oven Panasonic NN-GD566M. Samples briquette (20 g) were pyrolyzed in quartz flask at 900 Watt, during 30 minutes under nitrogen atmosphere (sample name: MWpyr WS:FF). After the pyrolysis, the sample are washed with deionized water and also dialyzed against to deionized water at neutral pH. Volume magnetic sucsceptibility (□) was measured by Kappabridge KLY-2 apparatus.

CHNS analysis was performed using an elementary analyzer Vario MACRO cube (Elementar Analysensysteme GmbH, Germany) equipped with a thermal conductivity detector. The combustion tube was set up at 1150ºC and the reduction tube at 850ºC. Sulfanilamide (C = 41.81%, N =16.26%, H = 4.65%, S = 18.62%) was used as a CHNS standard.

The ash content was determined by burning in muffle oven at 815ºC to a constant weight.

X-ray powder diffraction study was carried out using D8 Advance diffractometer (Bruker, Germany), working with Cu Kα radiation.

Surface properties of studied samples were determined from the adsorption and desorption isotherms measured with the NOVA 1200e Surface Area & Pore Size Analyzer (Quantachrome Instruments, USA) by the method of physical adsorption of nitrogen at –196ºC. Firstly, the samples were degassed at 100ºC in a vacuum oven under a pressure lower than 2 Pa for 16 hours.

Mössbauer spectra was measured by ^{57}Fe Mössbauer spectrometer in transmission mode and constant acceleration mode with a ^{57}Co(Rh) source at laboratory temperature.

The metal quantity in solutions was determined by AAS (Varian 240 RS/240 Z, Australia).

2.2 Sorption experiments

Sorption properties of magnetic chars after microwave pyrolysis were studied toward to As(V). Model solutions of As(V) (c = 100 mg/L) were prepared by dissolving of $AsHNa_2O_4.7H_2O$ in deionized water. The sorption properties were studied under batch type conditions at different pH. The sorbent concentration was 2 g/L. The initial concentration for As(V) was 100 mg/L. The experiments were performed at constant temperature 25°C in rotary shaker set at 30 rpm and equilibrium time 24 hours.

The sorption experiments were evaluated by Langmuir [7] and Freundlich [4] model, respectively.
The Langmuir isotherm is defined according to equation:

$$q_e = Q_m \frac{bC_e}{1+bC_e} \tag{1}$$

where q_e is an equilibrium adsorption capacity (mg/g), Q_m is the maximum adsorption capacity (mg/g), C_e is the equilibrium metal concentration, b is a Langmuir constant characterizing the affinity between the adsorbed molecule and the adsorbent (L/mg).

The Freundlich isotherm is defined according to:

$$q_e = K_F C_e^{\frac{1}{n}} \tag{2}$$

where K_F [L/g] and n are the constants of the isotherm. Q_m, b, K_F and n were determined from the experimental values using the linearized form of previous equations.

3 Results and discussion

3.1 Characterization of the samples

Elemental analysis of samples is shown in Table 1. WS:FF contained 17.4% of iron (Fe_{total}). The magnetic nanoparticles were responsible for the high value of volume magnetic susceptibility (493,150·10–6 SI units). After pyrolysis, iron content considerably increased, a loss of magnetic properties was recorded. Nevertheless, both samples are still strong magnetic materials, which enable them to be easily removed from water by applying a low-intensity magnetic field.

Table 1 Elemental (CHNS) analysis, ash content, total iron content and volume magnetic susceptibility (κ) of initial samples as well as magnetic biochar (MWpyr WS:FF).

Sample	**A^d [%]**	**C^d [%]**	**H^d [%]**	**N^d [%]**	**S^d [%]**	**O^d [%]**	**Fe_{Total} [%]**	**κ [unit SI]**
Wheat straw	7.5	43.1	6.1	0.6	0.5	45,7	-	-
WS:FF	30.1	38.3	5.6	0.4	0.7	24.9	17.4	$493\ 150\cdot10^{-6}$
MWpyr WS:FF	-	26.4	1.0	0.2	1.0	-	40.4	$188\ 995\cdot10^{-6}$

Abbreviations: A = Ash, d – dry basis, O^d – by difference – according to 100 – ($A^d + C^d + H^d + N^d + S^d$).

Before the mixing of the WS with FF, the FF was studied in detail in order to know the particular phase composition. In general, FF is a colloidal suspension of magnetite or maghemite. Fig. 1a shows the X-ray diffraction patterns and Mössbauer spectroscopy study of dried FF. X-ray diffraction is not able to differentiate between maghemite/magnetite phase, especially when the particles are extremely small (several nanometers) and the recorded peaks are relatively broad. Therefore, Mössbauer spectroscopy was applied in order to discern between magnetite/maghemite phases (Fig. 1b-d, Table 2). The room temperature Mössbauer spectrum (Fig. 1B) shows a sextet with broad spectral lines, therefore the low temperature Mössbauer spektra were measured. Both spektra measured at T=5 K, T=150 K were evaluated by two sextet components (Table 2) corresponding to tetrahedral and octahedral Fe atoms in the cubic spinel structure of maghemite. The ratios of the sub-spectrum areas slightly differ from those for stoichiometric maghemite, i.e. Tetrahedral/Octahedral = 37.5%/62.5%.

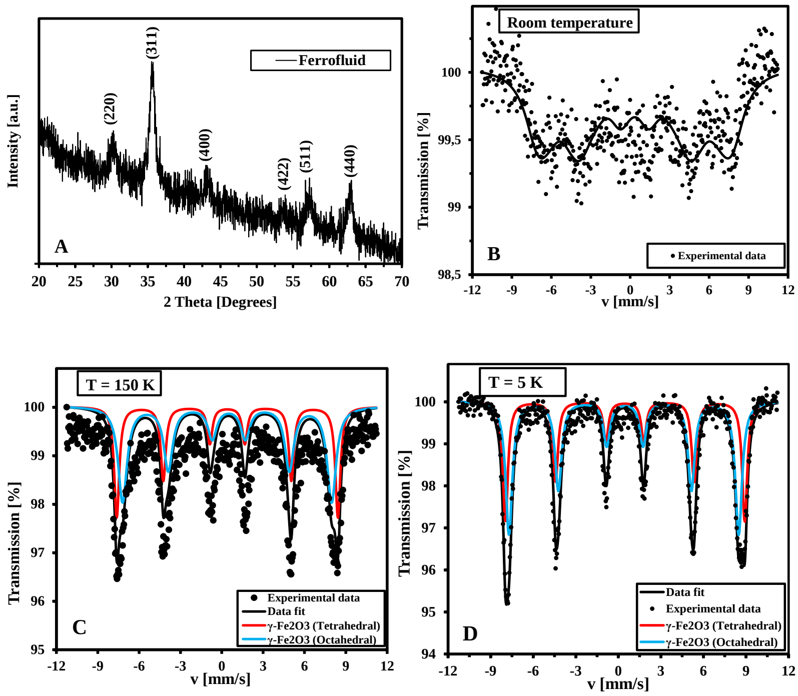

Fig. 1 X-ray powder diffraction (a) and ^{57}Fe Mössbauer spectroscopy study (b, c) of dried ferrofluid. ^{57}Fe Mössbauer spectra were taken at room temperature (b), 150 K (d) and 5 K (d), respectively.

Table 2 Mössbauer parameters for dried ferrofluid. Mössbauer spectra are presented at Figure 1C-D.

Sample	**IS (mm/s)**	**QS (mm/s)**	**H (T)**	***I* (%)**	**Component**
Ferrofluid (T = 150 K)	0.40	-0.00	49.9	39.5	γ-Fe_2O_3 - sextet (Tetrahedral position of Fe^{3+})
	0.45	-0.12	47.1	60.5	γ-Fe_2O_3 - sextet (Octahedral position of Fe^{3+})
Ferrofluid (T = 5 K)	0.47	0.01	52.4	40.4	γ-Fe_2O_3 - sextet (Tetrahedral position of Fe^{3+})
	0.42	-0.07	50.3	59.6	γ-Fe_2O_3 - sextet (Octahedral position of Fe^{3+})

According to X-ray powder diffraction (Fig. 2A), it is clearly visible that initial sample (composite of WS and ferrofluid) contained broad peaks assigned to maghemite nanoparticles. Microwave pyrolysis caused chemical changes of ferrofluids. Only few peaks were recorded. Peaks of magnetite nanoparticles disappeared and X-ray diffraction indicated that α–Fe, γ–Fe, and non-stoichiometric Fe_3C could be generated. The existence of metallic iron resulted from high temperature and presence of reduction atmosphere during the pyrolysis. The formation of new substances (carbides) related with the phenomena of microwave heating, where pyrolysis process is very fast and high temperature is generated. It was confirmed by ^{57}Fe Mössbauer spectroscopy [19] that during microwave synthesis new iron-phases were synthetized: [super] paramagnetic Fe_2O_3, α-Fe, non-stoichiometric Fe_3C, γ-Fe_2O_3, γ-Fe.

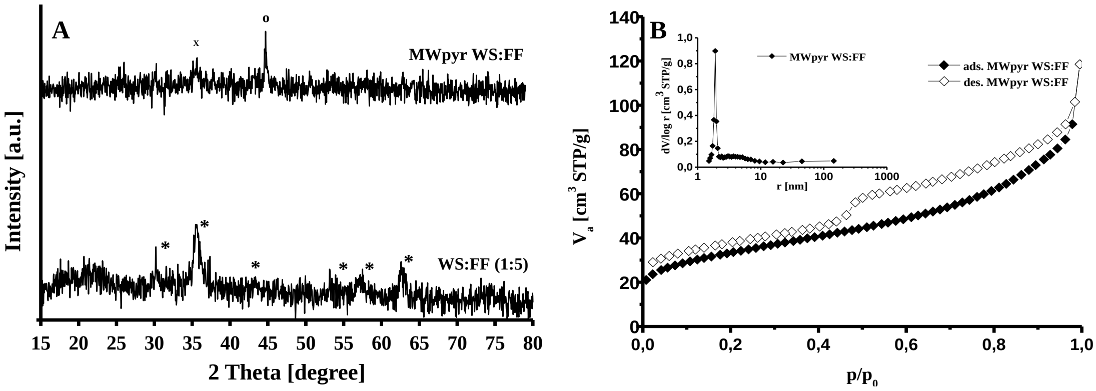

Fig. 2 X-ray powder diffraction analysis (A) (Symbols: * = Maghemite; o = α-Fe or γ-Fe; x = non-stoichiometric Fe_3C) and adsorption and desorption isotherm (B) of magnetic biochar (Top left B inset: pore size distribution curve, STP means standard temperature and pressure, t=0°C, p=101.325 kPa).

Low-temperature nitrogen adsorption was used for characterization of textural properties of samples after microwave pyrolysis (Figure 2B). The isotherm was of type IV with a hysteresis loop corresponding with the capillary condensation in the mesopores. The hysteresis loop is open (decrease of desorbed gas volume is observed for relative pressure $p/p_0 \approx 0.45$). Textural parameters of sample MWpyr WS:FF are as follows: $S_{BET} = 119.3\ m^2/g$; $C_{BET} = 884$; $V_{Tot.} = 0.1832\ cm^3/g$; $V_{micro} = 0.0108\ cm^3/g$; $S_{ext} = 93.3\ m^2/g$. Notably, the value of external surface was lower than S_{BET} what corresponds with the presence of micropores in the structure. Moreover, significant value of V_{micro} was obtained from the t-plot analysis.

3.2 Adsorption of arsenic (V) oxyanion

The structure, porosity and chemical composition have a significant effect on the adsorption properties of prepared materials. The sample MWpyr WS:FF was tested from the viewpoint of sorption properties towards As(V). Adsorption of As(V) is strongly depended on pH (Fig. 3A). The best sorption was reached at acidic pH (45 mg of As per gram of MWpyr WS:FF). On the other hand, iron leaching from the magnetic bio-char (MWpyr WS:FF) under pH 3.5 was observed. Therefore, experiments based on the determination of maximum sorption capacity (Q_m) were carried out pH = 4 (Fig. 3B). In comparison to the Freundlich model (R^2= 0.777; K_F = 7.87 L/g; n = 4.85), the adsorption process is better characterized by the Langmuir model (R^2=0.998; b = 0.08224 L/mg; Q_m = 25.6 mg g^{-1}).

Different magnetic adsorbents were studied for the removal of arsenic. Zhang et al. [16] reported that the Q_m of biochar/γ-Fe_2O_3 composite was 3.147 mg g^{-1}. In this paper, the authors compare the sorption properties of several iron oxides as well as iron composites of As(V): γ-Fe_2O_3, 4.643 mg g^{-1} [11]; hydrous iron oxide, 8.0 mg g^{-1} [14]; iron modified activated carbon, 1.92–6.57 mg g^{-1} [5]. In a review of Mehta et al. [9], different magnetic adsorbents were compared for the removal of diverse pollutants. For As(V) removal, several magnetic adsorbents showed a high value of Q_m (e.g., multi-walled boron nitride nanotubes functionalized with Fe_3O_4 nanoparticles, 32.2 mg g^{-1} [1]; cetyltrimethylammonium bromide modified Fe_3O_4 particles, 23.07 mg g^{-1} [6]; ascorbic acid-coated Fe_3O_4 nanoparticles, 16.56 mg g^{-1} [3]; superparamagnetic ultra-fine magnesium ferrite nanoparticles, 83.2 mg g^{-1} [12]; copper ferrite from PCB sludge, 45.66 mg g^{-1} [13]; and Fe_3O_4-loaded activated carbon from waste biomass, 204.2 mg g^{-1} [8].

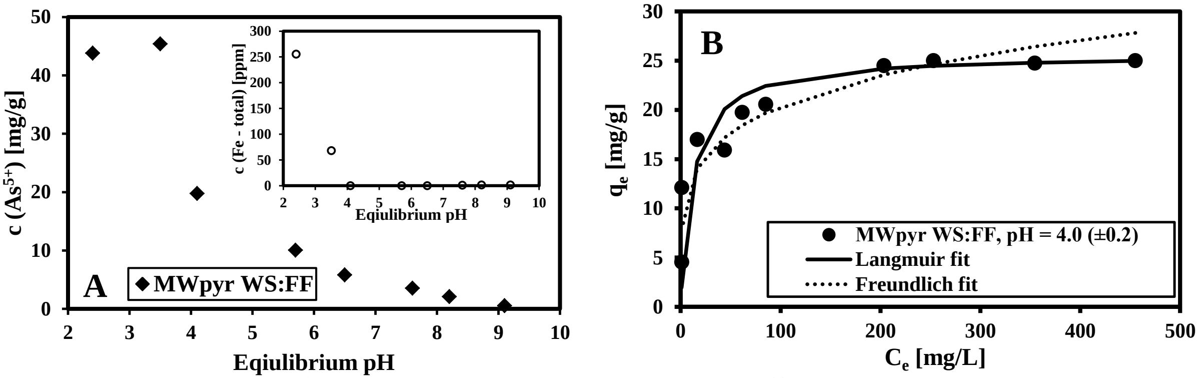

Fig. 3 (A) - The sorption capacity of magnetic bio-chars towards As(V) at different pH (A) ($c_{(As)\ initial}$ = 100 mg/L, Top A inset – leaching of iron during the sorption at different pH). (B) Langmuir and Freundlich adsorption isotherms for As(V) at pH = 4.

4 Conclusion

The magnetic bio-char from agricultural waste biomass (wheat straw) and ferrofluid was prepared by microwave pyrolysis (MWpyr WS:FF). Microwave conversion allowed obtaining material with different chemical composition (various Fe-bearing phases identified by Mössbauer spectroscopy), more developed porosity and texture. The prepared material were tested as a potential sorbents of heavy metal As(V) oxoanion. Magnetic biochar showed excellent sorption properties (Q_m(As)=25.6 mg/g). Moreover, solid/liquid magnetic filtration as a rapid and effective technique can be applied in removal of used magnetic biochar from aqueous solution after sorption process.

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- Application of theoretical knowledge from presented areas for detailing the top technologies principles in the following fields: rock disintegration, mineral processing, monitoring of selected components of working and living environment, monitoring of environmental, chemical and geological changes in the waste repositories with the aim of ecological revitalizations.
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- Department of Destructional and Constructional Geotechnics
- Department of Environment and Hygiene in Mining
- Department of Mineral Biotechnologies
- Department of Mechanochemistry

Department of Mineral Biotechnologies

Research program in the field of mineral biotechnologies at the Institute of Geotechnics SAS was founded by Prof. Dr.h.c. Ing. František Spaldon, DrSc. in 1985. In 1992, a detached Department of Mineral Biotechnologies was established and led by assoc. prof. Ing. Mária Kušnierová, PhD. The department currently employs a group of experts in various fields of this interdisciplinary research. From 2006 is department working under leading MVDr. Daniel Kupka, PhD.

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- ***Environmental biotechnology*** **-** the development and application of biotechnology methods for mineral processing and waste recycling, improving the quality of raw materials for ceramic and glass industry, remediation of old environmental burdens and sites contaminated by antropogenic activities, synthesis of advanced (bio)materials and bio-composites for selective recovery of usable components from solid and liquid municipal and industrial wastes, prediction and prevention of bio-corrosion of construction composite materials.

Research experiences:

- ***Bioleaching & Metal Mobilization*** - dissolution of minerals by bacterial oxidation of iron and/or reduced inorganic sulfur compounds (RISC), bacterial reductive dissolution of iron oxides, liberation of iron impurities from nonmetallic by bacterial by-products.
- ***Biocorrosion & Biodeterioration of Synthetic and Industrial Materials*** - bio corrosion of building composite materials, bio-deterioration of underground storage structures.
- ***Bioremediation of Mine and Industrial Waste Effluents*** - biodegradation, biosorption, bioprecipitation, biovolatilization, bacterial dissimilatory reduction of iron, sulphate, nitrate, (per)chlorate, ion exchange, advanced oxidation processes (AOPs), toxicity and biodegradability assessment (respirometric methods).
- ***Degradation of Organic Pollutants in Waters and Soils*** - biodegradation of organic pollutants in soils, groundwaters and wastewaters, contaminant-specific monitoring of the degradation based on the extraction of the analyte from the matrix before analysis, contaminant-non-specific monitoring of organic matter mineralization based on oxygen and carbon dioxide gas analysis (respirometric methods).

Research infrastructure:

- UHPLC liquid chromatography Dionex Ultimate 3000 with DAD detector and tandem mass spectrometry Q-TOF (micrOTOF-Q II™ Bruker Daltonics)
- two-channel ion chromatography Dionex ICS 5000 for parallel separation and analysis of anions, cations and low molecular compounds
- fluorescence microscopy LEICA DM 6000
- GC-MS Gas chromatography Agilent 7890A with mass spectrometry Agilent 5975C
- inductively coupled plasma – mass spectrometry ICP-MS Agilent 7700
- TOC/TNb analyzer Vario TOC Cube Elementar Analysensysteme GmbH,
- Beckman Coulter ® Multisizer IV. Analyzer of particle size, volume and number distribution
- surface area and pore size distribution analyzer Quantachrome NOVA e 1200
- water polishing system Elga-PureLab-Option + Elga PureLab-Ultra for preparation of 18.2 MΩ ultra-pure water
- microfluidic chip bio analyzer Agilent Bioanalyzer 2100 for the electrophoretic analysis of DNA, RNA, and proteins
- real-time PCR (Q-PCR) systém Light Cycler 2.0 Roche
- BBL Crystal bacterial identification system, Becton-Dickinson
- paramagnetic oxygen analyzer by Sable Systems
- carbon dioxide analyzer with IR sensor Sable Systems
- 8-channel gas multiplexer RM-8 Sable Systems
- respirometric equipment for the measurements of metabolic activity based on flow through gas analysis
- vibratory sieve shaker AS 200 RETSCH, mesh size of sieves 2 mm, 1 mm, 800 μm, 600 μm, 400 μm, 250 μm, 100 μm, 63 μm, 40 μm
- sample splitters RT 6.5 RETSCH
- titration workstations TitraLab®854, radiometer analytical
- rotary vacuum evaporator INGOS
- high-temperature laboratory furnace VP 02/16 with a programmable temperature controller for heat treatment of the samples to a temperature of 1600°C

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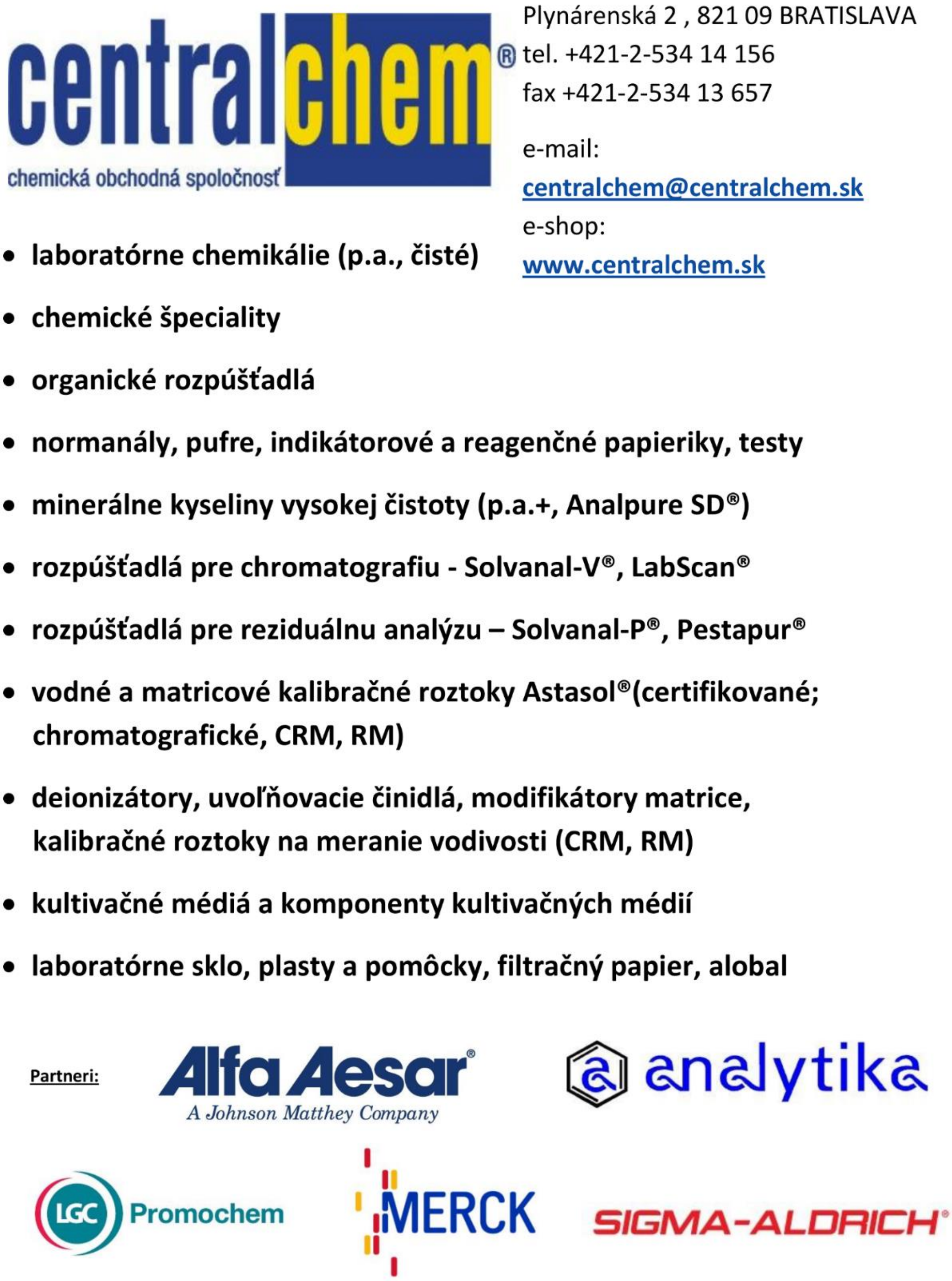

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