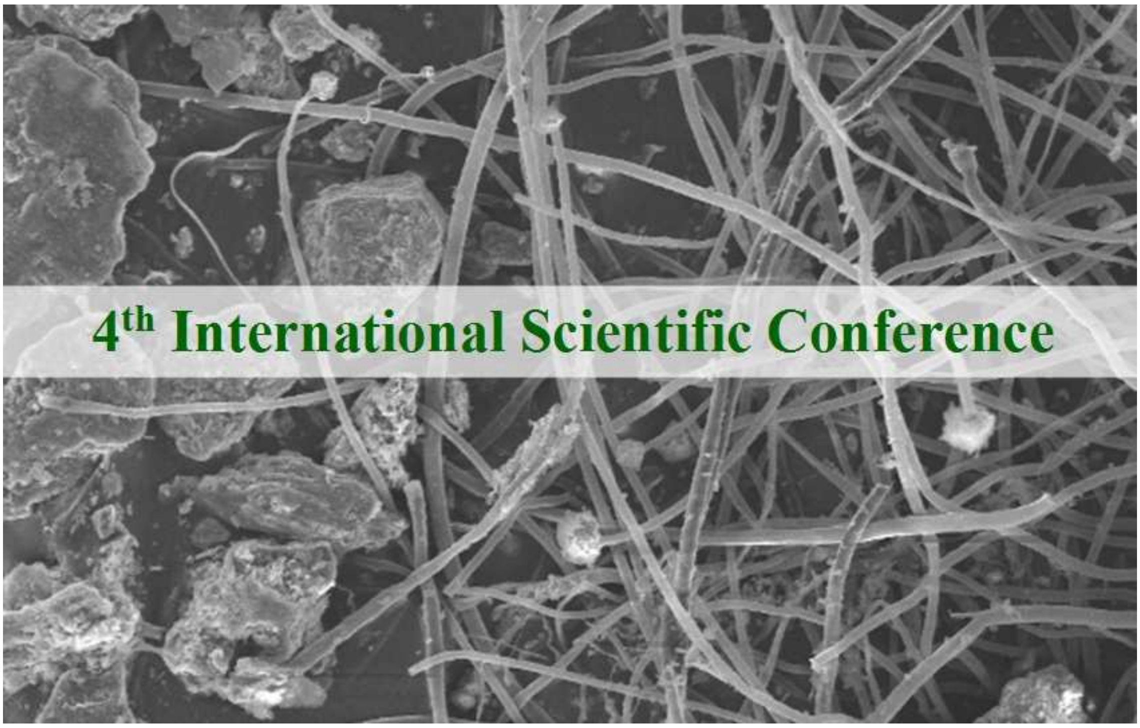
Biotechnology & Metals

Košice 2016

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



4th International Scientific Conference

Editors:
Jana Jenčárová, Alena Luptáková, Jana Kaduková

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Preface

Dear colleagues and friends,

it is honour to welcome you at the 4th International Conference on Biotechnology and Metals.

Biotechnology is a broad discipline in which biological processes, organisms, cells or cellular components are exploited to develop new progressive technologies. European Union considers biotechnology as one of the key scientific disciplines of the 21st century. New tools and products developed by biotechnologists are useful in research, agriculture, industry and medicine.

Environmental biotechnology is the branch of biotechnology that addresses environmental problems, such as the removal of pollution, renewable energy generation or biomass production, by exploiting biological processes. At present time environmental biotechnology have increasing importance in the metal recovery from low grade ores, industrial residues and secondary metal-bearing materials, in pollution classification and protection of natural environment. By application of biotechnological methods such as bioleaching, biosorption, bioprecipitation, phytoextraction etc., they provide a convenient alternative to conventional industrial methods.

Our reaction to rapid development of aforementioned biotechnological methods in the field of metal processing and metal removal from the environment is organising the conference "Biotechnology and Metals". Its main aim is to provide a forum for experts to knowledge, ideas and results exchange in a broad range of topics relevant to the biotechnology application in the treatment and processing of mineral raw materials and their waste.

Conference topics are following:
1. Biotechnological processes of metal and metalloids extraction from soil, water and air.
2. Biotechnological treatment of metal-bearing waste.
3. Application of biotechnological methods in practice.

The conference will be held in honour of Assoc. prof. Mária Kušnierová, PhD. the life jubilee. She is one of the first researchers in the field of application of biotechnology methods for the treatment and processing of mineral raw materials in Slovakia and later founder of the Department of Mineral Biotechnology of Institute of Geotechnics, Slovak Academy of Sciences.

The conference is organized by two institutions – Institute of Geotechnics, Slovak Academy of Sciences and Faculty of Science, Pavol Jozef Šafárik University in Košice.

We hope you will enjoy your visit to the conference as well as to Košice – European city of sport 2016.

We believe that we will meet again also at next years at the conference Biotechnology and Metals.

Alena Luptáková and Jana Kaduková

Biotechnology & Metals Košice 2016

CONTENT

INTAKE OF POTENTIALLY TOXIC ELEMENTS TO REPRESENTATIVES OF GENUS *PINUS* L. AND *QUERCUS* L. AT SELECTED SLOVAK, ITALIAN AND PORTUGUESE CLOSED COPPER DEPOSITS

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Abstract

The research was realized at four abandoned copper deposits: at Ľubietová (Podlipa dump-field, Slovakia), Libiola and Caporciano (Italy) and São Domingos (Portugal). The highest Fe contents in soil are at Libiola (x = 13.75 %). For this deposit are characteristic also high Cu (x = 3654 mg.kg^{-1}) and Ni (x = 717 mg.kg^{-1}) contents. Similary high Cu contents (x = 0.73 %), were described also at Caporciano. In anthrosol at São Domingos are extraordinary high Pb (x = 2219 mg.kg^{-1}), Zn (x = 765 mg.kg^{-1}) and As (x= 1072 mg.kg^{-1}) contents. The results of the potentially toxic elements contamination study of the representatives of the genus *Pinus* L. and *Quercus* L. at four abandoned but historically important European Cu-deposits Ľubietová (Slovakia), Libiola and Caporciano (Italy) a São Domingos (Portugal) are relatively complicated. The plant contamination at mining areas in comparison with reference areas substantially differ. At the mining areas show the plants much more bioaccumulation of potentially toxic elements in their tissues. The bioavailability of individual potentially toxic elements is varied but generally relatively limited. Most of the studied plants are excluders (BCF<1). Only Ag shows extraordinary high degree of bioconcentration. Also BCF values in *Pinus* sp. from Ľubietová indicate high level of Pb and Zn bioaccumulation. Zinc is accumulated in needles at all four Cu-deposits. The rest of the metals is accumulated preferentially in roots.

Keywords: soil, plants, bioconcentration factor, translocation factor, enrichment factor

1 Introduction

The results of research were obtained at four abandoned copper deposits: at Ľubietová (Podlipa dump-field, Slovakia), Libiola and Caporciano (Italy) and São Domingos (Portugal).

The ore field **Podlipa** (Figure 1) at Ľubietová (NE from Banská Bystrica) in Starohorské Mts. consists of residual spoil material from mining activity in 18 galleries. The deposit is situated in sandstone cupriferous shales and conglomerates of lower Permian age on southern slopes of Vysoká Hill above Zelená Valley [1, 2]. The economically most important minerals were chalcopyrite, Ag-tetrahedrite and pyrite, as well as accesoric galena [3]. The oxidation and cementation zone was developed very well. In this zone were the masin Cu-minerals cuprite and native copper. For this deposit is typical also the wide range of rare secondary minerals.

Fig. 1. Podlipa dump-field at Ľubietová
Fig. 2. Abandoned open-pit mining activities at Libiola

Copper deposit **Libiola** (Figure 2) is situated in northern Italy (Liguria) close to Sestri Levante. In the past it belonged to the important deposits with great economical significance [4]. The exploitation activity stopped in 1962 and since this time is the mining polygon abandoned. The stratiform VMS (volcano-sedimentary massive sulphidic) form lenses and irregular bodies [5] associated with bazaltoid rocks of pillow-lavas character as well as with ofiolites of internal Ligurian sequences of Val di Vara serie [6].

The Tuscan **Caporciano** copper deposit (Figure 3) at Val di Cecina was in 19th century one of the most important European Cu-mineralizations [7]. In period between 1830 and 1907 was at the deposit exploited 30 000 tons of copper. The mine was closed in 1963 [8]. The mezothermal mineralization was formed in ofiolites by remobilization of ore components from rock material [9]. The most important ore minerals were chalcopyrite, bornite and chalkocite [10].

Fig. 3. Erosion on the slopes of the dump-field at Caporciano (Val di Cecina)

Fig. 4. Acid mine drainage water in open pit mining polygon at São Domingos

São Domingos (Figure 4) in Iberian Pyrite belt was exploited since pre-Roman times for pyrite which was used for sulphur production. From 1867 to 1966 was here exploited 25 Mt of copper ore, including 9.9 Mt of Cu-bearing pyrite which was used (as in pre-Roman times) for sulphur production. Within range of years 1913 – 1932 was from the deposit exploited 3 445 533 tons of copper. The resources og the ore are still estimated to 1 700 Mt. The mining was realized predominantly by surface open pit system [11, 12].

2 Material and methods

2.1 Material

The individual parts of *Pinus* spp. and *Quercus* spp. (roots, branches and needles/leaves) were sampled at the studied localities. At Ľubietová was sampled *Pinus sylvestris* and *Quercus petraea*, at Libiola *Pinus pinaster* and *Quercus rotundifolia* (at Libiola dump-field was found only *Pinus pinaster*). The sampling was realized in August 2012 and in August 2015. The similar sampling was realized also at the suitable traced polygons of reference areas (with exception of locality São Domingos).

2.2 Analytical procedure and methods of evaluation of results

The dry antrsoil and plant samples were analysed in ACME Laboratory (Vancouver, Canada) by ICP-MS using weight of 2 g. The samples were steamed off on sand-bath in H_2O-HF-$HClO_4$-HNO_3 solution prepared in rate: 2 : 2 : 1 : 1. After adding of 10 ml 50 % HCl was the sample heated on the water-bath. The coolled solution was analyzed by ICP-MS for set of 38 elements.

The **Bioconcentration factor (BCF)** represents the allotment of the potentially toxic element (PTE) content in assimilations organs (leaves or needles) vs. PTE in soil [13]. It is possible distinguish according to BCF values the next plant strategies [14]:

- **excluder (**BCF < 1**)** plants accumulate PTE from soil into their roots and immobilize them in such a way that they are no able entry to their aerial parts (needles/leaves) [15].
- **indicator** type plants (BCF = 1) accumulate PTE in the aerial parts and their PTE content is equal to those in soil [15].

- **accumulators and hyperaccumulators** (BCF > 1) accumulate 100- to 1000-times more PTE in their aerial parts [16, 17].

Translocation factor (TF) shows the efficiency of the plant ability to translocate PTE to selected plant organs [18]. It reflects allotment of the PTE concentration in needles/leaves vs. their content in roots [12]. According to [19] the plants characterized by TF and BCF >1 are suitable for phytoextraction aplication.

Enrichment factor (EF) was calculated as a PTE fraction in anthrosol/plant from the contaminated site divided by PTE content from the reference area [20].

3 Results and discussion

The highest average values of potentially toxic elements (metals) in anthrosol at Podlipa dumps in **Ľubietová** were described for Fe (2.415 %), Cu (3654 mg.kg^{-1}), As (224 mg.kg^{-1}), Co (44 mg.kg^{-1}), Sb (33 mg.kg^{-1}), Ni (29 mg.kg^{-1}), Pb (21 mg.kg^{-1}), Zn (20 mg.kg^{-1}), Ag (1.15 mg.kg^{-1}) and Cd (0.1 mg.kg^{-1}).

Both tree species (*Pinus sylvestris* and *Quercus petraea*) are predominantly contamined by Fe. In *Pinus sylvestris* continue Cu > Pb > Zn >Ag > Ni > Co > As > Sb > Cd and in *Quercus petraea* Zn > Pb > Ag > Ni > Cu > Co > As > Sb > Cd (Table 1).

The average PTE contents in *Pinus sylvestris* and in *Quercus petraea* are given in Table 1. The highest Cu, Zn, Pb, Ag, Cd, Ni, Co contents in *Pinus sylvestris* were found in *Quercus petraea* in roots. Only in case of As was the highest content described in leaves. The Fe contents and less markedly also the Pb contents at reference area are substantially lower as at the contamined area.

Table 1. Average ICP-MS analyses of PTE in *Pinus sylvestris* and *Quercus petrea* from dump-fields and reference areas of studied deposits

Tree sp.	**Fe**	**Mn**	**Cu**	**Zn**	**Pb**	**Ag**	**Cd**	**Ni**	**Co**	**As**	**Sb**
	%		**mg.kg^{-1}**								
ĽUBIETOVÁ - PODLIPA											
dump-field											
Pinus sylvestris	150.1	544	28.53	24.32	26.95	3.53	0.05	3.34	2.03	0.30	0.19
Quercus petraea	204.9	1896	4.90	33.77	28.33	22.01	0.07	5.73	1.63	0.54	0.14
reference area											
Pinus sylvestris	0.01	694	5.21	24.63	1.43	20.97	0.04	1.27	1.66	0.45	0.25
Quercus petraea	0.01	2064	1.90	35.70	2.32	14.28	0.03	3.25	3.04	0.48	0.14
LIBIOLA											
dump-field											
Pinus pinaster	890	94	68.11	189.02	11.00	44	121	4.8	0.6	0.88	1.57
reference area											
Pinus pinaster	314	112	19.08	22.00	1.21	12	11	2.2	0.3	0.40	1.76
VAL DE CECINA – CAPORCIANO											
dump-field											
Pinus pinaster	0.047	43	162	0.70	82.02	125	2.75	1.3	0.7	0.1	0.09
Quercus petraea	1128	104	1.03	104	1128	88	3367	4.3	2.63	0.1	0.17
reference area											
Pinus pinaster	79	16	17	0.7	38	10	0.08	0.6	0.3	0.1	0.04
Quercus petraea	77	16	20	0.2	28	9	0.20	0.9	1.0	0.1	0.07
SÃO DOMINGOS											
dump-field											
Pinus pinaster	0.117	361	19.00	72	40	540	0.83	3	20	25	0.91
Quercus rotundifolia	0,076	665	23.00	66	20	172	0.32	4.1	2.2	6.6	0.81

The highest average values of potentially toxic elements at **Libiola** dump-field anthrosol were recognized for Fe (13.75 %), Cu (6226 mg.kg^{-1}), Mn (1812 mg.kg^{-1}), Ni (717 mg.kg^{-1}), Zn (439 mg.kg^{-1}), Co (212 mg.kg^{-1}), Pb (56 mg.kg^{-1}), As (11 mg.kg^{-1}), Sb (3.4 mg.kg^{-1}). The average metal contents in *Pinus pinaster* decrease in range: Fe (890 mg.kg^{-1}) > Zn (189 mg.kg^{-1}) > Mn (94 mg.kg^{-1}) > Cu (68 mg.kg^{-1}) > Pb (11 mg.kg^{-1}). The highest Fe, Zn, Cu, Pb accumulation was described in roots. The only exception is Mn, which is predominantly concentrated in needles.

At **Caporciano** dump-fields was the average content of some metals in anthrosol very high: Fe (5.79 %), Cu (0.73 %), Mn (809 mg.kg^{-1}), Zn (582 mg.kg^{-1}) a Ni (115 mg.kg^{-1}).

The average contents of PTE in *Pinus pinaster* and in *Quercus rotundifolia* decrease in range: Mg > Fe > Al > Cu. In *Quercus rotundifolia* is the Mn vs. Zn and Pb vs. Cd rate opposite: Mn > Zn > Pb > Cd. The highest metal accumulations are in roots. Only Mg contents in needles of *Pinus pinaster* are higher as those in roots. The PTE contents in soil at reference area are substantially lower. Also their sequence is different (Table 1).

The highest average PTE contents in anthrosol samples from **São Domingos** were contents of Fe (9.84 %), Pb (2219 mg.kg^{-1}), As (1072 mg.kg^{-1}) > Cu (890 mg.kg^{-1}) > Zn (765 mg.kg^{-1}) > Mn (488 mg.kg^{-1}) > Sb (71 mg.kg^{-1}) > Bi (27 mg.kg^{-1}) > Ni (26 mg.kg^{-1}) > Co (25 mg.kg^{-1}).

The average PTE contents in *Pinus pinaster* decrease in range: Fe > Mn > Ag > Zn > Cu > Pb > As > Ni > Co > Sb. It is most heavily contamined by Fe and Ag (Table 1). Also Mn and Zn contents, as well as Pb contents are plumbless. The As, Co, Cu, Ni and Sb contents are lower. The highest Fe, Cu, Pb, Ni, As and Sb contents are in roots, the highest Ag contents in branches and the highest Zn, Co and Mn accumulations in needles. The highest accumulation of PTE (with exception of Zn and Mn) were in *Quercus rotundifolia* in roots. Zn is accumulated mainly in leaves.

The research showed relatively important differencies in ability of *Pinus* L. and *Quercus* L. at various ore deposits accumulate PTE to their organs (Table 2). The calculated BCF values of studied tree species indicate that with exception of Ag and Cd in *Pinus* sp. all of them are excluders (BCF<1). Both *Pinus* sp. and *Quercus* sp. are accumulators of Ag. *Pinus sylvestris* at dump-field Podlipa is also accumulator of Zn and Pb. The same trend was proved also in area of abandoned São Domingos deposit in *Pinus rotundifolia.* It seems to be accumulator of Mn, Ag, Cd and Co. The bioaccumulation and translocation trends at Libiola and Caporciano deposits are presented in Table 2.

Table 2. Bioconcentration, translocation and enrichment factors calculated for *Pinus* L. and *Quercus* L.

Deposit	Tree sp.	Fe	Mn	Cu	Zn	Pb	Ag	Cd	Ni	Co	As	Sb
Bioconcentration factor – BCF												
Ľubietová	*Pinus s.*	0.010	0.11	0.27	**2.22**	**1.60**	**5.00**	**1.00**	0.00	0.05	0.00	0.00
	Quercus p.	0.000	0.17	0.16	0.51	**1.19**	**18.93**	0.60	0.12	0.02	0.00	0.00
Libiola	*Pinus p.*	0.002	0.12	0.01	0.68	0.02	**66.36**	0.69	0.01	0.04	0.01	0.02
Caporciano	*Pinus p.*	0.002	0.07	0.01	0.29	0.04	**73.75**	0.45	0.01	0.02	0.05	0.01
	Quercus r.	0.006	0.14	0.01	0.08	0.05	**66.59**	0.07	0.03	0.15	0.52	0.34
S. Domingos	*Pinus p.*	0.003	**4.97**	0.01	0.42	0.01	**57.51**	**1.85**	0.24	**5.62**	0.01	0.00
	Quercus r.	0.004	**3.54**	0.24	0.41	0.01	**43.79**	0.27	0.16	0.11	0.01	0.01
Translocation factor – TF												
Ľubietová	*Pinus s.*	**0.940**	**1.1**	**2.14**	**2.95**	**0.18**	**11.43**	**3.33**	**1.81**	**1.9**	**0.36**	**0.08**
	Quercus p.	0.770	0.99	0.87	0.50	0.50	0.75	0.60	0.31	0.29	**1.54**	0.44
Libiola	*Pinus p.*	0.170	**1.1**	0.18	**1.6**	0.14	0.23	0.20	**3.75**	0.31	0.09	0.06
Caporciano	*Pinus p.*	0.590	**4.26**	0.19	**2.37**	**1.46**	**1.59**	0.67	**1.00**	**1.75**	**1.00**	0.36
	Quercus p.	0.400	**3.82**	0.11	**1.36**	0.94	**2.29**	0.23	**1.27**	**3.60**	**1.00**	**1.94**
S. Domingos	*Pinus p.*	0.103	**5.20**	0.14	**2.49**	0.11	0.45	0.70	0.67	**16.4**	0.23	0.30
	Quercus p.	0.300	**5.56**	0.21	**1.36**	0.32	0.45	0.41	0.99	0.51	0.45	0.10
Enrichment factor – EF												
Ľubietová	*Pinus s.*	8466		0.26	0.75	8.29	0.00	0.05	1.48	1.65	1.28	0.42
	Quercus p.	32200		0.21	1.40	14.71	0.11	0.21	6.36	0.40	0.53	0.12
Libiola	*Pinus p.*	1.77	7008	1.28	0.36	5.25			5.76	2.64	1.86	3.90
Caporciano	*Pinus p.*	2.54	4.10	6.13	9.00	8.61						
	Quercus r.	26.52	7.00	17.67	5.57	10.00		20.67				

Explanations: *Pinus s. – Pinus sylvestris; Pinus p. – Pinus pinaster; Quercus p. – Quercus petraea; Quercus r. – Quercus otundifolia;* the BCF and TF values >1 are shown in bolt fonts.

The TF values, which reflect ability of plants translocate PTE (contaminants) from roots to shoot, are also relatively low. The value 1 is exceeded only for Mn, Zn, Ni and Co (Table 2). The highest TF values are in *Pinus pinaster* and *Quercus rotundifolia* from Caporciano dump-field and for Co in *Pinus pinaster* from São Domingos deposit (Table 2).

In majority of the tree species are the PTE accumulated in roots [14] and only in several few cases are able tranfer to the aeral parts (needles/leaves). Uncomplete set of EF data indicates great difference in soil and plants contamination (mainly for Fe, Mn, Cu and Pb) at reference areas vs. dump-fields (Table 2).

In the areas with extraordinary high PTE contamination, e.g. dumps are the plants used more usually for phytostabilization. The purpose of phytostabilization is to control broadering of polutants to surrounding [21]. The phytostabilization stabilize polutants by redox reactions and both transform them to insoluble forms and incorporate them to the plant organs [22]. The migration of contaminants in soil is controlled and limited by absorption and accumulation of polutants in roots, eventually their adsorption on root-system, precipitation, formation of complex compounds or reduction or bonding on organic mater [23]. Mainly plats with low ability accumulate contaminants in biomass [24, 25].

4 Conclusions

The most contamined technosoils are at dump-fields at São Domingos and Libiola. The highest Fe contents in soil are at Libiola. For this deposit are characteristic also high Cu, Ni and Co contents. Similarly high Cu contents were described also at Caporciano. In anthrosol at São Domingos are extraordinary high Pb, Zn, As and Sb contents.

Differencies in the degree of the trees contamination at reference areas is in comparison with those at contamined sites are significant. The bioaccumulation of the PTE to the tree organs is very limited. Most PTE are accumulated preferentially in roots so the studied tree species *Pinus* spp. and *Quercus* spp. are excluders (BCF<1). The excluders are not suitable for phytoextraction application which removes the PTE from soil. It is possible to use them only for phytostabilization. This method is oriented on immobilization of PTE by their influence on chemical, biological and physical conditions of the environment (soil).

Acknowledgements

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COMPARISON OF SORPTION PROPERTIES OF BENTONITE AND KAOLIN SAMPLES BEFORE AND AFTER BIOLEACHING

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Abstract

The work deals with the study of adsorption properties of natural raw materials bentonite and kaolin as potential adsorbents of Cu(II) and Zn(II) cations. These natural raw materials are generally not pure, often associated with iron hydroxide and oxy-hydroxide impurities usually in the form of Fe^{3+} phases adsorbed onto the mineral surface. The effect of bioleaching (using iron-reducing bacteria *Bacillus spp*.) onto the sorption properties of the samples were investigated and compared. The values of specific surface area were studied by the low temperature nitrogen adsorption measurement. In the introductory experiments in static regime, the flask contain samples of bentonite and kaolin with Cu(II) and Zn(II) model solutions. The results from the batch adsorption experiments of Cu(II) and Zn(II) removal were processed by the column graph. Obtained results from experiments indicate slight decrease in sorption properties of samples after bioleaching (reducing of iron content), but the difference was not significant. We can summarize that the sorption of Cu(II) and Zn(II) ions onto the individual samples was effective in the following order B > B(bio) > K > K(bio). In the batch adsorption experiments both samples showed higher affinity to Cu(II) ions compare to Zn(II) ions.

Keywords: bentonite, kaolin, sorption

1 Introduction

Heavy metals discharged from various industries pose serious environmental problems and are dangerous to human health. In recent years the removal of heavy metals ions from waste water has received a great attention for global awareness of the underlying detriment of toxic metals in the environment [1].

Iron oxides, a common constituent of soils, sediments, and aquifers, have high surface areas and are dominant adsorbents in many environments because of their capability to be finely dispersed and act as coatings on other particles [2]. Reduction of iron also plays an important role in the geochemical transformation of iron-containing minerals and causes mobilization of metals in the environment. The concentration of some toxic metals is higher than permissible discharge levels in effluents. Toxic metal pollution in ecosystem is an important environmental problem encountered in many industrial areas. It, therefore, becomes necessary to remove these toxic metals from these wastewaters by an appropriate treatment before releasing them into the environment. Bentonite is one of the clay minerals, hydrated aluminium silicate. The application areas of bentonites vary depending on the amount of their constituents. Bentonites are used as selective adsorbents, ion exchangers, catalysts, catalysts support, for production of pillared clays and organoclays [3]. Since the clay fraction in soil is a preferential sorbent for organic matter, reference kaolinite is a useful model for studying the mechanism and the strength of sorption. Kaolin is an economically important raw material often used in wide variety of ceramic applications, from high quality tableware and sanitaryware to electrical porcelain, tiles and glasses. There are some less common uses including glass fibre, white cement and refractory insulation bricks [4]. These clay minerals were used in this study to determine and compare the affinity to toxic metal cations. The copper and zinc ions were chosen for the sorption studies with regard to their wide use in industry and potential pollution impact.

2 Material and methods

2.1 Kaolin and bentonite samples

In the laboratory sorption experiments we compared sorption properties of the natural bentonite originated from the Kadaň deposit with the chemical composition, (see Table 1) and natural kaolin sample from the Horná Bříza deposit with the chemical composition, (see Table 2) before and after bioleaching. The samples were denoted as natural kaolin (K) and natural bentonite (B) and after bioleaching as bentonite (B-bio) and kaolin (K-bio).

Table 1. The chemical analysis of the bentonite sample

Na [%]	Mg [%]	Al [%]	Si [%]	P [%]	S [%]	K [%]	Ca [%]	Ti [%]	Vn [%]	Cr [%]	Mn [%]	Fe [%]	Zr [%]	Zn [%]
0.27	2.06	18.6	53.2	0.9	0.01	0.98	2.91	5.11	0.13	0.01	0.10	15.2	0.04	0.02

Table 2. The chemical analysis of the kaolinite clay

Na [%]	Mg [%]	Al [%]	Si [%]	P [%]	K [%]	Ca [%]	Ti [%]	Vn [%]	Cr [%]	Mn [%]	Fe [%]	Co [%]	Zr [%]	Zn [%]
0.26	1.37	27.4	56.4	0.13	3.64	0.29	1.18	0.03	0.01	0.09	8.91	0.01	0.03	0.01

2.2 Adsorbents characterization

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 [5].

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.

2.3 Adsorption experiments

The sorption measurements were made by a batch technique at the ambient temperature using the rotary shaker (30 rpm). Through the study, the pH was adjusted to pH 5, Cu(II) and Zn(II) concentration in model solutions was 50 mg L^{-1}. The adsorbents dose was 1 g L^{-1}.

20 mg of adsorbents was added into the plastic tubes containing 20 ml of Cu(II) or Zn(II) solution. 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). To interpret the experimental data, the column graphs were used.

3 Results and discussion

The low temperature nitrogen adsorption was used to better characterize the textural properties of natural samples. Both isotherms were of type IV with hysteresis loop corresponding with the capillary condensation taking place in mesopores [6], Figure 1. Broader loop was observed for natural bentonite, what could point at the higher porosity in comparison to kaolin sample. For both samples, the adsorption and desorption branches are nearly horizontal and parallel over a wide range of relative pressure - Type H4 loop, often associated with narrow slit-like pores. The expressive increase of adsorbed gas volume at $p/p_0 \approx 0.9$ can be observed for both natural samples, what indicate the presence of macropores in their structures.

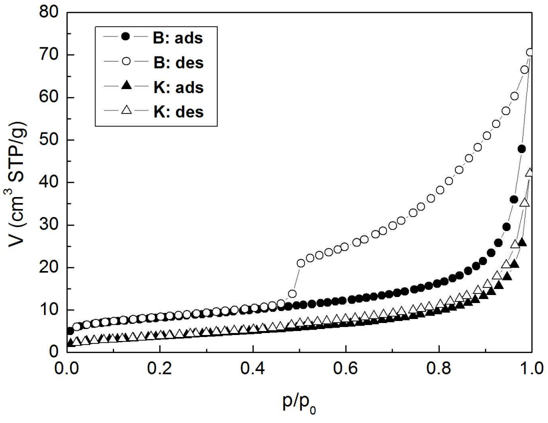


Fig. 1. Adsorption and desorption isotherms of natural samples

The higher value of specific surface area was calculated for the bentonite sample, what corresponds with its mineralogical composition and structure in comparison to kaolin, Table 3. While the value of external surface of kaolin is almost equal as its value of specific surface area, for the bentonite sample the difference in these values was obtained. The value of external surface of bentonite was lower than specific surface due to content of micropores. On the other hand, the kaolin sample is only meso-macroporous.

Table 3. 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}]
B	28.9	0.1092	0.0035	21.0
K	13.7	0.0652	-	15.2

The comparison of pore size distributions curves of natural samples are shown in Figure 2. Maxima for pore radius 1.9 nm correspond with the decrease on the desorption branches at $p/p_0 \approx 0.45$ and do not represent the real pores [7]. Both samples showed broad distribution in the range of mesopores without the expressive maximum. The distribution curve of bentonite sample is shifted left in comparison to kaolin, to lower values of pore radius. The bentonite showed distribution in the range of pore diameter from 4.5 to 30 nm. Larger pores, in the range 4.8 – 90 nm (in diameter), contains the kaolin sample, what corresponds with results presented above.

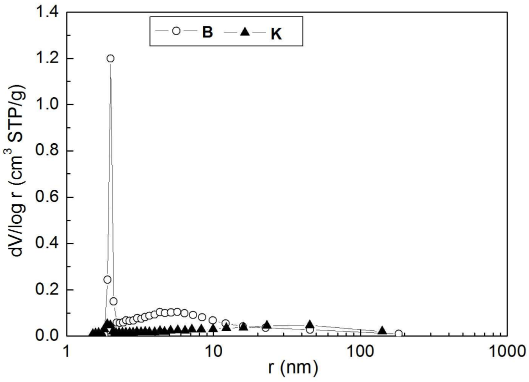


Fig. 2. Pore size distribution curves of natural samples

The scanning electron microscopic data for most samples of the bentonite can be summarized as follows: montmorillonite occurred either as ultrafine, thin, leaf-like crystals forming a dense aggregate or in a more open honeycomb texture [8]. The SEM micrograph of the sample used for the investigation showed their sheet structure, Figure 3 and 4.

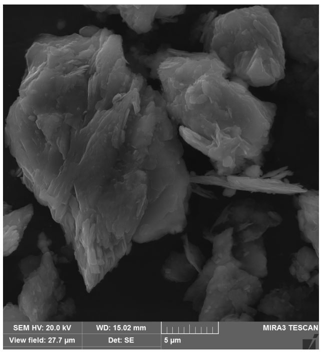


Fig. 3. SEM micrograph of the natural kaolin

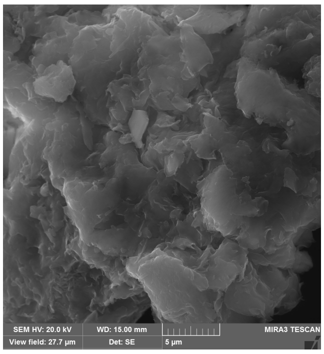
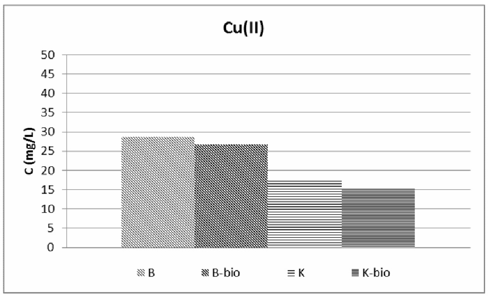


Fig. 4. SEM micrograph of the natural bentonite

The concentration of iron in the bentonite sample B after 30 days of bioleaching was decreased only by 5.33 %. The concentration of Fe in the kaolin sample K decreased by 9.29 % after 30 days of bioleaching process.

Figure 5 shows the adsorption of copper ions onto individual samples. In case of Cu(II) ions slightly decreased sorption properties of both samples after bioleaching process. Higher Cu(II) uptake was shown for B sample compare to K sample. Whereas the value of specific surface area to a certain extent relates with the adsorption properties it was expected, that the bentonite sample will be better sorbent for selected metal ions. The influence of the bioleaching process on sorption properties of the samples in Zn(II) removal was not significant, see Figure 6. Also higher affinity of Zn(II) ions to B sample than to K sample was observed and the copper ions demonstrated higher affinity onto both samples compare with zinc ions.



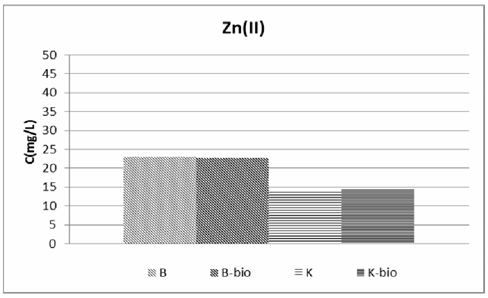


Fig. 5. Sorption of Cu(II) ions onto individual samples (left)

Fig. 6. Sorption of Zn(II) ions onto individual samples (right)

4 Conclusions

Equally often like zeolite, bentonite and kaolin have been used as model adsorbents because they have large specific surface area, cation exchange capacity and adsorptive affinity for organic and inorganic ions and are widespread in nature. Natural samples of bentonite and kaolin before and after bioleaching were used and compare in sorption properties of Cu(II) and Zn(II) ions from the model solution. We can summarize that the sorption of Cu(II) and Zn(II) ions onto the individual samples was effective in the following order B > B(bio) > K > K(bio). In the batch experiments both samples showed higher affinity to Cu(II) ions compare to Zn(II) ions. The influence of the bioleaching process of the samples (reducing of iron content) not enhance the sorption properties of samples, slightly decrease sorption ability in case of Cu(II). In case of Zn(II) the sorption efficieny was comparable. Based on metal uptake both metal ions showed higher affinity to bentonite sample compare to kaolin sample. However, the regeneration step needs to be perform to evaluate the economical aspect of sorption process.

Acknowledgements

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DESULPHURISATION OF WASTE CAR TYRES BY BIOSOLUBILISATION

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Abstract

The recycling of waste car tyres is a very important environmental task. The waste tyres usually are re-used or recycled by thermal processes like pyrolysis, gasification or incineration. The main problem associated with these processes is the sulphur content of waste tyres, which pollutes the environment and the valuable products as well.

In our research the desulphurisation of waste, mechanically pre-treated tyre sample was carried out by the biosolubilisation with *Acidithiobacillus thiooxidans*, strain Karitas.

It was found that sulphur does solubilise, the solubilised sulphur then forms precipitates. The precipitates form in the solution bulk, as well as on the surface of rubber particles. The origin of the precipitates was investigated using XRD and scanning microscopy with micro probe.

Special efforts were done to elaborate the proper separation of precipitate from rubber particles. Flotation process was applied for this purpose. The parameters were chosen based on the revealed surface properties of the precipitates. Finally, conclusions are drawn.

Keywords: tyre desulphurisation, bioleaching, flotation of precipitates

1 Introduction

Waste tyres are generating all over the world in a huge amount. In Europe the recycling of waste tyres is related to their reuse or thermal treatment under the target “Waste to Energy”. The sulphur content originated from the vulcanisation of rubber is the bottle-neck of the recycling. There are chemical techniques for the desulphurization of the waste tyres. In the scope of the creating of circular economy the possible application of bioprocessing techniques for this aim is a very promising. There are some attempts to develop a biotechnique for the rubber desulphurization: aerobic and anaerobic treatment, and both the metabolic pathways and associated enzymes degrading and recycling tyre rubber waste [1]. Autotrophic acidophilic microorganisms were also tested [2-5]. Nevertheless, there are still a lot of questions to answer including the mechanism of desulphurization by *Acidithiobacillus ferrooxidans*.

2 Materials and methods

A sample of waste rubber originated from not truck waste tyres was ground below 0.5 mm using Retsch cutting mill, then the steel wires were removed by magnetic separation and hand picking. The average composition of the prepared rubber sample is shown in Table 1 as it was revealed by micro probe, scanning electron microscope at Materials Science School, University of Miskolc.

Acidithiobacillus ferrooxidans strain Karitas obtained by courtesy of Slovak Academy of Sciences, Košice was cultivated at 25 °C for six days with 9K nutrition medium. To 2 L deionized water the following salts were added:

- 6 g $(NH_4)_2SO_4$,
- 0.2 g KCl,
- 1 g K_2HPO_4,
- 1 g $MgSO_4.7H_2O$,
- 0.0288 g $Ca(NO_3)_2.4H_2O$,
- 88.4 g $FeSO_4.7H_2O$.

pH was 2.5.

Table 1. Average composition of the rubber sample
(micro probe, the ICP measured S-content was as high as 1.75 %)

Element	**Wt %**	**At %**
C K	77.47	87.33
O K	10.18	8.61
Na K	0.11	0.07
Al K	0.26	0.13
Si K	1.93	0.93
S K	2.93	1.24
K K	1.24	0.13
Ca K	1.03	0.35
Fe K	0.88	0.21
Zn K	4.84	1.00
Total	100.00	100.00

The bioleaching experiments were carried out using Erlenmeyer flasks of 250 mL. Solid concentration was 10 g/L. All Erlenmeyer flasks were shaken at 30 °C and 150 rpm by Wise Cube shaker with adjustable heating. The residence time was 7 days. Parallel, the experiment using sterile 9K medium was also carried out under the adequate conditions. After that the solid-liquid separation by filtration was carried out. Since a yellow coloured precipitate was observable in both experiments, the solids were then separated by density using poly-tungstanate solution of $\rho = 1.5$ kg/dm^3, the light and the heavier fractions were investigated further. The experimental flow-sheet is shown in Fig.1. The micro probe (SEM, Materials Science School, University of Miskolc), the X-ray diffraction analysis (the Institute of Mineralogy and Geology, University of Miskolc), the optical microscopy and the ICP chemical analyses (Institute of Chemistry, University of Miskolc) were applied.

3 Results and discussion

3.1 Biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas and solubilisation with 9K

As it was mentioned above it was found after the bioleaching with *Acidithiobacillus ferrooxidans* Karitas, as well as the contacting with 9K that the surface of the rubber particles was covered with yellow-coloured precipitates, which was separated by density. The heavier product was a pure precipitate, although the light product (rubber) was not completely precipitate free. The mass balance of the experiments is shown in Table 2.

It can be seen that the mass of the solid phase after the biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas is higher by 24 %, while that after the solubilisation with 9K is lower by 22.5 % as compared with the initial sample mass (2 g). Nevertheless, the yield of the pure precipitate is higher by almost 10 % in case of solubilisation with 9K as compared with the bioleaching with *Acidithiobacillus ferrooxidans* Karitas.

Table 2. The mass balance of rubber biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas and solubilisation with 9K

Experiment	**Mass of solids after the solubilisation (g)**	**Separation density (kg/dm^3)**	**Light fraction (%)**	**Heavier fraction (%)**
KARITAS	2.48	1.5	93.55	6.46
9K	1.55	1.5	83.24	16.13

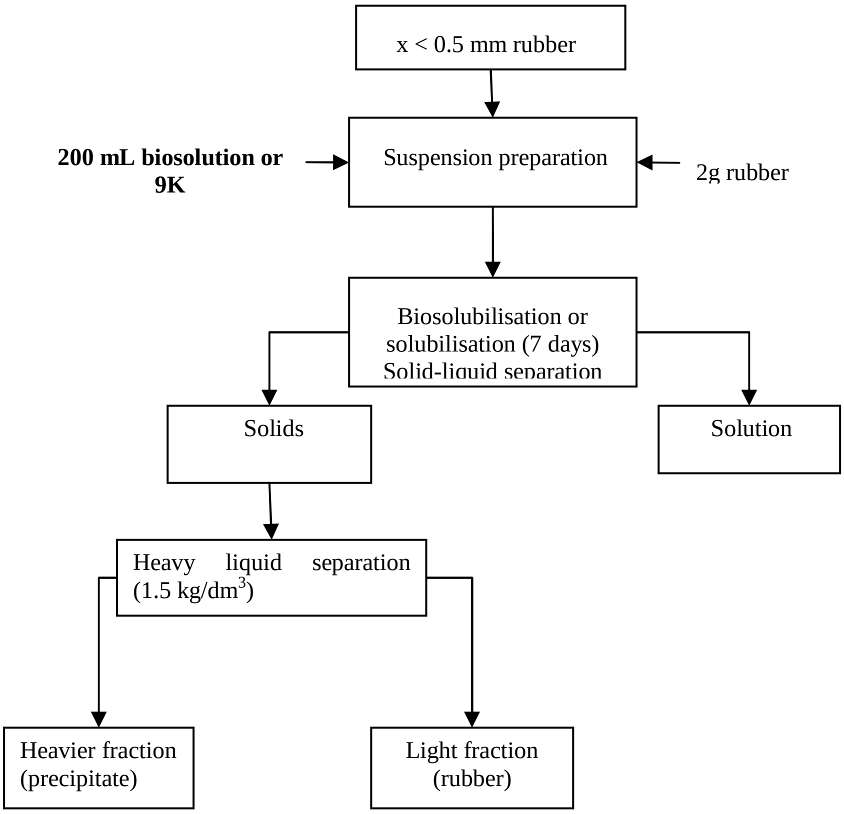

Fig. 1. The rubber bioleaching with *Acidithiobacillus ferrooxidans* Karitas and leaching with 9K, experimental flow-sheet

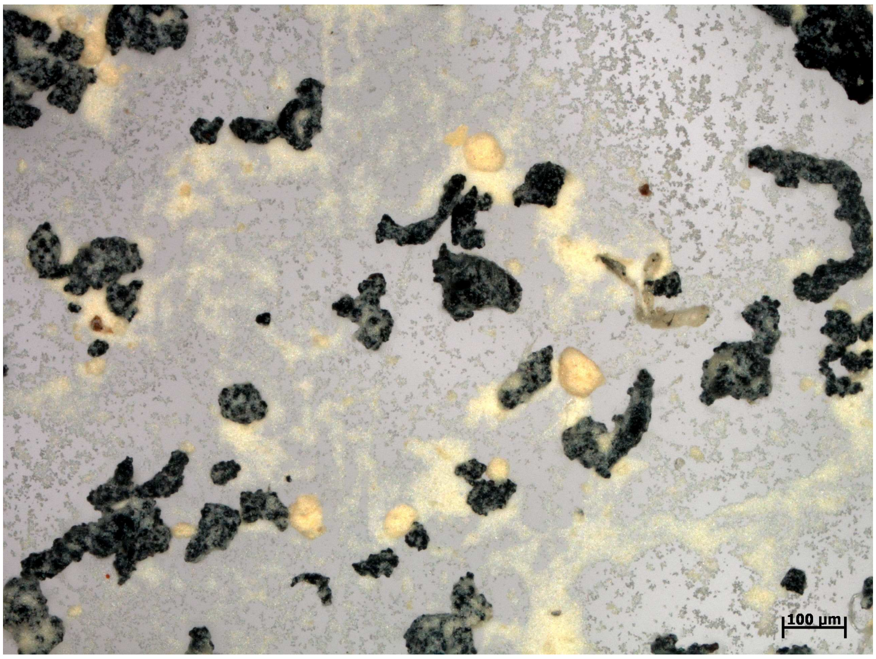

Fig. 2. The rubber and precipitate particles after the leaching with 9K

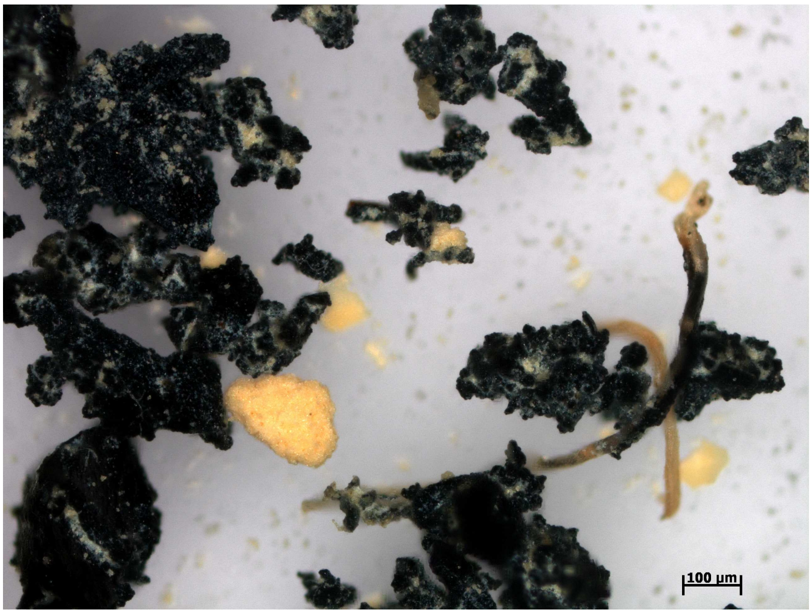

Fig. 3. The rubber and precipitate particles after the bioleaching with *Acidithiobacillus ferrooxidans* Karitas

The rubber and the precipitate particles are shown in Fig. 2 and Fig. 3, respectively for the leaching with 9K and the bioleaching with *Acidithiobacillus ferrooxidans* Karitas. In both cases the precipitates are yellow coloured. pH drop from the initial pH = 2.5 in case of the solubilisation with 9K was down to 2.0; in case of the biosolubilisation down to 1.99.

Fig. 4. Solid and liquid phases after the bioleaching with *Acidithiobacillus ferrooxidans* Karitas (left) and leaching with 9K (right)

As it can be seen from Fig. 4 the liquid phases separated after the experiments have different colours: after the bioleaching with *Acidithiobacillus ferrooxidans* Karitas the colour is darker, in case of the leaching with 9K it is turbid, which is the evidence of the not complete solid-liquid separation.

X-ray diffraction analysis was carried out in the Institute of Mineralogy and Geology, University of Miskolc. The X-ray diffraction patterns are shown in Fig. 5 and Fig. 6. It was revealed that while in the process of the sulphur solubilisation with 9K medium the jarosite ($KFe^{3+}_3(SO_4)_2(OH)_6$) forms, the process of the biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas is accompanied by the formation of the jarosite and the halotrichite ($FeAl_2(SO_4)_4.22H_2O$) as well. The density of the minerals differs: 3.09 as opposite 1.89 kg/dm^3. That can be an explanation of the different precipitate yields shown in Table 2.

The ICP-measured sulphur content of the separated precipitate obtained in the solubilisation with 9K was 2.95 %, while that formed in the process of biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas was 4.15%!

3.2 Separation of rubber from precipitates by flotation

It was measured so far that the sulphur content of the precipitates is almost three times higher than that of the rubber. At the meantime, the rubber particles were still covered by the precipitates, that is why the sulphur content of solids was two times higher as the initial. Thus, the main problem is to find a technique for proper separation of the precipitates from the rubber particles.

A technique which is promising in this concept is flotation. Flotation is very versatile and promising selective recycling technique for the fine and ultrafine disperse wastes. Prior to the implementation of the recycling flotation process, it is essential to determine the surface properties of particles to be separated in different aqueous surroundings, as well as to establish the theoretical background for their control [6].

In order to do so the surface properties of synthetic jarosite were investigated using zeta-potential measurements carried out with Zeta PALS Brookhaven instrument. (The instrument was purchased within the framework of the GVOP-3.2.1.-2004-0219-3.0 National Project (Project Leader: Dr. L. Bokányi)) [7].

Fig.7 illustrates that the anionic oxhydryl Hostaflote M91 (mixture of dithiophosphate, mercaptobenzothiazole and thiocarbamate) adsorbs on the surface of the synthetic jarosite by chemisorption.

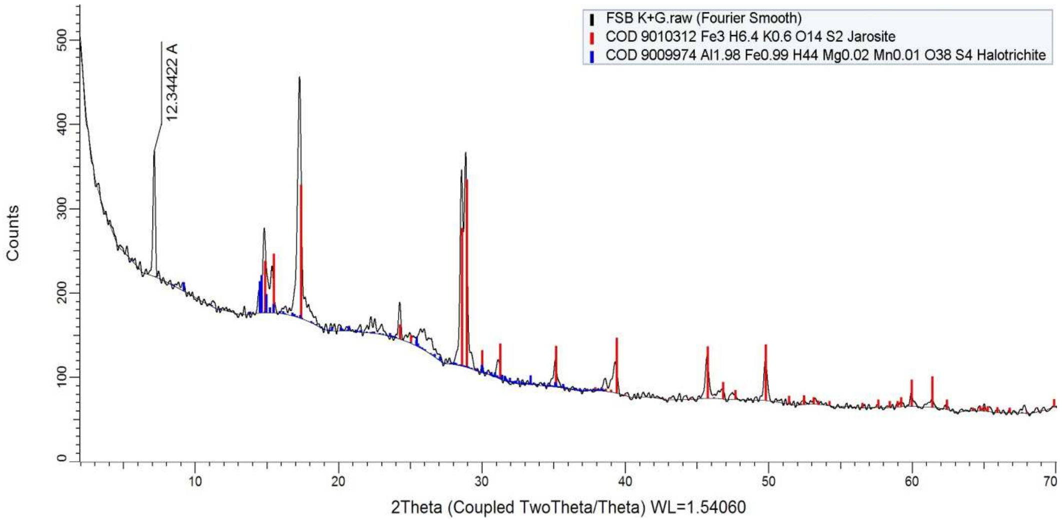

Fig. 5. X-ray diffraction pattern of precipitate originated from the bioleaching with *Acidithiobacillus ferrooxidans* Karitas

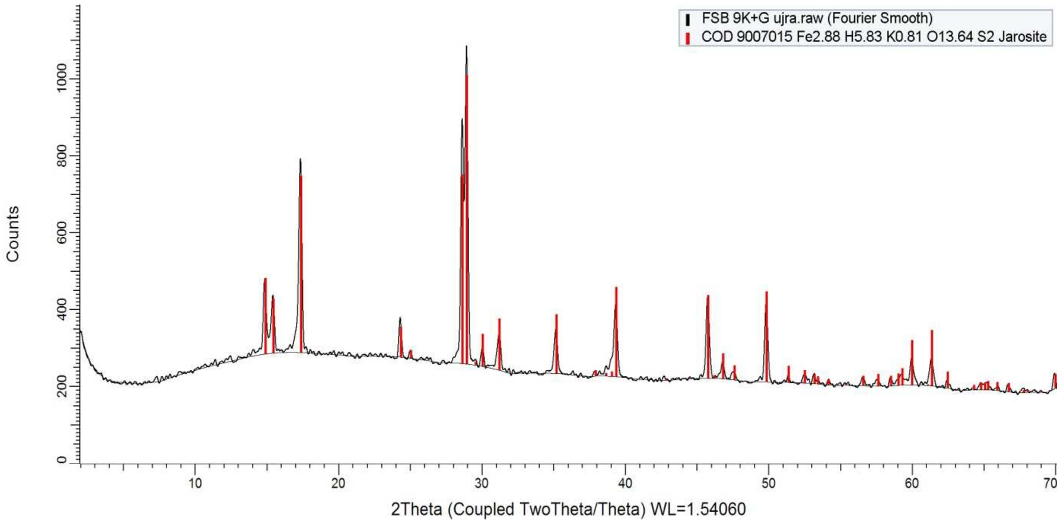

Fig. 6. X-ray diffraction pattern of precipitate originated from the leaching with 9K

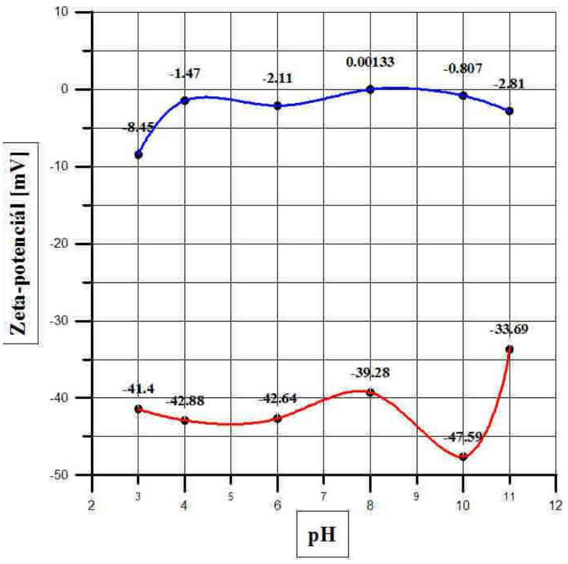

Fig. 7. Zeta-potential of artificial jarosite as a function of pH (upper curve) and in presence of 1000 mg/L anionic Hostaflote M91 (lower curve) [7]

The flotation experiments were carried out using this reagent at various pH after the attrition for 40 min. The aim of the latter was to detach the precipitate particles from the rubber ones.

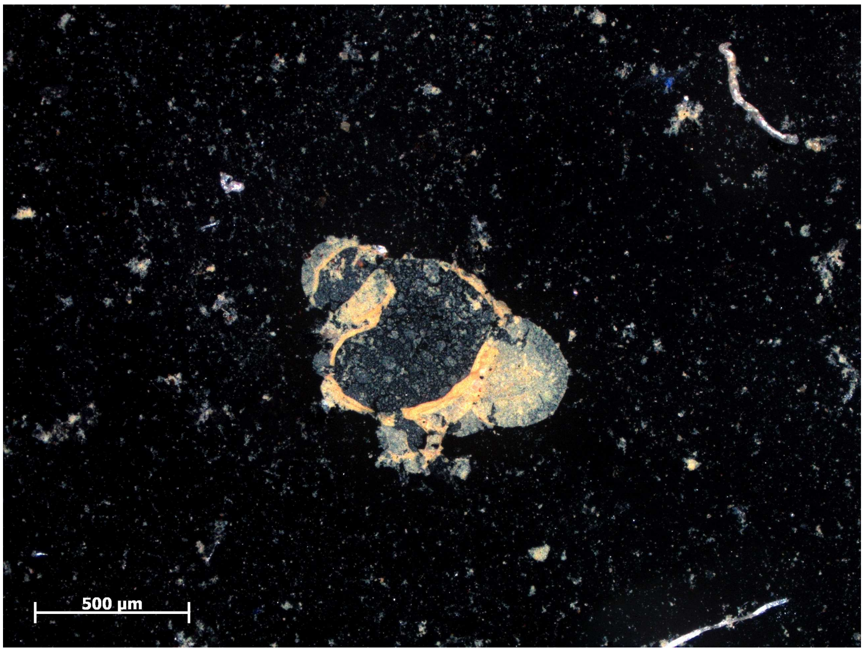

Fig. 8. Waste rubber after biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas and flotation at pH = 10, still covered with jarosite and halotrichite

It was found that the sulphur content of rubber cell-product obtained at pH = 10 decreased below the initial value, although the separation was not complete. Further investigations are needed to find the proper way to separate the rubber and the precipitates. The treatment of the biosolution is also in the scope of our future research.

4 Conclusions

Series of experiments were carried out to study the possibility of the desulphurisation of waste tires by bioleaching with with *Acidithiobacillus ferrooxidans* Karitas. Parallel, the solubilisation with 9K nutrition medium was also tested.

It was revealed that the sulphur does solubilise both due to the bio- and the chemical leaching.

The mechanisms of the bio- and chemical solubilisations are rather different as it was found and experimentally proved by several tests.

The solubilised sulphur forms precipitates. The precipitates form in the solution bulk, as well as on the surface of rubber particles. The origin of the precipitates is also different. It was revealed by XRD-analysis that while in the process of the sulphur solubilisation with 9K medium the jarosite ($KFe^{3+}_3(SO_4)_2(OH)_6$) forms, the process of the biosolubilisation with *Acidithiobacillus ferrooxidans* Karitas is accompanied by the formation of the jarosite and the halotrichite ($FeAl_2(SO_4)_4.22H_2O$) as well.

The idea was to separate the particles of precipitates from the rubber ones using flotation after the relatively long attrition to detach the precipitates. The tenzide for the flotation of jarosite was selected based on the investigation of the surface properties. Nevertheless, further research work is needed to fully reveal the dissolution mechanisms, to optimise the biosolubilisation process, to develop the proper treatment technology for the separated liquid phase, as well as to successfully separate desulphurised rubber from the sulphur-rich precipitates.

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STUDY OF HEAVY METALS REMOVAL FROM MODEL SOLUTIONS BY POPLAR WOOD SAWDUST

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Abstract

The application of natural organic materials obtained from plant wastes or by-product is very often used as a replacement of the costly conventional methods of removing heavy metal ions from wastewater. It is well known that cellulosic waste materials can be obtained and used as cheap adsorbents for removal of heavy metal ions in chemical treatment. Nowadays are used many kinds of natural organic materials as adsorbents including rice husks, spent grain, sugarcane bagasse, fruit wastes, weeds, and wood sawdust.

This study is aimed to the static and dynamic sorption experiments with using of organic natural sorbent, poplar sawdust, for removal Cu(II), Zn(II) and Fe(II) from model solutions. The presence of hemicelluloses, cellulose and lignin in structure of wood sawdust was studied by infrared spectrometry. Poplar sawdust had efficiency of metal cations removal from aquatic model solutions approximately of 80 % under static conditions. Under dynamic conditions efficiency of the sorption processes reached 80 % for all model solutions in 5 minutes. The highest efficiency of Cu(II) removal (98 %) was observed after 30 minutes. The best efficiency of zinc removal (88 %) and iron removal (85 %) were reached after 45 minutes. Changes of pH values confirmed a mechanism of ion exchange on the beginning adsorption process.

Keywords: heavy metals, absorption, poplar wood sawdust

1 Introduction

Heavy metals and their excessively released into the environment due to industrialization created contamination of water environment in wide scale [1]. Cadmium, zinc, copper, nickel, iron, lead, mercury and chromium are often detected in industrial wastewaters, which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, and many others branches of industries [2]. In comparison with organic wastes, heavy metals are non-biodegradable and they can be accumulated in living organisms what is causing various diseases and disorders. From these reasons, heavy metals must be removed before discharge into the aquatic environment [3]. In this times, researches have an interest on the production of cheaper adsorbents as a replacement most commonly wastewater treatment methods such as chemical precipitation, ion-exchange, membrane separation, reverse osmosis, electro-dialysis, etc. [1, 4]

Using of the physic-chemical processes based on adsorption on the natural organic materials is one treatment processes to cheap and effective removal of heavy metals from contaminated water. According to Bailey et al. [5] adsorbents can be used as easier, faster and cheaper replacements of industrially produced sorbents if they have at big abundance in nature, their origin is a by-product or waste material from industry.

Wooden materials or wastes are cheap due to a low costs on their producing and processing [1]. Researches, which are focused on the study of absorption properties by wooden materials, many times deals with authentic and untreated wooden products or wastes in many cases [6-8]. Some of the benefits of using of wooden by-products or wastes for wastewater treatment include simple technique, requires low costs and processing, potential at selective removal or good capacity for adsorption of heavy metal ions, sufficient amount and possibility of regeneration [1, 3]. Between often used wooden materials for absorption are straw, tree bark, peanut skins, wood sawdust, moss and peat. Wood sawdust, like low-costs adsorbents, are perspective for removing heavy metals, some types of acid and basic dyes, and some other unwanted compounds from waste water [6, 9].

Ahmad et al. [10] studied sorption properties of wood sawdust and they discovered that are formed complexes compounds with a metal cations using their ligand or functional groups. The sorption of metals by sawdust may be caused by lignin, cellulose and hemicellulose, carbohydrates, and phenolic compounds that have carboxyl, hydroxyl, sulphate, phosphate, and amino groups that can bind metal ions [11, 12]. The use of wood sawdust brings a potential as a replacement for current expensive methods. The using of sawdust for removing pollutants would be benefit also for the environment and timber industry. Contaminated water could be cleaned, and a new market would be opened for the sawdust [6].

The aim of this article is a study of sorption properties of poplar wood sawdust for removal heavy metals (copper, zinc and iron, respectively) from model solutions with initial concentration of cations 10 mg.L^{-1}. Poplar sawdust was analysed by infrared spectrometry for characterization of functional groups. Efficiency of heavy metals removal was analysed by colorimetric method and changes of pH values were also observed.

2 Material and methods

2.1 Wood sample

The sawdust of poplar, species of locally available wood, was sieved, and the fraction with particle size max. 2.0 mm was used for experiments. 1 g of dry poplar sawdust was mixed with 100 mL of each kind of the used model solution. FTIR measurements of poplar sawdust were carried out on Bruker Alpha Platinum-ATR spectrometer (BRUKER OPTICS, Ettingen, Germany). A total of 24 scans were performed on sample in the range of 4,000–400 cm^{-1}.

2.2 Adsorbate solutions

Adsorbate solutions of Cu(II), Zn(II) and Fe(II) (initial concentration c_0= 10 mg.L^{-1}) were prepared by dissolving $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$, respectively in deionised water. Concentrations of appropriate ions were determined by colorimetric method by Colorimeter DR890 (HACH LANGE, Germany) with appropriate reagents to determine concentration of dissolved cooper, zinc, and iron. Change of pH value was also measured by pH meter inoLab ph 730 (WTW, Germany).

2.3 Adsorption studies

Batch adsorption experiments were carried out on static and dynamic conditions of experimental set up. In both cases 1 g of poplar sawdust was mixed with 100 mL of model solutions containing of 10 mg.L^{-1} copper, zinc, and iron, respectively. In case of static conditions, after 24 hours reaction time, wood sawdust was removed by filtration through a laboratory filter paper for qualitative analysis, residual concentrations of appropriate ions were determined by colorimetric method and pH change was also measured.

In case of dynamic condition was poplar wood sawdust intensive mixed with model solutions containing of 10 mg.L^{-1} cooper, zinc and iron, respectively. The reaction time of these experiments was 5, 10, 15, 30, 45, 60 and 120 min, respectively. After the end of each reaction time, wood sawdust was removed by filtration through a laboratory filter paper for qualitative analysis, residual concentrations of appropriate ions were determined by colorimetric method and pH change was also measured.

In both cases the % removal was calculated using the following equation (Eq. 1):

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

where η is efficiency of ion removal (%), c_0 is the initial concentration of appropriate ions (mg.L^{-1}) and c_e equilibrium concentration of ions (mg.L^{-1}).

3 Results and discussion

3.1 Poplar sawdust infrared spectrum

The heavy metals adsorption capacity is influenced strongly by the surface structures of carbon–oxygen (functional groups) and surface behaviour of carbon [13]. Functional groups in poplar wood sawdust were determined using FTIR spectroscopy. The IR spectrum of poplar sawdust is shown in Figure 1. According to a literature [14], we can suppose that the structure of wooden sawdust is mainly formed by cellulose, hemicellulose, and lignin. As is clearly from Figure 1, the strong broad OH stretching (3,500–3,000 cm^{-1}) and C–H stretching of methyl and methylene groups (2,950–2,850 cm^{-1}) are present. The strong broad OH stretching at this area can be caused by the presence of water or damp too. For hemicelluloses are typical the stretching band at 1731 cm^{-1} (presence of C=O from the acetyl groups) [15]. Infrared spectra of lignin were observed by Zhang et al. [16]. The characteristic bands of lignin were confirmed at 1503 and 1452 cm^{-1} (aromatic skeletal vibrations of lignin) and at 1320 cm^{-1} (syringyl and guaiacyl condensed lignin). Deformations detected in range from 1421 to 894 cm^{-1} wavenumber appertain to cellulose that occurs in two forms (in crystalline and amorphous). Functional groups of aromatics, carboxylic acids, alkyl halides were found at 828 cm^{-1} [17]. At 554 cm^{-1} were also determined alkyl halides (C–Cl and C–Br stretch).

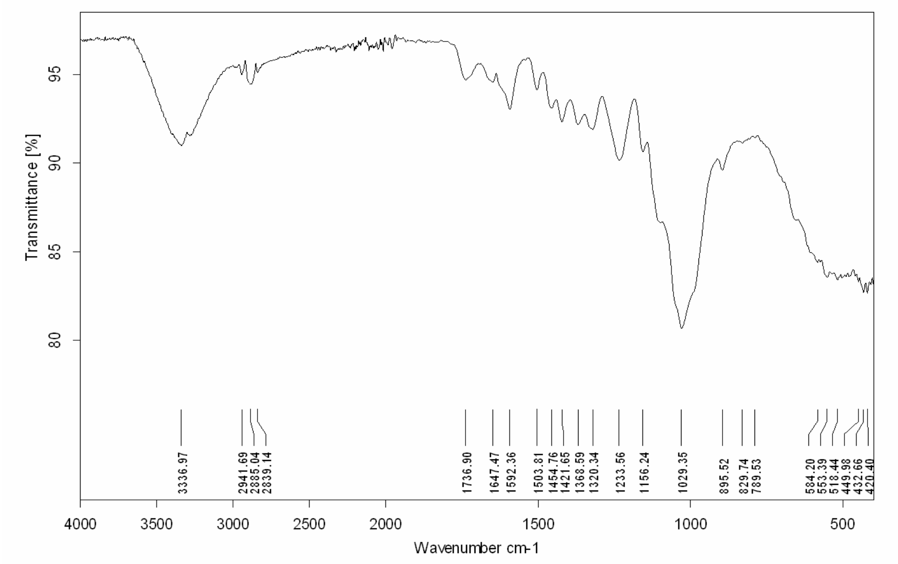

Fig. 1. Infrared spectra of poplar wood sawdust

3.2 Adsorption study of poplar wood sawdust - static conditions

Results of sorption experiments under static conditions for solutions with concentrations of 10 $mg.L^{-1}$ cation are shown in Table 1. The poplar sawdust used for sorption confirmed a capability to removal the ions. Šćiban et al. [7] investigated of heavy metals removal by poplar sawdust as promising adsorbent for metals removal from wastewater with potential more economical than conventional removal processes. Poplar sawdust exhibits the best effect on sorption of copper from solution with efficiencies above of 80 %. For zinc and iron removal the poplar sawdust was with efficiencies above of 70 %.

Changes of pH values in solutions were observed after sorption too. The pH value of the model solutions is an important controlling parameter in the adsorption process. The pH values have affected the surface charge of adsorbent, the degree of ionization and speciation of adsorbate during adsorption [18]. At lower pH, the positive charged metal ion species may compete with H^+ and be absorbed at the surface of the sawdust by ion exchange mechanism. At elevated pH, mainly neutral, metal cations may be absorbed by hydrogen bonding mechanism along with ion exchange. Due to different properties of Cu(II), Zn(II), and Fe(II) of model solutions, the adsorption took place in a slightly different pH range for different metals. Sorption of Cu(II) by a poplar sawdust increase of value pH. Possibility of ion exchange between dissolved Zn(II) and Fe(II) metal cations and H^+ from poplar sawdust was indicated by decreasing of pH.

Table 1. Results of static sorption experiments with poplar sawdust
(initial concentration of cations in solutions c_0=10 $mg.L^{-1}$)

Initial concentration $c_0(Cu^{2+})$=10 $mg.L^{-1}$ at pH=5.8		Initial concentration $c_0(Zn^{2+})$=10 $mg.L^{-1}$ at pH=5.4		Initial concentration $c_0(Fe^{2+})$=10 $mg.L^{-1}$ at pH=5.4	
$c_e(Cu^{2+})$ [$mg.L^{-1}$]	pH	$c_e(Zn^{2+})$ [$mg.L^{-1}$]	pH	$c_e(Fe^{2+})$ [$mg.L^{-1}$]	pH
1.42	5.3	2.6	5.8	2.2	5.7

3.3 Adsorption study of poplar wood sawdust - dynamic conditions

Comparison of sorption efficiency and changes pH values over time are shown in Figure 2. Using the poplar sawdust for absorption experiment in model solutions with dissolved ions Cu(II), Zn(II), Fe(II), the curve indicates the rapid progress of sorption. After only 5 minutes of the sorption process was achieved over 80 % efficiency in all cases. The residual time of experiment can be considered as a relative settled, only with little changes at efficiency of removal. In the case of dynamic absorption conditions of Cu(II) by poplar sawdust, after 30 minutes was obtained the highest efficiency of removal 98 %. At 45 minutes of reaction time, were reached the highest efficiency of Zn(II) (88 %) and Fe(II) (85 %).

Also in this case changes of pH values in solutions were observed after adsorption. The pH is one of the most important parameters controlling adsorption of heavy metals from aquatic solutions. Resulting from Figure 2, using of poplar sawdust in dynamic conditions is connected with decreasing (Cu^{2+}) and increasing (Zn^{2+} and Fe^{2+}) of pH values of the tested samples. Holub et al. [19] found, that with higher initial concentration of dissolved heavy metal is significant change of pH value, because the ion exchange is more intensive. In all cases were recorded markedly changes of pH values after 5 minutes of the adsorption experiments. The most significant change of pH values was in case Fe(II) removal, where at beginning was value of pH changed from 5.4 to 6.4. By intensive increasing of pH values, metal cations were absorbed by mechanism of ion exchange by hydrogen bonding. With the decreasing concentrations of dissolved metals cations in solutions, the ion exchange is not so dominant and is not affecting on the changing of pH value [20]. After the completion of the ion exchange, pH value began decrease slowly to approximately input value in all cases as is illustrated on Figure 2.

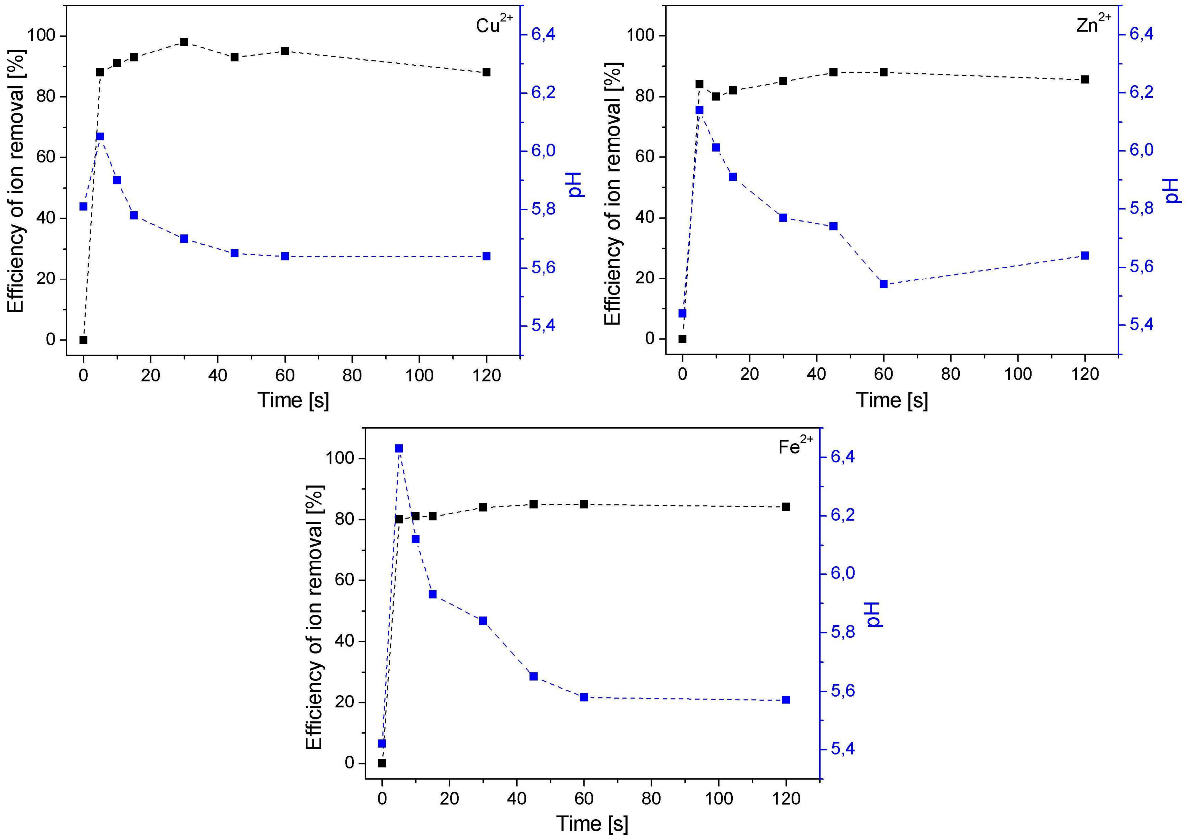

Fig. 2. Comparison of sorption efficiencies and changes of pH values over the experimental time

4 Conclusions

Despite of the existence of various technologies for heavy metal ions removal from wastewaters (chemical precipitation, ion exchange, membrane techniques, etc.), is still trend to discover further new researches with aim of getting more economically and environmentally sustainable technology that could provide the lowest level of heavy metal ions in a treated effluent that will be low enough to be safely discharged in a surface water stream. One from many interesting researches is directed to a study the adsorption of heavy metal ions from wastewaters by using various natural materials. The main benefit of using such materials as the conventional adsorbents or ion exchange resins is in their availability and their low price, so that they are also known as “low-cost” adsorbents. A special attention in these researches is dedicated towards bark and wood sawdust of different trees as by-products of timber and wood industry that could be used particularly for a passive treatment of wastewaters with potential for active treatment too.

Poplar sawdust shows as suitable product for removal metals from solutions. The function groups of poplar sawdust were characterized by infrared spectra that confirmed the presence of hemicelluloses, cellulose and lignin (aromatic skeletal vibrations of lignin syringyl and guaiacyl condensed lignin).

In case of static conditions, results of the adsorption experiments confirmed the poplar wood sawdust capability to removal various ions with best effect on sorption of copper from solution with efficiencies above of 80 %. At zinc and iron removal, the poplar sawdust was with efficiencies above of 70 %. Changes of values pH under static conditions showed the processes of adsorption and ion exchange.

Under dynamic conditions efficiency of the sorption processes reached 80% for all model solutions in 5 minutes. The highest efficiency of Cu(II) removal (98 %) was observed after 30 minutes. The best efficiency of zinc removal (88 %) and iron removal (85 %) were reached after 45 minutes. In all cases at dynamic conditions were recorded markedly changes of pH values after 5 minutes of the adsorption experiments. The most significant change of pH values was in case Fe(II) removal, where at beginning was value of pH changed from 5.4 to 6.4, where metal cations were absorbed by mechanism of ion exchange by hydrogen bonding.

The sorption experiments showed a potential of the poplar sawdust to remove cations from model acidic solutions. The results from experiments also provide promising perspectives for the utilization of poplar sawdust as a natural adsorbent for reducing metal pollution in wastewaters and they showed potential to be used on an industrial scale.

Acknowledgements

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MOBILIZATION OF ARSENIC FROM TECHNOSOLS UNDER LABORATORY DYNAMIC TESTS (MODEL ENVIRONMENTAL BURDEN)

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Abstract

This study describes the mobilization of arsenic from technosols through laboratory dynamic experiments. Studied environmental burden in Zemianske Kostoľany is the most polluted area in Slovakia, where in 1965 a dam failure of a coal combustion ash impoundment released approximately 3 million m^3 of power plant ash in the surrounding environment. The total content of arsenic in the studied soil samples (technosols) is in the range of 1100-1139 mg.kg^{-1}. Column experiments were performed in two glass columns. Using a peristaltic pump leaching solutions were flowing through the column (demineralized water, 1 mM citric acid and 0.1 M hydrochloric acid) from bottom to the top for 105 days, whereas every 35 days leaching agent was changed to compare the efficiency of solutions. Most arsenic during experiments with demineralized water released from the sample ZK2 from a depth of 60 cm (= 11.11 % from As$_{tot}$ 1100 mg.kg^{-1}), during the experiment with citric acid from a given sample of 16.58 % As was obtained and during the experiment with hydrochloric acid from a given sample of 5.18 % was obtained. From the results obtained we can find that the strongest extactant was citric acid. This work confirms that soil microorganisms producing organic acids play an important role in the environmental fate of arsenic in soils.

Keywords: technosol, Zemianske Kostoľany, arsenic, laboratory dynamic test

1 Introduction

The studied locality Zemianske Kostoľany is the most polluted area in Slovakia. In 1965 a dam failure of a coal ash impoundment released 3 million m^3 of coal fly ash with a high content of arsenic and other potentially toxic trace elements in the surrounding environment. Instead of remediation the ash layer was covered by 40 to 100 cm of different soil types. According to the new morphogenetic classification system we call the soil with a layer of power plant ash as technosol [1].

Soil is a medium through which the contaminants are getting from the earth's surface into the groundwater. During the movement of pollutants through the soil profile, these substances are subjects to complex physico-chemical and biological transformations, so it is important to understand the processes (environmental characteristics, physico-chemical properties, mineral composition of the ash, sediments and soils) controlling their fate in soil ecosystems [2, 3]. The rate of leachability of potentially toxic elements is related to their concentration in coal and ash, and depends on the conditions of coal combustion, on the processes of sorption/desorption, on the redox and dominantly on the pH conditions of the environment [4, 5].

On the basis of the results of numerous studies and observations, the main soil parameters that govern processes of sorption and desorption of potentially toxic elements are presented as follows:

- pH and Eh values
- Cation exchange capacity (CEC)
- Fine granulometric fraction (<0.02 mm)
- Organic matter
- Oxides and hydroxides, mainly Fe, Mn and Al
- Soil microorganisms [4, 6].

Living organisms, often referred to as the soil biota, composed of fauna and flora of various dimensions, occur abundantly in soils. Several species of biota are known to be useful indicators of soil contamination. However, growth, reproduction, and metabolic activities of soil biota are sensitive to other soil variables as well as to the concentrations of trace pollutants. The Eh-pH system and organic matter content are highly important. Humic substances are known to have both beneficial and deleterious effects on biota caused by mobilization of metals [7].

2 Material and methods

2.1 Study area and sample collection

Collection of soil samples (technosols) intended for laboratory (dynamic) experiments took place in location of the Upper Nitra region, in Zemianske Kostoľany (Figure 1). From soil probes there were two samples - ZK1 and ZK2 collected from the depth of 30 and 60 cm. The soil samples were processed in a standard manner (drying, homogenisation and sieving into a fraction smaller than 2 mm). Chemical analysis of the soil samples were performed at ACME Analytical Laboratories Ltd (Vancouver, Canada) by AES-ICP, AAS-F and AAS-HG.

Fig. 1. Sampling locations

2.2 Column (dynamic) experiments

Laboratory dynamic tests were performed in two glass columns (Figure 2). 25 cm long duplicate glass columns with a 2.5 cm internal diameter were packed with 70 g of dry solid soil samples. Before filling the columns with a dry sample, a filter paper (pore size 16 µm) and 1 cm of clean silica sand was added to the column top and bottom in order to prevent fine particles to penetrate into the plastic tubes. Using a peristaltic pump ISMATEC IPC 12 leaching solutions were flowing through the column from bottom to the top for 105 days (upward flow was regulated at a rate of 7.10 ml.min^{-1}), at which every 35 days leaching agents were changed to compare the efficiency of the solutions. There were used three leaching agents: deionized water – standard agent in the extraction experiments, 1 mM citric acid – organic acid naturally produced by microorganisms in soil ecosystem, 0.1 M hydrochloric acid – strong inorganic acid. Leachates were taken at intervals (day): 7, 14, 21, 28, and 35. After the collection, the basic physico-chemical parameters (pH, EC and temperature) were determined in leachates. Chemical analyses of the extracts were realized in the laboratory of EL spol. s.r.o., Spišská Nová Ves by AES-ICP, AAS and UV/VIS.

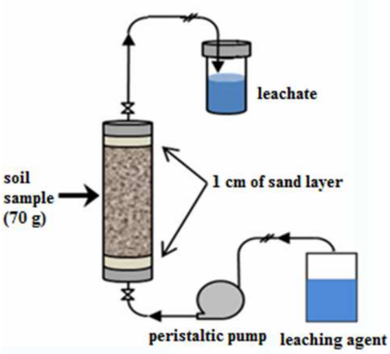


Fig. 2. Scheme of the column experiments [8]

2.3 Mineralogical analyses

The sieved soil samples were evaluated by quantitative X-ray diffraction analysis. Sample preparation for X-ray analysis was performed as follows: 1 g of soil sample was mixed with 0.25 g of corundum powder – Al_2O_3 (internal standard). The obtained mixture was then placed to the container with a corundum roller and added 4 ml denatured alcohol. At first the container was shaked by hand and after the shaking the container was put into a special mill (Micronizing McCrone Mill) and the sample was milled for 5 minutes. The obtained fraction was analyzed with a machine Bruker D8 Advance using CuKα radiation. The primary identification of minerals was conducted in the program DIFFRACplus EVA. Quantitative representation of mineral phases in X-ray diffraction pattern was determined in the program TOPAS.

3 Results and discussion

The total content of selected contaminants in technosols used in the column experiments confirm the fact that the area is highly contaminated with potentially toxic trace elements. In the case of arsenic and mercury limited value for agricultural soils (soil type – sandy loam to loamy) is 25 $mg.kg^{-1}$ As and 0.5 $mg.kg^{-1}$ Hg). The total content of arsenic in the studied samples is in the range of 1100-1139 $mg.kg^{-1}$. Table 1 shows the total content of selected chemical elements in the soil samples.

Table 1. The total content of selected chemical elements in the studied samples

Sample	As	Hg	Pb	Fe	Ca	Al
	$mg.kg^{-1}$			wt.%		
ZK1	1139	0.58	7.10	4.09	3.70	3.45
ZK2	1100	0.97	8.20	3.91	3.66	3.18

On the basis of the results it is stated that the most of arsenic released during the experiment with citric acid and the least with the agent of hydrochloric acid though the leachability of arsenic did not become persistent with none of the leaching agents (Figure 3). The results with deionized water show decreasing trend of arsenic leaching. According to the results, citric acid was the most effective leaching agent, which confirms the assumption that the organic acids have a great effect on the mobilization of arsenic and other potentially toxic elements (Figure 4). In the sample ZK2, most arsenic was released after application of citric acid 16.58 % of As_{Tot}. Demineralized water released 11.11 % of As_{Tot}. During the experiments with hydrochloric acid 5.18 % of As_{Tot} was released (Table 2).

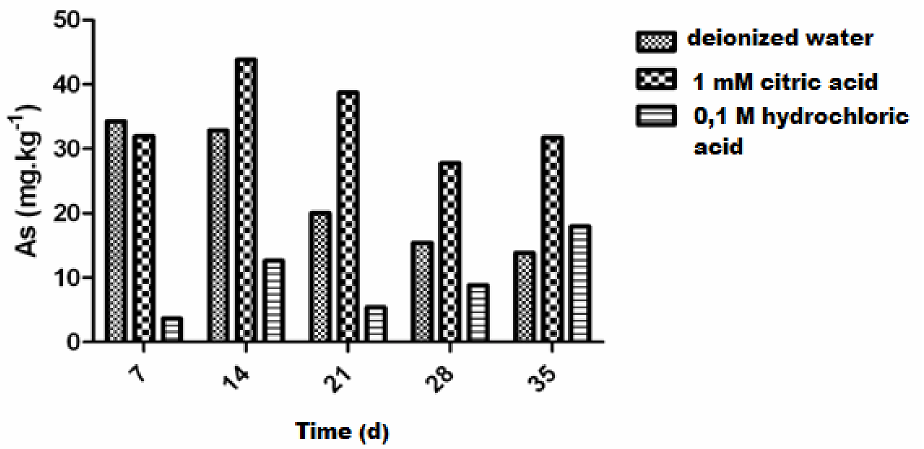


Fig. 3. The average content of arsenic ($mg.kg^{-1}$) in the leachates during the experiments

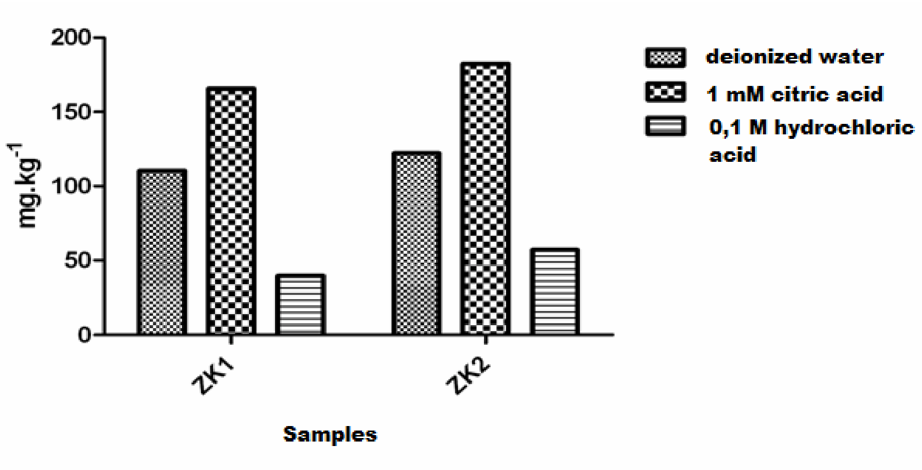
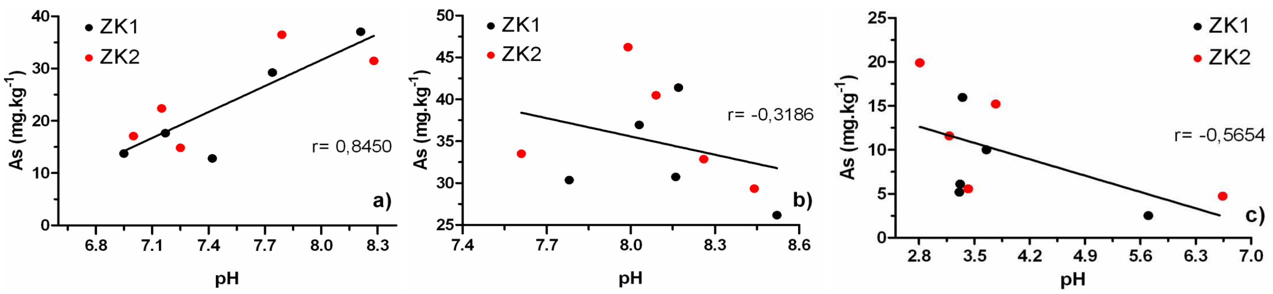


Fig. 4. The total content of As leached (mg.kg^{-1}) during the experiments

Table 2. The comparison of total content of arsenic (mg.kg^{-1}) in soil samples with the contents of As in leachates (wt.%)

Samples	Total content of As in the leachate (mg.kg^{-1}) – de. water	Total content of As in the leachate (wt. %) – de. water	Total content of As in the leachate (mg.kg^{-1}) – citric acid	Total content of As in the leachate (wt. %) – citric acid	Total content of As in the leachate (mg.kg^{-1}) – h. acid	Total content of As in the leachate (wt. %) – h. acid	Total content of As in soil samples (mg.kg^{-1})
ZK1	110.49	9.7	165.63	14.54	39.84	3.5	1139
ZK2	122.26	11.11	182.36	16.58	57.03	5.18	1100

Soil pH is one of the most indicative measurements of the chemical properties of the soil. All (bio)chemical reactions in soils are influenced by proton (H^+) activity, which is measured by the soil pH. The pH values of most natural soils vary between <3.00 (extremely acid) and 8.00 (weakly alkaline). The solubilities of various compounds (e.g. heavy metals) in soils are influenced by soil pH, as well as by microbial activity and the microbial degradation of pollutants. Optimum pH values for pollutant-degrading microorganisms range from 6.5 to 7.5. Soil pH is influenced by various factors: the nature and type of the inorganic and organic constituents that contribute to the soil's acidity, the soil/solution ratio, the salt or electrolyte content, and the CO_2 partial pressure [9]. Trace metal mobility depends on the following soil characteristics: soil pH and texture, the type and quantity of the oxy-hydroxides present, the content of organic matter, carbonates, phosphates and clays [10]. Arsenic is characterised by a pH-dependent leaching, and displays a plateau of maximum solubility in the pH 7 - 11 range [11]. Figure 5a, b, c shows the dependence of leached arsenic on the pH value during the column experiments. Figure 5a shows the increase of extracted arsenic with the increase of the pH value in the given extract. Figure 5b, c also shows the opposite trend of the fact in the relation of the leached arsenic in citric acid (negative trend), hydrochloric acid (negative trend) and demineralized water (positive trend).



a **b** **c**

Fig. 5. Dependence of As leached (mg.kg^{-1}) on the pH value during the experiments with deionized water (a), 1 mM citric acid (b) and 0.1 M hydrochloric acid (c)

In the studied samples the major component are the amorphous mineral phases. This group may also include unburned residues of coal and amorphous Fe oxy-hydroxides. Amorphous aluminosilicate glasses are the most common phases present in the sample (approximately 40 wt. %), also there are common quartz, calcite, mullite, plagioclases, magnetite, hematite, cristobalite, rutile, pyrrhotite, pyrite, montmorillonite, albite and biotite. Amorphous glasses are characteristic by highly variable content of Si, Al, Ca and Fe. They are able to accumulate a wide range of chemical elements and are the main mineral phases bearing arsenic. The second mineral phase with the ability to absorb As are less commonly occurring unburned coal particles [12]. Quartz is present in the all studied samples (cca 3 wt. %). It is the dominant primary mineral in the coal fly ash, because its melting point is 1710 °C [13]. Quartz particles in the power plant reactors can burst at higher temperatures (above 870 °C) to form cristobalite [14]. Mullite is strongly represented in the samples and arises from high temperature changes of clay minerals, especially kaolinite [13]. Figure 6a, b shows the quantitative representation of the mineral phases in the soil samples.

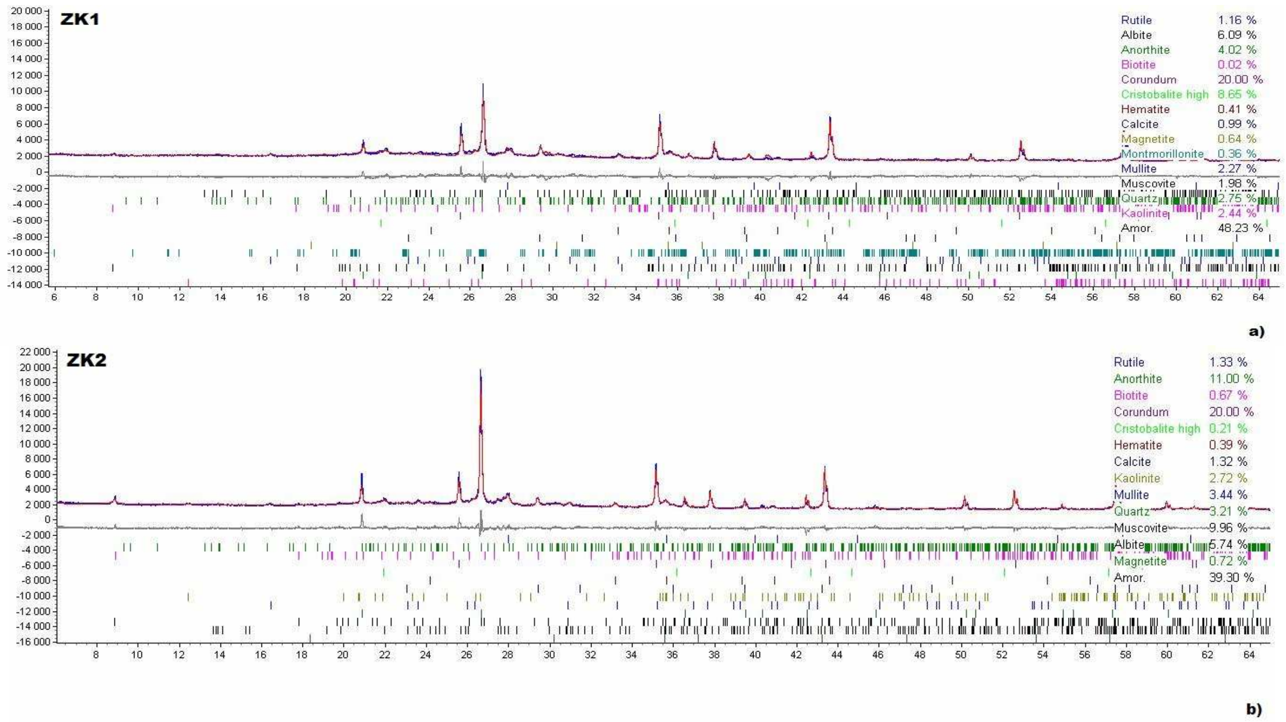


Fig. 6. Quantitative representation of the principal mineral phases in the sample ZKI and ZK2

4 Conclusions

The aim of the realization of column experiments was to assess the potential mobility and leachability of arsenic from the solid phase of the soil samples under influence of the chosen leaching agents (demineralized water, citric acid and hydrochloric acid) and compare their effectivity. The total content of arsenic in the technosols is in the range of 1100-1139 mg.kg^{-1}. After application of demineralized water most arsenic was released from the sample ZK2 (of the total As – 1100 mg.kg^{-1} leached 11.11 % of As). Experiments with citric acid showed that it was leached 16.58 % of As_{Tot} from sample ZK2. During the experiments with hydrochloric acid 5.18 % of As was released. In the studied samples the major mineralogical components are amorphous glasses, quartz, calcite, albite, rutile, montmorillonite and biotite. From the results we can obtain that after the experiments there were not any significant changes in the mineralogical composition of the samples. Study confirms that the soil microbiota which produce organic acids play an important role in the environmental fate of arsenic in soils.

Acknowledgements

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COMBINATION OF VARIOUS METHODS FOR ACID MINE DRAINAGE TREATMENT

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Abstract

The mining represents one the most common human activities that fundamentally impact not only the country itself but also have adverse effects on the fauna, flora and human beings. The negative impacts also include acid mine drainage which is formed by the dissolution of products resulting from the oxidation (chemically and microbiologically mediated) of sulphide minerals, mainly pyrite or iron disulphide. According to stringent European Union effluent discharge regulations it is necessary to look into innovative technologies to remove considerable amount of effluent rather than discharging into surface water.

Resulting from previous partial achievements, the article is focused on the combination of chemical precipitation, ion exchange and biosorption techniques for the acid mine drainage treatment. Concentrations of four different metal cations (copper, iron, manganese, aluminium) and sulphates were observed. First stage of treatment included chemical precipitation by combination of oxidation using 31 % hydrogen peroxide and subsequent precipitation with 0.1 M sodium hydroxide. After the first stage, the ion exchange using two different exchangers (PUROLITE MB400 resin and AMBERLITE MB20 resin) took place. The last stage of the experiments was focused of the biosorption study. Low-cost sorbents included in this case natural non-modified peat "PEATSORB" and hemp shives in modified state. Overall, the best results were observed after combination of MB20 resin in the second stage and subsequent using of modified hemp shives in the third stage of the treatment.

Keywords: sorption, precipitation, ion-exchange, heavy metals, sulphates, acid mine drainage

1 Introduction

Manufacturing processes, primarily mining activities, often result in the production of effluents containing high amounts of inorganic pollutants e.g. heavy metals or sulphates.

The problems connected with the extraction of sulphide ores are present throughout the whole process of mining, but arise especially during the last stage of mining, closure and abandonment of the extraction area, when the conditions for the generation of strong acid water are most likely to occur [1].

This generation consists from weathering of sulphide minerals under oxidation conditions (in contact with water, atmospheric oxygen, micro minerals and aerobic bacteria) and effluents are characterized with very low pH value of about 2 and limit concentrations of heavy metals (Cu, Zn, Cd, As, Mn, Al, Pb, Ni, Ag, Hg, Cr and Fe), toxic elements and sulphates [2-4]. Importantly, once acid mine drainage (AMD) has formed, it can remain for hundreds of years, and it is generally difficult and costly to control [5].

Currently used technologies for removal of inorganic compounds are result of a need by industry to achieve acceptable effluent quality standards set by the governments. These conventional and emerging treatment methods include precipitation, secondary co-precipitation, differential precipitation, ion exchange, membrane separation, evaporative recovery, reductive electrolysis, etc [6-9].

Precipitation has found its wide application for metals removal, particularly in industrial treatment technologies. The fact that precipitation can only reduce the dissolved metal concentration to the solubility product level, create the motivation for change away from these metal control technologies. Another limitation can be found in treatment of wastewaters containing low concentrations of elements, what makes in this case applications of common methods for removing of heavy metals either technically complicated or economically unfavourable. Precisely such problems are creating a space for new and innovative methods for treatment of heavy metals and sulphates contaminated wastewater. It is also possible to combine different treatment techniques in order to apply the positive properties of each method.

Adsorption or ion-exchange onto low-cost particulate materials such as biosorbents now offers an attractive, inexpensive option for the removal of colloidal and dissolved metals [10].

Ion exchange represents a very powerful technology for removal of toxic elements from water and other solutions. Undesirable ions are replaced by others which do not contribute to contamination of the environment. The method is technologically simple and enables efficient removal of even traces of pollutants from solutions [11].

The present paper is focused on the study of the chemical and physical methods combination for the heavy metals and sulphates removal from acid mine drainage out-flowing from the Pech shaft located on the deposit Smolník in the Slovak Republic. The treatment process includes oxidation and chemical precipitation in the first stage, followed by ion exchange using two different resins. Last stage is focused on the biosorption processes with the dead biomass (peat and modified hemp shives).

2 Material and methods

As mentioned above, the treatment process consists of three stages. Overall, concentrations of four different metal cations (copper, iron, manganese, aluminium) and sulphates were observed. Concentrations of metal ions before and after the experiments were determined by colorimetric method using Colorimeter DR890 (HACH LANGE, Germany) with combination of appropriate reagent. Concentrations of sulphates were analyzed by X-ray fluorescence analysis (XRF) using SPECTRO iQ II (Ametek, Germany) with silicon drift detector with resolution of 145 eV at 10 000 pulses.

pH values were determined by pH meter FiveGo FG2 (Mettler – Toledo AG, Switzerland) which was standardized using buffer solutions of different pH values (4.01, 7.00).

First stage is focused on the oxidation and chemical precipitation of iron cations due to their high concentration. Precipitation was followed by ion exchange and finally in the last stage AMD was contacted with two different biosorbents.

The percentage of efficiency (%) was calculated using the following equation:

$$Efficiency \quad = \frac{(C_0 - C_e)}{C_0} \; x\,100\% \,, \tag{1}$$

where c_0 is the initial concentration of ions (mg.L^{-1}), c_e is equilibrium concentration of ions (mg.L^{-1}).

2.1 Acid mine drainage samples

Real sample of AMD containing a high level of sulphates and heavy metals (concentrations of monitored metals are listed in Table 1). The experimental sample was collected from abandoned subsurface mine Smolnik (Slovakia).

Table 1. Properties of experimental AMD samples (input concentration)

	pH	Fe_{total}	Cu	Al	Mn	SO_4
mg/L	3.52	305	0.86	44.8	24.4	2400

2.2 Oxidation and precipitation

Chemical precipitation (combination of oxidation using 31 % hydrogen peroxide and subsequent precipitation with 0.1 M sodium hydroxide) was used for the removal of iron cations due to their high concentration which is several times higher than concentration of the others monitored metals. The sample of 1 L of AMD was continually stirred after adding of 4 mL H_2O_2 and sequentially neutralised by NaOH under continuous stirring and pH measuring. The resulting precipitate was filtered through laboratory filter paper and concentration of monitored metals and sulphates in filtrate was determined.

Following reaction takes place during the experiment:

$$2Fe^{2+} + H_2O_2 \rightarrow 2Fe^{3+} + 2OH^- \tag{2}$$

$$Fe^{3+} + OH^- + SO_4^{2-} \rightarrow Fe(OH)SO_4 \tag{3}$$

2.3 Ion exchange

For the removal of sulphates and metals by ion exchange process, two inorganic ion exchange resins PUROLITE MB400 and AMBERLITE MB20 were used.

PUROLITE MB400 is a high quality resin mixture for direct purification of water. It is suitable for use in regenerable or non-regenerable cartridges and in large ion exchange units. Passage of water at recommended flow rates through the resin as supplied can achieve almost complete reduction of total dissolved solids.

AMBERLITE MB20 resin is an ionically equilibrated mixed bed resin. It is a fully regenerated, ready-to-use mixture of a strong acid cation exchanger with a strong base type 1 anion exchanger. AMBERLITE MB20 resin has been developed for the production of high purity water. It can be used for all applications requiring totally demineralised water, free of silica and of carbon dioxide.

The cations removal efficiency by ion exchange process was tested at laboratory temperature (23±0.2 °C) under static conditions. 1 g of each resin was mixed with 100 mL of AMD sample after precipitation. After 24 hours reaction time, resins were removed by filtration through a laboratory filter paper for qualitative analysis and equilibrium concentrations were determined.

2.4 Biosorption

In the biosorption study two types of adsorbents were chosen. As a biosorption materials non-modified peat “PEATSORB” (provided by REO AMOS; Slovakia) and hemp shives (provided by Hempflax; Netherlands) were used. In experiments finer heterogeneous fraction of peat prepared by sieving through a 2 mm sieve was used.

The final fibre length of used technical hemp shives ranged between 4 mm and 0.063 mm. According the previous results, hemp shives were used only in the modified state. Hemp fibres were modified by chemical treatment in order to gradually remove either hemicelluloses or lignin. The progressive removal of the hemicelluloses and keeping the lignin content unchanged was brought by treating the fiber samples with 1.6 M NaOH solution, at room temperature, for 48 h, followed by neutralisation with 1 % acetic acid. Fibres were then washed with deionised water until the pH value was 7.0.

The adsorbents were dried at 105 °C for 2 h and then allowed to cool in the desiccators before using in experimental set-up.

For the purpose of removal efficiencies investigation, batch adsorption experiments were carried out. Each type of sorbent was mixed with AMD sample after precipitation and ion exchange. Batch experiments were carried out at room temperature (23±0.2 °C) in beakers by adding of a constant mass of sorbent (1.0 g) in 100 mL of AMD sample. After 24 hours reaction time, sorbents were removed by filtration through a laboratory filter paper for qualitative analysis, residual concentration metals and sulphates were determined by colorimetric method and pH change was also measured.

3 Results and discussion

3.1 Precipitation

The removal of sulphate ions from water environment is a complex problem due to the high solubility and stability of these anions in aqueous solutions [12]. The hydrogen peroxide, in acid solution, oxygenates from ferrous ions to ferric ions. During this oxidation is mainly formed the ferric hydroxy-sulphate that is insoluble precipitate (see Fig. 1). A substantial part of sulphates is remaining in the solution due to stability of sulphate anion [13].

Fig. 1. Precipitate of $Fe(OH)SO_4$

The removal efficiency was 99.8 % and 25.1 % for iron and sulphates, respectively. Removal of the other monitored metals was insufficient what created the space for the application of ion exchange in the next step. Summary changes in concentrations and removal efficiencies are shown in Table 2.

Table 2. Concentrations of heavy metals and sulphates after precipitation

	pH	Fe_{total}	Cu	Al	Mn	SO_4
mg/L	3.66	0.71	0.79	42.2	20.4	1820
Efficiency [%]	-	**99.8**	**8.1**	**5.6**	**16.4**	**25.1**

3.2 Ion exchange

Samples of AMD after chemical oxidation were subsequently treated by ion exchange. The main goal was reduction of sulphates and metals that were not effectively removed in the first phase of treatment. From the results (listed in the Tables 3 and 4) it is evident that both MB400 and MB20 resins exhibit similar results. The most significant decreases in concentrations are observed in the case of aluminum and manganese. pH values are stable at the level between 2.5 and 2.6. MB20 resin is partially more effective than MB400.

Table 3. Concentrations of heavy metals and sulphates after ion exchange by PUROLITE MB400

	pH	Fe_{total}	Cu	Al	Mn	SO_4
mg/L	2.52	0.63	0.39	12.1	8.8	1200
Efficiency [%]	-	**11.3**	**50.6**	**71.3**	**56.9**	**33.8**

Table 4. Concentrations of heavy metals and sulphates after ion exchange by AMBERLITE MB20

	pH	Fe_{total}	Cu	Al	Mn	SO_4
mg/L	2.60	0.69	0.39	11.3	8.2	1150
Efficiency [%]	-	**2.8**	**50.6**	**73.2**	**59.8**	**36.6**

3.3 Biosorption

The last stage of experiments consists of biosorption. Four different combination of adsorbate/adsorbent system were evaluated:

- AMD after precipitation and ion exchange by PUROLITE MB400 vs. peat;
- AMD after precipitation and ion exchange by PUROLITE MB400 vs. hemp shives;
- AMD after precipitation and ion exchange by AMBERLITE MB20 vs. peat;
- AMD after precipitation and ion exchange by AMBERLITE MB20 vs. hemp shives.

The results are presented in the Tables 5 and 6.

Table 5. Concentrations of heavy metals and sulphates after biosorption
(Input: PUROLITE MB400 pre-treated AMD)

		pH	Fe_{total}	Cu	Al	Mn	SO_4
Peat	mg/L	2.61	0.53	0.08	9.7	7.6	1190
	Efficiency [%]	-	**15.9**	**79.5**	**19.8**	**13.6**	**0.8**
Hemp shives	mg/L	3.08	0.09	0.03	8.0	7.8	1060
	Efficiency [%]	-	**85.7**	**92.3**	**33.9**	**11.4**	**11.7**

Table 6. Concentrations of heavy metals and sulphates after biosorption
(Input: AMBERLITE MB20 pre-treated AMD)

		pH	Fe_{total}	Cu	Al	Mn	SO_4
Peat	mg/L	2.77	0.50	0.14	6.7	7.6	1120
	Efficiency [%]	-	**27.5**	**0.64**	**40.7**	**7.3**	**2.6**
Hemp shives	mg/L	3.39	0.00	0.06	5.3	7.1	905
	Efficiency [%]	-	**100**	**84.6**	**53.1**	**13.4**	**21.3**

Overall, biosorption was more efficient by using of modified version of hemp shives which exhibit better results for all monitored elements. The results showed that biosorption could be powerful method in the case of trace concentration. Iron and copper cation were eliminated bellow 0.1 mg/L. On the other hand decrease in manganese concentration was minimal (only bellow 15 %).

Changes in pH values were also measured. Peat caused a small increase in pH value after sorption. Generally, peat as a product of humification causes increasing in pH values. Metals react with the carboxylic and phenolic acid groups of the acids to release protons or, at sufficiently high pH, with their anion sites to displace an existing metal [14]. In this case only small concentration of metals is adsorbed and cannot significantly influence pH value in already acidic conditions. Increase in the pH values caused by hemp shives may be result of the OH functional groups release into the solution in both cases of adsorbates. This theory is also supported by the results obtained from the previous sorption experiments at higher concentration levels.

3.4 Overall results

For the clear representation of the results, the partial achievements were summarized into the graphical form (Fig. 2).

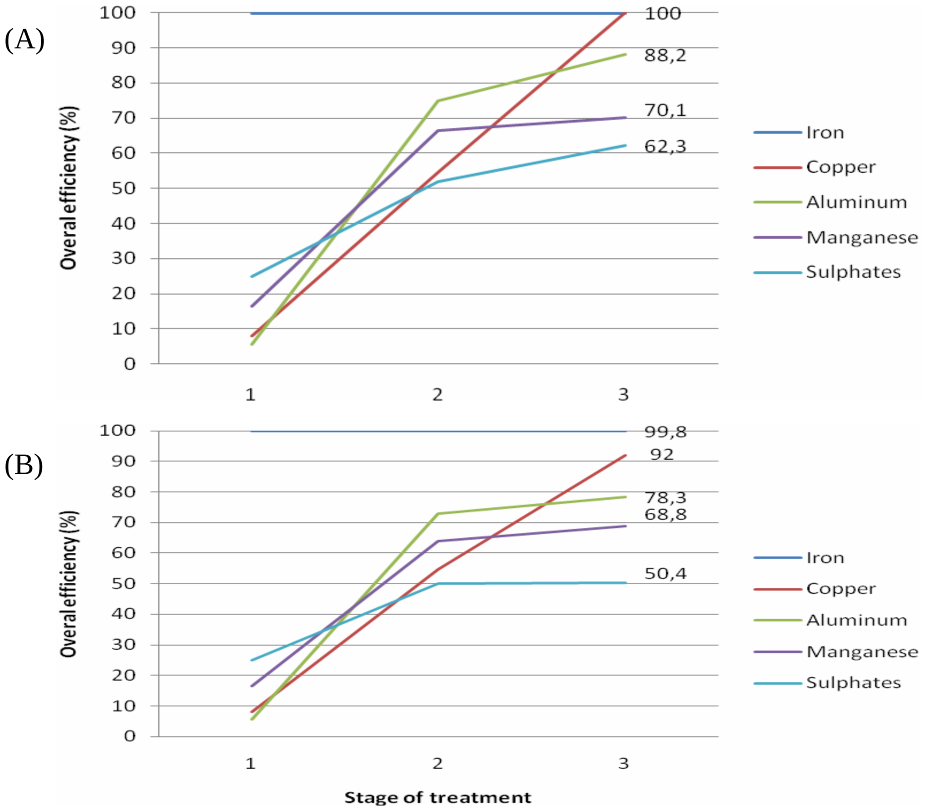

Fig. 2. Summarized results
(A – best option: combination of MB20 resin and hemp shives sorption;
B – worst option: combination of MB400 resin and peat sorption)

Two of the combination were chosen (A – best option and B – worst option). As the most efficient option, combination of precipitation, ion exchange using MB20 and hemp shives sorption has proved. The efficiency in this case is around 100 % for elimination of iron and copper cations. 88 % of aluminum was also removed. The sulphate removal reached the level of 62 %. The worst results were obtained using combination of precipitation, MB400 ion exchange and sorption by peat.

4 Conclusions

The experiments presented in this article were focused on the combination of chemical precipitation, ion exchange and biosorption techniques for the acid mine drainage treatment. Concentrations of four different metal cations (copper, iron, manganese, aluminium) and sulphates were observed.

Chemical precipitation (combination of oxidation using 31 % hydrogen peroxide and subsequent precipitation with 0.1 M sodium hydroxide) was used for the removal of iron cations. Another positive fact in this first stage of treatment is partial removal (around 25 %) of sulphates.

After the chemical precipitation, the ion exchange using two different exchangers (PUROLITE MB400 resin and AMBERLITE MB20 resin) took place. Decreasing in sulphates concentration was observed around 34 % and 37 % for MB400 and MB20 resins, respectively. The ion exchange also influenced the concentration of heavy metals; the most prominent was decreasing in manganese and aluminium concentration (around 60 % and 70 %, respectively).

AMD after precipitation and ion exchange was used as an input for the biosorption study. Low-cost sorbents included in this case natural non-modified peat “PEATSORB” (provided by REO AMOS, Slovakia) and hemp shives in modified state.

Generally, biosorption was more efficient by using of modified version of hemp shives. Hemp shives exhibit better results for all monitored elements. The results showed that biosorption could be powerful method in the case of trace concentration. Iron and copper cation were eliminated bellow 0.1 mg/L.

Overall, the best results were observed after combination of MB20 resin in the second stage and subsequent using of modified hemp shives in the third stage of the treatment.

Acknowledgements

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STUDY OF ZINC REMOVAL FROM AQUEOUS SOLUTIONS USING A STREPTOMYCES STRAIN

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Abstract

Bacteria *Streptomyces* strain K11 was isolated from highly alkaline aluminium brown mud samples. The obtained isolate was identified on the basis as of 16S rRNA gene sequence and it is probably a representative of a new streptomycete species related to the *S. wuyuanensis* and *S. variabilis.* High level of heavy metal resistance was reported for isolated bacterium with the highest resistance to zinc.

Biosorption experiments revealed that studied biomass is highly effective for the removal of Zn^{2+} ions from diluted solutions. More than 97 % of zinc was removed from solution at the lowest initial zinc concertation 6 mg/l when dead *Streptomyces* biomass was used. At initial concentrations up to 100 mg/l still 50 % of zinc was sequestered. However, very low biosorption efficiency (about 4 %) was found at the highest studied initial concentrations (1.2 g/l) suggesting that biosorbent derived from bacteria *Streptomyces* could be effective for the treatment of low-concentrated solution but not suitable for zinc recovery at higher Zn^{2+} ions concentrations.

Keywords: *Streptomyces*, zinc, biosorption, metal resistance

1 Introduction

Heavy metals in contrast to other pollutants represent specific group because there is no way how to destroy them so that after they enter the environment they circulate there posing a serious threat for various ecosystems [1]. To remove heavy metals from the environment different approaches based on physical and chemical methods are used. However, most of these methods are expensive, non-specific, and of limited effectiveness, especially when the concentrations of polluting metals are below 100 mg/l [2]. Utilisation of different kinds of biomass offers an attractive alternative for treatment of groundwater or wastewater by retaining heavy metals from dilute solutions. Biomaterials of microbial and plant origin interact effectively with heavy metals. Metabolically inactive dead biomass due to their unique chemical composition sequesters metal ions and metal complexes from solution, which obviates the necessity to maintain special growth-supporting conditions. The method of utilising biological materials for metal recovery, called biosorption, is based on passive adsorption of metal ions on the cell surface [3, 4].

While choosing biomaterial for metal biosorption, its origin is a major factor to be taken into account. As a natural part of soil ecosystem, microbial communities have some characteristics which greatly influence the interaction of microorganism with metal ions. For example, the high surface-to-volume ration provides a large contact area and a great number of functional groups to interact with metal ions; the resistance to extreme environment makes them successful colonizers of harsh metal-rich habitats.

The aim of the article was to characterise the biosorption abilities of the *Streptomyces* strain isolated from brown mud in Ziar nad Hronom, Slovakia.

2 Materials and methods

2.1 Sample collection and analysis

Brown mud samples were collected from three different sites at Slovalco co. disposal site near Ziar nad Hronom (Slovakia). The samples were stored at 4 ºC until microbiological analysis. According to the Slovalco data the average composition of mud is SiO_2 (11-14 %), Fe_2O_3 (30-35 %), TiO_2 (3-4 %), Al_2O_3 (10-12 %), CaO (24-26 %), Na_2O (3-6 %). Heavy metal content is 10 ppm for Hg, 220 ppm for Cu, 400 ppm for Cr, 700 ppm for V, 150 ppm for Pb and 800 ppm for As. pH of the sample was determined according to the ISO standard and was found to be 11.6.

2.2 Isolation and characterisation of bacteria

To the mixed sample of mud (0.5 g) 10 ml of sterile PBS solution were added and after 20 min of intensive mixing aliquots were spread on non-selective agar medium (Tryptone Soya Agar, Oxoid, USA). Cultivation was conducted under aerobic conditions at 22 °C for 72 h. The streptomyces-like isolates were selected visually and used for further analyses.

The total DNA from streptomycete isolates was extracted by Bacterial DNA Isolation Kit – BACTOZOL (Molecular Research Centre, Inc., Cincinnati, OH). Isolated DNA was used as a template for PCR amplification of 16S rRNA gene fragments. 16S rRNA sequences were identified using the RDP database and subsequently subjected to BlastN analysis at http://www.ncbi.nlm.nih.gov.

The minimal inhibitory concentrations (MIC) of Al, Li, Mn, Mg, Fe, Ag, Hg, Co and Zn for the bacterial isolates were determined by the agar dilution method [5].

2.3 Zinc biosorption experiments

Spores of *Streptomyces* K11 strain were inoculated to 100 ml TSB liquid media and cultivated for 2 days at 25 °C. Cultures were sterilised in autoclave at 120 °C for 15 min. Cells were harvested by vacuum filtration and washed twice with deionised water. The cells were re-suspended in 100 ml of deionised water and placed in shaker for 15 minutes. To remove the rest of cultivation media the procedure was repeated three times. The biomass was oven dried at 55 °C for 24 hours.

Dried bacteria were used for the biosorption experiments in concentration 1 g/l. Initial concentrations of zinc in studied solution ranged from 0 – 1200 mg/l. Experiments were carried out according to the protocol for equilibrium biosorption experiments [3]. pH value of used solutions was 6.5. Experimental solutions were stirred during 24 hours. After this time, solution samples were withdrawn for metal analysis. The concentration of zinc ions was measured by atomic absorption spectroscopy (Varian AA20+). The metal uptake q was calculated from the mass balance equation as follows:

$$q = \frac{V(C_0 - C_e)}{m} \qquad (1)$$

where q - the quantity of metal uptake by biomass [mg.g^{-1}]
C_0 - the initial metal concentration [mg.dm^{-3}]
C_e - final (after sorption at equilibrium) metal concentration [mg.dm^{-3}]
V - the volume of solution [dm^3]
m - dry weight of the biomass added [g].

3 Results and discussion

3.1 Identification and characterization of isolate

Brown mud sample from disposal site near Ziar nad Hronom was analysed for the presence of cultivable bacteria. Upon cultivation on non-selective TSA plates number of bacterial colonies were observed with frequency of about 3500 cfu per gram of mud. Based on colony morphology isolate K11 with colonies showing typical streptomycete morphology was isolated. Streptomycete-like isolates represented less than 5 % of cultivable bacteria on TSA medium. The isolate was characteristic by white aerial mycelium and produced dark brown diffusible pigment during growth on TSA medium. For many heavy metal contaminated soils it has been shown that streptomycetes are a dominant group of bacteria in the soil and that growth of soil bacteria had positive effects on bioremediation. From alkaline soils several alkaliphilic and alkalitolerant streptomycetes were described [6, 7]. There are no data available on occurrence of streptomycetes in brown or red mud environment.

Due to very high number of validly described *Streptomyces* species phylogenetic analysis of amplified 16S rRNA gene was used for correct identification and phylogenetic placement of obtained isolate. The sequence analysis confirmed that the isolate belongs to the *Streptomyces* genus. The 16S rRNA sequence of K11 isolate showed the high similarity to the 16S rRNA of recently described *S. wuyuanensis* (98.1 %, KF730778) and *S. variabilis* strain NRRL B-3984 (97.9 %, DQ442551), when compared against 16S ribosomal RNA database of archae and bacteria. When compared against non-redundant database the K11 16SrRNA sequence showed the highest similarity against several 16S rRNA sequences of *S. variabilis,* however multiple sequence alignments indicated that K11 isolate is probably a representative of a new streptomycete species (data not shown). All related 16SrRNA sequences have origin in soils mainly from

China. There is no report on occurrence of *S. variabilis* related bacteria from extreme alkaliphilic or heavy metal contaminated environments.

The K11 isolate showed high minimal inhibitory concentrations to several heavy metals. Heavy metal resistance is frequently occurred in streptomycetes [8]. The resistance level against Zn^{2+} is even higher those observed for *S. zinciresistens* [9] isolated from soil from a zinc and copper mine in China.

3.2 Study of Zn^{2+} biosorption

The efficiency of zinc biosorption on dead cell of *Streptomyces* K11 strain was determined as a function of initial Zn^{2+} ions concentration using initial concentration from 0 – 1200 mg/l. The equilibrium sorption increased from 5.3 to 54.2 mg/g. The results show that *Streptomyces* biomass is very efficient biosorbent of Zn^{2+} ions from diluted solutions. At the lowest initial zinc concentration (6 mg/l) 97 % removal was observed (Fig. 1).

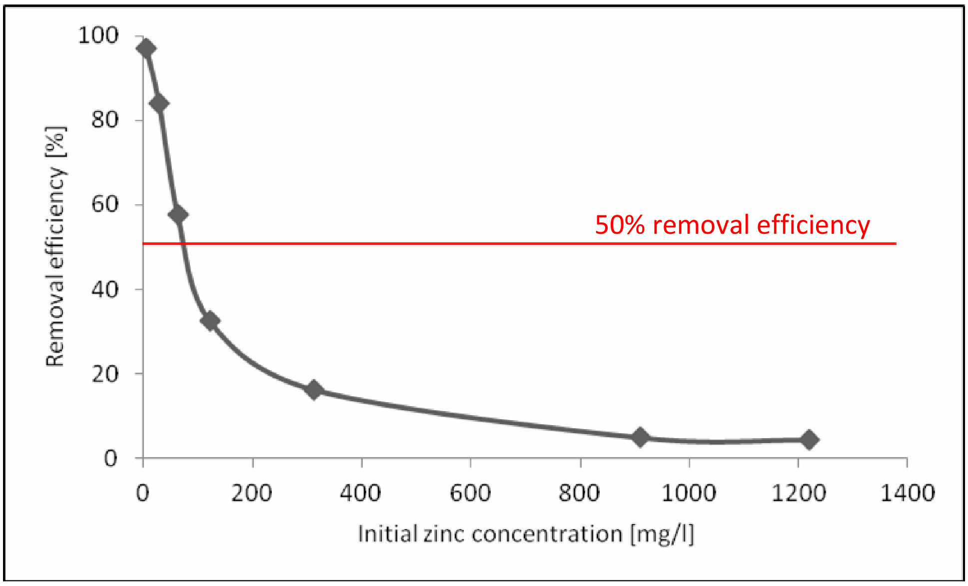

Fig. 1. Zinc removal efficiency at different initial Zn^{2+} ion concentrations

With the increase of initial Zn^{2+} ions concentrations, the decrease of zinc removal efficiency was observed, however up to initial concentration of 80 mg/l still more than 50 % of zinc was removed. When the initial zinc concentrations were higher than 900 mg/l, the biosorption efficiency was 4 % and no further change in the efficiencies was observed with the increase of the initial zinc concentration.

The maximum uptake calculated from experimental data was 48.9 mg/g of dry biomass. It was found to be comparable with the highest measured biosorption capacities found for different bacteria from *Streptomyces* genus (Table 1).

Table 1. Comparison of zinc biosorption capacity of different *Streptomyces* species

Bacterial strain	**Zn uptake [mg/g]**	**Reference**
Streptomyces aureofacienes	0.73	[10]
Streptomyces rimosus	27.4	[11]
Streptomyces ciscaucasicus	75.85	[12]
Streptomyces zinciresistens	165.38	[13]
Streptomyces noursei	1.6	[14]
Streptomyces lunalinharesii	13.64	[15]
Streptomyces sp. K11	48.9	This study

4 Conclusions

Biosorption is a process which offers many advantages when applied in the industry such as low operation costs, high efficiency, low waste production, usage of biodegradable materials etc. but there are still many studies necessary to understand its mechanism. Biosorption of zinc using *Streptomyces* derived biomass was evaluated in the article. From the presented results it is obvious that zinc can be sequestered from diluted aqueous solutions with very high efficiency when dead biomass is used. Thus, biosorption offers good way for studying zinc recovery from solutions with zinc concentrations under 100 mg/l.

Acknowledgements

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MOLECULAR MODELLING OXIDANT METAL SULPHIDE SYNTHESIZED BACTERIA *Acidithiobacillus ferrooxidans*

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Abstract

Using the method of computer molecular modelling determined the structural formula and the quantum-chemical characteristics of the energetically most probable conformation of the molecule oxidant metal sulphides synthesized mesophilic iron-oxidizing bacteria. Based on the analysis of the quantum-chemical characteristics theoretically proved experimentally established biooxidants large oxidation activity relative to iron sulphate (III) by reacting with metal sulphides.

Keywords: computer molecular modelling, biooxidant, quantum-chemical characteristics, structural formula, oxidation activity, partial atomic charge

1 Introduction

Leaching non-ferrous metals from sulphide ores, concentrates and enrichment technogenic raw polysaccharide is used, which is synthesized by autotrophic bacteria mesophilic *Acidithiobacillus ferrooxidans* in the oxidation of iron ions (II) in a solution of sulphuric acid [1]. It is found that the oxidant biooxidants molecule consists of iron ion (III) and the three acidic residues glucuronic acid - $Fe(S_6H_9O_7)_3$ [2]. According to the results of experimental studies biooxidants possesses greater oxidative activity by reacting with sulphide minerals than iron sulphate (III) [3]. To study the properties of the molecules being effectively used method of molecular computer modelling [4]. Identified and analyzed the quantum-chemical characteristics of the molecule biooxidants and evaluated the effectiveness of its interaction with the sulphides compared with iron sulphate (III).

2 Material and methods

For the research used method frontier molecular orbitals, a semi-empirical molecular modelling package ChemBio3D programs specialized complex ChemOffice corporation «Cambridge Soft» and software module MOPAC 2012.

3 Results and discussion

Minimize the potential energy of the possible structures of molecules biooxidants found that the most probable energy, stable conformation of the molecule contains acid residues of glucuronic acid non-cyclic structure, the potential steric energy is almost 4 times less than the cyclic (Table 1).

Table 1. Quantum-chemical characteristics of stable conformations of molecules, kJ/mol

Molecule	Minimum steric energy	Heat of formation	1/4-VDW interaction	No 1/4-VDW interaction	Interaction of dipole-dipole	Stretching valence bonds	Bending bond angles
$Fe_2(SO_4)_3$	0.31	-55.166	-0.834	-0.01355	0.628	0.0023	0.53
$Fe(S_6H_9O_7)_3$ structure:							
cyclic	220.488	-193.606	10.755	18.796	8.302	65.785	48.302
a noncyclic	55.298	-194.232	9.914	77.852	6.817	1.533	20.06

Characteristics that affect the efficiency of interaction - the volume, heat of formation, steric energy and its components: Van der Waals interaction (VDW), stretching valence bonds, etc., biooxidants molecules are many times more than the molecule $Fe_2(SO_4)_3$ (Table 1, 2).

The ionization potential of the energetically most probable conformation of the molecule biooxidants less, and the electron affinity 5 times lower than the molecule $Fe_2(SO_4)_3$ (Table 2).

Table 2. Quantum-chemical characteristics of stable conformations of molecules, eV

Molecule	Cosmo volume, Å^3	Total energy	Electronic energy	Core-core repulsion	Ionization potential	Electron affinity
$Fe_2(SO_4)_3$	267.76	-4861.14	-26123.08	21261.95	10.511	-2.535
$Fe(S_6H_9O_7)_3$ structure:						
cyclic	602.79	-9173.06	-79238.51	70065.45	9.808	-0.803
a noncyclic	642.52	-9172.3	-92354.61	83182.38	9.918	-0.503

Calculated in accordance with the theorem of Koopmans energy of the Highest Occupied Molecular Orbital (HOMO) and energy of the Lowest Unoccupied Molecular Orbital (LUMO) biooxidants smaller than a molecule $Fe_2(SO_4)_3$ (Table 3). Biooxidants molecule is characterized by a greater absolute rigidity η and at the absolute electronegativity χ, than a molecule of iron sulphate.

Table 3. Energy characteristics of molecules, eV

Molecule	Energy of the Highest Occupied Molecular Orbital E_{HOMO}	Energy of the Lowest Unoccupied Molecular Orbital E_{LUMO}	Absolute rigidity η	Absolute electronegativity χ
$Fe_2(SO_4)_3$	-10.511	-2.535	7.976	6.523
Biooxidant	-9.918	-0.503	9.005	5.306

The partial charge of the iron atom in the molecule biooxidants 1.15 eV larger than the molecule $Fe_2(SO_4)_3$ – 0.54, and 0.91 that it defines a large oxidation activity.

Contact of iron with oxygen atom biooxidants less strong (bond length 1.85 Å) than one atom of iron to oxygen atoms in the ferrous sulphate (length 1.75 and 1.76 Å), but firmer than the second iron atom oxygen atoms in the ferrous sulphate (1.99 and 2.025 Å). The dipole moment of the molecule biooxidants 3.240 D (debye) 2 times less and less polar molecule, than a molecule of iron sulphate (III) – 6.523 D. Bioreagents in accordance with the principle of Pearson relates to a rigid Lewis acid, electron acceptor than iron sulphate (III), less polarized bioreagents molecule and characterized at absolute electronegativity and a 2-fold larger volume.

4 Conclusions

Molecular modelling method for the first time established the most probable energy, stable conformation of molecules and quantum-chemical oxidant characteristics, synthesized by bacteria *Acidithiobacillus ferrooxidans*.

Analysis of the quantum-chemical characteristics allow theoretically justify greater oxidative activity bioreagents respect to iron (III) sulphate in contact with sulphide minerals, due to the large values of the amount of the heat of formation, steric energy and its components bioreagents molecules and large partial charge of the iron atom and lower energy lower unoccupied molecular orbital.

Acknowledgements

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BIODEGRADATION OF PAINT STRIPPER WASTE

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Abstract

The paper presents comparison of using the bacteria *Desulfovibrio* and activated sludge to biodegradation waste generated in the paint stripping process. The paint stripper used in study consists of esters and alcohol solvent, dioxolane, thickener agent and an inorganic peroxide. In study we used sulphate-reducing bacteria (SRB) *Desulfovibrio* sp. isolated from natural mineral water Gajdovka from Kosice in Slovakia. Second medium used to biodegradation was activated sludge from sewage treatment from Tarnów in Poland. During the study the change of chemical oxygen demand (COD) was measured. Biodegradation products were analysed by the gas chromatography.

Keywords: paint stripper, *Desulfovibrio*, activated sludge, biodegradation

1 Introduction

Care of the environment is important aspect of each technology in the today's world. Therefore the non-waste technologies are sought. However, sometimes we can't eliminate all waste which is formed during process. One of such process is the paint stripping process. The waste which is formed during this process can be incinerated or landfilled.

The studies on biodegradability tests of waste generated during paint stripping process were conducted. Arquiaga et al. [1] in their work studied decomposition of wastewater after removing coatings. The wastewater contained methylene chloride at the concentration of 5000 mg/dm^3, phenol at the concentration of 1800 mg/dm^3 and other organic compounds, such as paraffin, cellulose derivatives, petroleum sulphonates and naphthalene at total concentration of 2200 mg/dm^3. In the study the activated sludge was used. The authors conducted the identification of bacteria present in the tested activated sludge. They studied the ability of isolated bacteria to growth in the presence of phenol and wastewater after removing coatings. Moreover, they determined if tested compounds can be a source of carbon for the isolated bacteria.

The most common bacteria isolated from the studied wastewater was Gram-negative bacteria, among which were dominated by *Pseudomonas*. The Gram-positive bacteria represented by *Bacillus* occurred less frequently. The study showed that the majority of isolated bacteria have the ability to biodegradation of phenol and paint stripping wastewater. However the most important role in biodegradation paint stripping wastewater have bacteria of the genus *Pseudomonas* and *Bacillus* [1].

The same bacteria are used by authors of patent US8202424. Almadidy et al. described the process of waste biodegradation obtained after the paint removement. The waste contained a high concentration of organic compound. They used two types of bacteria *Pseudomonas fluorescens* and *Bacillus subtilis* and one type of fungus *Cunningham elegans*. In their work they studied changes of the waste samples COD which were exposed to the activity of microorganisms. The research showed that it is possible to obtain even 47 % decrease of COD by using selected strains of bacteria and fungi [2].

The attempts of solvents biodegradation used in paint strippers were conducted by Vanderberg-Twary et al. [3]. In their work they studied biodegradation of typical solvents used in paint strippers. They prepared two kinds of preparations: preparation 1 contained toluene, acetone and dichloromethane, preparation 2 contained dichloromethane, 2-propanol and methanol. In the research they used two kinds of bacteria: *Hyphomicrobium sp.* and *Rhodococcus rhodochrous* [3].

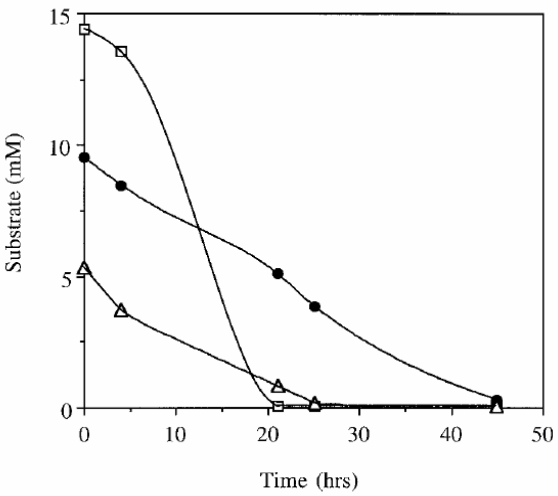

Fig. 1. Degradation of a surrogate paint stripper 1 by the combined culture of *R. Rhodochrous* and *Hyphomicrobium* sp. Open triangles: toluene; closed circles: dichloromethane (DCM); open squares: acetone [3]

The study showed that selected strains of bacteria are able to degrade solvent present in paint strippers [3].

We deal with the paint stripping process from many years. We work out the recipe of paint stripping preparation on the base of waste fraction from some technologies. Our recent studies focus on the biodegradability tests of waste generated during paint stripping process. This work presents attempt of using the anaerobic bacteria *Desulfovibrio* and aerobic bacteria in the activated sludge for biodegradation of waste generated in paint stripping process.

2 Material and methods

2.1 Paint stripper

The paint stripper was obtained from by-products which are produced in installation for cyclohexanone production. The process is a catalytic oxidation of cyclohexane. The paint stripper used in this study consists of esters and alcohol solvent. The alcohol solvent consists mainly of n-pentyl alcohol and small amount of such alcohols as n-propanol, isopropanol, n-hexanol and cyclohexanol. The ester solvent consists mainly of pentyl alcohol esters and pentyl alcohol. Moreover it consists of small amounts of ethylcyclohexane and dipentyl ether. Moreover the paint stripper used in the study contained dioxolane, thickener agent, surfactant and hydrogen peroxide [5].

2.2 Bacteria and growth conditions

In study we used sulphate-reducing bacteria (SRB) *Desulfovibrio* sp. isolated from natural mineral water Gajdovka from Kosice in Slovakia. As the medium we used selective culture medium for sulphate-reducing bacteria DSM-63, with a pH value of 7.5.

Second medium used to biodegradation was activated sludge from sewage treatment from Tarnów in Poland. The activated sludge was adapted for one week. For adaptation we used a medium consist of 75 % of distilled water, 25 % of tap water, peptone (750 mg/dm3) and 4 cm^3/dm^3 of phosphate buffer of pH 7.5.

2.3 The sample preparing

Bacteria Desulfovibrio

To the study the three samples were prepared. The sample PA consists of 400 cm^3 of selective growth medium, 50 cm^3 of waste and 50 cm^3 of active bacteria. The sample PA-DLNa consists of 400 cm^3 of selective growth medium with additional carbon source, 50 cm^3 of waste and 50 ml of active bacteria. The sample PA-K consists of 450 cm^3 of selective growth medium, 50 cm^3 of waste. As the medium we used selective culture medium for sulphate reducing bacteria DSM-63, with a pH value of 7.5. As an additional carbon source we used sodium lactate (DL-Na). The study was conducted at the temperature of 30 °C, without oxygen access in glass bottle [6].

Activated sludge

The study was conducted at the room temperature, in aquarium with aeration. To the aquarium were added 400 cm^3 of activated sludge and 2 dm^3 of growth medium with 0.2 % of paint stripper waste.

2.4 Methods

In the work the change of chemical oxygen demand was studied. The chemical oxygen demand (COD) was measured by method of Hach Chemical Company number HACH 8000 [4].

Biodegradation products were analysed by the gas chromatography. The procedure of preparation of the sample for chromatographic analysis was shown on Figure 2. Gas chromatography was performed using SRI 8610C chromatograph. The initial temperature was 80 ºC and it was rising 10 ºC per minute up to 120 ºC, next temperature was rising 20 ºC per minute up to 200 ºC. Temperature of 200 ºC was maintained for 15 minutes.

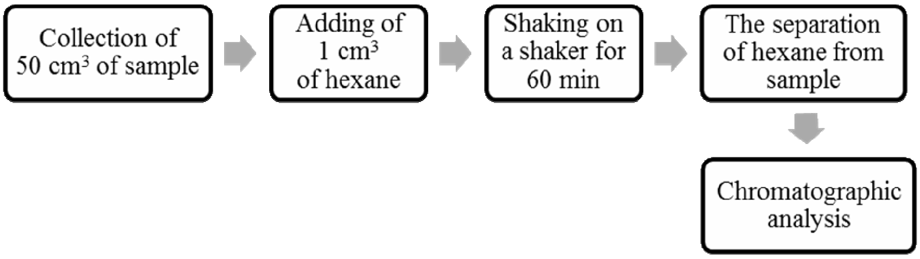

Fig. 2. Preparation of the sample to chromatographic analysis

3 Results and discussion

Bacteria Desulfovibrio

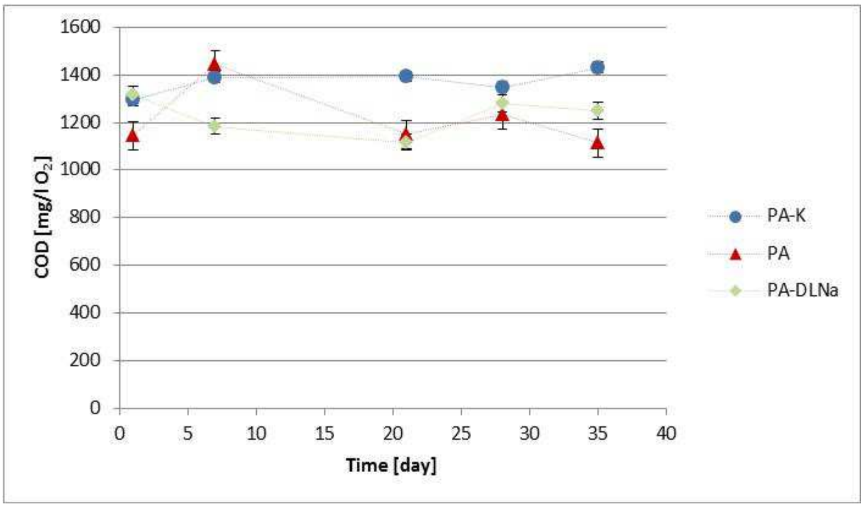

Fig. 3. The change of COD for sample PA, PA-K, PA-DLNa during the experiment

The experiment was conducted by five weeks. The Figure 3 shows the change of chemical oxygen demand during the experiment. For the sample PA we observed dispersion of results. After one week the COD increased from level of 1150 mg/dm^3 to 1440 mg/dm^3 for sample PA. After two weeks COD of samples PA decreased to the same level like at the beginning of experiment. Next, after three weeks the COD again increased and after four weeks the COD decreased to the output level. For the sample PA-DLNa we observed the decreasing of the COD level for two weeks from 1317 mg/dm^3 to 1118 mg/dm^3. For the next two weeks the COD level was increased from 1118 mg/dm^3 to 1250 mg/dm^3. In the control sample we observe maintenance of the chemical oxygen demand on a constant level. Comparing all sample we observe decrease of COD level for samples PA and PA-DLNa compared to control sample.

The chromatograms of samples PA, PA-DLNa, PA-K are shown on the Figures 4 - 6. The chromatograms of other samples are similar. We don't observe significant differences between chromatograms of control samples and test samples. Regardless of the type of sample we observe on the chromatograms peaks which derived from esters and alcohols.

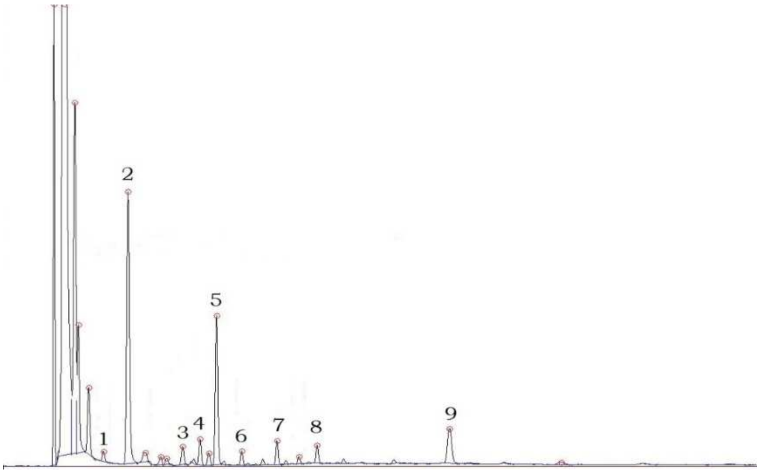

Fig. 4. The chromatogram of sample PA-K; (1) cyclohexene; (2) amyl alcohol; (3) pentyl formate; (4) 2-methylcyclopenthanone; (5) cyclohexanone; (6) pentyl acetate; (7) pentyl butyrate; (8) pentyl valerate; (9) unidentified peak

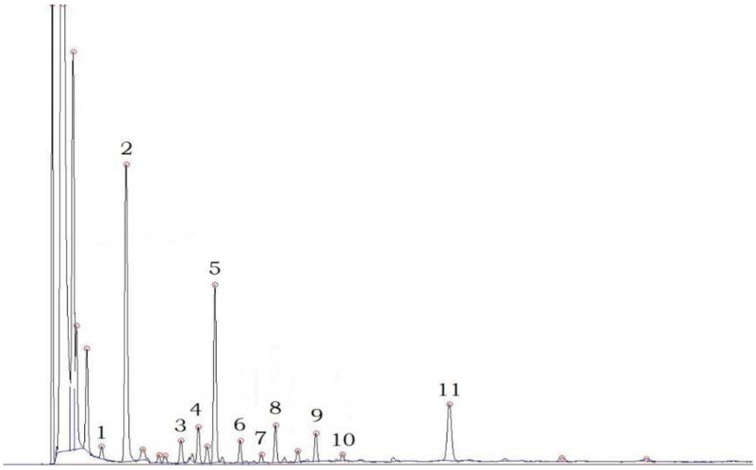

Fig. 5. The chromatogram of sample PA-DLNa; (1) cyclohexene; (2) amyl alcohol; (3) pentyl formate; (4) 2-methylcyclopenthanone; (5) cyclohexanone; (6) pentyl acetate; (7) pentyl propionate; (8) pentyl butyrate; (9) pentyl valerate ; (10) pentyl caproate; (11) unidentified peak

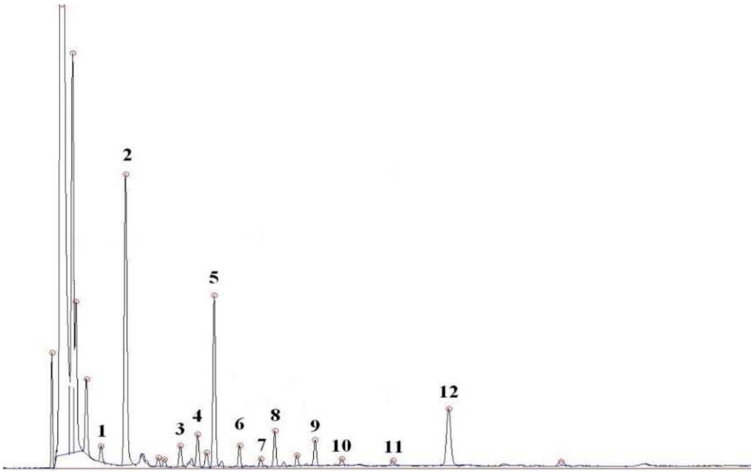

Fig. 6. The chromatogram of sample PA; (1) cyclohexene; (2) amyl alcohol; (3) pentyl formate; (4) 2-methylcyclopenthanone; (5) cyclohexanone; (6) pentyl acetate; (7) pentyl propionate; (8) pentyl butyrate; (9) pentyl valerate; (10) pentyl caproate; (11) unidentified peak; (12) unidentified peak

Activated sludge

The experiment was conducted by twenty four hours. As you can see at the Figure 7, after the two hours the COD decreased from 1245 mg/dm^3 to 777 mg/dm^3. After 24 hour the COD decreased to the level of 309 mg/dm^3. The experiment was conducted three times. Each time we observed decrease the COD level respectively from 1782 mg/dm^3 O_2 to 260 mg/dm^3 O_2 for the first time, from 1473 mg/dm^3 to 370 mg/dm^3 for the second time and from 1245 mg/dm^3 to 309 mg/dm^3 for the third time.

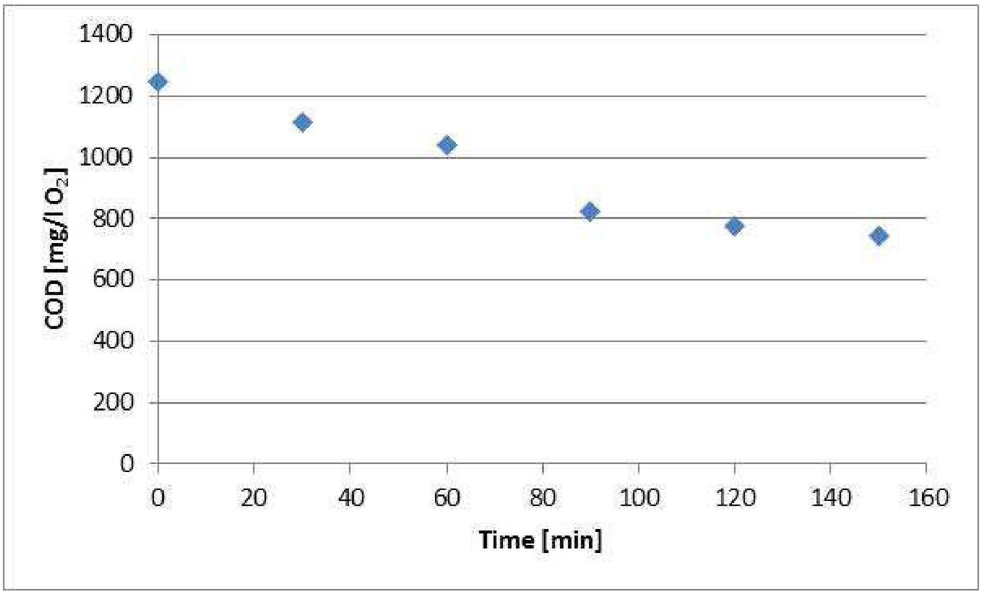

Fig. 7. The change of COD during the experiment of activated sludge

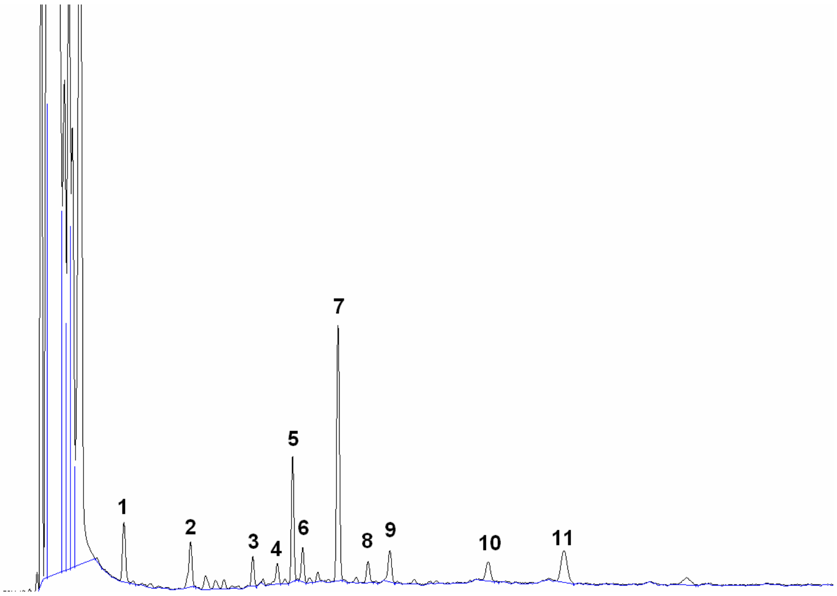

Fig. 8. The chromatogram of sample before treated a activated sludge; (1) amyl alcohol; (2) pentyl formate; (3) pentyl acetate; (4) pentyl propionate; (5) pentyl butyrate; (6) unidentified peak; (7) pentyl valerate; (8) unidentified peak; (9) pentyl caproate; (10) unidentified peak; (11) unidentified peak

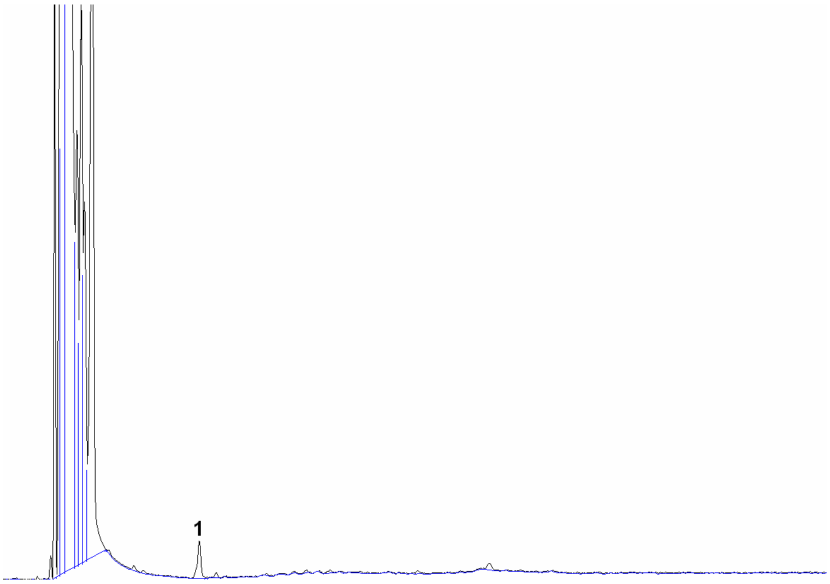

Fig. 9. The chromatogram of sample after treated activated sludge; (1) pentyl formate

The conducted study of gas chromatography confirmed the ability of activated sludge to biodegradation of paint stripper waste. In the chromatogram of sample before treated activated sludge we observe the 11 peaks which derived from esters and alcohols. However in chromatogram of sample after treated activated sludge we can observe only one peak which derived from pentyl formate. The same situation took place in all three experiments. Such results can attest to effectiveness of activated sludge to biodegradation of paint stripper waste.

The obtained results, in particular lack of a clear reduction of COD, can attest to the ineffectiveness of bacteria *Desulfovibrio* in biodegradation of this type of waste. In the results of gas chromatography we don't observe change in samples subjected to biodegradation in comparison to the control sample. It can attest to the ineffectiveness of a selected strain of bacteria in biodegradation of this type of waste.

The opposite situation we observe in the case of activated sludge. The reduction of COD level and results of gas chromatography can attested to effectiveness in biodegradation of paint stripper waste.

4 Conclusions

The conducted studies show the ability of activated sludge for biodegradation of paint stripper waste. The research showed that it is possible to obtain even 70 % decrease of COD by using activated sludge. At the same time the studies shows the ineffectiveness of bacteria *Desulfovibrio* in biodegradation of this type of waste.

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ACCUMULATION OF ARSENIC IN PLANTS UNDER DIFFERENT FERTILIZATION REGIMES AND ITS IMPACT ON PHOTOSYNTHETIC PARAMETERS OF WHEAT

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Abstract

The presence of both arsenic and high concentrations of nitrates is a negative phenomenon in the environment. Different fertilization regimes influence the uptake of toxic elements by plants but the mechanisms are not clear and results of different studies inconclusive. We studied the effect of different nitrogen concentrations (0-35 mM of N applied as ammonium nitrate) on accumulation of arsenic in wheat plants grown in hydroponics. Arsenic at the dose of 5 mg.l^{-1} affects the function of the photosynthetic apparatus under each of eight different nitrogen conditions (0, 0.75, 5.25, 7.5, 15, 25, 30 and 35 mM of N). Chlorophyll fluorescence and pigment content varied with dose of nitrogen in the medium. At the same time, nitrogen availability affected arsenic accumulation in plant tissue. Lowest amount were accumulated in both leaves and roots under starvation conditions, highest at N doses close to optimal. Excess of nitrogen in shoots but not in roots apparently restricted the metalloid accumulation. Thus, nitrogen nutrition plays a key role in the photosynthetic capacity of the plant to grow in contaminated soil as it determines the availability of resources for growth as well as for activation of defense reactions. However, our present data show that at excessive N doses the transfer of metalloid to shoots is limited, and the lower content of accumulated metal in tissue is not the result of commonly accepted dilution effect.

Keywords: arsenic, metal pollution, nitrogen excess, starvation, *Triticum*

1 Introduction

In cereals up to 90 % of the nitrogen can be remobilized from the vegetative plant parts to the grain. This strongly influences yield and also bread quality. As a result, nitrogen (N) is a very important nutrient that limits plant productivity. A considerable amount of knowledge is available on mechanisms of nitrate uptake, transport and N remobilization, especially under growth in soils with limited N. More recently, however, the excess of N fertilizers is becoming a serious problem due to intensive use in agriculture. All together only 30-40 % of N applied as fertilizer is utilized by plants; the rest remains and is decomposed in soil by microbial and results in a series of environmental and economic problems [1].

The effects of N deficiency and of excess on plants are described very well. Deficiency impairs photosynthesis and limits the growth and productivity of plants. Longer period of N starvation negatively interferes with basic metabolism of plants [2, 3]. Similarly, N concentrations above 10 mmol/l also hamper plant growth and higher values can be even toxic to plants. The plants have damaged root system, suppressed assimilation and mineral uptake, but also affected flowering and reproduction. In addition, non-optimal N conditions can strongly affect the ability of plants to defend themselves from environmental stresses. For example, various phenolic compounds that have protective effects in plants are synthetized in lover amounts under conditions of limited nitrogen, although the opposite has also been observed e.g. for terpenes or alkaloids [3]. Unfortunately, the number of reports on effects of broader range, non-optimal nitrogen availability on plants is rather limited.

Nitrogen dose and the form in that it is applied impact the uptake of metals by plants. It can cause soil acidification, which alters heavy metal mobility and availability for plants. Previously it has been concluded that N supply can be used as a means of decreasing metal toxicity to soybean seedlings since it can decrease accumulation and development of oxidative processes in roots [4]. In contrast, a mere dilution effect in plants with larger biomass under favouring nutrition has also been suggested [3].

The aim of this study was to study and compare the effects of a range of N conditions, starting with starvation (0 mM N) up to toxic doses (35 mM N) on metalloid uptake by wheat in hydroponic conditions. Arsenic (As) is a non-essential element that is largely phytotoxic and seriously affects the growth and development of plants. In the environment, As can exist in inorganic and organic form, while of the inorganic forms oxidized arsenate is more prevalent in aerobic conditions and the more reduced arsenite is typical for anaerobic environment [5]. Plants are able to take up As^V through phosphate (P^V) transporters, while As^{III} through aquaporin channels [6]. They act in plants by different mechanisms either by replacing phosphate and restricting ATP production (As^V) or by binding sulphhydryl groups and interfering with plant metabolism (As^{III}) [6].

Here we studied the effect on N concentration on photosynthetic performance of wheat leaves and the rate of arsenic uptake in both leaves and roots to conclude on the effects of studied parameters individually and in combination.

2 Material and methods

2.1 Plant material and growth conditions

Seeds of wheat (*Triticum aestivum* L. cv. Genoveva) were surface sterilized in 10 % (v/v) sodium hypochlorite solution (Savo) for 15 minutes, then rinsed three times with distilled water and germinated on Petri dishes (Ø 15 cm) with a double layer of filter paper (Whatman no. 1) in sterile distilled water. When the roots reached the length of 6-8 mm, germinated seeds were transferred to hydroponic containers with standard Hoagland solution in that N was supplied solely in a form of $(NH_4)NO_3$ to final concentrations od 0, 0.75, 5.25, 7.5, 15, 25, 30 and 35 mM N. The plants were cultivated for 5 days under 25 °C, humidity 60-70 %, of the light period of 12 hours light / 12 hours dark, the maximum radiation intensity of 500 $\mu mol.m^{-2}s^{-1}$. As stress was applied to the 2 containers after 4 days at a concentration of 1 $mg.kg^{-1}$ As^{3+} (in form of As_2O_3 solution) for 24 hours. After 5 day of growth control and stress plants were analysed. After determination of fresh weight shoots of wheat plants were dried at 105 °C to constant weight, and then weighed on an analytical balance.

2.2 Qualitative detection of H_2O_2

Hydrogen peroxide was detected histochemically directly on the leaf tissue using solution of 1 $mg.ml^{-1}$ (pH 3.8) 3,3'-diaminobenzidine-HCL (DAB-HCL). Controls and stress shoots were placed into DAB-HCl and incubated for 3 hours in the dark. Afterwards shoots were washed in distilled water and photographed.

2.3 Measurement of maximal quantum yield

Measurement on intact leaves was performed with a Handy FluorCam FC 1000-H fluorometer [7]. Prior to fluorescence measurements the plants were dark-adapted for 1 h at room temperature (22 °C). Measuring flash duration was 10 μs – 33 μs. Continuous actinic light was adjustable in duration and power (up to 660 μmol photons $m^{-2}s^{-1}$). Fluorescence signal was detected by a high sensitivity CCD camera.

2.4 Content of arsenic in the tissue

The measurement was done ICP MS (mass spectrometry with inductively coupled plasma) on a Perkin Elmer Elan 6000. The procedure has been validated using two CRM products: NCS NCS DC73349 and DC73350 (China).

2.5 Statistical analyses

All experiments were performed in three independent biological replicates. The obtained data were subjected to statistical analysis using MS Excel. Statistically significant differences were determined by Student's t-test.

3 Results and discussion

In this work we observed changes in the growth of shoots as well as roots of wheat in relation to the N availability in media. When combined with presence of metalloid as a stressor, we observed strong reduction of growth by means of dry biomass (Fig. 1) compared to the control as described previously [8]. The observed effect was more obvious in roots since stunted growth was typical regardless on nutrient availability (Fig. 1A). Thus, the presence of metalloid is harmful to roots as to the first tissue (organ) that comes into contact with, while the upper plant parts of wheat are apparently better protected.

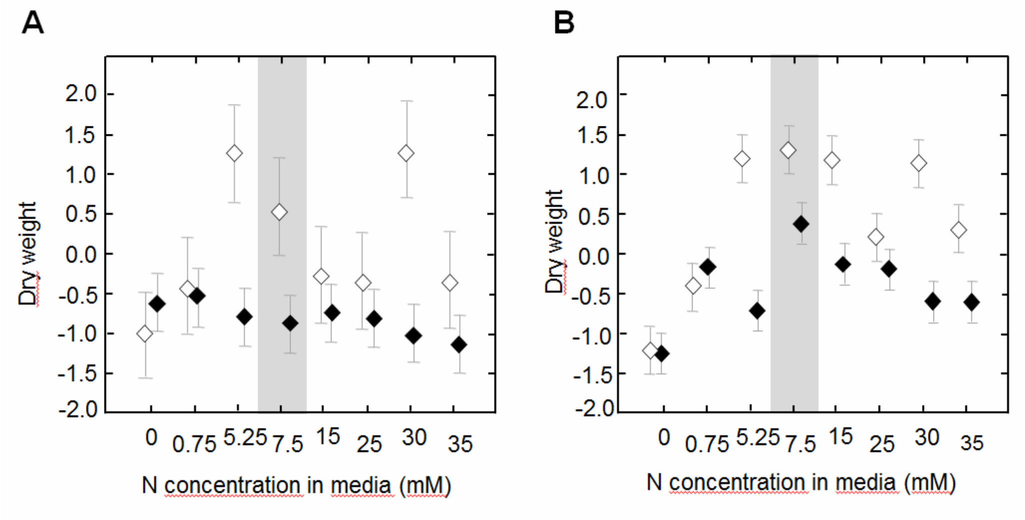

Fig. 1. Growth parameters of wheat plants
Impact of arsenic presence (black symbols) on dry weight of wheat roots (A) and leaves (B) under variable nitrogen (N) availability (in mM). The values correspond to the arithmetic average ± confidence intervals (95 %) (n = 5 for B and C).

The growth patterns indicate that the plants are at the extreme N doses (starvation, toxic doses) stressed. This is apparent also when the formation of hydrogen peroxide is observed in leaf tips (Fig. 2). The presence of hydrogen peroxide is to certain levels normal in plants, but as any other reactive oxygen species is very carefully controlled in plants. Disbalance causes stress since accumulation overveils the detoxifying mechanisms. In the experimental plants obvious accumulation was detected only in the plant set exposed to metalloid, however, only under extreme N conditions. Low (0 and 0.75 mM) and excess (25, 30 and 35 mM) nitrogen in media triggered in combination with As stress (Fig. 2).

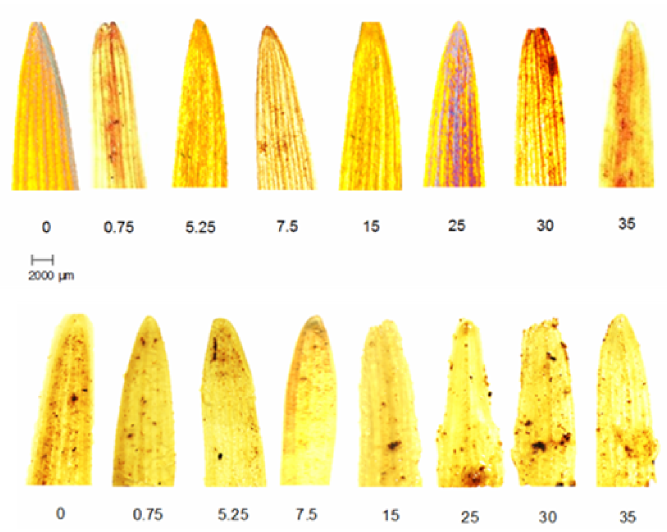

Fig. 2. Formation of hydrogen peroxide complexes in wheat
The complexes (brown dots) indicate stress in wheat leaf tips grown in absence (upper panel) or presence of arsenic (lower panel) under variable nitrogen (N) availability (in mM).

Levels of F_{max} and F_v /F_m were under optimum conditions and higher nitrogen nutrition unchanged as observed by some others [9], probably due to preservation of the Photosystem II integrity. However, the presence of arsenic significantly influenced the parameters of photosynthetic apparatus. The values of examined photosynthetic parameters showed significant reduction caused by the effect of arsenic compared with corresponding controls. However, this effect was less pronounced at low doses of the nutrient. The maximum value of chlorophyll fluorescence and F_{max} maximum quantum yield of photosynthesis F_v/F_m were significantly increased in the presence of arsenic under nutritional deficiencies (0 mm, 0.75 mM N) compared to control plants (Fig. 3). At optimal doses (7.5 mM) and excess nitrogen in the media the metalloid exerted almost no effect on these parameters. The interaction between the effects of metalloid and different doses of nitrogen in the medium was statistically significant for the values of F_v/F_m. In contrast, the values of minimum chlorophyll fluorescence F_0 remained unaffected by the action of arsenic on wheat plants (data not shown); for this parameter we did not demonstrate interaction between metalloid and nutrition conditions. Zhang et al. [10] suggest that nitrogen acts as a detoxifying factor in stress because in excess allows unlocking the biosynthesis steps for the conversion of Urogene $^{3+}$ to Coprogen^{3+} that is blocked under the influence of heavy metal(loid). At low doses of nitrogen, on the other hand, higher photosynthetic yield is probably due to the compensation mechanism photosynthetic apparatus in order to acquire energy sources for growth.

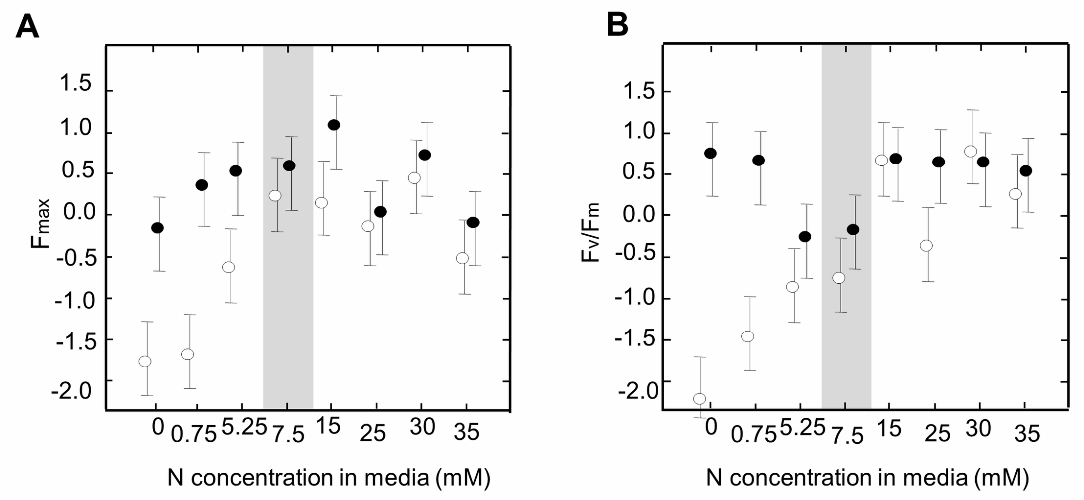

Fig. 3. Photosynthetic parameters of wheat plants exposed to metalloid under variable nutrition conditions
Impact of arsenic presence (black symbols) on chlorophyl fluorescence of wheat leaves under variable nitrogen (N) availability (in mM) is shown on the values of maximum chlorophyll fluorescence (A) and the maximum quantum yield Fv/Fm (B).
Data correspond to the arithmetic average ± confidence intervals (95%) (n = 5 for B and C).

In our experiment, the amount of accumulated arsenic was lower in the shoots and roots grown at low concentrations of nitrogen comparing to optimal conditions (Fig. 4). Arsenic amounts in the roots growing under optimal (7.5 mM) and supra-optimal nitrogen doses were comparable. This suggests a more effective retention of toxic elements in the roots at sufficient nutrition, which is a prerequisite for tolerance of plants against metal toxicity [11]. Variable distribution of Cd^{2+} between roots and shoots depending on nutritional conditions have also been described by Chen et al. [12] in Arabidopsis as a result of the regulation of nitrate transporters.

Recently it has been shown [10] that ethylene, jasmonate and low affinity nitrate transporters (ET/JA/NRT) form a signalling module that enables a cross-talk between stress-initiated nitrate allocation to roots and the environment. This module further coordinates nitrate allocation and the trade-off between the growth and environmental adaptation. Thus, optimization of fertilization management can greatly affect the mineral uptake (including toxic metals) in plants.

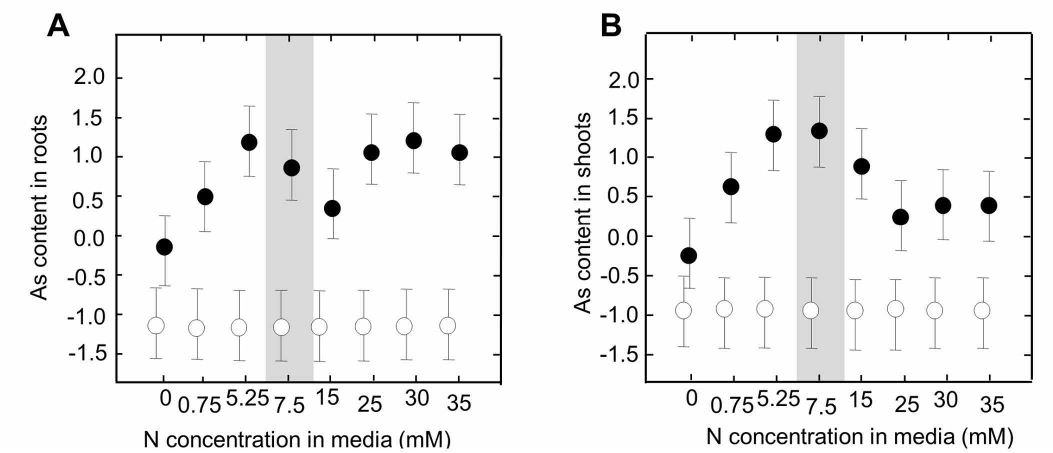

Fig. 4. Accumulation of metalloid in experimental wheat plants
Values for arsenic (black symbols) comparing to control plants (empty symbols) in wheat roots (A) and roots (B) leaves under variable nitrogen (N) availability (in mM) correspond to the arithmetic average ± confidence intervals (95%) (n = 4).

4 Conclusions

Nitrogen nutrition plays a key role in the photosynthetic capacity of the plant to grow in contaminated soil as it determines the availability of resources for growth as well as for activation of defence reactions. Our present data show that at excessive N doses the transfer of metalloid to shoots is limited, and the lower content of accumulated metal in tissue is not the result of commonly accepted dilution effect.

Acknowledgements

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HEAVY METALS RESISTANT BACTERIA FOUND IN THE DRAINAGE WATER AND THE FLOTATION SLUDGE FROM THE SLOVINKY SLUDGE BED

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Abstract

Cultivable bacteria from the heavy metal polluted sludge bed Slovinky in the north-eastern part of Slovakia were isolated and characterized. Using cultivation on non-selective media followed by MALDI-TOF typing and identification 3 taxonomic groups of bacteria which dominated the microflora of the drainage water and the flotation sludge in Slovinky sludge bed were recognised. Significantly different bacterial population were detected in the drainage water compared to the solid flotation sludge. Bacteria of *Bacillus* genus (Firmicutes) were found predominantly in solid sludge. Bacteria of *Corynebacterium* genus (Actinobacteria) were found exclusively in the drainage water. Gammaproteobacteria, represented by *Pseudomonas*, and *Aeromonas* genera, were found in both drainage water and the flotation sludge. The results of the heavy metal resistance tests show that 100 % of the selected isolates were resistant to Mn at the concentration 10 mM and 96 % to As at the concentration 10 mM. The prevalence of heavy metal resistance to other heavy metals were lower, for instance 40 % of isolates showed resistance towards Mn (25 mM) and only 12 % were resistant to Cu (5 mM) and zinc (5 mM). Since these bacterial species can grow in the presence of heavy metals, we assume their potential applicability in removing of toxic compounds from our environment, in bioremediation.

Keywords: sludge bed, heavy metals, bioremediation, *Pseudomonas*, *Bacillus*, *Corynebacterium*

1 Introduction

Environmental pollution represents one of the serious threats to the ecosystem and human health. Sources of pollution are introduced into our environment during various human activities like mining, refining ores, industrial processes, combustion of fossil fuels, etc. what causes the accumulation of contaminants in soils, ground waters and subsequently in the food chain. Those anthropogenic activities have led to the emission of heavy metals such as Cd, Cu, Ni, Co, Zn and Pb into the environment and these toxic elements negatively impact all biological systems.

Because of their chemical composition, heavy metals are not biodegradable and therefore difficult to remove from the environment. When heavy metals are present in higher concentrations, they accumulate in living organisms and can cause serious health problems and a threat to the environment. Of course, heavy metals are also toxic to bacteria and normally, heavy metals can cause serious cell damage, e.g. change or damage of their proteins, cell membranes or DNA. Heavy metals can modify functions of some enzymes and cause oxidative stress too [1, 2]. Heavy metals produce reactive oxygen species (ROS) by auto-oxidation, which can cause peroxidation of lipids, oxidation of proteins, inactivation of enzymes, DNA damage [3, 4]. Taken together, negative effects of heavy metals result in changes of metabolism, growth and morphology of bacterial cells [5-8].

Long-term exposure of bacteria to heavy metals and selection pressure usually lead to the development of several mechanisms which help bacteria to survive in extreme toxic environmental conditions via keeping metal homeostasis in balance. The heavy metal resistance can be either acquired or inherited. Bacteria acquire resistance by mutation of genes or by transferring and subsequent accepting of resistance genes from other bacteria via mechanisms, such as conjugation, transformation and transduction [9-11].

In recent years, researchers have paid more and more attention to this worldwide problem of the environmental pollution and have tried to develop appropriate methods to remove toxic compounds from the environment. Some interactions and strategies which are used in the mechanisms of bacterial resistance may be exploited as a basis for possible strategies for the eco-friendly method of removing pollutants from the environment - bioremediation [12].

One of those heavy metals polluted ecosystem in Slovakia is represented by Slovinky sludge bed. Slovinky is a village and municipality in the Spišská Nová Ves District in the Košice Region of Slovakia. In the past, tailing impoundments Slovinky was used for deposition of flotation sludge from the processing and mining of ores (siderite-sulphidic). The mine tailing Slovinky is currently not being used and referred to the so-called „dry tailing pond“ [13]. Tailing impoundment Slovinky contains high concentrations of several toxic elements and heavy metals (i.e. As, Sb, Hg, Cu, Fe, Mn, Ba, Al, Pb, Cd, Ni, Zn) [13-16]. The aim of this study was to identify and to characterize cultivable bacteria outlast in the Slovinky sludge bed.

2 Material and methods

2.1 Isolation of bacteria

The drainage water and sludge samples were obtained and collected from Slovinky sludge bed in October 2014. Bacteria were isolated using spread plate method, when 20 µl of drainage water/sludge sample were transferred to the centre of the sterile TSA (Merck, Germany) agar plate and spread over the surface with a sterile L-shaped rod. The plates were incubated at 25 °C and 37 °C for 18 hours to obtain individual bacterial colonies.

2.2 MALDI-TOF analysis

The method of MALDI–TOF (Matrix – assisted laser desorption ionization time-of-flight) mass spectrometry is a method used for classification and identification of microorganisms based on the analysis of protein profiles. Single bacterial colonies were prepared for MALDI-TOF analysis according to Ferreira et al. [17]. MALDITOF MS was performed using a Microflex LT instrument (Bruker Daltonics GmbH, Leipzig, Germany) with FlexControl software (version 3.0). The spectra were recorded and analysed automatically with Biotyper 2.0 software (Bruker Daltonics), containing reference database.

2.3 Heavy metal resistance test

The bacterial isolates were tested for their resistance to heavy metals on sterile Nutrient agar medium (Conda, Spain) supplemented with different concentrations of $CuSO_4.5H_2O$ (5 mM, 10 mM), $ZnCl_2$ (5 mM, 10 mM), $MnCl_2$ (10 mM, 25 mM), and $Na_2HAsO_4.7H_2O$ (1 mM, 2 mM, 5 mM, 10 mM). The plates were inoculated with the individual bacterial samples and incubated for 24 hours at 25 °C.

3 Results and discussion

In our work the bacterial isolates from the drainage water and sludge from Slovinky sludge bed (Slovakia) were characterized, analyzed and classified. At the beginning of our experiment, 78 pure bacterial isolates were obtained and subjected to identification by MALDI-TOF analysis. Using this approach 3 taxonomic groups were identified which dominated the microflora of the drainage water and the flotation sludge (Table 1). Significantly different bacterial population were detected in the drainage water and the solid flotation sludge. Bacteria of *Bacillus* genus (Firmicutes), represented by *B. thuringiensis* and *B. cereus* species, were found predominantly in solid sludge. Bacteria of *Corynebacterium* genus (Actinobacteria), represented by single species *C. callunae,* were found exclusively in the drainage water. Gammaproteobacteria, represented by *Pseudomonas,* and *Aeromonas* genera, were found in both drainage water and the flotation sludge. *P. chlororaphis* dominated in the flotation sludge while other species, *P. brassicacearum P. koreensis, P. brenneri, P. azotoformans, P. extremorientalis, P. veronii* and *Aeromonas spp.* dominated in drainage water.

In heavy metal resistance test the bacterial isolates were tested for their ability to grow on Nutrient agar plates supplemented with four heavy metals – Cu, Zn, Mn and As. The frequence of resistance to each heavy metal is presented in the Figure 1. The results show that most of the bacterial isolates grew well in the presence of low concentrations of heavy metals such as Cu, Mn and As while in the presence of Zn no isolates grew. Strains *Pseudomonas thivervalensis*, *P. azotoformans, P. trivialis, P. extremorientalis, P. rhodesiae, P. brenneri, P. chloroaphis, P. koreensis, P. veronii, Aeromonas popoffii, A. salmonicida, Corynebacterium callunae, Bacillus thuringiensis* and *B. cereus* showed multiple-metal resistance to Mn (10 mM, 25 mM) and As (1 mM, 2 mM, 5 mM, 10 mM). Moreover, isolates *P. brassicacearum, P. jesenii* and *P. chlororaphis* were resistant to three heavy metals – Cu (5 mM), Mn (10 mM) and As (1 mM, 2 mM, 5 mM, 10 mM).

However, some differences between heavy metal resistant strains in the sludge and water were observed. For instance, in case of copper-resistant bacteria only species from the sludge had the ability to grow in the presence of this heavy metal, namely *P. brassicacearum* and *P. chlororaphis*. Of the total number of ten Mn-resistant strains (25 mM), only one resistant strain *B. cereus* (L58) was found in the sludge. Moreover, there was only one arsenic sensitive species *P. brenneri* (P11) in the water samples.

Table 1. Incidence of bacterial strains in drainage water and flotation sludge by MALDI-TOF analysis

Taxonomic group	Species	Water		Sludge	
Corynebacterium	*C. callunae*	11x	12x	-	
	Corynebacterium sp.	1x			
Bacillus	*B. cereus*	1x	2x	8x	23x
	B. thuringiensis			14x	
	Bacillus sp.	1x		1x	
Pseudomonas like	*P. azotoformans*	2x	24x	-	17x
	P. brassicacearum	-		4x	
	P. brenneri	2x		-	
	P. chlororaphis	1x		9x	
	P. extremorientalis	2x		-	
	P. jessenii	-		1x	
	P. kilonensis	1x		-	
	P. koreensis	2x		1x	
	P. rhodesiae	1x		-	
	P. thivervalensis	1x		-	
	P. trivialis	1x		-	
	P. veronii	1x		-	
	A. bestiarum	2x		-	
	Aeromonas eucrenophila	-		1x	
	Aeromonas salmonicida	1x		-	
	A. popoffii	1x		-	
	Pseudomonas sp.	6x		1x	

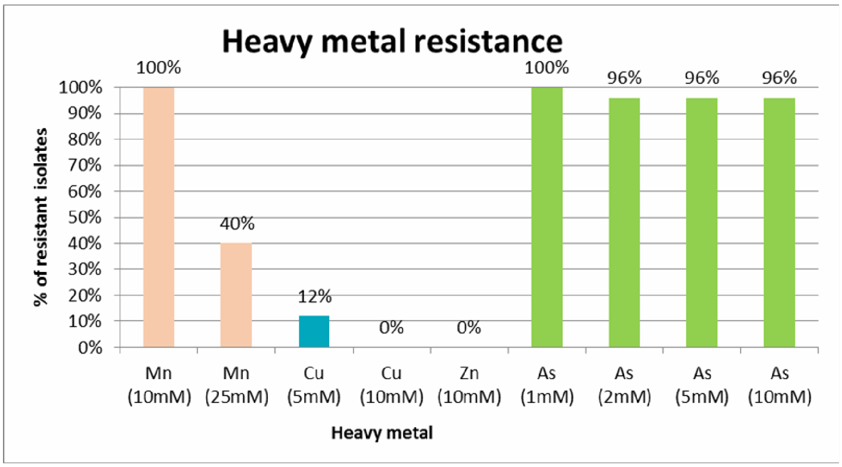

Fig. 1. Heavy metal resistance

4 Conclusions

In recent years, the environmental contamination has become a serious problem and researchers do efforts to discover and develop new and efficient strategies of toxic compounds removal from the ecosystem. The most common physic-chemical processes such as chemical precipitation, electrochemical treatment or reverse osmosis processes and their cost-effectiveness are limited. In addition, these processes have a lot of disadvantages, e.g. the contaminating reagents are used for desorption which results in toxic sludge and secondary environmental pollution. Bioremediation using bacteria could be an effective and successful biotechnological method. Heavy metal resistant bacteria are able to take up heavy metals from the aqueous ecosystems or produce biosurfactants which can enhance biodegradation of petroleum products and therefore they could be used in the process of biodegradation.

In our work the bacterial isolates from heavy metal polluted ecosystem were identified, classified and characterized. Some of the determined species could be potentially applied in bioremediation due to their ability to survive in such extreme environmental conditions. They are able to tolerate heavy metals and thank to this nature we consider them a successful contribution to bioremediation of toxic agents distributed in our environment. Further investigation and characterization are still required which could be the subject of following research experiments.

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ENVIRONMENTAL FACTORS AND THEIR INFLUENCE ON PHYSIOLOGICAL PROPERTIES AND PELLETIZATION OF FOUR *Aspergillus niger* WILD TYPE STRAINS IN WATER SUSPENSIONS WITH FINE-GRAINED MONTMORILLONITE

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Abstract

Wild type strains of the *A. niger* microscopic filamentous fungus originated from soils on the sites of Banská Štiavnica-Šobov, Pezinok-Kolársky vrch, Gabčíkovo and Slovinky, marked here as (An-S), (An-P), (An-G) and (An-Sl), respectively, were used. The substrates obtained from these sites differ in various pH values (pH/H_2O 3 - 8.3) and contents of organic matter (% C_{ox} 0.3 - 7.2) and chemical elements (As, Sb, Al, Fe, Cd, Cu, Pb and Zn). According to observation of changes in physiological properties such as growth and formation of colonies, enzymatic activity, production of organic acids and degree of pelletization of four *Aspergillus niger* wild type strains, significant effects of factors of the environment representing original substrate of these strains, were found out.

A. niger species are capable to effectively degrade complex organic pollutants to simple organic acids as non-harmful compounds. Moreover, other microorganisms can utilize these substances as a source of carbon or energy. Application of obtained results includes recycling of wastes with higher contents of metal and metalloid pollutants, especially bioleaching of mining wastes, which are often very poor in organic carbon.

Keywords: environmental factors, microscopic filamentous fungi, *Aspergillus niger*, organic acids, pellets, fine-grained montmorillonite

1 Introduction

Aspergillus niger belongs to the most widespread microscopic filamentous fungus not only in the soil [1, 2] but also in the other environments. Environmental factors of soil, which is the primary environment of the occurrence of microscopic filamentous fungi, such as pH, contents of organic matter and heavy metals and potentially toxic elements; all these represent primary indicators with very fast influence on microorganisms [3]. Physiological properties of microorganisms changed in dependence on these factors. Four wild type strains of *A. niger* originated from variable environments and their influence on growth, colony size, enzymatic activity, production of organic acids and their pelletization in water suspensions with montmorillonite was studied.

2 Material and methods

2.1 Microscopic fungi

All wide type of *Aspergillus niger* strains were isolated from terrestrial substrates or stream sediment using the dilution plating method (dilution 10^{-4} CFU) from 10 g of substrate. Dilutions were plated on Sabouraud Maltose Agar (SAB) (Himedia, Mumbai, India) for isolation. *Aspergillus niger* strains were isolated from mixed culture of soil microscopic fungi. Pure cultures of *A. niger* strains were cultivated in an incubator at 25 °C for 5 - 7 days on SAB. All strains were identified according to micromorphological features and PCR analyses [4, 5].

2.2 Growth and colony size of *A. niger* strains

The growth and the size of colonies of all studied *A. niger* wild strains were observed by cultivation on SAB agar in temperature-controlled oven at 25 °C for 5 - 7 days in three replicated runs. Influence of the original substrates (Šobov, Pezinok, Gabčíkovo and Slovinky) was studied by adjustment of pH values of SAB agar to pH 3 - 5 - 7 and 9.

2.3 Enzymatic activity of *A. niger* strains

Enzymatic activity of the strains was studied using diagnostic culture media as follows: cellulase activity (CE) on CongoRed medium, esterase activity (EA) on Tween 80 medium, lipase activity (LA) on Spirit Blue medium and protease activity (PA) on Gelatine P3 medium as so-called "halo" effect [6, 7].

2.4 Production of organic acids of *A. niger* strains

Pre-treatment of the samples

The samples were stabilised at -4 °C. The samples were defrosted in water bath at 20 °C before HPLC analysis. Then, all samples were homogenised and filtered through regenerated cellulose membrane filters with pore size 0.45 μm.

HPLC analysis

Analysis of the samples was realized using HPLC device with PDA detector (YoungLin 9100). The mobile phase used to analysis included methanol and water; their concentration during analysis was changed from initial ratio 10:90 up to 90:10. Analysis of the samples was carried out at 25 °C. The column (GraceSmart, RP 18, 150 mm length, OD 4.6 mm) for selective separation of analytes was used. The flow rate was 1 $ml.min^{-1}$ and the wave length of PDA detector ranged from 222, 210, 200 to 235 nm [8].

2.5 Pelletization of *A. niger* strains

The fungal pellets were prepared in 60 ml SAB medium with adjusted pH to 3 - 5 - 7 - 9 enriched with a 10 ml suspension of conidia from each strain and 1 g of montmorillonite. Controls at each adjusted pH value were without montmorillonite. Cultivation was carried out using the shaker Unimax 2010 (Heidolph, Germany) at 135 rpm. Then, the pellets were washed with a huge amount of distilled water and stored in Petri dishes where number and size of the pellets were measured. Microstructure of the pellets was recorded using digital stereomicroscope Leica DMD 108.

3 Results and discussion

3.1 Microscopic fungi

Wild strains of *A. niger* originate in soil and river sediment from various sites in Slovakia (Table 1).

Table 1. Studied sites with different environmental factors

Strain	Locality	Substrate/environmental factors
An - S	Banská Štiavnica-Šobov	Dystric Cambisol (contaminated and eroded without vegetation) pH H_2O = 3.12 (ultra acid); % C_{ox} = 0. 49; Al = 727 - 506 mg/kg
An - P	Pezinok	Stream sediment; pH H_2O = 5.3 (strong acid); % C_{ox} = 7.2; As = 363 mg/kg; Sb = 93 mg/kg; Fe = 82.8 mg/kg
An - G	Gabčíkovo	Eutric Fluvisol; pH H_2O = 7.7 (slightly alkaline); % C_{ox} = 6.3; without any contamination, vegetation is *Salici-Popuetum*
An - Sl	Slovinky	Technosol without vegetation; pH H_2O = 8.5 (strongly alkaline); % C_{ox} = 0.3 - 0.8; As = 305 - 511 mg/kg; Cd = 8.6 - 13.4 mg/kg; Cu = 7 372 - 9 227 mg/kg; Pb = 2964 - 8 078 mg/kg; Zn = 24 786 - 47 291 mg/kg

The first strain An - S were isolated from Dystric Cambisol (contaminated and eroded) on the locality Šobov, near Banská Štiavnica. The ultra acid soil is without vegetation and very poor on organic material. From stream sediment on the locality Pezinok was isolated second strain An - P. Substrate is strong acid, rich on organic material, but contaminated with very high amount of As, Sb and Fe. From of Eutric Fluvisol, slightly alkaline and rich on organic material, on the locality Gabčíkovo was isolated third strain, An - G. This strain was as a control strain, because of the locality is without any contamination. Strongly alkaline

Technosol without vegetation was as a source of fourth strain An - Sl. Amount of organic material is on the minimum, but the soil is enriched on very toxic elements as As, Cd, Cu, Pb and Zn.

3.2 Growth and colony size of *A. niger* strains

The smallest colonies in size produced those strains where the difference between the original pH of the substrates and adjusted pH of the media was significant (An - G 1.4 ± 0.2 at pH 3; An - S 1.6 ± 0.1 at pH 5; An - P 1.5 ± 0.1 at pH 7 and 0.8 ± 0.1 at pH 9). On the other hand, the highest diameters of the colonies were achieved in the environment, which was the most similar to their original environment. The average diameters of the colonies on the growth medium with adjusted pH value were very similar at pH 3, 5 and 7 (2.37 cm at pH = 3; 2.42 cm at pH = 5 and 2.32 cm at pH = 7). It is interesting that the *A. niger* strain originally isolated from strongly alkaline substrate (Slovinky site) always produced the smallest colonies at all examined pH values. Likewise, the average diameters of the colonies of other strains were always the lowest at pH = 9 (Tab. 2). Even though microscopic filamentous fungi occur in soil and other terrestrial substrates under various pH values, it is obvious that they prefer acidic environmental conditions compared with alkaline conditions [9].

Table 2. Colony size of all *Aspergillus niger* wild type strains

Strain	pH of original substrate	Colony size (cm)			
		pH 3	pH 5	pH 7	pH 9
An - S	3.0/2.7	2.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	1.4 ± 0.2
An - P	5.3/4.8	3.1 ± 0.1	3.7 ± 0.2	1.5 ± 0.1	0.8 ± 0.1
An - G	7.7/7.4	1.4 ± 0.2	2.4 ± 0.1	4.0 ± 0.1	1.2 ± 0.1
An - Sl	8.9/8.6	2.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.8 ± 0.1
Diameter of colony (cm)		**2.37**	**2.42**	**2.32**	**1.55**

3.3 Enzymatic activity of *A. niger* strains

Enzymatic activity is very important because all reactions in living organisms are catalysed by enzymes. Relatively low growth of all species were recorded on the substrate assigned for esterase activity assessment and very low growth, almost negligible, was recorded on the substrate assigned for protease and cellulase activities. Though, the growth of all *A. niger* strains was very good on the substrate assigned for lipase activity. The so-called "halo" effect was recorded for all strains (Fig. 1).

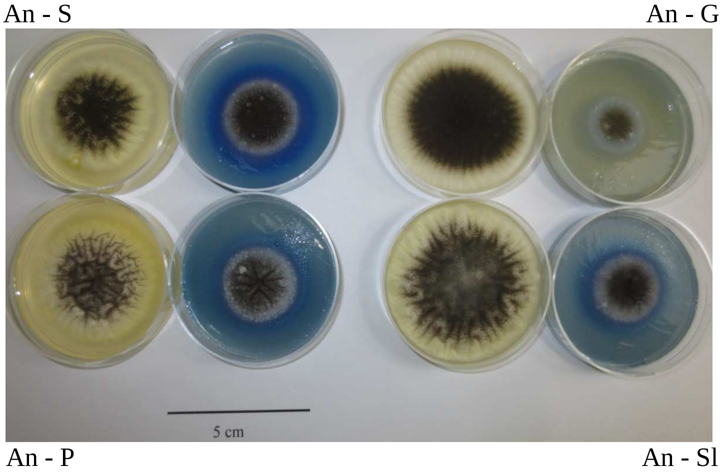


Fig. 1. Growth of the *A. niger* strains on SAB Agar (as a control) and activity of lipase on Spirit Blue Agar after 5 days of cultivation

The obtained results confirm the higher lipase activity for the An - G strain compared with the other studied strains. Inhibition of metabolism as well as enzymatic activity is likely due to metal(loid) pollutants (As, Sb, Al, Cd, Cu, Pb and Zn), which occur in all other substrates besides the Gabčíkovo substrate. Inhibition of enzymatic activity in the samples from the Šobov and Slovinky sites was significantly affected not only by higher contents of potentially toxic elements but also by extremely low content of organic carbon. The higher content of organic carbon in the substrate (Tab. 1) does not cause higher enzymatic activity in case of comparison of the An - P and An - G samples.

3.4 Production of organic acids of *A. niger* strains

According to production of organic acids by *A. niger* strains (Tab. 3), achieved results of HPLC analysis refer to the ability of all strains to decompose organic pollutants in contaminated soil samples. Such decomposition products can include lower acids, notably oxalic, formic and acetic acids. Their ratio in the samples vary, likely due to enzymatic activity of the particular *A. niger* strain. The highest concentrations of organic acids, especially of oxalic acid were recorded in pre-treated sample of the control strain originated from Gabčíkovo (An - G) as a probable consequence of the highest enzymatic activity of the microorganism (Fig. 1).

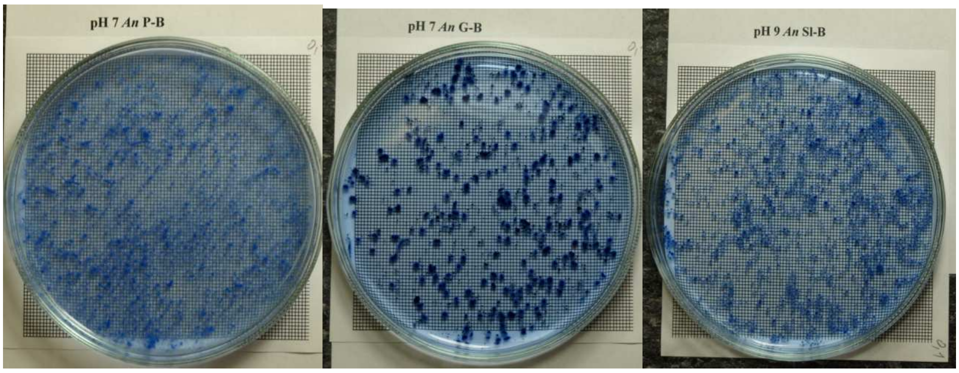
Table 3. Production of organic acids in mg/l of all *Aspergillus niger* wild type strains

Sensitivity mg/l	0.095	0.1	0.1
Strain	formic acid	acetic acid	oxalic acid
An - S	0.108	0.143	0.173
An - P	0.144	0.1	0.175
An - G	0.238	0.1	0.228
An - Sl	0.164	0.11	0.138

Partial intermediates of decomposition of organic acids during bioleaching gradually separated organic acids due to disruption of the equilibrium in term of lysis of the cells of *A. niger* strains. Due to disruption of the equilibrium, the complete degradation of organic acids e.g. to acetone, methanol, aldehyde etc. was reached [10]. The *A. niger* strains effectively degrade pollutants to simple acids with no significant negative ecological impacts to the environment. Subsequently, such produced substances can be utilized by other microorganisms as a source of carbon or energy. These processes can stimulate decomposition procedures of resistant types of pollutants occurred in landfills confirmed by seepages [11]. Metabolites of microscopic filamentous fungi such as organic acids and amino acids affect pH of the medium. Acidification of the medium supports the mobility of metals, especially the transport mechanism of metal from the environment into the fungal cell and backwards [12].

3.5 Pelletization of *A. niger* strains

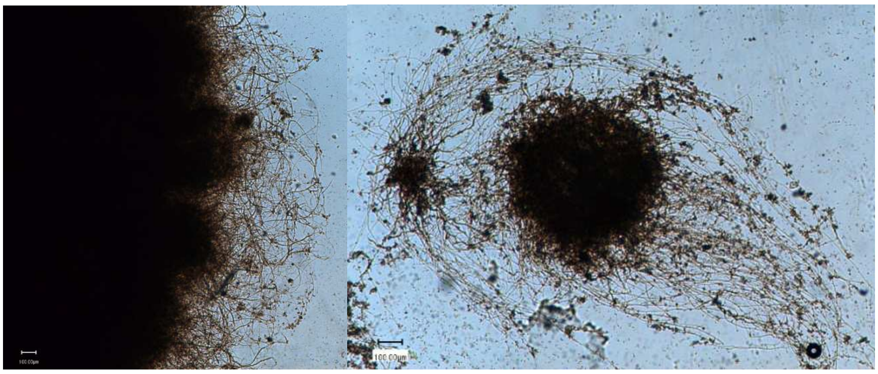
Significantly higher number of pellets in the presence of montmorillonite was produced; however, diameter of the pellets was in all growth stages lower (Fig. 2a-c).



2a **2b** **2c**

Fig. 2. Significantly higher number of pellets with the lower diameter as a consequence of the montmorillonite presence using *A. niger* isloted from Pezinok at pH = 7 (2a), *A. niger* from Gabčíkovo at pH = 7 (2b) and *A. niger* from Slovinky at pH = 9 (2c)

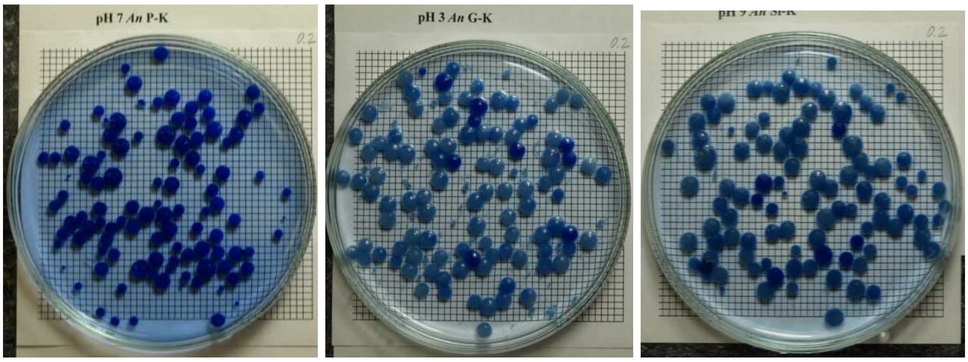
This can be explained by several reasons. The most probable one is that higher amount of nucleation sites is responsible for increasing of the number of pellets and spherical inhibition of fungal growth expressed in the diameter changes. Another possible reason is a relative increase in the length of fungal filaments in pellets (Fig. 3).



3a **3b**

Fig. 3. Changes in length of filaments of pellets in the control sample An - G (3a) and their relative lengthening in the presence of montmorillonite (3b)

According to control experiments, the biggest pellets were produces as follows: by the An - S strain at pH = 3 (2.67 mm) and pH = 9 (3.04 mm), An - P at pH = 5 (5.28 mm) and pH = 7 (4.7 mm), An - G at pH = 3 (4.39 mm) and pH = 5 (3.65 mm) and An - Sl at pH = 9 (5.35 mm), (Fig. 4).



4a **4b** **4c**

Fig. 4. Pellets sizes in the control samples. Strain An - P at pH = 7 (4a) with a diameter 4.7 mm, strain An - G at pH = 3 (4b) with a diameter 4.39 mm and strain An - Sl at pH = 9 (4c) with a diameter 5.35 mm

The changes in size of the pellets and shape and length of their mycelia of *Cladosporium cladosporioides* and *Humicola grisea* was also confirmed by other authors [13]. Fungal pellets have many advantages of easy harvest, low fermentation broth viscosity and high yield of some proteins [14]. It was discovered, that mycelial pellets are effective as a biomass carrier for the immobilization of bacteria for the degradation of target pollutants [15], for degradation of lignin in water and bioremediaton of soil contaminated with PCB [16].

4 Conclusions

Obtained results confirm the direct effects of environmental factors such as pH values of the substrate, % C_{ox}, contents of potentially toxic elements on physiological properties of *Aspergillus niger* strains, especially to colony growth and metabolism (production of enzymes and organic acids). Higher lipase activity using the An - G strain if compared with other studied strains, was found out. Inhibition of metabolism as well as enzymatic activity is likely due to presence of pollutants (As, Sb, Al, Cd, Cu, Pb and Zn), which occur in all other substrates besides the substrates from the Gabčíkovo site. Inhibition of enzymatic activity in the samples from the Šobov and Slovinky sites was significantly affected not only by higher contents of potentially toxic elements but also by extremely low content of organic carbon. The higher content of organic carbon in the substrate (Tab. 1) does not cause higher enzymatic activity in case of comparison of the An - P and An - G samples. The production of pellets was significantly reduced in the presence of montmorillonite. In this case, all strains produced a lot of pellets with small sizes. The obtained results can be applied in remediation of landfills, where the contents of metal(loid)s are higher, especially during bioleaching of mining wastes with low contents of organic carbon.

Acknowledgements

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COMPARISON OF BIOSORPTION OF LEAD AND CADMIUM ON SUNFLOWER SEED HULLS, SOYBEAN HULLS BY-PRODUCTS AND ALGAE

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Abstract

Biosorption is a promising process regarding the removal of toxic and non-toxic metals from different kinds of liquids. Numerous organic and inorganic materials have been examined as potential biosorbents for the last few decades. Enormous sorption ability is one of the most important factors in the selection of biosorbents. Besides it, they shall be easily separable and cheap to obtain. Establishing the circular economy is crucial, as well. Due to the latter mentioned issue, different materials generated as biowaste or by-products deserve extraordinary attention of researchers. Sunflower seed hulls and soybean hulls represent this kind of by-products, because they are cheap and available in large quantities. In the paper presented the sorption abilities of sunflower seed hulls and soybean hulls are being determined and compared with the sorption ability of *Undaria pinnatifida* macro algae and *Lyngbya taylorii* micro algae in case of Cd^{2+} and Pb^{2+}. Although the mentioned algae have enormous sorption ability towards more metals, they are unfortunately not appropriate to be operated in aqueous environment for a long term causing difficulties in case of their treatment and possible industrial applications.

Keywords: biosorption, sunflower seed hulls, soybean hulls, algae, zeta-potential, FT-IR

1 Introduction

Biosorption is a promising process for removing toxic metals from different kinds of solutions, e.g. contaminated groundwater, industrial effluents, etc. Although there are countless research reports on this biosorption process topic, the majority of them are still at laboratory scale. Nevertheless, finding the appropriate, cheap and effective industrial biosorbents is also an encouraging challenge.

Many kinds of biological materials have an affinity for binding inorganic and organic pollutants, so that there is an enormous biosorption potential within the countless types of biomaterials [1, 2].

Micro- and macro algae have outstanding toxic metal sorption ability [3, 4] and they are available in large quantities, but several other circumstances should be considered as well in case of a possible usage as an industrial bisorbent. Macro algae *Undaria pinnatifida* and micro algae *Lyngbya taylorii* can only be effective when the pH value of the initial solution is adjusted between pH = 4...6. To achieve the effective separation the micro algae must be immobilized on another carrier materials. It was revealed that *Lyngbya taylorii* immobilized on zeolites has higher sorption capacity than that of biomass and the carrier zeolite together due to the synergy [5]. The place of origin and ways of previous treatment of the biomass - especially in case of macro algae - are also important to know, because it can be contaminated with pollutants (e.g. heavy metals) from the environment during its growth and from the treatment processes after harvesting, as well. Interactions (e.g. swelling) with liquids should be also considered [6].

Different by-products and their biosorption capacity are also widely investigated, and could be applied in the development of biosorbents. Sunflower seed hulls (SFH) and soybean hulls (SBH) are the by-products of vegetable oil processing and occur in great abundance in Hungary, too. According to our estimation more than 100,000 tons/year of SSH are generated as a by-product in Hungary. Most of the hulls are used as a fuel in biomass power plants or as commercially available briquettes. New uses for these agricultural by-products are possible, because they can be converted into effective biosorbents (potentially value-added products) when used in such applications as metal-ion adsorbents for wastewater treatment. It would be important to create the circular economy as well.

Several previously reported studies confirmed that SFH [7, 8] and SBH [9, 10] are promising potential biosorbents for different metals, e.g. Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+} or Cr^{6+}. The optimal pH for biosorption in case of both biosorbents is around 5.0, as far as adsorbing capacity is concerned [7, 8, 10].

The objective of this study was to investigate the sorption ability of SFH and SBH generated in a Hungarian vegetable oil production plant for cadmium and lead. This paper also contains comparison of their sorption ability with that of *Undaria pinnatifida* macro algae and *Lyngbya taylorii* micro algae. Several examinations like BET specific surface, FT-IR and ξ-potential measurements were also performed to understand the mechanism of metal uptake process.

2 Material and methods

2.1 Biosorbents preparation

SFH and SBH were supplied by a biomass power plant (KISERŐ Ltd.) and originated from a vegetable oil production company located in SVIP Sajóbábony Industrial Park, Hungary.

The hulls were ground in Retsch SM 2000 cutting mill equipped with a 2 mm screen and afterwards sieved. The particles with size between 200 and 315 µm were stored for further experiments. These fractions were repeatedly washed three times in deionized water (50 g of biomass with 3 times 200 mL of deionized water) in order to remove surface contaminations (metal content of the first washing water was also analysed). The hulls were then dried at 80 °C for 24 h and cooled in a desiccator.

2.2 Biosorption experiments

Biosorption of cadmium and lead onto SFH or SBH were investigated in mono-cationic systems.

Lead(II) solutions were prepared by adding different mass of lead(II) nitrate powder into deionized water to reach the following desired concentrations: $C_{0,\,Pb}$ = 20; 50; 80; 100; 200; 300; 400; 600; 800 and 1000 mg/L. This metal salt was obtained from VWR Chemicals company.

Cadmium(II) solutions were prepared by adding different mass of cadmium nitrate tetrahydrate powder into deionized water to reach the following desired concentrations: $C_{0,\,Cd}$ = 20; 50; 80; 100; 200; 300; 400; 600; 800 and 1000 mg/L. This metal salt was obtained from SIGMA-ALDRICH.

The initial pH values of the working solutions (before the biosorption) were adjusted between 5.0 and 5.2 by adding 0.1 M NaOH or 0.1 M HCl using pH meter METTLER TOLEDO SevenEasy.

Batch equilibrium sorption experiments were performed in 250 mL Erlenmeyer flasks. The concentration of biomass was 1 g/L (100 mg biomass in 100 mL solution). The suspension was shaken at 150 rpm for 18 h and at the temperature of 25 °C using adjustable incubator shaker WiseCube WIS-20.

2.3 Determination of sorption isotherm

Suspended solid particles were removed after the biosorption experiments by using filter paper (grade: 1,289; diameter: 150 mm; 84 g/m^2). After the filtration some drops of HNO_3 were added to the separated liquid samples. Prior the testing in ICP-OES, all the samples were refrigerated at approximately 4 °C.

The concentrations of the metals in liquid phase were subsequently determined by using inductively coupled plasma optical emission spectrometer (ICP-OES), type SPECTRO CIROS VISION at KISANALITIKA Laboratory Services Ltd.

The biosorption capacity at equilibrium conditions (q_{eq}) was calculated by using the following equation (V: volume of sample solution; m: dry mass of used biosorbent):

$$q_{eq} = \frac{(c_0 - c_{eq}) \cdot V}{m} \tag{1}$$

The highest initial concentration (C_0) was 600 mg/L (2.9 mmol/L) in case of lead and 300 mg/L (2.7 mmol/L) in case of cadmium regarding the isotherms.

2.4 Other measurements on the biosorbents

Zeta-potential of SFH and SBH was measured before and after conditioning with magnetic stirrer for 10 minutes by Zeta Potential Analyzer (Brookhaven Instruments Corporation ZetalPALS, purchased by GVOP-3.2.1-2004-04-0219/3.0. Project; Project Leader: Dr. Ljudmilla Bokányi) in deionized water and in mono-cationic model solutions ($C_{0,\,Pb}$ or $C_{0,\,Cd}$ were 800 mg/L) at the Department of Bioprocessing and Reaction Techniques, Institute of Raw Material Preparation and Environmental Processing, University of Miskolc.

FT-IR analysis was performed by JASCO FT/IR-4200 Spectrometer before and after biosorption experiments with $C_{0,\,Pb}$ = 1000 mg/L or $C_{0,\,Cd}$ = 1000 mg/L at our Institute.

Specific surface area of SFH and SBH was measured by TriStar 3000 Micromeritics at Institute of Metallurgy, University of Miskolc.

3 Results and discussion

It was experimentally established that both the SFH and SBH do have sorption ability towards dissolved cadmium and lead-ions as it can be seen from Fig. 1. The sorption capacity of SFH at equilibrium can reach 0.30...0.35 mmol/g, while the SBH reaches slightly higher q_{eq}-s with 0.35...0.45 mmol/g in case of Pb^{2+} and Cd^{2+} in mono-cationic systems, if the maximum C_{eq} is not higher than 3 mmol/L. It can be stated that there are no significant differences between the sorption ability of SFH and SBH. Remarking that these q_{eq} values cannot be considered as maximum uptake values (q_{max}), further examinations are required on higher C_{eq} values to determinate q_{max}.

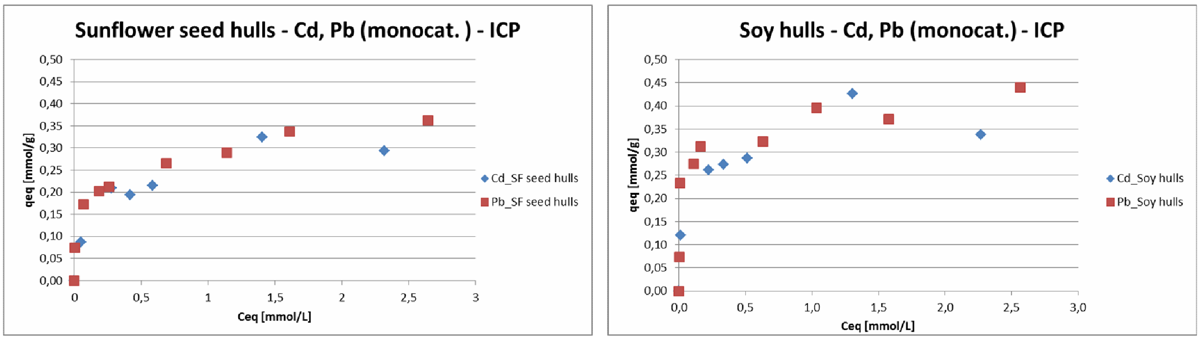

Fig. 1. Sorption isotherm measured points of SFH (left) and SBH (right)

Fig. 2 contains the results of our previous experiments with *Undaria pinnatifida* macro algae (9 mg biomass in 9 mL solution) [12] and *Lyngbya taylorii* micro algae (0.02 mg biomass in 9 mL solution) [11]. The higher q_{eq} values were measured with the two tested algae species compared with these by-products (Fig. 1) in mono-cationic systems. Nevertheless, *Lyngbya taylorii* has similar sorption ability as the SFH or SBH regarding the cadmium.

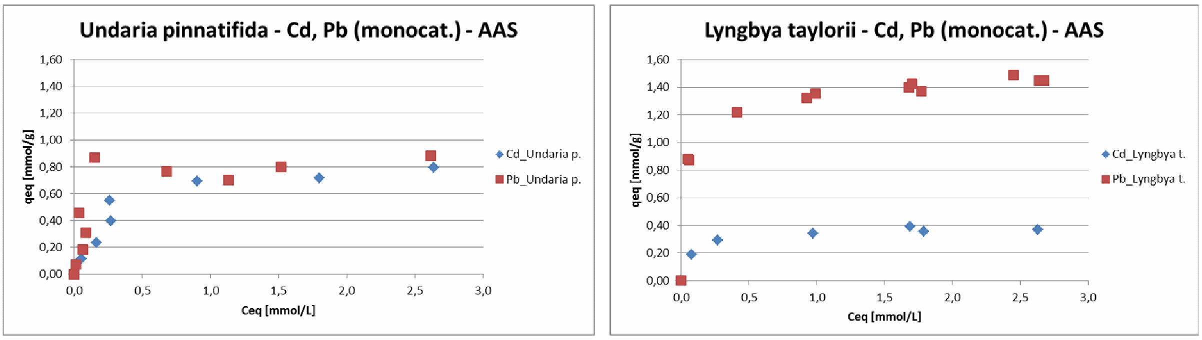

Fig. 2. Sorption isotherm measured points in case of *Undaria pinnatifida* [12] and *Lyngbya taylorii* [11]

Although the different algae species could have much higher sorption ability, the chance of their applicability as effective industrial biosorbents is less practical in comparison with the by-products, which are cheap and can be obtained easily in Hungary, as well. The main problems with the micro algae are that they require even light in the rector volume for their cultivation, which is expensive. Furthermore, their separation after the biosorption is difficult to solve, thus immobilization process is also necessary on solid carrier. The latter may cause the significant decrease in the adsorption capacity due to the blockage of the active centers. Exceptions are if, the q_{max} of the immobilized algae can be very high, e.g. the immobilized *Lyngbya taylorii* on A4 zeolite can have higher sorption capacity towards cadmium and lead than that of biomass and the carrier zeolite together [11].

Our previous experiments carried out with *Undaria pinnatifida* macro algae showed very promising results regarding metal uptake, but its excessive swelling occurred when it was applied in column systems [6]. Thus its application is not recommended as sorption material of column beds. Besides that, the macro algae can be contaminated with pollutants (e.g. heavy metals) before the biosortion process from the environment during its growth and from the treatment processes after the harvesting as well [6]. The investigating the initial metal concentration of macro algae purchased as food stuff is highly recommended before using them in biosorption process.

In order to remove surface contaminations of SFH and SBH, the biosorbents were repeatedly washed three times in deionized water before biosorption experiments. Metal content of the first washing effluent was also analysed (Table 1) with ICP-OES.

Table 1. Metal concentration in liquid phase remained after washing the biosorbents before biosorption process

Metals in washing water	**Metal concentration [mg/L]**		**Metals in washing water**	**Metal concentration [mg/L]**	
	SFH	**SBH**		**SFH**	**SBH**
As	< 0.002	< 0.004	Hg	0.000	0.000
Cd	**0.027**	**0.040**	Ni	0.004	0.025
Cr	0.001	0.000	**Pb**	**0.009**	**0.248**
Cu	0.071	0.040	Zn	0.119	0.297

It is clearly observable that the cadmium concentrations are very low in the washing water, but the lead concentration is higher in case of SBH, and it can reach 0.248 mg/L. The source of this contamination is unclear and needs to be investigated further. Zn concentrations of the washing effluents are the highest in case of both biosorbents, but it is still under 0.3 mg/L. However, washing effluent of *Undaria pinnatifida* (as food stuff) could contain more than 43 mg/L zinc. These data state that the initial metal concetration of biosorbent should be investigated before the biosorption, because the eq. 1. assumes that q_0 equals zero, thus sorpition isotherms can be inaccurate, as well as the process itself can be disturbes without the pretreatment (e.g. washing) of biosorbents.

Table 2 summerizes the results of zeta-potential measurements.

Table 2. Zeta-potential of the biosorbents before and after biosorption

	ξ-potential average [mV]	**pH**		**ΔpH**
		Before conditioning	**After conditioning**	
SBH in deionized water	-4.72	5.13	6.23	-1.1
SBH in Pb(II) solution ($C_{0,\,Pb}$ = 800 mg/L)	-2.42	5.01	4.12	0.89
SBH in Cd(II) solution ($C_{0,\,Cd}$ = 800 mg/L)	-4.60	5.03	4.73	0.3
SFH in deionized water	-13.29	5.13	5.72	-0.59
SFH in Pb(II) solution ($C_{0,\,Pb}$ = 800 mg/L)	-1.97	5.01	4.23	0.78
SFH in Cd(II) solution ($C_{0,\,Cd}$ = 800 mg/L)	-4.84	5.03	4.90	0.13

The SFH shows the highest increase of zeta-potential in case of both metal-ions, thus it can be concluded that there is an electrostatic interaction between Pb and Cd cations and the negatively charged SFH surface. The SBH uptakes them by a specific sorption mechanism, since there are only slight changes in ξ-potential. Additionally, the SBH having a near zero zeta-potential is able to agglomerate spontaneously. This phenomenon can be later used for the development of an immobilisation technique.

The specific surface area of SFH and SBH is presented in Fig. 3 and Table 3. All the three calculation methods proved that the SFH has more than double higher specific surface area than the SBH in case of the applied fraction (between 200 and 315 µm). The SFH has 0.52 m^2/g, while SBH has 0.21 m^2/g specific surface area according to multipoint BET method. Nevertheless, this more than twice higher specific surface of SFH does not respond with its Pb and Cd uptakes, and the SBH can reach some higher q_{eq} values (see Fig. 1).

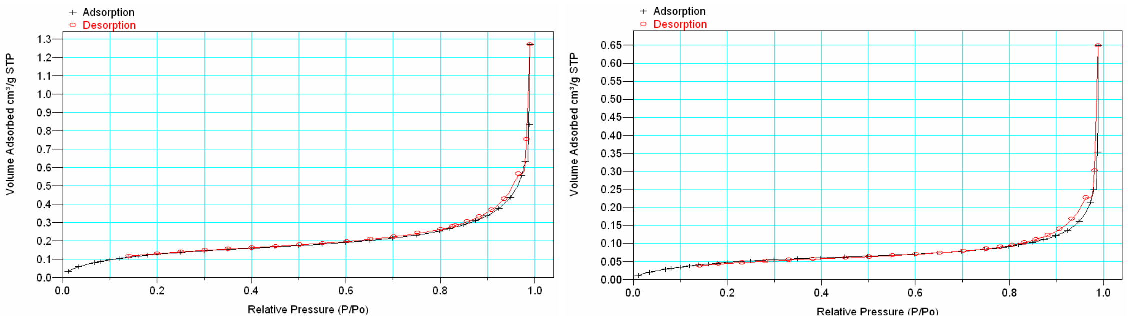

Fig. 3. BET-isotherm plot of sunflower seed hulls (left) and soybean hulls (right)

Table 3. Specific surface area of SFH and SBH

Method of calculation	**Specific surface area, m^2/g**	
	SFH (between 200 and 315 μm)	**SBH (between 200 and 315 μm)**
Singlepoint BET	0.44	0.17
Multipoint BET	0.52	0.21
Langmuir-type	0.78	0.32

Fig. 4 shows the FT-IR spectra of SFH and SBH before and after biosorption of lead and cadmium. Both SFH and SBH have peaks at similar wavenumbers and when the biomasses were loaded with Pb and Cd, the FT-IR spectrum showed some changes.

The highest deviation from the unloaded curve of biomasses appears at around 1030 cm^{-1}, which corresponds to the –C–O alcohols and carboxylic acids [7]. The broad peak observed at 3444 cm^{-1} corresponds to the O–H stretching vibrations of cellulose, pectin, absorbed water, hemicellulose, and lignin [9]. These infrared measurements also revealed the presence of such functional groups as amide groups (1651 cm^{-1}), –C=O from carboxyl or ester groups (1739 cm^{-1}) [7].

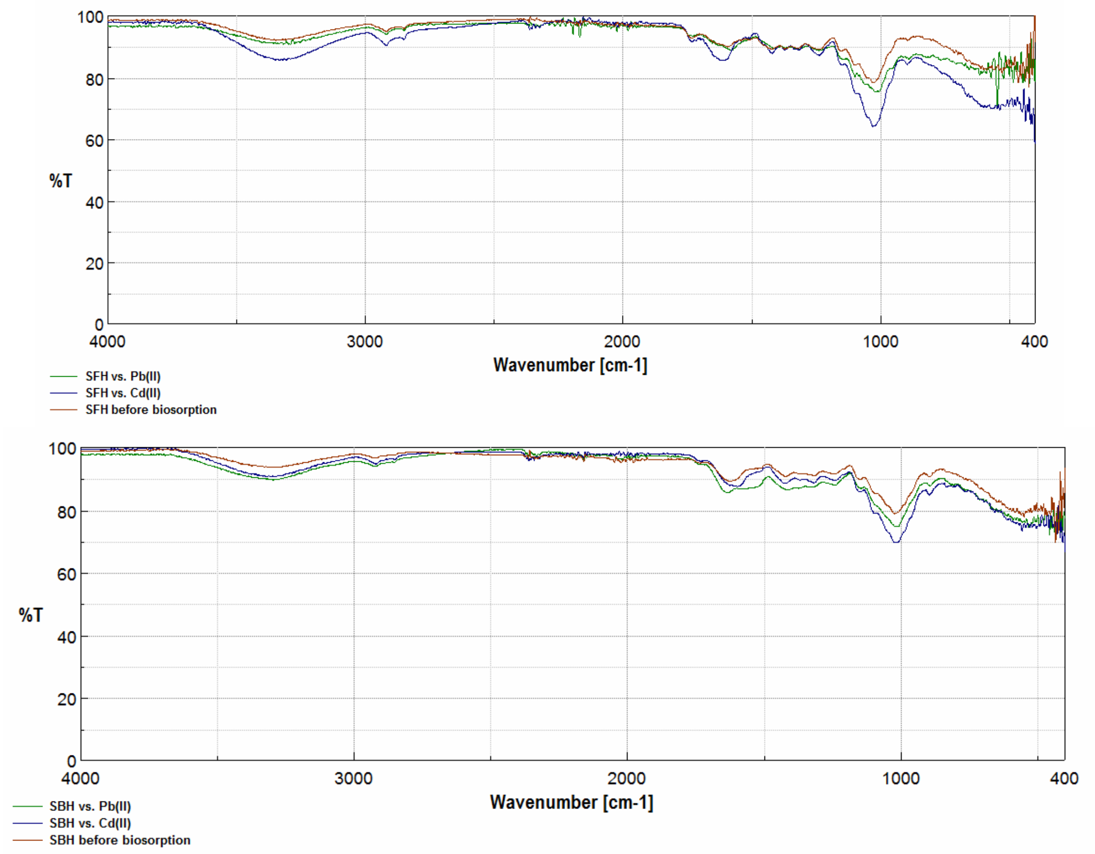

Fig. 4. FT-IR spectra of SFH (upper) and SBH (lower)

Fig. 5 shows the photos obtained by SEM of the surface of biosorbents according to [7] and [9]. The SBH shows a regular surface with robust consistency [9], while the surface of SFH is irregular and porous [7]. Porous character of the surface of the SFH can explain the more than twice higher specific surface area of it.

The higher sorption ability of SBH towards lead and cadmium cannot be directly deduced from the FT-IR and SEM measurements.

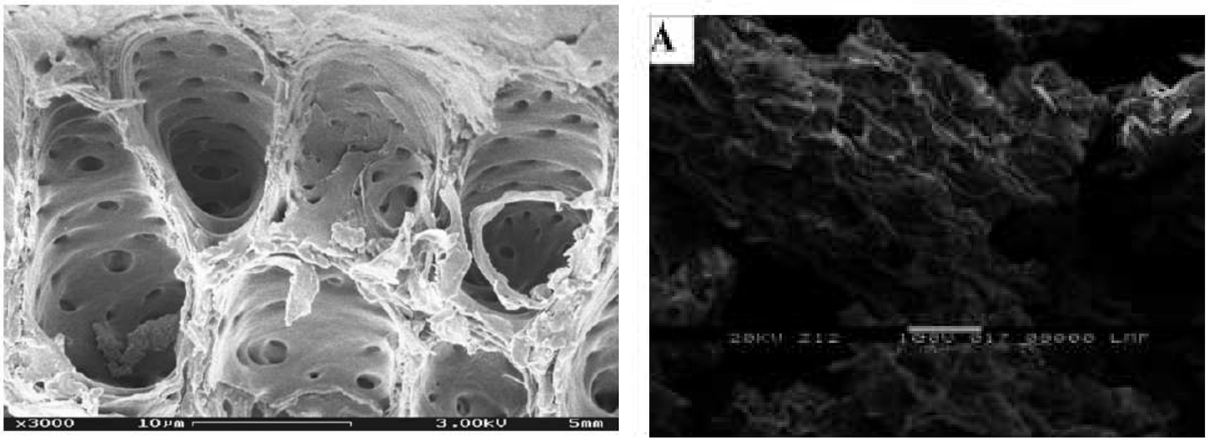

Fig. 5. The SEM images for SFH [7] (left) and SBH [9] (right) before metal sorption

The sorption ability strongly depends on the pH of the solution. Table 4 and Table 5 contain the pH values before and after biosorption process. In most of the cases there is some increase or decrease of pH, but these changes are typically under 0.5. In some cases with Pb there are changes close to 1, but Cd represents changes under 0.5 in all the measurements.

Table 4. pH values before and after biosorption in case of Pb^{2+}

Before biosorption process			**After biosorption process**		**Difference**	
$C_{0, Pb}$ [mg/L]	**Original pH**	**Adjusted pH**	**SBH**	**SFH**	**SBH ΔpH**	**SFH ΔpH**
20	4.43	5.15	5.75	5.74	-0.6	-0.59
50	4.42	5.13	5.6	4.72	-0.47	0.41
80	5.02	-	4.73	4.66	0.29	0.36
100	5.08	-	4.9	4.51	0.18	0.57
200	4.92	5.13	4.61	4.24	0.52	0.89
300	4.92	5.05	4.51	4.14	0.54	0.91
400	4.76	5.05	4.43	4.19	0.62	0.86
600	4.69	5.09	4.39	4.07	0.7	1.02

Table 5. pH values before and after biosorption in case of Cd^{2+}

Before biosorption process			**After biosorption process**		**Difference**	
$C_{0, Cd}$ [mg/L]	**Original pH**	**Adjusted pH**	**SBH**	**SFH**	**SBH ΔpH**	**SFH ΔpH**
20	5.52	5.09	5.47	5.49	-0.38	-0.4
50	5.23	5.02	5.36	5.52	-0.34	-0.5
80	5.31	5.01	5.50	5.48	-0.49	-0.47
100	5.19	-	5.49	5.53	-0.3	-0.34
200	5.21	5.07	5.32	5.02	-0.25	0.05
300	5.18	-	5.22	5.18	-0.04	0

4 Conclusions

Batch biosorption experiments showed that micro and macro algae e.g. *Lyngbya taylorii* and *Undaria pinnatifida* have higher sorption ability towards lead and cadmium than the investigated by-products such as sunflower seed hulls (SFH) and soybean hulls (SBH). Nevertheless, it can be worth studying their applicability as a biosorbents, since they represent a huge potential occurring in a great abundance in Hungary, too. Additionally, the capabilities of these by-products for adsorbing metals can be enhanced by citric acid modification as it was reported in literature by [13, 14]. Our future experiments will be carried out in this direction.

Our zeta-potential measurements proved that there is an electrostatic interaction between Pb and Cd on SFH. Moreover, SBH uptakes these metals by specific sorption mechanism.

Although the SFH has more than twice higher specific surface area than the SBH in case of particles between 200 and 315 µm, this characteristic of SFH is not reflected in the sorption ablility towards lead and cadmium. SBH has slightly higher capability towards them.

FT-IR spectra showed that SFH and SBH have similar functional groups on their surface, for example –C–O alcohols and carboxylic acids, O–H groups, amide groups and –C=O from carboxyl or ester groups.

Initial metal content of the biosorbents always should be investigated before biosorption, because it is not necessarily equals zero and can cause disturbances on the isotherms and in the process itself.

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CHARACTERISTICS AND UTILIZATION OF ALGAE IN BIOMETALLURGY AND WASTEWATER TREATMENT

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Abstract

Large amounts of water used for municipal, agricultural and industrial purposes result in issues due to the large volumes of wastewater generated. Wastewaters contain high levels of various substances – nutrients, heavy metals and organic pollutants. In recent years, there has been a growing interest in developing biotechnology based on algae. Phycoremediation is a process of use of algae for the removal or biotransformation of pollutants from wide variety of wastewater. Biosorption and bioaccumulation are the most frequently used mechanisms. Laboratory research focuses on the use of algae in biometallurgy and wastewater treatment, indicated by the amount of published scientific literature. However, currently there is no comprehensive and transparent system describing algae studies and used in this area. The present work contains 36 information lists of algal species and genera used in treatment processes of pollutants from wastewaters, and it provides the first comprehensive systematic review of algae studied in wastewater treatment in laboratory or semi / pilot - scale.

Keywords: Algae, wastewater, treatment, nutrients, heavy metals, organic pollutants

1 Introduction

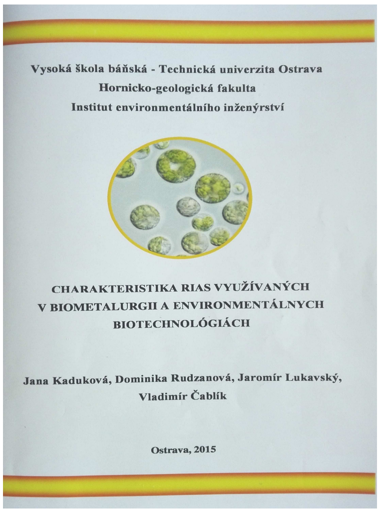
One of the main problems of the society in the 21st century is an environmental pollution. Wastewaters contain high levels of various substances and when they get into the environment, they can cause major problems. The untreated municipal wastewaters have high concentration of nutrients, mainly nitrogen and phosphorus, causing eutrophication of water bodies and can upset the balance of the aquatic ecosystem. Heavy metals are the important environmental pollutants, many of them are toxic at very low concentration [1-3]. Many industrial activities, such as metal plating, mining operations, fertilizer industry introduce heavy metals to environment via their waste effluents. The most conventional wastewater treatment technologies are based on chemical and physical methods. However, application of these methods is often restricted because of technical or economical constraints. Organic pollutants are introduced into the aquatic environment as a result of human activities involving fuel use, textile industry, agricultural industry, oil industry and many more [1, 2].

In recent years, there has been a growing interest in developing biotechnology based on algae, that are less expensive and more "environmental friendly technologies". Phycoremediation may be defined as the use of algae for the removal or biotransformation of pollutants, including nutrients, heavy metals and xenobiotics from wastewater. Algae have been proven to be efficient in removing nitrogen, phosphorus, toxic metals and organic pollutants from wide variety of wastewaters. Biomass obtained in this way may serve as a source of biofuel or feed [1, 2]. Biosorption and bioaccumulation are the most frequently used mechanisms. They include interactions between the particular pollutant (toxic metal or organic pollutant) and biomass, either living (bioaccumulation) or dead (biosorption). Also, algae play an important role in controlling and monitoring of pollutants in aquatic environments [1].

Laboratory research focuses on the use of algae in biometallurgy and wastewater treatment. The aim of present study is summarize all available information about characterization and utilization of algae used in wastewater treatment and biometallurgy from scientific literature.

2 Book review

Laboratory research focuses on the use of algae in biometallurgy and wastewater treatment indicated the amount of published scientific literature. However, currently there is no comprehensive and transparent system describing algae studied and used in this area. The present work is focused on the summarization of all available information about characterization and utilization of algae used in wastewater treatment and biometallurgy from scientific literature. This work has been written in collaboration with VŠB – Technical University of Ostrava and it is available as a book in print version for students and scientists. The present work provides the first comprehensive systematic review of algae studied in wastewater treatment in laboratory or semi / pilot - scale.



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CHARAKTERISTIKA RIAS VYUŽÍVANÝCH
V BIOMETALURGII A ENVIRONMENTÁLNYCH
BIOTECHNOLÓGIÁCH

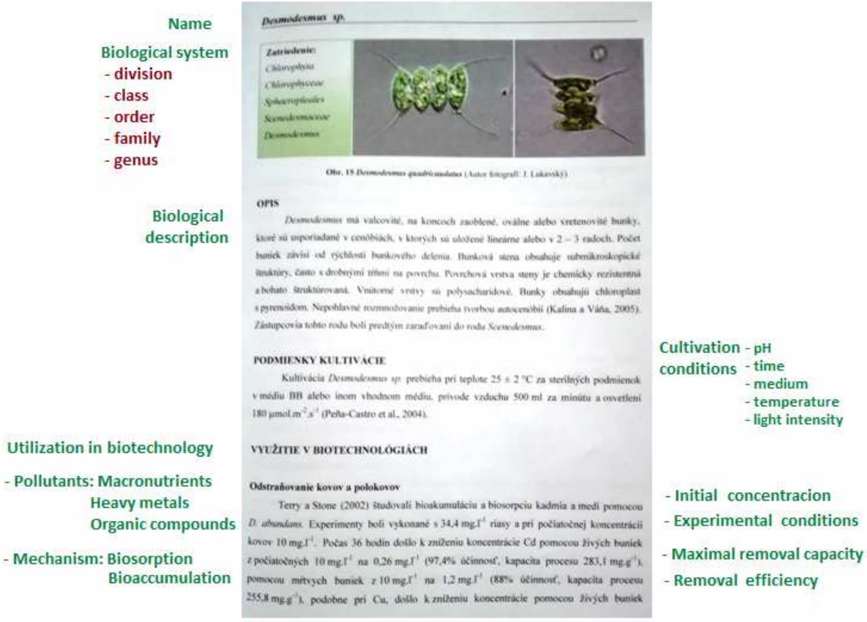
Jana Kaduková, Dominika Rudzanová, Jaromír Lukavský,
Vladimír Čablík

Ostrava, 2015

Fig. 1. Characteristics and utilization of algae in biometallurgy and biotechnologies in print version [1]

The present work is divided into three major sections:

- a general biological description, description of bioaccumulation and biosorption, a short general characterization of removal of pollutants and utilization of algae used in wastewater treatment and biotechnologies,
- "Atlas of algae" - 36 information lists of algae species or genera, which contain basic biological information, cultivation conditions, utilization of algal genera or species for removal of pollutants. An example of information list is shown at Fig. 2.



Desmodesmus sp.

Zatriedenie:
Chlorophyta
Chlorophyceae
Sphaeropleales
Scenedesmaceae
Desmodesmus

Obr. 15 *Desmodesmus quadricaudatus* (Autor fotografií: J. Lukavský)

OPIS

Desmodesmus má valcovité, na koncoch zaoblené, oválne alebo vretenovité bunky, ktoré sú usporiadané v cenóbiách, v ktorých sú uložené lineárne alebo v 2 – 3 radoch. Počet buniek závisí od rýchlosti bunkového delenia. Bunková stena obsahuje submikroskopické štruktúry, často s drobnými tŕňmi na povrchu. Povrchová vrstva steny je chemicky rezistentná a bohato štruktúrovaná. Vnútorné vrstvy sú polysacharidové. Bunky obsahujú chloroplast s pyrenoidom. Nepohlavné rozmnožovanie prebieha tvorbou autocenóbií (Kalina a Váňa, 2005). Zástupcovia tohto rodu boli predtým zaraďovaní do rodu *Scenedesmus*.

PODMIENKY KULTIVÁCIE

Kultivácia *Desmodesmus sp.* prebieha pri teplote 25 ± 2 °C za sterilných podmienok v médiu BB alebo inom vhodnom médiu, prívode vzduchu 500 ml za minútu a osvetlení 180 µmol.m^{-2}.s^{-1} (Peña-Castro et al., 2004).

VYUŽITIE V BIOTECHNOLÓGIÁCH

Odstraňovanie kovov a polokovov

Terry a Stone (2002) študovali bioakumuláciu a biosorpciu kadmia a medi pomocou *D. abundans*. Experimenty boli vykonané s 34,4 mg.l^{-1} riasy a pri počiatočnej koncentrácii kovov 10 mg.l^{-1}. Počas 36 hodín došlo k zníženiu koncentrácie Cd pomocou živých buniek z počiatočných 10 mg.l^{-1} na 0,26 mg.l^{-1} (97,4% účinnosť, kapacita procesu 283,1 mg.g^{-1}), pomocou mŕtvych buniek z 10 mg.l^{-1} na 1,2 mg.l^{-1} (88% účinnosť, kapacita procesu 255,8 mg.g^{-1}), podobne pri Cu, došlo k zníženiu koncentrácie pomocou živých buniek

Fig. 2. An example of information list [1]

- summary tables of algae used in wastewater treatment with original source of literature for quick search.

3 Phycoremediation

The ability to survive in polluted wastewaters containing high quantities of various pollutants makes algae a sustainable platform for wastewater treatment. Phycoremediation may be defined as the use of algae for the removal or biotransformation of pollutants from wide variety of wastewater and it is the eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling [1, 2].

Phycoremediation comprises several applications: nutrient removal from municipal wastewaters, nutrients and xenobiotic compounds removal with the aid of algae-based biosorbents, treatment of the metal wastewaters, transformation and degradation of xenobiotics and detection of toxic compounds with the aid of algae-based biosensors [2].

The advantages of algae are that they are present in all existing earth ecosystems, they are cheap sorbents and need cheap substrates (sunlight and CO_2) for their growth, what enable them to produce compounds with added value. The algal biomass from the treatment of wastewaters can be easily converted into added value products. Depending by the species used for this purpose, the resulting biomass can be applied for different aims, including the use as additives for animal feed, as biofuel [2] or bioenergy, used in medical, cosmetics or food industry [1].

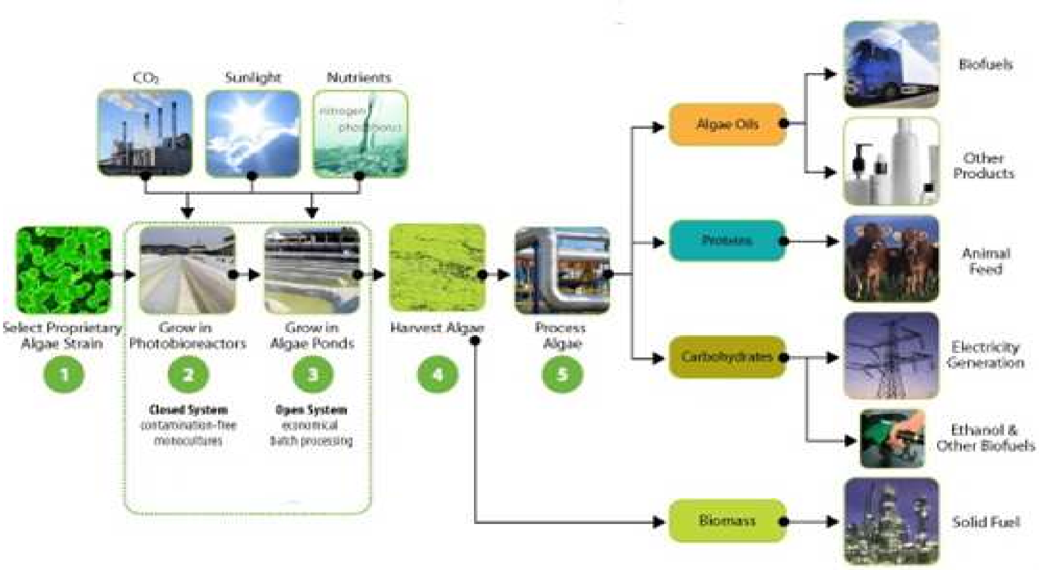


Fig. 3. Utilization of algae [4]

4 Removal of macronutrients (N and P)

The treatment of wastewaters by algae to remove nitrogen and phosphorus was proposed over 50 years ago by Oswald and Gotaas. Municipal and agricultural wastewaters contain high concentration of nitrogen and phosphorus, which lead to eutrophication in water bodies. Eutrophication causes uncontrolled growth of certain aquatic plants and undesirable algal blooms that cause a shift the aquatic environmental equilibrium by negatively affecting the natural nutrient recycling process and can upset the balance of the aquatic ecosystem. However, under controlled conditions the algae offer a cost-effective approach to removing these nutrients from wastewaters [1-3]. Algae can be efficiently used to remove significant amounts of nitrogen and phosphorus for protein (45 - 60 % of algae dry weight), nucleic acid and phospholipids synthesis [1, 2]. Nutrient removal with aid of algae compares very favourably to other conventional technologies [2].

Gonzáles et al. used *A. dimorphus* for phosphorus removal from industrial wastewaters, with removal efficiency of 20 – 55 %, after 9 days and initial concentration 112 $mg.l^{-1}$ [1, 5]. Lavoie and De la Noue studied the ability of *A. Obliquus* in nutrient removal and reported a nutrient efficiency of 79 – 100 % for NH_4^+ and 47-98 % for P [1, 6]. Lau et al. used *Ch. vulgaris* and reported an elimination of NH_4^+ (90 %) and PO_4^{3-} (80 %) in municipal wastewaters during 10 days [1, 7]. Khan and Yoshida observed removal of 99 % NH_4^+ in solution with addition of L – glutamic acid [1, 8]. Other algae used for nutrient removal are in Table 1 [1].

Table 1. Removal of nutrients – nitrogen and phosphorus by some algal species [1]

Macronutrients	Algae	Initial concentration [mg.l^{-1}]	Removal efficiency [%]
NH_4^+	*Acutodesmus obliquus*	27	79 - 100
	Desmodesmus sp.	14 - 44	30 - 100
	Chlorella minuttisima	37	99
	Chlorella vulgaris	3 - 8	60 - 78
		-	> 90
		-	99
		13 - 410	23 - 100
		3 - 36	30 - 95
	Trentepohlia aurea	262	5
NO_3^-	*Chlorella minuttisima*	350	58
	Desmodesmus sp.	14 - 44	30 - 100
	Parachlorella kessleri	168	8 - 19
	Trentepohlia aurea	504	20
PO_4^{3-}	*Desmodesmus sp.*	1.4 - 6	30 - 100
	Dunaliella salina	-	64.7
	Chlorella minuttisima	12.8	99
	Chlorella vulgaris	5 - 8	46 - 94
		112	20 - 55
	Trentepohlia aurea	158	35
Total nitrogen	*Chlorella pyrenoidosa*	267	87 - 89
	Chlorella sp.	100 - 240	76 - 83
Total phosphorus	*Acutodesmus obliquus*	12	47 - 98
	Chlorella pyrenoidosa	56	70
	Chlorella sokoriniana	22	45 - 72

4.1 Cultivation of algae

Basically there are two main commercial cultivation systems for algae: open raceway ponds and closed photobioreactors.

Algal high-rate ponds (HRAP) were developed by Oswald, beginning in the 1950s for municipal wastewater treatment and have been used for over 60 years [1, 2, 3, 9]. HRAPs were designed to promote the symbiosis between algae and aerobic bacteria in a wastewater treatment processes. Open ponds are simple open tanks or ponds and they are relatively simple in construction and operation compared to closed photobioreactors. Algae growth in open raceway ponds is cheap and amenable to nutrient removal in domestic wastewaters [2]. There are 2 - 3 m wide and 0.1 – 0.3 m depth [3]. The circulation is done with the help of a paddle wheel. Algal biomass can be harvested for animal feed or used as biofuel [2]. There are in Mexico, California, Hawaii, Japan or Australia [1].

Fig. 4. Large scale commercial algae production in outdoor ponds [10]

The photobioreactors or PBRs are used to cultivate algae under controlled conditions in closed systems. These systems can be constructed as tubular or flat plate and horizontal or vertical column types. The cultures are less susceptible to contamination and evaporative losses are very low as compared to open ponds [9].

Fig. 5. Closed systems for algae cultivation: flat-plate, horizontal tubular and polybags [10]

There is a wide array of harvesting methods that can be employed to harvest algal biomass such as centrifugation, flocculation, sedimentation, filtration and any combination of these [2].

5 Removal of heavy metals

Heavy metals are the important environmental pollutants, many of them are toxic at very low concentration. Many industrial activities, such as metal plating, mining operations, fertilizer industry introduce heavy metals to environment via their waste effluents. Algae can be efficiently used to remove heavy metals from wastewaters.

There is a long list of sorbates which can be removed by biosorption and bioaccumulation: Ag, Al, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Mo, Ni, Pt, Se, U, V, Zn [1]. The removal of heavy metals by algae is therefore well described in the literature (Table 2).

Luptáková and Kaduková studied biosorption and bioaccumulation of Cu from model solutions using *P. kessleri*. Maximal biosorption capacity for dry algae was 10.87 mg.g^{-1} (86.5 %) and for bioaccumulation from 1.7 mg.g^{-1} (89.3 %) to 7.6 mg.g^{-1} (99.6 %) [1, 11]. *H. valentiae* has been successfully used for biosorption of Cs. The maximal sorption capacity of Cs was 71.9 mg.g^{-1} [1, 12].

Table 2. Removal of heavy metals by algae [1]

Metal	Algae	Initial concentration [mg.l^{-1}]	Capacity [mg.g^{-1}]	Removal efficiency [%]
Ag	*Parachlorella kessleri*	48.5	12.1	70
B	*Caulerpa recemosa*	8	0.78	78
Cd	*Acutodesmus incrassatulus*	3.5	0.84	24.1
	Caulerpa lentilifera	11.2	0.95	90.9
	Parachlorella kessleri	26.5	8.7	65.8
	Sargassum muticum	250	78.7	78.7
Cr	*Acutodesmus incrassatulus*	1.2	0.63	52.7
	Laminaria digita	250	42	33.6
Cs	*Hypnea valentiae*	20-500	71.9	-
	Padina australis	20-500	16.2	-
	Caulerpa lentilifera	6.35	0.2	69.9
	Ecklonia maxima	30-300	92.6	-
	Parachlorella kesslerri	15.4	10.9	86.5
		0-5000	1260	-
Eu	*Sargassum polycystum*	460	151	65.7
La	*Sargassum polycystum*	460	138	60
Pb	*Caulerpa lentilifera*	20.7	9.67	90.9
	Pithophora oedogonia	10	9.1	91
Rh	*Ulva lactuca*	-	$5.2.10^{-3}$	-
U	*Acutodesmus obliquus*	5.5	12,4	-
Yb	*Sargassum polycystum*	460	121	52.6
Zn	*Caulerpa lentilifera*	10	-	88
	Ulva fasciata	100	26.8	89.2

Biosorption and bioaccumulation are the most frequently used mechanisms for removal of metals from the solutions and they differ in that in the first process pollutants are bound to the surface of cell wall and in the second, they become also accumulated inside cell. Biosorption is a metabolically passive process [1, 13]. Bioaccumulation is metabolically active and is performed by living cells, and goes further, the first step is biosorption and then subsequent stages occur, related with transport of pollutants into inside of cells and eventually the concentration of cells increases [13].

5.1 Biosorption

Biosorption is metabolically passive process and is performed by not living biomass. The process is simple in operation and very similar to conventional adsorption or ion exchange, except that sorbent of biological origin is employed. Biosorption is a quick process. The equilibrium is usually reached within few minutes. Biosorption can be performed by immobilized biosorbents. Biosorbents are selective and regenerable and a process is in particular highly effective in the treatment of dilute effluents [1].

The factors which influence biosorption performance include the type of biomass, pH, temperature, presence of other competing ions [13]. The advantages of the process include low operation costs if low-cost sorbents are used, low quality of sewage sludge disposed; COD of wastewater does not increase. Disadvantages include additional costs, higher mechanical diffusion resistance, lower capacity, interaction of carrier with active sites.

There are some commercial biosorbents available on the market: AlgaSORB and AMT-BIOCLAIM. AlgaSORB is produced by the company Biorecovery Systems. The other commercial available biosorbent is BIOCLAIM [1, 13].

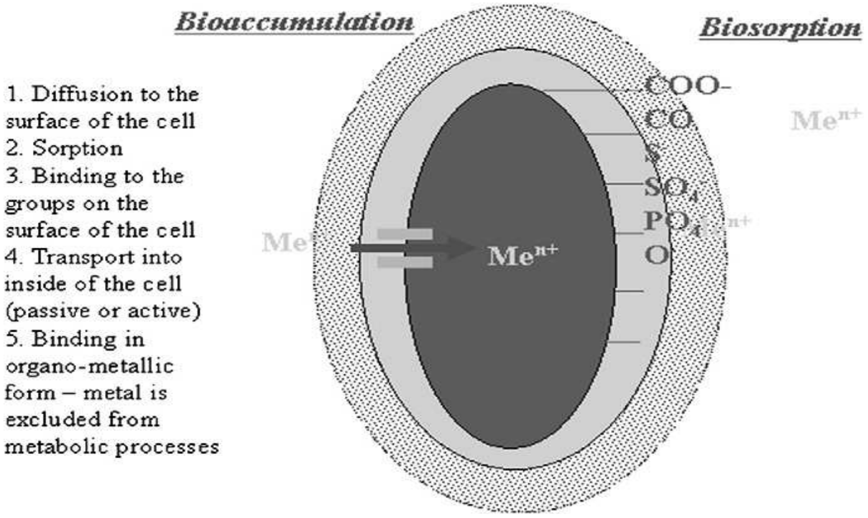


Fig. 6. The mechanism of biosorption and bioaccumulation [13]

5.2 Bioaccumulation

Bioaccumulation can be defined as the cultivation of an organism in the presence of sorbate. It is the intracellular accumulation of sorbate, which occurs in two stages: the first identical with biosorption which is quick, and the subsequent, which is slower and includes transport of sorbate into inside of cells. The process is more complex than biosorption [1, 13].

The advantage of bioaccumulation is that it is not necessary to include a separate biomass cultivation mode or harvesting biomass from the environment. The process of removal of pollutants is highly affected by the operational conditions, in particular by the presence of pollutants in the growth medium which can inhibit the growth of the cells and also bioaccumulation itself. This is a limitation of the process, because it makes it impossible to treat solution with high load of pollutants. Moreover, it is necessary to supply external source of energy to growing cells [13].

6 Removal of organic pollutants

Organic pollutants are introduced into the aquatic environment as a result of human activities involving fuel use, textile industry, agricultural industry, oil industry and many more. In some cases, the algae do not participate on biodegradation of organic pollutants, directly, but they are suitable medium for bacteria. In system algae – bacteria, the algae are responsible for removal of macronutrients, heavy metals or pathogens and they supply bacteria with oxygen. Bacteria are responsible for degradation of organic compounds and produce CO_2, which is used by algae during photosynthesis [1].

Ozer et al. studied the biosorption of dyes Acid Red 274, methylene blue, Acid Red 337 and Acid Blue 324 from wastewaters from textile industry by green algae *E. prolifera* [1, 14, 15]. Green algae *M. braunii* is promissing species for the treatment of wastewaters which contain Bisphenol A [1, 16]. The algae *C. indica, S. glaucescsens* and *S. marginatum* showed great removal efficiency for Acid Black 1 – 91 %, 94 % and 96 % [1, 16].

Marungrueng and Pavasant have investigated the adsorption of dyes Astrazon Blue, Astrazon Red and methylene blue by *C. lentilifera*. *C. lentilifera* exhibited greater sorption capacity than activated carbon, for Astrazon Blue 38.9 mg.g^{-1}, Astrazon Red 47.9 mg.g^{-1} and for methylene blue 417 mg.g^{-1} [1, 17]. The ability of algae to removal organic pollutants is shown in Table 3 [1].

Table 3. Removal of organic pollutants by algae [1]

Organic compounds	Algae	Initial concentration [mg.l^{-1}]	Capacity [mg.g^{-1}]	Removal efficiency [%]
Acid Black 1	*Cystoseira indica*	10	8.39	91
	Sargassum glaucescens	30	4.7	94
	Stoechospermum marginatum	30	5.78	96
Acid Blue 9	*Turbinaria conoides*	100	21.3	63.8
Acid Blue 25	*Jania rubens*	20	17.2	68.6
Acid Blue 324	*Enteromorpha prolifera*	100	160.6	80.3
Acid Red 274	*Enteromorpha prolifera*	300	244	40.7
Acid Red 337	*Enteromorpha prolifera*	100	210.9	100
Astrazon Blue	*Caulerpa lentilifera*	-	38.7	-
		1800	94.3	87
Astrazon Red	*Caulerpa lentilifera*	-	47.6	-
		1800	113.6	100
Bisphenol A	*Monoraphidium braunii*	2	-	39
		4	-	48
		10	-	35
Methylene blue	*Caulerpa recemosa*	50	5.6	93
	Enteromorpha prolifera	50	10	100

7 Conclusions

Many research reports and articles described many advantages of using algae in environmental biotechnologies. Algae can be successfully used in wastewaters treatment. They are able to remove various types of pollutants, such as nutrients, heavy metals and organic compounds from domestic and industrial wastewaters. Algae are easy to cultivate and can growth almost anywhere, they are cheap biosorbents and their biomass can have many commercial applications, such as biofuel, animal feed or be used in pharmaceutical, medical, food or cosmetics industry. Also, algae play an important role in controlling and monitoring of pollutants in aquatic environments.

Laboratory research focuses on the use of algae in biometallurgy and wastewater treatment indicated the amount of published scientific literature. However, currently there is no comprehensive and transparent system describing algae studied and used in this area. The present work contains 36 information lists of algae used wastewater treatment with 137 notes of nutrients (16), heavy metals (98) or organic compounds (23) removal from domestic and industrial wastewaters and provides the first comprehensive systematic review of algae studied in wastewater treatment in laboratory or semi / pilot - scale.

Acknowledgements

The work has been supported and written in collaboration with VŠB – Technical University of Ostrava and it is available as a book in print version for students and scientists.

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BIOHYDROMETALLURGY – PRACTICAL APPLICATION

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Abstract

Methods of microbiological leaching enable to experimental exploitation of poor sulphides ores of cobalt, molybdenum, nickel or zinc. However, on the industrial scale such methods are used only to process the ores of copper, gold, uranium, zinc and nickel. In the article short characteristics and processing parameters of such methods are presented. Biological processes are also used to recover metals from different kinds of waste. Biohydrometallurgical treatment of waste has a lot of advantages such as: limitation of the need for natural sources, energy or waste landfill. Bioleaching is cheaper and simpler than the conventional methods. The possible metals that could be recovered by bioleaching can be divided into four groups: basic metals (Cr, Pb, Zn, Cu, Ni), precious metals (Au, Ag and Pt), special metals (rare earth metals, Li) and radionuclide. In the article characteristics concerning the possibilities of recovery of the mentioned metals from different types of waste was shown.

Keywords: biohydrometallurgy, bioleaching, waste treatment.

1 Introduction

Since the fifties of the twentieth century it have been investigating the recognition and industrial application of bacterial leaching processes – they forming the basis of the modern biohydrometallurgy used to utilize metals from poor ores, waste or off-balance sheet resources. The earliest reports talk about the unconscious using of the bacteria to obtain copper(II) sulphate(VI) from ores containing sulphur compounds, (to obtain copper the solution is then precipitated by iron). Such technique was applied in China in II century BC, although unconsciously biohydrometallurgical recovery of copper from ores was used significantly earlier – in XI BC by Phoenicians. The area of south-west Spain with the mines in Rio Tinto is seems to be the cradle of biohydrometallurgy. Such area was exploited from early ancient times as deposits rich in copper, silver and gold. From the eighteenth century copper bioleaching in Rio Tinto was conducted on an industrial scale [1-3]. The low quality ores, containing 0.75 % of Cu, was formed in heaps (10 m of height) and left in that the atmospheric factors influence on them from one to three years. Using this method 20 to 25 % of copper was recovered yearly. Table 1 presents short historical reports concerning biohydrometallurgy.

Table 1. Breakthroughs in the biohydrometallurgy [1-3]

Period/year	Happening
1000 BC	Phoenicians – copper recovery
100-200 BC	China – recovery of copper from mine drainage probably using the method of precipitation by iron
23-79 AD	Gaius Plinius II (the Older) – copper recovery from drainage water coming from mines
166 AD	Galen (Claudius Galenus) – in situ bioleaching on Cypress
500-1200	Aztec, Maya – using the plants juice to dissolved copper oxides
1493-1541	Paracelsus – copper bioleaching from ores in the area of formerly Hungary (today Slovak Republic)
1494-1555	Agricola – in situ bioleaching, crystallization of metals sulphtes
1752	Spain – copper bioleaching in Rio Tinto
1922	Waksman, Joffe – isolation of bacteria oxidizing sulphur (*Acidithiobacillus thiooxidans*) from soil
1947	Colmer, Hinkle – isolation of the bacteria of *Acidithiobacillus* strain, giving proofs of bacterial catalytic iron oxidation and sulphuric acid production
1970	Toromocho, Peru – bioleaching in heap and dump
1985	Minera Pudahuel, Chile – first plant basing completely on bioleaching technology
1994	Youanmi mine, Australia – thermophilic bacteria was used in industrial scale to treat gold concentrates
1995	The commercial bioleaching of chalcopyrite was first used

2 The main information about biohydrometallurgy

2.1 Characteristics of the process

Biohydrometallurgy has been developed basing on the interdisciplinary studies taking a lot from different scientific disciplines such as: biochemistry, physical chemistry, geochemistry. Today it includes bioleaching and biosorption processes for metal recovery. Bioleaching is mainly based on microbiological oxidation of insoluble metals sulphides and transferring them into soluble sulphates ($MeS \rightarrow MeSO_4$), oxidation of the elemental sulphur and other reduced sulphur compounds, oxidation of iron ions Fe^{2+} and the formation of organic and inorganic compounds enabling the mineral disintegration due to dissolution or creation of complex compounds. Biosorption allows to concentrate metals from solutions by microorganisms inside the cell (heavy metals) [4].

The choice of bioleaching methods depends on the ores quality or the size of ore particles. For low quality ores (containing 0-2 % of metal) the following methods are commonly used: in situ, bioleaching in heaps and dumps. For concentrates and high quality ores (2-5 %) the more expensive way of bioleaching is applied – in tanks, tanks with stirring, in tanks linked in series. Fig. 1 shows the main characteristic features of the mentioned methods.

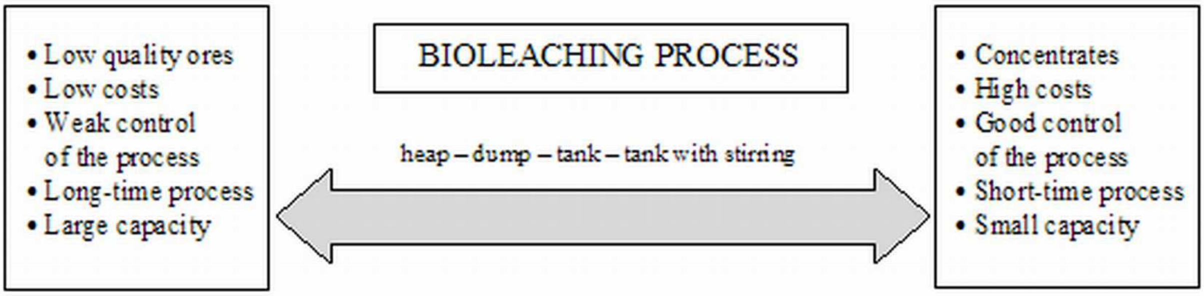

Fig. 1. Main characteristic features of bioleaching methods [2]

Bioleaching process depends on many physicochemical factors such as: bacteria concentration, oxidizing agent concentration, temperature or pH. The more details presents Table 2.

Table 2. Factors influencing the bioleaching process [1, 2]

Factor	Parameter
Physicochemical and microbiological factors	Temperature, pH, redox potential, carbon dioxide, oxygen accessibility, availability of nutrients, mass transfer, surface tension, presence of inhibitors, pressure, iron ions concentration, variety of microorganisms, activity of bacteria, tolerance for metals and their adaptation.
Properties of the mineral	The type of mineral, composition, particle size and its release, the size of particles and surface porosity, water repellency, the presence of additional minerals, the speed of leaching is increased with the decreased size of particles (the bigger surface, the higher speed of mass transfer and oxidation, higher adhesion of bacterial cells).
Bioleaching	Procedure of bioleaching, pulp density (bioleaching rapidity decreases with the increase of pulp density – lower mass transfer speed, high effect of attrition), stirring speed (reactor), geometry of dump/heap.

2.2 Advantages and disadvantages

Bioleaching is a cheap method, especially direct in situ methods, and needs substantially lower investments taking into account the traditional refining and melting processes. The rest advantages of the process are following:

- it does not require large amount of energy;
- it has a lower technological requirements, there is no need to apply expensive, complicated apparatus;
- it is cleaner and more environmental friendly, there is no emission of sulphur dioxide in the process of sulphides leaching;
- there is no need of high pressure or introducing and keeping high temperature;

- continuous regeneration of leaching agent;
- the remains after bioleaching are less chemical active than in physicochemical processes;
- easy to expand the further piles in case of dump and heap;
- ideal for poor sulphides ores and waste, typical composition of solutions after leaching is: 2-3 g/dm^3 iron, several g/dm^3 other useful metals such as Zn, Ni, Cu, 20-30 g/dm^3 sulphates, population – 106 bacteria per dm^3, the obtained solutions are treated by classical hydrometallurgical methods to recover metals.

Apart from the advantages also drawbacks should be mentioned, for example the time of process course (slow process compared to traditional methods), which could be several years. Other disadvantages are the following:

- climate dependence;
- toxic influence of heavy metal on the microorganisms;
- possibilities of leakage and migration of acid solution and metals to the environment.

3 Practical application of biohydrometallurgy

Up to now, the worked out technologies of bioleaching enable to extract many metals from waste materials or low quality ores. Bioleaching process is applied for treatment of such metals as: copper, uranium, zinc and nickel. Biological oxidizing is used to process pyrite and arsenopyrite gold minerals that are hardly leached. Apart from technologies, which find practical applications, there are constant works, which aim is to look for new methods and develop existing technical solutions of metals bioleaching. Table 3 presents the most important technologies applied in industrial scale and being in experimental or pilotage phase. Table 4 shows the characteristics of bioleaching process of ores and concentrates of such metals as: copper, nickel, cobalt, gold and uranium; whereas Table 5 presents the example of industrial plants using bioleaching processes.

Table 3. Biohydrometallurgical technologies applied over the world [2, 5-8]

Technology owner	Name	Application – kind of mineral / technique	
BHP Billiton Ltd.	BioCOP™ Process	Cu	Copper sulphides Oxidation and leaching in tank with stirring
	BioNIC™ Process	Ni	Nickel sulphides Oxidation and leaching in tank with stirring
	BioZINC™ Process	Zn	Zinc sulphides Oxidation and leaching in tank with stirring
Newmont Mining	BIOPRO® Process	Au	Ores that are hard processed Heap leaching
Gold Fields, Ltd	BIOX® Process	Au	Ores that are hard processed Oxidation in the tank with stirring
BacTech Environment	BacTech/Mintek Process	Cu	Copper sulphides Oxidation and leaching in tank with stirring
GeoBiotics, Inc.	GEOCOAT® Process	Au Cu	Sulphides ores and concentrates Heap leaching

4 Possibilities of using biohydrometallurgy to recover metals from waste

Together with bioleaching the primary materials conducted on the industrials scale scientists teams are carrying out research [13-18], which main aim is to find out possibilities and effectivity of metals bioleaching process from many secondary materials (waste). Such big interest results in advantages of biological methods. Biohydrometallurgical treatment of waste is similar to natural geochemical cycles of metals, decreases the demand for natural sources (ores of metals), energy and places of waste deposits.

Research finding the possibility of metals extraction from waste materials using microorganism (see Table 6) were carrying out for a 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) [18]. Such waste is treated as a complex material containing not also a basic metals but also precious metals as well as dangerous substances. Metals, that were analyzed taking into account their possible recovery, can be divided into the following categories:

a) basic metals (e.g. Cr, Pb, Zn, Cu, Ni),
b) precious metals (Au, Ag, Pt),
c) special metals (Li, rare earth elements REEs),
d) radionuclides (Th, U).

Table 4. Bioleaching of the chosen ores of metals [2, 5-12]

Metal	Characteristics
copper	The oldest bioleaching plants were used to process very poor copper ores; later the bioleaching process was also adopted to the copper concentrates; process was conducted in heaps or appropriate bioreactors; in 2006 there was more than 20 installation to obtain copper via bioleaching; these plants produce totally more than 2 million Mg of copper; oxidation process is carried out mainly in the presence of bacteria: *A. ferrooxidans*, *Leptospirillum ferriphilum*, *A. thiooxidans* and *L. ferrooxidans*; *A. caldus*, which introduction to the leaching system considerably improves the kinetics of oxidation by the participation in reaction of iron (III) and sulphuric acid (VI); the bioleaching conditions: relatively high Eh potential (about 650 – 700 mV), pH (more than 2) can promote the precipitation of iron in the form of hydroxide, iron hydroxy-sulphates, including jarosites.
gold	Biological oxidation of sulphides minerals belongs to the processes of primary treatment of very heavy leachate gold ores carried out for example in such countries like: Australia, Brazil, Ghana, Peru or RSA. This technology is basing on processing the arsenopyrite and pyrite ores with chemoautotrophic bacteria (in 2001 there was more than 10 industrial plants applying such technologies), it replaced roasting oxidation processes, which were in many cases very harmful for the environment (emission of sulphur oxides and arsenic). Bioleaching can be also an effective alternative for treatment of nonconventional materials and waste containing gold, which processing in traditional way is not profitable: – ponds located near mines gathering through many years waste, which can contain even 1 g gold per 1 Mg of material, – lower quality gold ores containing less than 1-2 g Au per 1 Mg of material, – very hardly treated gold materials, in which small gold particles are dispersed and captured in the matrix of sulphides minerals, that is way their treatment is not effective using direct method of cyanide solving.
nickel and cobalt	The extraction of cobalt using biological methods was commercialized and introduced into market in 1999 as a pilotage project Kasese in Kilembe mine (Uganda). In this technology bioleaching is carried out in reactors with continuous stirring using mesophilic bacteria oxidizing iron. The plant is using the solvent extraction and electrolysis to recover cobalt from solution after bioleaching. Nickel is found in nature mainly in the form of sulphides or laterite ores. Both forms can be bioleached in heap or bioreactors (for nickel concentrates). In Finland in 2005 there was introduced a bioleaching technology Talvivaara to process sulphide nickel ores. This technology is based on bioleaching in heap using endemic bacteria settling the heap and well-adjusted to the existing atmospheric condition (subarctic climate). Process is conducted in two stages containing primary leaching lasting from 15 to 18 months (80 % of Ni recovery) and then secondary leaching lasting additionally 3.5 years (Ni recovery more than 90 %).
uranium	Commercial bioleaching of uranium has been conducted from 1957 – in Urgeirica in Portugal it was initialized obtaining this metal using poor uranium ores. During the next years many other countries such as: Canada, Australia, Nigeria, Russia, Namibia, Uzbekistan developed the technologies of uranium leaching using microorganism. Today two biggest suppliers of uranium (Canada and Australia) have obtained more than 50 % of the total uranium production. The extraction process is conducted periodically by watering in heap, dump or pouring the underground tunnels by leaching agent. The used microorganism consortium consists of mixture of acidophilus, autotrophic and heterotrophic strains. In the microbiological leaching bacteria *A. ferrooxidans* oxidizing the pyrite phase (additives to uranium ore) to iron (III) and in the same time sulphur to sulphuric acid solving in that way the uranium from ore.

The more and more attention is paid to Rare Earth Elements (REEs). REEs are identified as the most critical raw materials – they world demand in 2015 was about 210000 tones. Additionally, almost 97 % of total output is in one country – China. Better understanding of the biological role of REEs and their acquision by microorganism can allow the development of bioremediation strategies for cleanup of REE mining sites and biorecovery of REEs from waste (water) [19-21]. Table 7 shows the microbe-metal interaction for immobilization of REEs.

Table 5. Plants applying bioleaching processes for copper ores, gold concentrates and uranium ores [2, 5-12]

Metal	Mine, country, date of introduction, applied bioleaching method, production
Cu	Cerro Colorado, Chile (1993) heap, 115 000 tones Cu/year
	Ivan Zar, Chile (1994) heap, 12 000 tones Cu/year
	Zaldivar, Chile (1998) heap/dump, 150 000 tones Cu/year
	Cerro Verde, Peru (1977), heap, 54 200 tones Cu/year
	Nifty Copper, Australia (1998), heap, 16 000 tones Cu/year
	Morenci, Arizona USA (2001), leaching in mine, 380 000 tones Cu/year
	Whim Creek and Mons Cupri, Australia (2006), heap, 17 000 tones Cu/year
	Phoenix deposit, Cyprus (1996), heap bioleaching, 8 000 tones Cu/year
Au	Fairview, Barberton, South Africa (1986), BIOX, 62 tones
	Wiluna, Australia (1993), BIOX, 158 tones
	Ashanti, Obuasi, Ghana (1994), BIOX, 960 tones
	Beaconsfield, Tasmania, Australia (2000), BacTech Bacox, ~ 70 tones
	Laizhou, Shandong Province, China (2001), BacTech Bacox, ~ 100 tones
	Suzdal, Kazakhstan (2005), BIOX, 196 tones
	Kokpatas, Uzbekistan (2008), BIOX, 1069 tones
Co	Kilembe, Uganda, 241 tones/day
Ni	Talvivaara Mining, Finland, 33 000 tones Ni
U	Rio Algom, Canada, heap
	Olympic dam, Australia, heap
	Agnew Lake, Canada, mine
	Ranger, Australia, dump
	Denison, Canada, mine
	Beverley, Australia, heap
	Figueria, Brazil, dump
	Stepnogorsk, Kazakhstan, dump
	St. Pierre, France, dump
	Southern ISL, Kazakhstan, heap

Table 6. Examples of waste being bioleached [2, 18]

Group of metal	Kind of waste	Leachate metal	Using microorganism
Basic metal	Fly ashes	Zn, Al, Cd, Cu, Ni, Cr, Pb, Mn, Fe	*A. niger, A. thiooxidans* + *A. ferrooxidans*
	Tannery sludge	Cr	*A. thiooxidans*
	Plating sludge	Cu, Ni, Zn, Cr	Bacteria oxidizing iron
	Slag from steel production (convertor), slag from Cu production	Zn, Fe, Cu, Ni	*Acidithiobacillus* and *Leptospirillum* bacteria strain
	Electronic slag	Cu, Ni, Al, Zn	*A. ferrooxidans* *A. ferrooxidans* + *A. thiooxidans*
	Ni-Cd batteries	Ni, Cd	*A. ferrooxidans*
	Spent catalyst from cracking	Al, Ni, Mo, V, Sb	*A. niger, A. thiooxidans*
Precious metal	Jewellery waste, spent auto catalyst, electronic scrap	Ag, Au, Pt	*C. violaceum, P. fluorescens, P. plecoglossicida*
Special metal	Li-ion batteries	Li, Co	*A. ferrooxidans*
	Bauxite residue	As, Ba, Cr, Cu, Ni, Pb, Zn	*A. niger*
	Bauxite residue	Sc, Y, La, Nd, Ga, Yt	*Penicillium tricolor*
Radionuclides	Bauxite residue	U, Th	*Penicillium tricolor*

Among the possibilities of metals bioleaching in acid environment the more and more role in biohydrometallurgical techniques play microorganisms able to create cyanic acid (HCN) [2]. Bacteria *Chromobacterium violaceum* or *Pseudomonas fluorescens* have such ability. On the contrary of using microorganisms having ability to oxide sulphur compounds and create sulphuric acid or heterotrophic microorganisms creating organic acids when using cyanogenic bacteria it is possible to mobilize metals from solid phase in basic environment. For solving metals alkaline conditions can be favorable – the behavior of metals comparing with the acidic environment can be totally different than in basic environment for example in the range of solubility, mobility, sorption, precipitation or creation of secondary minerals. Especially taking into account the cyanide it is easier to recover metals e.g. by sorption on active carbon. Additionally, the created eluates with basic character are less aggressive to the environment. Table 8 presents the results of experimental research of bioleaching process of precious metals using cyanogenic bacteria.

Table 7. Microbe-metal interaction form immobilization of REEs [19]

Metal	Organism/mixed culture	Metal source	Biochemical process / remarks
Dy	*Penidiella* sp.T9	$DyCl_3$	Bioaccumulation / this fungi strain showed high bioaccumulation of Dy from acidic solutions and selectivity for REEs
	P. aeruginosa	$DyCl_3$	Biosorption / capacity of 1000 mmol Dy /g at pH5
La	*Pseudomonas aeruginosa, Bacillus subtilis, Myxococcus xanthus, Myxococcus smegmatis, E. coli, Pseudomonas sp.*	$La(NO_3)_3$	Biosorption / La(III) removal from aqueous solutions. *Pseudomonas* sp. showed loading as high as 950 mg La per g of dry biomass at pH 5
Nd	*P. aeruginosa*	$NdCl_3$	Biosorption / removal of Nd(III) from pH 5 water
Sc	*Saccharomyces cerevisiae, Rhizopus arrhizus, Aspergillus terreus*	$ScCl_3$	Biosorption / Sc(III) removal from low pH (0.6 to 3) water
Eu	*P.aeruginosa, Myxococcus xanthus*	$EuCl_3$	Biosorption / Eu(III) removal from waters with low pH
Yb	*M. smegmatis*	$YbCl_3$	Yb(III) removal from acidic waters

Table 8. The level of leaching the precious and basic metals from waste using iron, sulphur and cyanogenic bacteria [2]

Using microorganism	Waste	Level of metals leaching
A. ferrooxidans + *A. thiooxidans*	Electronic scrap	Cu, Ni, Al, Zn > 90 %
A. ferrooxidans	Electronic scrap	Cu - 99 %
Sulfobacillus thermosulfidooxidans	Electronic scrap	Ni - 81 %, Cu - 89 %, Al - 79 %, Zn - 83 %
C. violaceum	Electronic scrap	Au -14.9 %
C. violaceum, P. fluorescens, P. plecoglossicida	Electronic scrap, jewellery waste, spent auto catalyst	Ag - 5 %, Au - 65.5 %, Pt - 0.2 %
C. violaceum	Printed circuit boards (phones)	Au - 10.8 %, Cu - 11.4 % with H_2O_2: Au -11.31 %, Cu - 24.6 %
C. violaceum	Printed circuit boards (phones)	Au - 11 % (pH 11) Cu - 11.4 % (pH 10)
C. violaceum	Electronic scrap	Cu - 79 %, Au - 69 %, Zn - 46 %, Fe - 9 %, Ag -7 %
C. violaceum + *P. aeruginosa*	Electronic scrap	Cu - 83 %, Au - 73 %, Zn - 49 %, Fe – 13 %, Ag – 8 %
C. violaceum	Electronic scrap	Au - 30 % (adopted strain) Au - 11 % (wild strain)
C. violaceum	Electronic scrap	Au - 22.5 % (adopted strain) Au - 11 % (wild strain)

5 Conclusions

Exploitation and primary treatment of ore minerals is connected with the generation of great amount of waste, which are deposited on the special landfills. Such waste contains many useful metals, which content is almost no more than 0.5 % (0.1-0.5 % for Cu). Extraction of metals from such waste using physicochemical methods is not profitable. The economic alternative for processing and recovery the precious metals from such waste is to apply the biological leaching methods.

Main argument for applying biohydrometallurgical method is economical factor. 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 sulphur dioxide, low energy consumption or possibilities of applying for not easily accessible sources containing metals. Apart from advantages there are also some disadvantages such as time of process course, which can reach even some years.

Methods of microbiological leaching enable to experimental exploitation of poor sulphides ores of such metals as: cobalt, molybdenum, nickel, zinc. However, today on the industrial scale these methods are used to process ores of copper, uranium, gold, and sometimes even zinc and nickel.

In the biological leaching of waste the identical mechanisms and natural abilities of microorganism are used to transform metals present in the solid matrix of waste to the dissolved form, similarly like in the case of leaching the ore minerals. Bioleaching process is carried out in the acidic environment, therefore the key role in biohydrometallurgical techniques plays the acidophilic microorganisms. 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.

In the last years more and more attention will be paid to research which concerns the microbiological methods of metals recovery from waste. Although these methods are only in experimental stage they still are considered as the most promising and in the same time it is expected they can lead to development of more effective and costless processes. Perspectives of practical using of microorganism potential in leaching of basic and precious metals from secondary waste material require further advanced research work with the participation of microbiologist and specialist from hydrometallurgy.

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SYNERGY OF SCIENCE AND PRACTICE IN REMEDIATION

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ABSTRACT

Starting with original contaminated area until the end of overall site remediation is a lengthy and expensive process, consisting of a large set of interlinked work including main procedures like geological survey for range detection of contamination - risk analysis of pollution - feasibility study of remediation - remediation works - risk analysis after decontamination. Completion of each of these sub-tasks is evaluated by the final report showing general view of the required range and intensity of remediation actions.

All of this can be implemented also in remediation where critical contaminants are complex organic substances with a massive content of PAHs (polycyclic aromatic hydrocarbons). Possible dilemma occurs here due to conventional comparative diagrams and calculations for determining the environmental and health risks applicable throughout the whole developed world are not counting with all individual PAHs but only with selected ones. This point of view may in certain situations distort the calculated risks and mitigate the degree of danger of pollution.

To assess the possibilities of optimizing biodegradation and determine the potential removal speed of contaminants from the site polluted by various organic pollutants laboratory respiratory tests were carried out. In addition to the continuous measurement of O_2 consumption and CO_2 production including calculating the respiratory quotient, during the whole set of tests samples were examined before and after biodegradation for quantitative and qualitative analysis of organic compounds.

Sample for measuring respiration rate was extremely polluted. Therefore, in the first part of the contribution respiratory characteristics of biodegradation tests are considered. The second part focuses on the qualitative characteristics of composition of degraded substances, including the determination of the potential options for individual contaminant removal. Evaluation of the results in terms of their use to optimize the decontamination in rock environment in case of remediation is discussed in conclusions and recommendations.

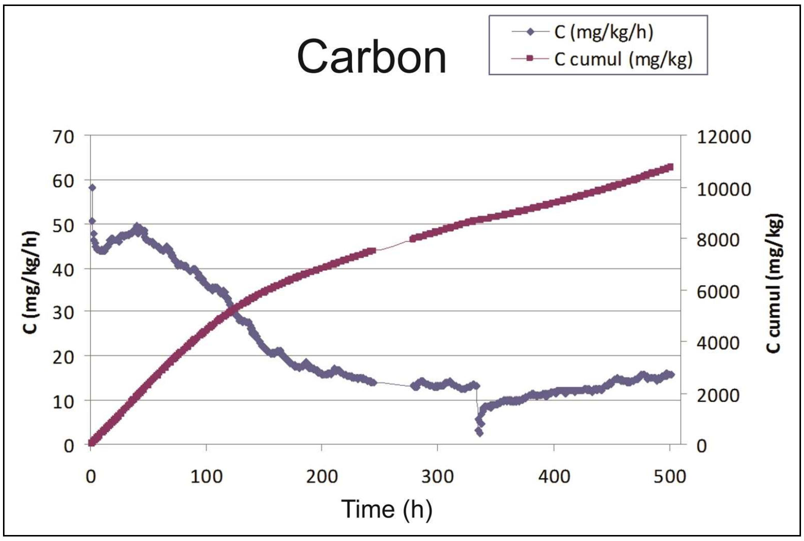

Fig. 1. Instant C degradation and cumulative C degradation in column

During the testing (500 hours), all methods of monitoring the decomposition of organic matter have confirmed more than 10 % decrease of pollution (in cumulative respiratory tests 18,000 mg/kg and more than 18,000 EXL_{GC} in chemical analysis) which presents a high degree of contaminant degradation. However, these

indicators are only quantitative and in terms of individual assessment of organic matter degradation are little selective. Specific issue is to determine the degradation or transformation of those PAHs, where there are no monitoring requirements in terms of the actual methodics.

Proven results indicate risks, where despite the clear observance of the established methods of analysis evaluation and calculation of the health and environmental risks, resulting assessment may neglect the various hazards. Therefore, an essential part of analysis should be toxicity tests before and after biodegradation. This indicator helps to objectify the efficiency of real or model remediation works with complex organic pollution.

Keywords: contamination, remediation, risk, PAHs, pollution

METAL POLLUTION INDEX FOR WATER AND SEDIMENT IN SMOLNIK CREEK

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Abstract

The environmental problems caused by mining have attracted a great deal of research attention. In recent years, numerous water and sediment heavy metal contamination assessment methods have been developed, including the index method, the dynamic method, the synthesis methods in chemistry, ecology and toxicology, etc. The present work is aimed to investigate the pollutants levels of some heavy metals (Ca, Mg, Fe, Mn, Al, Cu, Zn, As, Cd, Pb) in water and sediments relate to acid mine drainage (AMD) producing from abandoned sulphide mine in Smolnik in eastern Slovakia. Metal pollution index was used to compare the total content of metals at five sampling stations.

Keywords: heavy metal, metal pollution index, acid mine drainage

1 Introduction

Heavy metals are considerable environmental concern due to their toxicity, wide source, non-biodegradable properties and accumulative behaviours [1]. With rapid industrialization and economic development, heavy metals are continuing to be introduced to soils and sediment via several pathways, including fertilization, irrigation, rivers, runoff, atmospheric deposition, and point sources, where metal mining, refining and refinishing by products. Sediments can be sensitive indicators for monitoring contaminants in aquatic environments [2]. They are polluted with different kinds of hazardous and toxic substances including heavy metals and are important for the water quality [3]. Heavy metals are important environmental pollutants threatening the health of human and natural ecosystems [4]. Although sediments act as one of the ultimate sinks for heavy metals input into the aquatic environment, they cannot fix heavy metals permanently [1]. Taking into account the importance of sediments and the toxicity of heavy metals in them, related researches have been done to understand the effects of heavy metals on ecological systems [5]. Specifically, opencast mining activities have a serious environmental impact on soils [6] and water streams, having generated millions of tons of sulphide-rich tailings [7]. Runoff from mining operations can have negative impacts on the surrounding aquatic environment including heavy loads of suspended solids, decreased pH levels and increased levels of heavy metals. Acid mine drainage (AMD) is recognized as one of the more serious environmental problems in the mining industry, its cause, prediction and treatment have become the focus of a number research initiatives commissioned by governments, the mining industry, universities and research establishments, with additional inputs from general public and environmental groups [8]. In Slovak republic there are some localities with existing AMD generation conditions. The most critical values were observed in the abandoned deposit Smolnik [9]. Because sediments are responsible for transporting a significant proportion of many hazardous contaminants, not only research and modelling of erosion phenomena is very acute, but also consideration should be given their quality and their impact on the aquatic environment. For better knowledge about migration, transformation behaviour and rules of heavy metals in sediment, it is necessary to make an accurate assessment of contamination level and extent at each site. This paper describes the consistency in the metal pollution indices of the various stations in the water and sediment of the contaminated Smolnik creek.

2 Material and methods

2.1 Study area

The Smolnik stratiform deposit, historically one of the best-known and richest Cu – Fe ore deposits in Slovakia, was exploited from the 14th century to 1990. Mining was primarily focused on the extraction of copper ore. At the beginning of the 19th century, technology for obtaining copper by cementation was installed. Pyrite, which was mined from about 1900 until the 1960s, was used to produce sulphuric acid. In 1954, flotation plants for the treatment of copper ores and the tailings pond was completed. Lack of interest in pyrite and copper ores led to the cessation of mining at Smolnik [10]. The mine was flooded until 1994, when a breakthrough caused a local environmental catastrophe in Smolnik Creek, which annihilated fish populations. The mine is represented by a partially open geochemical system into which rain and surface water drain [11]. More than 6 million tons of pyritic ore of various qualities were abandoned in the mine [12]. An analysis of water in the deserted mine and in the broader area surrounding the mine was made after the ecological accident in 1995. Surface water that penetrates the mine had their metal content increased and pH values decreased [13].

The Pech shaft is currently the lowest located outfall of the flooded Smolnik mine and serves as the main drainage conduit. The Pech shaft collects most of the water draining from the flooded mine area and discharges them as AMD (pH = 3 - 4, Fe 400 - 500 mg/L, Cu 1 - 3 mg/L, Zn 8 - 13 mg/L, and Al 70 – 110 mg/L). The water acidifies and contaminates Smolnik creek, which transports the pollution into the Hnilec River catchment [14].

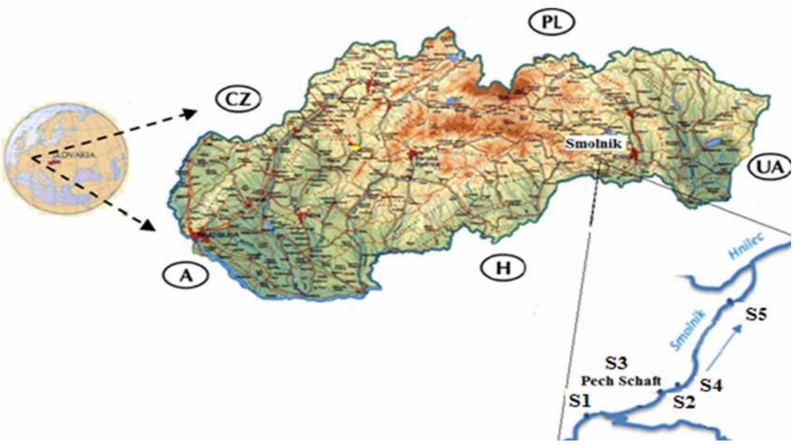

Fig. 1. Water and Sediment samplings of Smolnik Creek

2.2 Sampling preparation

Sediment was taken from five sample site during 2006 -2015. Two localities were in the upper part of the Smolnik creek without contamination by acid mine waters from shaft Pech (1 – outside the Smolnik village, 2 - small bridge - crossing to the shaft Pech) and another two sampling localities were located under the shaft (4 – approx. 200 m under the shaft Pech, 5 – inflow to the Hnilec river). The outflow of AMD from shaft Pech (Smolnik mine) has number 3.

The samples of sediment were air-dried and ground using a planetary mill to a fraction of 0.063 mm. The chemical composition of sediments was determined by means of X - ray fluorescence (XRF) using SPECTRO iQ II (Ametek, Germany). Sediment samples were prepared as pressed tablets with diameter of 32 mm by mixing 5 g of sediment and 1 g of dilution material (Hoechs Wax C Micropowder - M – HWC – $C_{38}H_{76}N_2O_2$) and compressing them at a pressure of 0.1 MPa/m^2.

The total concentrations of Fe, Mn, Al, Cu, Zn, As and Pb in water and sediment of Smolnik creek are presented in Table 1.

2.3 Metal pollution index

The Metal Pollution Index as a mathematical model which could solve some of the highlighted problems. MPI has been calculated to enable presentation of all results from the metal concentrations as one value if a possible, yet overcoming the difficulties with both application and understanding of demanding statistical analysis [15, 16]. To compare the total content of metals at the different sampling station, the MPI was used. The MPI was obtained with the equation:

$$MPI = (Cf_1 \times Cf_2 \ldots \ldots Cf_K)^{1/K} \tag{1}$$

Where; Cf_1 = concentration value of the first metal, Cf_2 = concentration value of the second metal, Cfk = concentration value of the k_{th} metal.

3 Results and discussion

Surface sediment and water pollution is considered by many regulatory agencies to be one of the largest risks to the aquatic environment. The mean levels of the metals in the surface water and bottom sediments of Smolnik creek as presented in Table 1 indicated Zn to be highest in water and As in sediment. Cd is the least in both media.

Table 1. The mean levels of the metals in the surface water and bottom sediments of Smolnik creek

	Fe	Al	Cu	Zn	As	Cd	Pb
	[mg/L]						
W1	0.13	0.05	0.0043	0.0039	0.0019	0.0003	0.0050
W2	0.96	0.17	0.0133	0.0469	0.0014	0.0005	0.0045
W3	341.67	69.64	1.6788	7.5979	0.0318	0.0011	0.0558
W4	14.92	1.42	0.1163	0.4061	0.0017	0.0006	0.0053
W5	6.49	0.42	0.0506	0.2959	0.0012	0.0006	0.0050
	[mg/kg]						
S1	42400	74900	138.56	153.78	46.22	0.50	45.22
S2	51100	61400	248.81	179.44	82.22	5.33	98.88
S3	338700	22900	493.78	126.78	1973.56	1.22	714.22
S4	82800	65100	420.78	197.44	149.11	0.50	177.89
S5	104000	61600	518.78	237.78	98.00	0.50	109.22

For the seven metals studies in the surface sediments and water (Fe, Al, Cu, Zn, As, Cd and Pb) significant differences were not established among stations means. The pattern of the metal levels in all the stations studied followed W3 > W4 > W5 > W2 > W1 for surface water (Fig.2) and S3 > S2 > S4 > S5 > S1 (Fig.3). This may be due to continuous dilution of the water body from the lower and upper reaches of the creek, similarity in physical conditions of the sediments, particle composition and organic matter of the sediments between the stations. The MPI of the surface water in station W3 (Pech shaft) was higher than that of the other stations while that of the sediments, stations S3 (Pech shaft) was higher, too. Although these difference between other stations were not significant. The high MPI in this station could be due to acid mine drainage runoff.

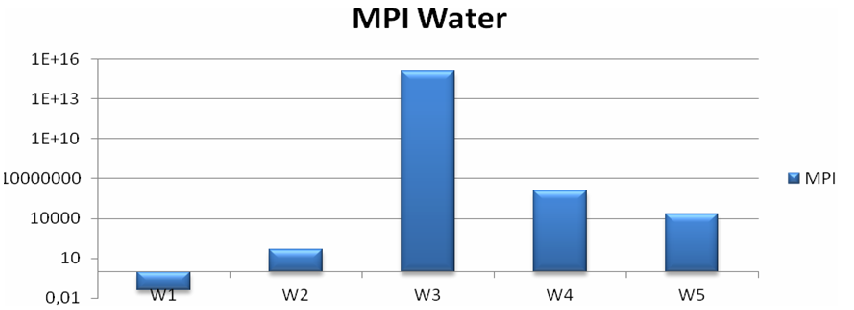

Fig. 2. Water Metal pollution index (MPI) for each sampling station

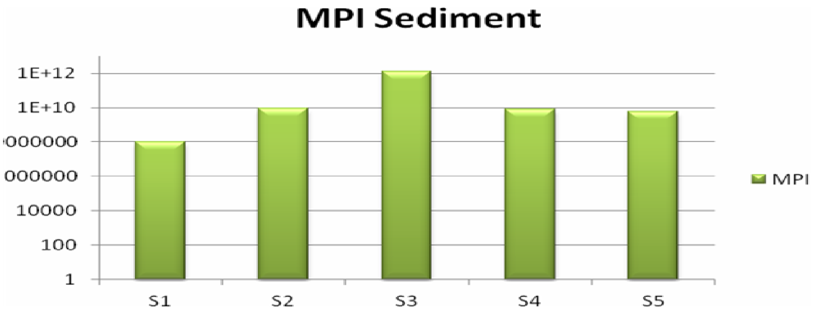

Fig. 3. Sediment Metal pollution index (MPI) for each sampling station

4 Conclusions

The data obtained in this study has showed consistency in the metal pollution indices of the five stations of the study area. This may be due to the continuous dilution of the water body from the lower and upper reaches of the river, similarity of the physical conditions of the sediments, particle composition and organic matter of the sediments may have also played major role. The stations with higher pollution indices are related to areas of intense activities such as the S4, S5 for the surface water and for the sediments.

The pattern of the metal levels in all the stations studied followed W3 > W4 > W5 > W2 > W1 for surface water and S3 > S2 > S4 > S5 > S1 for sediment. These results of MPI can be used to certain the heavy metal toxicity among the sampling sites.

Acknowledgements

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DETERMINATION OF SEDIMENTS GENOTOXICITY IN THE AREA HORNÁD AND HNILEC RIVERS

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Abstract

In the present study, the screening method, genotoxicity of sediments collected from the water reservoir Ružín No.I (Hornád and Hnilec Rivers) was used to establish the potential genotoxic risks on the aquatic environment. This area is well-known by contamination of heavy metals coming from mining and metallurgical activities. Genotoxicity of bottom sediment was evaluated by test SOS-ChromoPadTM 3.0 for solid samples without extraction. The mentioned test represents simple, quick, inexpensive, direct sediment phase toxicity testing procedure. In this test bacterial strain *Escherichia coli K12 PQ37* was used. The results of SOS-ChromoPadTM 3.0 showed that sample 1Hornád has genotoxic potential effect on the environment. It was determined on the basis of blue colouration of chromogenic paper at the point of sediment application. The sample 2Hnilec was negative. This test allows significantly reduce the time for obtaining information about the sediments genotoxicity and accept necessary security proceeding in time.

Keywords: sediments, heavy metals, genotoxicity, *Escherichia coli K12 PQ37*, environmental risk

1 Introduction

Research on sediment quality has been in focus during the last decade. The current problems of environmental protection largely related with contamination of watercourses with wide spectrum of chemicals, degradation of agricultural land, and loss of retention ability for water and high drain of torrential rains, loss of self-cleaning abilities. Thus, analyses of sediment samples are an important tool for assessing impact of anthropogenic activities on aquatic systems [1, 2]. The water reservoir Ružín No.1, branches of the Hornád and Hnilec Rivers drained a former mining area, have been polluted in the long-term by heavy metals (Hg, Cu, Cd, Cr, Ni, As, Sb), which significantly contributed to environmental degradation [3]. Many of these metals have genotoxic effects that act directly or indirectly on organisms (talking about mutagens and carcinogens). Action of matter with mutagenic effects leads to damage of genetic information stored in nucleic acids. In cells non-lethal genetic changes are formed, that are of permanent character. These show itself with defective offspring of cell. In some cases, the process of cell replication as a result of genetic damage information can completely overthrow cells transformation originate and develop, for example: cancerous process-carcinogenicity. For testing genotoxicyty organisms or community of organisms *Escherichia coli* (e.g. SOS-ChromoTest) or *Salmonella typhimurium* (e.g. ChromoPlate kit) are most commonly used [4, 5]. The tests are commercially available in the form of sets involving concentrated solutions, reference materials, test organisms in the form of latent eggs, algae embedded in alginate, which allow detecting genotoxic material even in non-laboratory [6, 7]. The genotoxicity tests were developed for the detection of chemical mutagens and carcinogens in environmental samples. A result of genetic damage material of an organism can thus show up for a long time, sometimes in the next generation [8-10].

The purpose of the present study was to use genotoxicity as useful screening method to assess toxicity of polluted bottom sediments from Eastern Slovakia (concretely from the water reservoir Ružin No.I).

2 Material and methods

2.1 Material for sediment genotoxicity testing

The samples of the sediment were collected from the water reservoir Ružín No.I (Eastern Slovakia). The first sample (1) is from the locality Hornád branch and the second (2) is from the Hnilec branch. The sampling was performed in the year 2012. The samples of bottom sediments were sampled into plastic bottles by sample device „Multisampler“ at a depth of 50 cm. The samples were first dried at room temperature (25 °C), and then were mechanically homogenized and quartered. The sediments comprised sand, silt and clay fractions. The silt and clay fractions were determined as the percentage of the sediments passing through a sieve with an opening size of 63 μm. The total extractable metal concentrations in the sediments were determined after mineralization with a mixture of acids (HCl/HNO_3/HF) in a microwave pressure digestion system (MWS-3, Berghof, Germany). The metal concentrations in the sediments were determined by atomic absorption spectrometry (AAS -VARIAN, Australia). The certified reference material LGC6187 was river sediment (Control), which was collected from a monitoring station lagoon on the river Elbe close to the Czech-German border. This material (Control) was systematically used to control the analytical precision and bias. Incubator-drier Memmert 100-800 (incubation at 37 °C), were used for acute genotoxicity study. The used chemicals and media of the SOS-ChromoPad^{TM}3.0 test contains: a nutrient medium for bacterial growth, lyophilized bacteria *Escherichia coli K12 PQ37* (non-pathogenic strain), a Petri dish with blue chromogenic substrate, 4NQO (4-Nitro-Quinoline-oxide-for positive control), disposable plastic pipette, test tubes, plastic bags for incubation and ampicillin. Sample (0.1 g) treatment before determining genotoxicity was performed by the following procedures: SOS-ChromoPad^{TM} version 3.0-fresh-solid. Sediment samples without extraction were used.

2.2 Methods of sediment genotoxicity testing

Preparation of bacterial strain *Escherichia coli K12 PQ 37*: For sediments genotoxicity testing, bacterial strain *Escherichia coli K 12 PQ 37* in test SOS-ChromoPadTM was used. Lyophilized bacteria *Escherichia coli K12 PQ 37* were 24 hours before each test mixed with 10 ml of culture medium and incubated in a thermostat for 16-18 hours at temperature 37 °C. On the next day, the concentration of grown bacteria of indicated strain was modified by absorbance measurement and by dilution with nutrient medium to a final concentration of 20 µl/ml. The concentration of bacteria was measured by the UV-Vis Spectrophotometer HELIOS-Y at a wavelength of 620 nm. Required bacteria concentration corresponded to the absorbance value of 0.07.

The principle of the test SOS-ChromoPad^{TM} version 3.0 is that genotoxic agents cause DNA damage and immediately thereafter, the cell tries to repair this damage with activation reparation system, which is called SOS. The result of SOS reparations affects the further development of cell. At incomplete replacement of cell, it leads to permanent changes of the cell genetic structure, which may lead to genetically portable mutation or carcinogenic cell transformation. Induction of SOS functions is evaluated using sfiA gene [10]. In this test is possible to use bacterial strain *Escherichia coli K12 PQ 3*.

Working scheme of SOS-ChromoPadTM 15 version 3.0: Preparation of test tubes for each sample: The bacterial suspension and sediment in the tube 1 by mixing 1 ml active bacterial culture with 0.1 g weighed portion of sediment was prepared. Table 1 shows dilution of prepared suspension in tubes 2-5 of concentrations: 0.5n (50 %) - 0.25n (25 %) - 0.125n (12.5 %) - 0,0625n (6.25 %) - 0,0313n (3.13 %); 6 - negative control (only bacteria/0.5 ml), 7 - positive blue control (4NQO/0.050 ml). Incubation of the tubes was in a thermostat during 4 hours at temperature 37 °C. Then 0.020 ml of a bacterial sludge in the form of dots on a Petri dish with blue chromogenic substrate was applied. Arrangement of dots was as follows: in the middle was a positive control, a negative control and diluted samples were on the edges (7 dots on chromogenic plate). Incubation of the Petri dishes with active bacterial culture was in a thermostat for 20 hours at 37 °C. After incubation wash of the sediment particulate from the surface of chromogenic paper was followed. Evaluation was realized by photographic documenting and visual evaluating of the intensity of blue colour dots versus positive indicator (dark blue) using colour-index. The scale is 5 colour tones. White colour corresponds to colour-index 0 (non-genotoxic) and the darkest blue colour corresponds to the colour-index 5 (the most genotoxic sample). Finally, the minimum effective concentration in a volume of the sample was determined.

Table 1. Colour Index SOS-ChromoPad and their associated % dilution

NC	**PC**	**1** /50	**2** /25	**3** /12.5	**4** /6.25	**5** /3.13
○	●	●	●	●	●	○

3 Results and discussion

The pH of the sediment samples (1 and 2) was in the range 7.25 - 7.59, which indicate an alkaline nature of sediments. Organic matter of the sediments ranged from 8.1 to 14.2 % [3]. The sediments comprised sand, silt and clay fractions. The silt and clay fractions were determined as the percentage of the sediments passing through a sieve with an opening size of 63 µm. Table 2 summarizes the results of the chemical analyses of the metals Cu, As, Sb and Hg in the sediments, revealing significant contamination with arsenic and mercury according to the law of the Methodological Instruction of the Ministry of Environment of the Slovak Republic No. 549/1998-2 for Assessment of Risks from Pollution of Sediments of Streams and Water Reservoirs. In terms of the environmental risk, Cu, As, and Hg exceeded the permitted concentrations in all samples.

Table 2. Metal concentration (average ± standard deviation) in the sediments 1 (Hornád) and 2 (Hnilec) and Control (certified reference material)

Sample	Cu	As	Sb	Hg
	(mg/kg) dry weight			
1	198±3.4	39±2.7	27±3.6	6.5±0.4
2	359±5.1	59±4.3	52±5.7	1.9±0.5
Control	82±2.7	25±1.9	14±2.5	1.6±0.3
Law used for comparison (mg.kg^{-1}) dry weight				
TV	36	29	3	0.3
MPC	73	55	15	10
IV	190	55	-	10

Norm No. 549/1998-2: TV-Target Value (Negligible Risk), MPC–Maximum Permissible Concentration (Max. Tolerable Risk), IV-Intervention Value (Serious Risk)

SOS-ChromoPad test has the great advantage that the sediments were used in solid state and it was not necessary to use an extraction method of sample preparation. Because of the rapid indicative determination of acute toxicity in monitored sediment samples, from the short-term indicative genotoxicity tests, this test was used. The endpoint of the bioassay is an easy to interpret colour reaction. In Figures 1, 2, the results of the visual – colour evaluation of two parallel tests of sediments according to colour-index, which is described in the Table 1 are shown. Dots on chromogenic paper induce a toxicity state of sediment samples compared to negative and positive signal light. The basic of scores is intensity of blue colour of positive signal light test and its comparing with samples according to % dilution. Figure 1 shows colour evaluation of sample 1 (Hornád) at plate SOS-ChromoPad. From Figure 1 it is evident, that the sample 1 (Hornád) is non-toxic only at 3.13 % dilution. Only in this dilution to sample 1 (Hornád) does not have genotoxic potential effect on the environment. The Figure 2 shows the results of sediment 2 (Hnilec) genotoxicity. The sample 2 (Hnilec) has not got the blue colour at chromogenic paper at the point of application. It confirms that the sample 2 (Hnilec) is negative - non-toxic in this test.

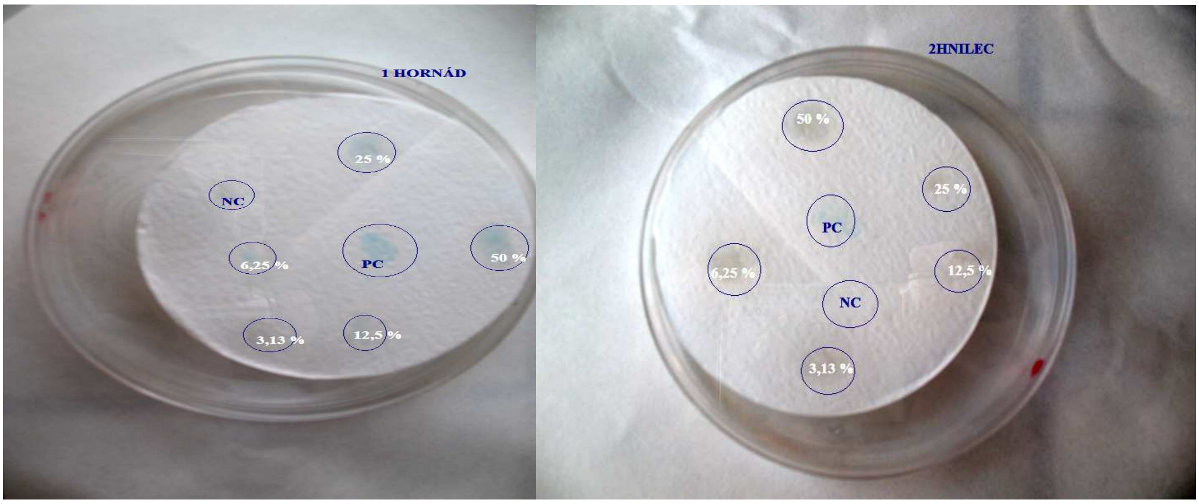

Fig. 1. - 2. The colour evaluation of samples 1 (Hornád) and 2 (Hnilec) at plate SOS-ChromoPad (NC-negative control - without sediment and PC-positive control with 4NQO)

4 Conclusions

The purpose of this study was to describe SOS-ChromoPad^{TM} 3.0 test for testing of bottom sediments from the water reservoir Ružín No.I (in the area Hornád and Hnilec Rivers). This test was used to establish the potential genotoxic risks on the aquatic environment. The results of acute genotoxicity were for sample 1 (Hornád) potential genotoxic and for sample 2 (Hnilec) non-toxic in used SOS-ChromoPad test. The test SOS-ChromoPad is usefulness because is easy to perform, faster, require minimal amount of sample, environmentally friendly and cost effective. Obtained information from this study can be useful for hazard identification and risk assessment of sediment-associated contaminants.

Acknowledgements

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ACINETOBACTER SPP. FROM HEAVY METAL POLLUTED ENVIRONMENTS

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Abstract

Bacteria of *Acinetobacter* genus isolated from different anthropogenically polluted areas of Slovak Republic: aluminium plant brown sludge dump near Žiar nad Hronom, landfill waste sludge from nickel smelter near Sereď and mine tailing Slovinky were characterised. Analysis of cultivable bacteria population of environments with elevated levels of heavy metals, realised in 2010 (Žiar nad Hronom), 2013 (Sereď) and 2014 (Slovinky), indicated that *Acinetobacter* spp. constitutes 18.75 % from bacterial population of brown sludge dump near Žiar nad Hronom, 30 % from bacterial population of landfill waste sludge near Sereď and 5.26 % from drainage water population of mine tailing Slovinky. MALDI-TOF MS confirmed that the prevalent bacterial species is *A. calcoaceticus* (K6, K13, NHL1, NHL2, NHL3, NHL4, P19, and P20) and the others are *A. lwoffii* (K1) and *A. johnsonii* (NHL11). Heavy-metal tolerance of bacterial strains to 5 selected heavy metals (Cu, Zn, Ni, Co, Mn) in concentrations 1 mM, 2 mM, 4 mM, 8 mM, 15 mM, 30 mM, 60 mM shown that *A. calcoaceticus* (K6, K13, NHL1, NHL4, P19, P20) exhibited higher tolerance to Mn (30 mM), Zn (15 mM), Cu (8 mM) compared to *A. lwofii* (K1) or *A. johnsonii* (NHL11). Despite differences in heavy metal content of tested environments all isolates exhibited similar levels of resistance to cobalt (1 mM) and nickel (2 mM). Extremophiles and *Acinetobacter* spp. often adapt to environmental conditions due to the plasmid DNA and genetic information carried by them. Surprisingly, no plasmid DNA was detected in *A. calcoaceticus* isolates. On the other hand, multiple plasmid population was detected in both *A. lwoffii* and *A. johnsonii* isolates.

Keywords: *Acinetobacter*, heavy metals, resistance, bioremediation, plasmids

1 Introduction

Species of the genus *Acinetobacter* are wide-spread in nature. They can be regularly isolated from human skin or mucous membranes of healthy people [1, 2] but presence of these species in the hospital, especially in the Intensive Care Units, as a source of various nosocomial infections, for example sepsis, urinary tract infections, meningitis, endocarditis, osteomyelitis, peritonitis, pneumonia, ambustion and wound infections was reported [3, 4]. *Acinetobacter baumanii* represents three quarters of clinical isolates and infections caused by them have worse course than infections caused by another species [5-7]. In addition to antibiotic resistance [8, 9] which is one of the reasons that allow their survival in a hospital, *Acinetobacter* spp. species are often heavy-metal resistant and are present in various naturally or anthropogenically polluted environments. Typical environmental representative of the genus is *A. calcoaceticus*. It has been shown that this species could remove phosphate or phenol from the environment [10-14], produce platinum nanoparticles by bio-reduction from hexachloroplatinic acid [15], and synthesize silver nanoparticles which can be used for biofilm disruption [16]. This information shows that the biotechnological applicability of these bacterial species is quite extensive.

Heavy metal pollution of soil, water and environment is serious problem of today's society and in recent years increasing the tendency of use more environmentally friendly approaches to clean our nature. Study of metallotolerant extremophiles is important because heavy metal resistant genes, present on chromosomal DNA or on bacterial plasmids, can be used in bioremediation or biosensors construction [17-19].

2 Material and methods

2.1 Sampling and identification of bacteria

Samples were collected from aluminium plant brown sludge dump near Žiar nad Hronom in 2010, from landfill waste sludge from nickel smelter near Sereď in 2013 and from mine tailing Slovinky in 2014.

The samples were collected in sterile boxes and immediately brought to the laboratory. Isolated bacteria were identified by using MALDI-TOF MS (Matrix – assisted laser desorption ionization time-of-flight mass spectroscopy) method [20].

2.2 Test for heavy metal resistance of bacterial isolates

All *Acinetobacter* spp. isolates (K1, K6, K13, NHL1, NHL4, NHL11, P19, P20) were checked for metal tolerance and for their ability to grow on TSA plates supplemented with five different heavy metal salts ($CuCl_2.2H_2O$, $ZnCl_2$, $NiCl_2.6H_2O$, $CoCl_2.6H_2O$, $MnCl_2$) in concentrations 1 mM, 2 mM, 4 mM, 8 mM, 15 mM, 30 mM, 60 mM (pH=7.3). Isolates were incubated for 24 hours at 25 °C.

2.3 Test for bacterial ability to grow at alkaline pH

Soil samples from brown sludge near Žiar nad Hronom show high alkaline pH with value 10.2. Alkaline nature is also characteristic for soil samples from landfill waste sludge near Sereď (pH=8.1) and for drainage water samples from Slovinky (pH=7.8). Bacterial isolates were tested for their ability to grow on TSA plates with controlled pH=9 and 10.5. Bacteria were incubated for 24 hours at 25 °C.

2.4 Plasmid isolation and gel electrophoresis

The plasmid DNA occurrence in studied isolates was determined using alkaline lysate method. Bacterial strains were inoculated in 5 ml LB (Luria-Bertani) liquid medium (pH=7) and cultivated for 24 hours at 25 °C in a shaker. The grown cultures were added to Eppendorf tubes and centrifuged for 3 min at 13 000 RPM at room temperature. This step was repeated twice. Supernatants were removed and 250 µl of solution P1 (50 mM Tris-HCl, 10 mM EDTA) and lysozyme were added to bacterial pellets. Pellets were re-suspended, mixed by vortexing and incubated for 15 min at 37 °C. The next step was addition of 250 µl lysis solution P2 (0.2 M NaOH, 1 % SDS) to the tubes and gently mixing by inverting. Then a neutralisation solution P3 (3 M potassium acetate, pH = 5) in volume 350 µl was added to bacterial lysates and gently mixed by inverting. After 15 min incubation and 10 min centrifugation at 13 000 RPM at 4 °C, the supernatants were transferred to new Eppendorf tubes. The next step was an RNA removal with 1 µl of RNase which was added to the supernatants. Cleavage mixtures were incubated at 37 °C for 30 min. After incubation, a half volume of chloroform was added to the tubes and centrifuged at 4 °C for 3 min. The top aqueous phases were removed and transferred to new sterile Eppendorf tubes. Then a ¾ volume of isopropanol was added to the tubes for precipitation and centrifuged at 4 °C for 10 min. The supernatants were carefully removed and 500 µl of 70 % ethanol was added to bacterial pellets and centrifuged for 5 min. The supernatants were removed again and pellets were dried in a thermostat at 37 °C. Dried pellets were dissolved in 50 µl of TE solution (10 mM Tris-HCl, 1 mM EDTA). Bacterial plasmids DNA were separated by agarose electrophoresis on a 1 % agarose gel which was stained with ethidium-bromide. After electrophoretic separation, we visualised DNA in the agarose gel under UV light.

3 Results and discussion

Variable population of bacteria was identified in soil and water samples from analysed polluted areas using MALDI-TOF MS. From brown sludge dump near Žiar nad Hronom 16 isolates were obtained and three of them belong to *Acinetobacter* spp. (18.75 %): *A. lwoffii* (1x) and *A. calcoaceticus* (2x). From landfill waste mud from nickel production near Sereď 20 bacterial isolates were obtained and six of them belong to *Acinetobacter* spp. (30 %): *A. johnsonii* (1x) and *A. calcoaceticus* (5x). From drainage water of mine tailing Slovinky 38 bacterial isolates were obtained and two of them belong to *Acinetobacter* spp. (5.26 %): *A. calcoaceticus* (2x). The results from bacterial identification show that the dominant species in polluted environments is *A. calcoaceticus* which corresponds to its frequent presence in anthropogenically polluted industrial areas and its high applicability in biotechnology [15, 16, 18, 19]. *A. calcoaceticus* was isolated from every cultivable bacterial population of analysed areas and represents 75 % of *Acinetobacter* spp. isolates. This strain exhibit higher tolerance to Mn (30 mM), Zn (15 mM) and Cu (8 mM) compared to other analysed species of *Acinetobacter* genus. No differences in nickel (2 mM) and cobalt (1 mM) tolerance were observed among studied isolates. The results are summarized in Tab. 1. Heavy-metal tolerant *Acinetobacter* species were isolated from different polluted areas. From sewage treatment plant in Guheswori (Nepal) were isolated *Acinetobacter* spp. resistant to cadmium (MIC 150µg/ml), copper (MIC 200µg/ml) and cobalt (MIC 180µg/ml) [21]. From Madurai district sewage water (South India) was obtained *A. radioresistens* BC3 with minimum inhibitory concentration to arsenic - 13 mM, cadmium - 4 mM and chromium - 0.7 mM [22].

A. baumanii N2S4 and *A. rhizosphaerae* BIHB 723 were present in samples from field which is localised 1 m away from landfill waste sludge of heavy metals near Sereď (Slovak Republic). These strains contain resistant genes czcA and nccA [23]. *A. baumanii* HAF-13 isolated from industrial area Hafar Al-Batin (Saudi Arabia) showed high tolerance to several heavy metals with maximum tolerable concentration for mercury 100 mg/l, lead 175 mg/l, cadmium 200 mg/l, chromium and arsenic 250 mg/l [24]. The analysed *Acinetobacter* spp. isolates are obtained from environments with different alkaline pH values: soil samples from Žiar nad Hronom have pH 10.2, soil samples from Sereď have pH 8.1 and drainage water samples have pH 7.8. Test for bacterial ability to grow on alkaline pH show that all these isolates grow on pH 9 but not on pH 10.5 indicating that despite different origin, all isolates probably possess similar adaptation mechanism to alkaline pH.

Table 1. Characterization of bacterial isolates

Sample	MALDI-TOF identification	sampling point			growth at pH		Minimum inhibitory concentration (mM)				
		locality	environment	pH	9	10.5	Mn	Zn	Cu	Ni	Co
K1	*A. lwoffii*	Žiar nad Hronom	soil	10.2	+	-	4	2	2	2	1
K6	*A. calcoaceticus*	Žiar nad Hronom	soil	10.2	+	-	30	15	8	2	1
K13	*A. calcoaceticus*	Žiar nad Hronom	soil	10.2	+	-	30	15	8	2	1
NHL1	*A. calcoaceticus*	Sereď	soil	8.1	+	-	30	15	4	2	1
NHL4	*A. calcoaceticus*	Sereď	soil	8.1	+	-	30	15	4	2	1
NHL11	*A. johnsonii*	Sereď	soil	8.1	+	-	8	4	4	2	1
P19	*A. calcoaceticus*	Slovinky	water	7.8	+	-	30	15	8	2	1
P20	*A. calcoaceticus*	Slovinky	water	7.8	+	-	30	15	8	2	1

Heavy metal resistance is frequently plasmid encoded in acinetobacters [25]. Figure 1 shows plasmids occurrence of *Acinetobacter* spp. populations from Žiar nad Hronom, Sereď and Slovinky. Despite greater tolerance to Mn, Zn and Cu than K1 (*A. lwoffii*) and K6 (*A. johnsonii*), isolates K6, K13, NHL1, NHL4, P19, P20 (*A. calcoaceticus*) don't contain plasmid DNA. K1 and NHL11 isolates contain several plasmids some of them of similar size. These differences in minimum inhibitory concentration of heavy metals and plasmid content may mean that isolates K1 (*A. lwoffii*), NHL11 (*A. johnsonii*) and K6, K13, NHL1, NHL4, P19, P20 (*A. calcoaceticus*) have different heavy metal resistance mechanisms. The ability of bacterial strains to grow in the presence of heavy metals or other contaminants would be helpful in the environment treatment with bioremediation or in monitoring the cleanliness of the environment through various bioluminescent biosensors. Heavy metal or antibiotic resistant genes may be present on bacterial chromosomal or plasmid DNA. But only mobile genetic elements as plasmids have ability to transfer to other bacterial species and genera by horizontal gene transfer which allows transfer of various resistance genes and provides higher bacterial adaptability in extreme environment [26-28].

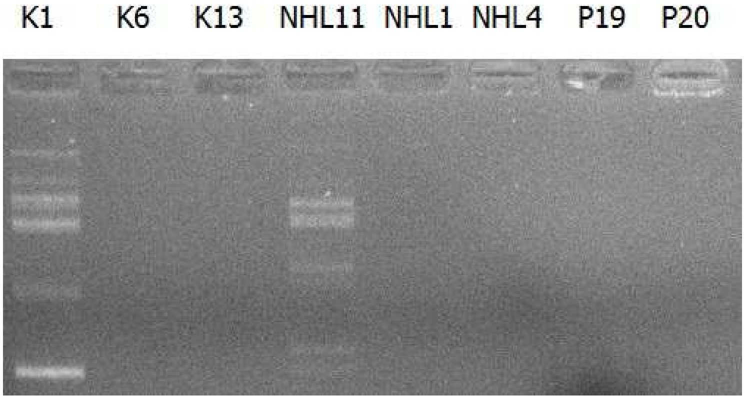

Fig. 1. Agarose gel electrophoresis of plasmids DNA from *Acinetobacter* spp.

4 Conclusions

Our data indicate that extremophile metallotolerant and alkali-tolerant bacteria of *Acinetobacter* spp. are frequently encountered from anthropogenically polluted areas of Slovak Republic. Minimum inhibitory concentration of Mn (30 mM), Zn (15 mM) and Cu (8 mM) is higher in *A. calcoaceticus* isolates compared to *A. lwofii* and *A. johnsonii*. On the other hand, minimum inhibitory concentration of Ni (2 mM) and Co (1 mM) is same. Multiple plasmids were detected in *A. lwoffii* and *A. johnsonii* isolates but not in *A. calcoaceticus*. The putative role of the plasmids in heavy metal resistance was not elucidated yet.

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IMPACT OF PERCOLATION AND STABLE CONDITIONS ON BIOLEACHING EFFECT AND EXTRACTION OF Cu, Zn, As, Fe FROM SOIL AND SEDIMENT

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Abstract

The soil and sediment samples with different content of metals were investigated during heterotrophic bioleaching. Heterotrophic bacteria caused decomposition of iron binding deposition as is adsorption on clay with followed dissolving of Fe mainly by the sediment bioleaching. The concentration of iron decreased by precipitation with As sorption after 19 days of sediment bioleaching. The Cu and Zn extraction was inhibited by bioleaching during the iron and arsenic dissolution and precipitation. By contrast, the additives 3 mM Cu and 3 mM Zn were applied into medium and thus affected the activity of soil resistant heterotrophic bacteria with followed increasing of the iron and arsenic extraction by the soil bioleaching. Therefore, this study confirmed the soil and sediment bioleaching in Fe or As releasing efficiency under different conditions regulated by indigenous bacteria. To examine the effects of chelant and biological leaching on the mobilization of Cu, Zn, As and Fe we conducted a batch and column next test during laboratory conditions. The use of bioleaching to enhance chelate extraction techniques is one method being tested to make biochemical extraction efficient enough to be used as a biochemical remediation technique. We performed the batch tests with stabile solution condition to investigate the use of the biochemical remediation in saturated zone of polluted soil and sediment and the column tests with percolation of solution to investigate the use the biochemical remediation in unsaturated zone. The biochemical leaching can be a suitable technology for As removal from the untreated soil and sediment by stimulation of the resistant bacteria activity in water environment.

Keywords: bioleaching, toxic elements, soil, sediment

1 Introduction

Soil and sediment are major reservoirs for contaminants as they possess an ability to bind various metals. In general many of them contain a wide range of heavy metals with varying concentration ranges depending on the surrounding geological environment and anthropogenic and natural activities occurring or once occurred [1]. Bioremediation is an option that offers the possibility to destroy contaminants or remove them innocuous using natural biological activity [2].

The mining waste, mineral processing and industrial treatment processes represent potential source of heavy metals in the soil in close proximity of the industrial area KOVOHUTY Krompachy with production of Cu and Zn. The soil contamination investigated here is located close to the river Hornád which flows into reservoir Ružín and contaminated sediment.

This study was conducted to determine the distribution of heavy metals in two different contaminated samples of soil and sediment. The purpose of the present test was to verify the resistance of bacteria to heavy metals and to investigate the effect of indigenous bacteria on mobilization of Fe, Cu, Zn, As by bioleaching in percolation and stable conditions.

2 Material and methods

2.1 Sediment and soil analyses

The soil and sediment used in this study were collected close to the industrial area of Kovohuty Krompachy (source - Z) and at border of the reservoir area of Ružín near town Margecany in Slovakia (outfall - O), where the river Hornád flows into a reservoir.

After the manual homogenization, the soil and sediment samples were sieved through a 2 mm sterilized stainless steel sieve (No. 1) in order to keep indigenous bacteria. The samples were deep frozen (at – 40 °C) before the laboratory tests. The separation of the clay fractions (< 2 μm) were performed from the homogenized 4 samples of different depth (soil – ZC, sediment – OC) by sedimentation in the sterilized physiological solution (8.5 g NaCl per liter). The clay fractions (< 2 μm) were obtained only from the samples of 30 cm depth from the sediment (OC3), because the soil contained very low amount of clay fraction (< 0.1 %) for laboratory tests. The separation of the silt fractions without the clay fraction (< 63 µm) were also performed from samples of 30 cm depth (soil – ZS3, sediment – OS3) and from homogenized 4 samples of different depth (soil – ZS, sediment – OS) by sedimentation in the sterilized physiological solution (8.5 g NaCl per liter).

The elements of the solid samples were analyzed on the fused beads using an energy dispersive X-ray fluorescence spectrometer (Spectro X-LAB 2000, Germany). The XRD analyses of the clay fraction from sediment and soil were carried out using a Phillips PW 1710 diffractometer (35 kV, 20 mA) with CuKα radiation and a graphite monochromator. All samples were scanned with a 0.02° 2θ step.

2.2 Bioleaching of sediment and soil

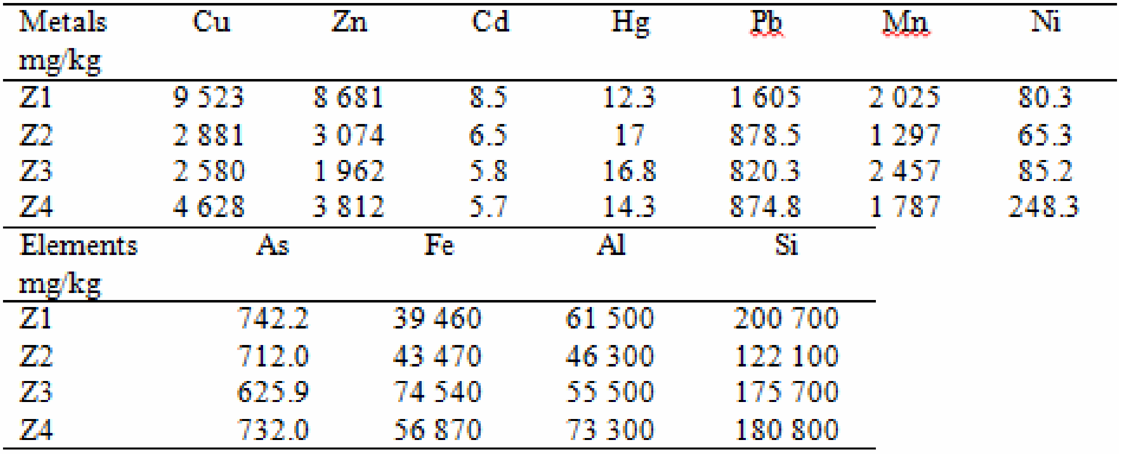
Bioleaching experiments were carried out in 250 ml culture flasks containing 50 g samples and 150 ml medium without metals (denoted as M1, M2, M3) and with 0.2 g/l sodium azid (denoted as A1, A2, A3). Sodium azid was used as biocides in chemical controls. The each media for the next bioleaching contained the metal salt solution 3 mM $CuSO_4$ (190 mg/l Cu) and 3 mM $ZnSO_4.7H_2O$ (196 mg/l Zn). Medium M1 contained (per liter) N and P fertilizers (DAM 390, Pekavit), chelating agent 0.59 g Na_3EDDS, 0.5 g $MgSO_4$, 0.14 g $CaCl_2$, 0.075 g NaCl, 0.35 g KCl, 2 g Na_2SO_4, 0.05 g yeast extract, 20 g glucose and 0.01 g nystatine as a fungal growth inhibitor; pH 6.0 (adjusted with NaOH). Medium M2 and M3 contained besides the listed chemicals 0.28 g SDS and 0.53 g $Na_4P_2O_7$, respectively. The experiments with metals additions were performed to assess the potential of indigenous resistant heterotrophic soil and sediment bacteria to metals accumulation. Two replicates were run per experimental onset. The initial pH of all experiments was adjusted to 7.00 ± 0.04 by addition of ultrapure 1 M NaOH before the bioleaching. The batch bioleaching of the soil and sediment test was performed under the static conditions at 24 °C during 14 - 21 days. The culture solution was separated from the samples after 10 minutes of centrifugation at 10 000 rpm and by means of 0.2 µm pore size membrane filtration. The liquid supernatant was collected for the chemical analyses.

3 Results and discussion

3.1 Toxic elements composition of sediment and soil

The soil is approximately 15 times more contaminated by heavy metals than sediment (Table 1, 2). The content of heavy metals in the clay fraction is a very good indicator of environmental pollution. In the finest fractions of soil and sediments due to the active surface of their components a continuous enrichment with heavy metals takes place. The XRD analysis of the fine clay fraction revealed no significant changes in the mineral composition between soil and sediment. It followed from the qualitative determination that the soil (Z) and sediment (O) consist predominantly from quartz, micas and clay minerals [3].

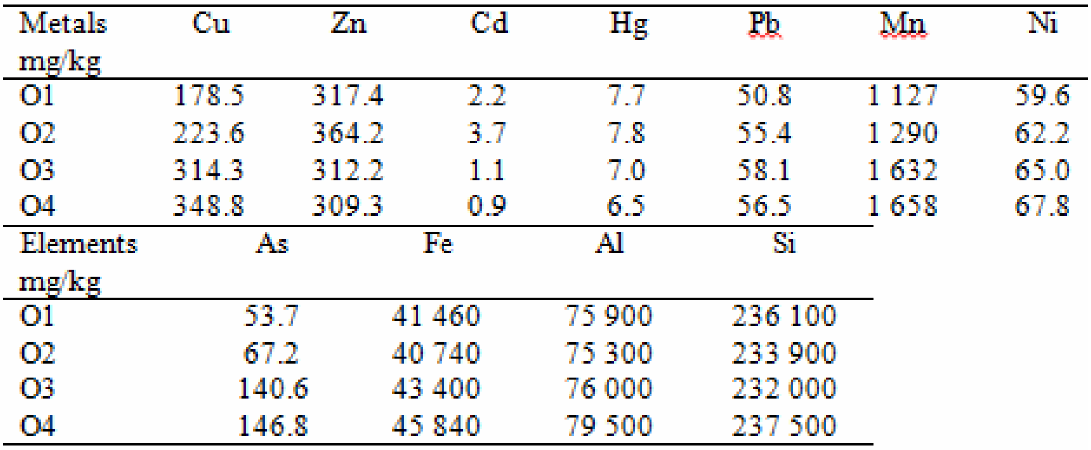
Table 1. Chemical composition of soil (Z) from different depth 10 cm (Z1), 20 cm (Z2), 30 cm (Z3), 40 cm (Z4)



Metals mg/kg	Cu	Zn	Cd	Hg	Pb	Mn	Ni
Z1	9 523	8 681	8.5	12.3	1 605	2 025	80.3
Z2	2 881	3 074	6.5	17	878.5	1 297	65.3
Z3	2 580	1 962	5.8	16.8	820.3	2 457	85.2
Z4	4 628	3 812	5.7	14.3	874.8	1 787	248.3

Elements mg/kg	As	Fe	Al	Si
Z1	742.2	39 460	61 500	200 700
Z2	712.0	43 470	46 300	122 100
Z3	625.9	74 540	55 500	175 700
Z4	732.0	56 870	73 300	180 800

Table 2. Chemical composition of sediment (O) from different depth 10 cm (O1), 20 cm (O2), 30 cm (O3), 40 cm (O4)



Metals mg/kg	Cu	Zn	Cd	Hg	Pb	Mn	Ni
O1	178.5	317.4	2.2	7.7	50.8	1 127	59.6
O2	223.6	364.2	3.7	7.8	55.4	1 290	62.2
O3	314.3	312.2	1.1	7.0	58.1	1 632	65.0
O4	348.8	309.3	0.9	6.5	56.5	1 658	67.8

Elements mg/kg	As	Fe	Al	Si
O1	53.7	41 460	75 900	236 100
O2	67.2	40 740	75 300	233 900
O3	140.6	43 400	76 000	232 000
O4	146.8	45 840	79 500	237 500

3.2 First bioleaching test

The ability of indigenous heterotrophic bacteria to solubilize Cu, Zn cations was investigated in the sediment (O3) and soil (Z3) bioleaching experiments in stable conditions with medium M1, M2, M3 and these results were compared with chemical controls A1, A2 and A3 [4].

The concentration of metal cations in the sediment was lower than in the soil. That is why a relatively low extent of Zn cations leached from the sediment sample was observed mainly in the chemical controls (Fig.1, A1 > A3 > A2) which decreased by the presence of indigenous heterotrophic bacteria.

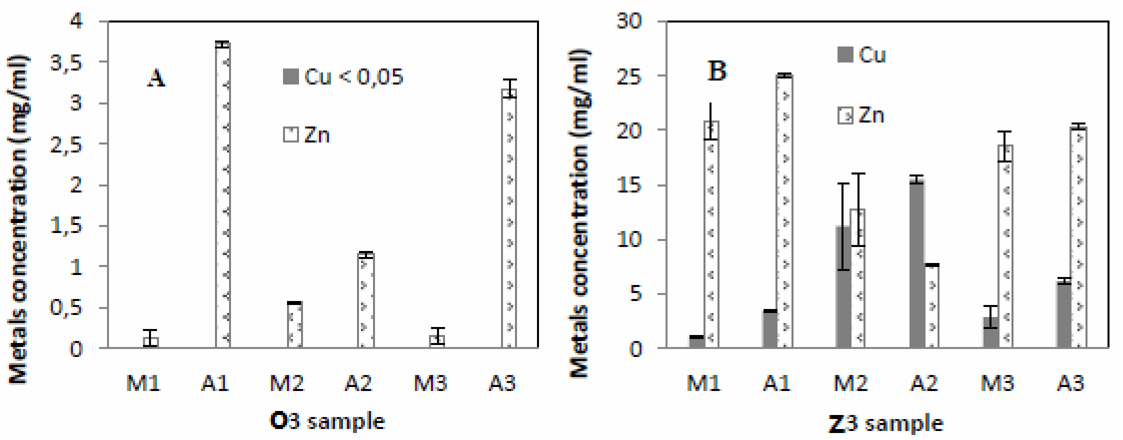


Fig. 1. Extraction of metals after 21 days of A - sediment (O3) and B - soil (Z3) bioleaching in stable conditions

The significance of Cu and Zn cations releasing by medium M1, M2 and M3 leaching was the decrease by the presence of heterotrophic resistant bacteria during the soil and sediment bioleaching and not only by the presence of dissolved organic source in abiotic control (A1, A2, A3). These results indicated that live heterotrophic microorganisms can affected the mobilization of Cu and Zn cations from soil and sediment in the presence of organic matter in natural environment as well as in the chemical remediation processes by EDDS and $Na_4P_2O_7$.

Iron (Fe^{+3}) bound in clay minerals should be considered an important electron acceptor supporting the growth of bacteria in soils or sedimentary environments. Along with microorganisms, clays provide some of the most catalytic surfaces in sedimentary environments, which are important to a variety of biogeochemical cycles [5].

Ferric minerals also cover clay minerals in sediment and soil, the interaction of ferric minerals and clay minerals is disturbed by bacteria as a result of dissolution of ferric minerals. These processes occurred during the bioleaching and the Fe concentration in the leachate was highest during the bioleaching of sediment OC sample in medium M1 > M3 > M2 than during the leaching of soil sample ZC (Fig. 2). In both cases of bioleaching the difference in the amount of iron in solution between the sediment sample and soil sample is probably caused by bacterial amount of resistant bacteria because greater amount of bacterial cell was in the clay sediment samples [3].

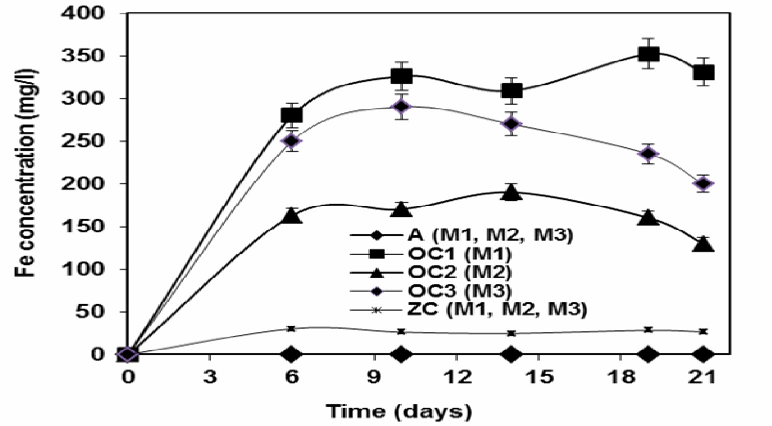


Fig. 2. Total iron concentration in leachates during iron dissolution and bioleaching of the clay fraction from soil and sediment

3.3 Second readsoption test after bioleaching

The ability of indigenous heterotrophic bacteria to uptake Cu, Zn cations was investigated in the next step of soil (Z) and sediment (O) bioleaching in stable conditions with medium M1, M2, M3 amended with 3 mM Cu and 3 mM Zn [3]. The soil was more contaminated by heavy metals than sediment (Table 1) and contained less clays than sediment. Thus, the yield of cumulated Cu, Zn was 100 % from the amended media M1, M2 and M3 by sediment after 19 days biotic and abiotic leaching. By addition of Cu and Zn cations into medium of sediment bioleaching, Fe dissolution partially decreased and As concentration was below 1 mg/l (data not showed). In the soil leaching by bacteria and by media without bacteria (A1, A2, A3) was observed the difference of qualitative analysis of leachates. This difference in the yield of accumulated Cu, Zn was detected only during soil bioleaching. This immobilization process was negligible in the abiotic samples and the soil bioleaching enabled mainly the Cu cation. It is also observed in Zn accumulation during the soil bioleaching (Fig. 3).

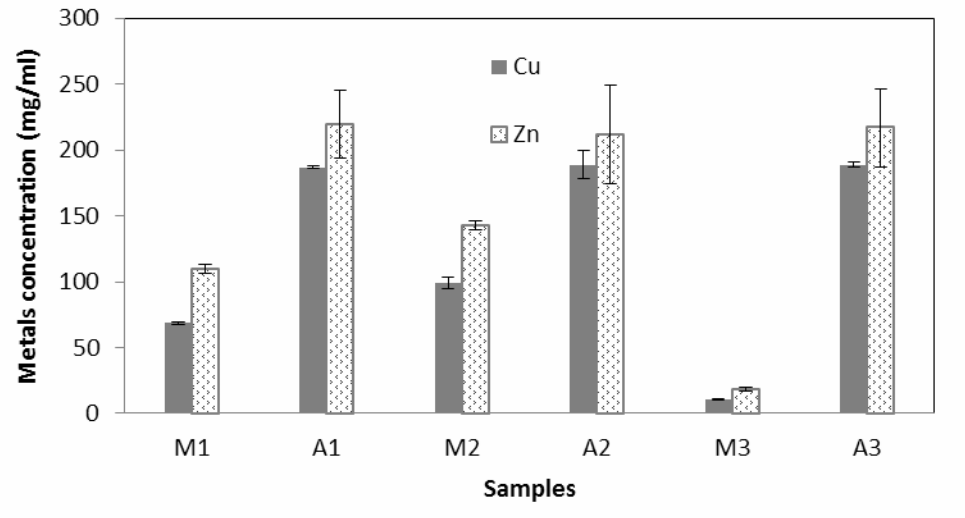


Fig. 3. Concentration of Cu, Zn in leachate of biotic sample (A) and abiotic samples in medium M1, M2 and M3 after 19 days bioleaching in stable conditions of the clay fraction from soil (ZC)

The decreasing of Cu and Zn concentration of added cations in medium M3 > M1 > M2 was caused by bacteria because the soil contained the low concentration of clay minerals than the sediment. Cu is immobile in samples with bacteria because of its sequestration with organic matter, precipitates by metabolites or cellular assimilation is taken up for metabolic purposes, or and cell sorption adsorbs onto bacterial surfaces. The bioleaching caused pH change from 5 to 4 during Cu and Zn accumulation and decomposition of iron minerals in the next soil and the sediment bioleaching.

However, the concentrations of Fe and As in the soil leachates were significantly higher in the presence of added cations than in the abiotic controls (Fe and As < 1 mg/l) or during the first soil bioleaching without Cu and Zn addition (Fig. 4, Fe < 30 mg/l and As < 1 mg/l). The bacterial dissolution of Fe from soil sample was affected by the addition of Cu in medium M3 and by the addition of Zn in medium M1 a M3 (Fig. 4).

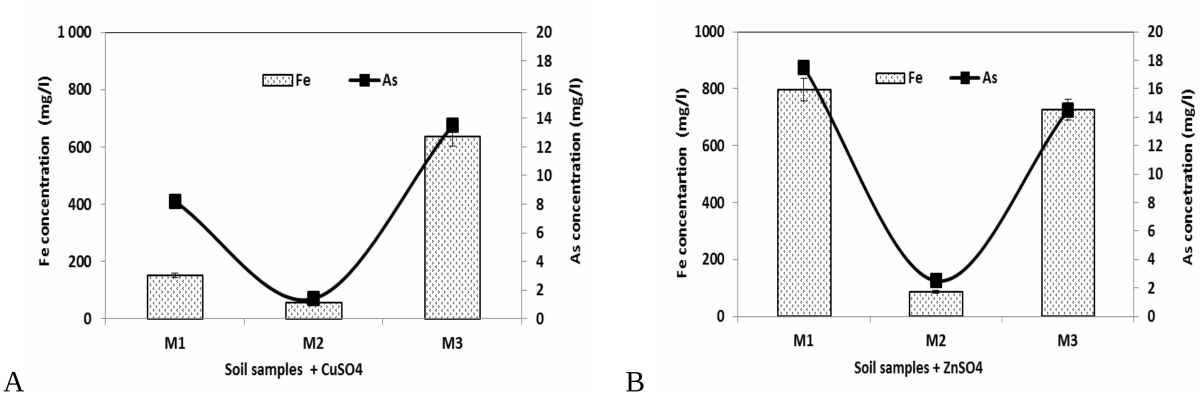


Fig. 4. Concentration of Fe, As in leachate of the clay fraction from soil (ZC) affected by the addition of Cu (A) and Zn (B) in medium M1, M2 and M3 after 19 days bioleaching

The gradual bacterial decomposition of iron minerals caused the As extraction from the soil sample. Probably the metabolic activity of soil indigenous bacteria is inhibited by the Cu and Zn additions in medium and only resistant bacteria are responsible for the extraction of As.

In the presence of added chelators, the removal of iron in the bacteria soil test was lower. In the presence of chelators, the bioleaching by indigenous bacteria and strains of Bacillus clearly produce the highest yield of iron dissolution. The effectiveness of As mobility in treated soil removal is dependent from addition of chelators, fertilizers and metals to activation of resistant bacteria.

3.4 Third bioleaching batch and column test

The third 1 month bioleaching experiment with the mixed soil (Z1, Z2, Z3, Z4) and sediment (O1, O2, O3, O4) was performed in columns (percolation conditions) and also in flasks (stable conditions) with parallel samples and biochemical leaching was compared with the untreated samples before leaching (Table 3).

Table 3. The difference toxic element concentrations in samples between batch (A) and column (B) bioleaching tests

Sample	Cu	Zn	As	Fe
	(mg/kg)			
Soil (Mixed)	5 340	6 132	717	78 620
Soil (A)	4 617	3 210	522	69 110
Soil (B)	4 453	3 469	644	70 565
Sediment (Mixed)	267	352	82	42 090
Sediment (A)	253	200	49	31 070
Sediment (B)	287	323	76	40 355

Chemical analyses of solid sediment samples after 1 month bioleaching show that static flasks bioleaching was very active in 40 % As and 26 % Fe removal. The same removal effectivity was in flask bioleaching in 43 % Zn removal. Columns bioleaching under percolation of medium was not very effective. Cu was very stable metal in bioleaching heterotrophic system. The difference between columns and flasks bioleaching was also similar in soil, so there was a higher removal of elements except Cu in flasks. However, columns bioleaching under percolation of medium was more effective than in sediment. Flasks bioleaching removed 27 % As and 12 % Fe. Zn removal from soil was similar in flasks and columns bioleaching with 48 % and 43 % removal. Cu removal was also similar, 14 % in flasks and 16 % in columns bioleaching.

4 Conclusions

Soil is a major source of metals for contamination of sediments. The concentration of dissolved Fe in the soil clay leaching experiment did not exceed 30 mg/l Fe. The iron was solubilized mainly by the sediment clay bioleaching. The bioleaching inhibited Cu and Zn dissolution and the addition of Cu and Zn cations increased Fe dissolution and As extraction from the soil clay sample. The dissolution of Fe by resistant bacteria leads to the extraction of sorbed As from the soil. In batch test, heterotrophic bioleaching increased the removal As and Zn from the soil and the sediment in the comparison with chemical leaching. Chelating agents are active in extracting heavy metals from contaminated soils and sediments and it can help in the increasing of As removal from soil by bioleaching. The addition of resistant sedimentary bacteria and Zn cations in media increased Fe dissolution and As extraction from the soil sample by flasks bioleaching. Organic matric or heterotrophic bacteria can caused readsorption and immobilization of Cu during chemical leaching. Mixed chelators and resistant heterotrophic bacteria systems with other additives – nutrients and organic sources increased As removal from the contaminated sediment. The heterotrophic bioleaching can be a suitable technology for Zn and As removal from the sediment and the soil by the stimulation of resistant heterotrophic bacteria in the isolate lake environment. The presence of heavy metals in the soils represents a significant environmental hazard, and one of the most important problems of contamination to solve.

Acknowledgements

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A BIOLEACHING PROCESSING PERSPECTIVE: TREATMENT OF NON-METALLIC RAW MATERIALS FOR THE GLASS AND CERAMIC INDUSTRY

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Abstract

Bioleaching of non-metallic raw materials is the method of biological conversion the insoluble iron compound to a water soluble form. For treatment specialized heterotrophic microorganisms are used in order to remove unrequested iron from silicate industrial minerals via bioleaching with consumption of organic source. An associated process is biological reduction of ferric iron to ferrous iron in leachates. In perspective bioleaching three technical processes are differentiated: tank bioleaching, heap bioleaching, *in situ* or *on place* bioleaching. The laboratory tests verified the activity of microorganisms during the imitation of tank or heap bioleaching, the suitable medium with various carbon - nitrogen sources and the influence of different parameters on iron dissolution. The biological reduction of iron minerals is associated with chelation necessarily for the intensification of iron removal. The chelating reagent also prohibited precipitation of secondary ferrous compounds in leachates. Bioleaching of non-metallic is ecological process which is perspective for commercial application to the quality improvement of industrial minerals such as quartz sands, kaolin and feldspars together with magnetic separation.

Keywords: bioleaching, quartz sands, kaolin, feldspars, iron minerals

1 Introduction

Kaolin, feldspar rocks and quartz sands are largely used as a raw material in the ceramic and glass industry, however, are often associated with iron and titanium impurities that decrease their economic value and hinder their application. Iron is a major element, substituting for aluminium in silicate structures, or present as iron oxyhydroxides, influences the refractoriness and whiteness of non-metallics. To remove Fe(III) impurities from the non-metallic ores, physical and chemical methods such as flotation, magnetic separation, and chemical treatments have been used [1]. However, these methods have many disadvantages. The efficiency of the removal of iron from clay minerals by the first two methods is low because either it is present in the structure or it is strongly adsorbed [2]. It has been suggested that iron-reducing microorganisms could be used effectively for microbial refinement of non-metallics [2-4].

Bioleaching is also a technology applicable to metal extraction from low grade ores and ore beneficiation. The technology is environmentally sound it may lower operational cost and energy requirement. Non-metallic ores such as oxides, carbonates and silicates contain no energy source for the microorganisms to utilize. Such ores may be leach by using heterotrophic bacteria and fungi, which require an organic carbon source as a source of energy and carbon for their growth. Heterotrophic bacteria have the potential for producing acidic metabolites that can solubilize oxide, silicate, carbonate and hydroxide minerals by reduction, acid attack, and complexation mechanisms [5]. The application of the bioleaching to the improvement of quality of non-metallic materials has yet to lead to commercial-scale processes.

Recovery or removal of iron from non-metallic raw materials using heterotrophic microorganisms is a poorly studied area of biotechnology. Iron extraction with oxalic acid produced by fungi has been reported in several papers. This is a two-step process: metabolite production and leaching by the metabolite. Thus fungi were first cultured under conditions optimized for metabolite production and then the spent medium was used as leaching agent. High concentrations of oxalic acid can be produced by some fungi and a kaolin whiteness index of 80 % has been obtained following the leaching [6]. In other research; oxalic, citric and glycolic acids which are principal organic acids of microorganisms to be used in leaching were used in these slime sized (-75 µm) feldspar ores in order to reduce iron amount. As a result of the tests, effective parameters were determined and discussed. The best result was obtained as 67.90 % iron removal from feldspar by using oxalic acid [7]. Oxalic acid has also been tested for iron removal from quartz sands. Yields of iron extraction were 35–45 %, obtained on treating quartz sand with 3 kg oxalic acid/t and 2 kg sulphuric acid/t at 90 °C for 4–5 h [8].

The purpose of the present study was to investigate the feasibility of bioleaching in the deferritization of non-metallics. The experiments were designed to determine: (a) the effect of Fe-containing minerals in iron removal in the samples, (b) whether a combination of bioleaching and magnetic separation can be used to increasing the quality of samples, and (c) the effect of bioleaching conditions on the extent of Fe removal from samples.

2 Material and methods

2.1 Characteristics of bioleaching and separation

The heterotrophic bacterial cells were subsequently centrifuged at 4000 rpm for 15 min, subsequently washed twice with saline solution (0.9 % NaCl) and added in a concentration of 10^7 cells per ml to modified different liquid medium according to the publications [9-14]. pH value of medium was adjusted before the beginning of experiments at 7.

The batch experiments were carried out in flasks, each containing 1: 10 from sample and medium. The column-leaching tests were conducted using 44 mm diameter glass columns, with a height of 160 mm. Silica filter paper was placed on the bottom and of the top of the columns. During the 60 days of a bioleaching test, a 25 ^{o}C solution was pumped from flask containing same medium into the top of the sample bed. Leachates were discharged from the bottom of the sample bed.

Dry electromagnetic separation was carried out using a laboratory high gradient magnetic separator with the induction of magnetic field at 1.3 T.

3 Results and discussion

Experimental studies of the bioleaching on iron decomposition rates and reactions are commonly performed in closed (batch) and open (flow-through) reaction vessels. In batch experiments, minerals and solutions are placed in a container, solution composition is monitored over time, and reaction rates are calculated from the evolution of solution chemistry. In flow-through experiments, solution passes through reaction vessels (columns) containing mineral samples and reaction rates are calculated from the effluent concentration and flow rate. These laboratory tests imitated tanks and heap leaching (Fig. 1 A, B).

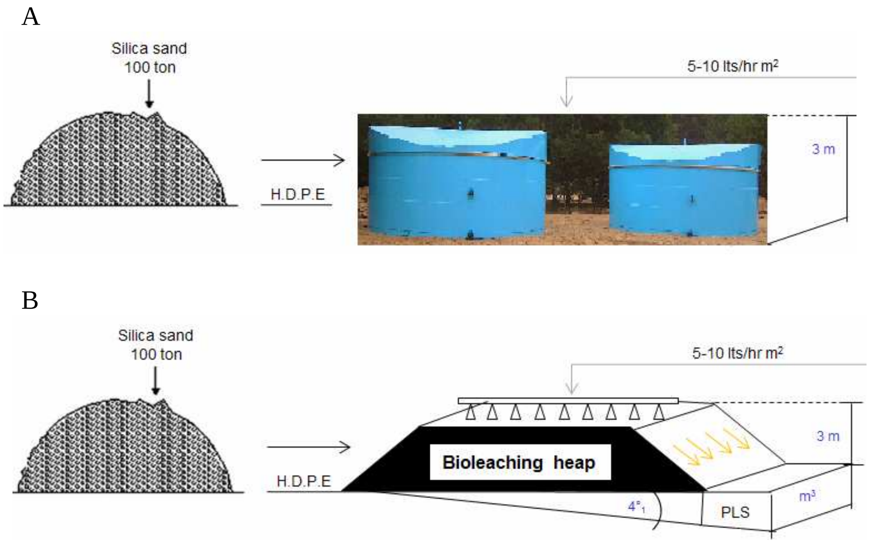

Fig. 1. Tanks (A) and heap bioleaching (B)

Bioleaching is effective for the removal of surface layers of fine iron minerals and fine-grained micaceous fraction. Visual comparison of colors was affected by mineralogical composition of all samples before bioleaching and after bioleaching showed decomposition and release of iron and clay minerals. This fact confirms combination of silicate destruction connected with iron dissolution detected by chemical analyses of leachates. Discrete iron minerals were removed by established process such as magnetic separation. This could be combined technology to increase the quality of non-metallics for ceramic and glass industry.

It was tested ability and activity of heterotrophic bacteria at Fe removal from various non-metallics, from various deposits under laboratory conditions. The overall effect of batch and columns bioleaching on

Fe removal was different in individual samples. Particle size of iron minerals, mineralogical and chemical composition of non-metallics and iron bearing minerals are major factors in determining the rate and extent of any bioleaching effect before magnetic separation (Table 1). The extent of iron removal from kaolin was 36 %, although from feldspars and quartz sand moved approximately 79-93 % in combination of bioleaching and magnetic separation. Bioleaching of kaolin was according to analyses of solid phases less effective and decrease of Fe content was in range 17 % because sample contained mica and pyrite admixtures where bacterial destruction was slow and magnetic separation was not effective in the iron removal. The decreased effect in iron removing was evident in bioleaching of feldspar (58 %) and quartz sands (59 %) sample dependent also to iron mineral composition and their size in the samples.

Table 1. The Fe concentration of non-metallic raw materials after treatment by bioleaching and magnetic separation

Sample	Untreated	Bioleached	Bioleached and Magnetic separated
	(%)		
Quartz sands (Šaštín)	0.32	0.13	0.02
Feldspar (Rudník)	0.29	0.12	0.06
Kaolín (Rudník)	1.10	0.91	0.70

The bioleaching steps dissolve Fe(III) impurities coating the surface of quartz grains, thereby increasing the amount of iron removal by subsequent magnetic separation. The removal of Fe(III) impurities could be performed by indigenous microorganisms but is greatly enhanced by addition of chelator and heterotrophic bacteria [13]. Bacterial respiration changed aerobic into anaerobic conditions, thus preventing the iron oxidation and reversible contamination of surface particles. The bioleaching samples in columns were less effective than in flasks and the difference in the Fe-removal was about 50 %. The heap bioleaching with heterotrophic bacteria took two times more time than bioleaching in tanks. The bioleaching laboratory tests with stationary flasks had significantly higher activity during partial imitation of tanks bioleaching because the organic source and nutrients were more available for heterotrophic bacteria in water environment than in percolate water environment. The dissolution of Fe was distinctly higher in the presence of heterotrophic bacteria than in the corresponding abiotic control where the content of Fe was below detection limit (< 0.1 mg/l). The heterotrophic bacteria are ubiquitous in the soil and non-metallic deposits and may accelerate the iron dissolution and decomposition of iron minerals such as goethite. Bioleaching is considerably slower than conventional techniques of removing iron impurities, and this needs additional optimization and possibly even input of other forms of microorganisms. The removal of Fe(III) impurities is possible with indigenous bacteria, but is greatly enhanced by augmentation with iron-reducing bacteria. These bioleaching results could be very important for the quality improvement of non-metallics.

4 Conclusions

Non-metallics contain iron minerals as coatings on grains or impregnations in the matrix. The extent of iron removal from samples depends on the mineralogy and distribution of iron in material. For this reason, bioleaching tests with industrial minerals for their quality improvement have examined the kinetics of iron dissolution from the sample by batch or column test. The feasibility of the bioleaching treatment has to be tested specifically to each type of non-metallics. This bioleached treatment may be applicable to the extraction of surface layers of fine iron minerals from non-metallic materials such as quartz sands, kaolin, and feldspars.

Acknowledgements

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EXTRACTION OF IRON FROM BENTONITE AND KAOLIN BY BIOLEACHING

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Abstract

The non-metallic raw materials as kaolin and bentonite are industrial minerals widely and extensively used as fillers and coating agents in the manufacture of paper, ceramics, glass, paint and cosmetics or as a sorbents for dissoluted toxic metals. Kaolin and bentonite are generally not pure, often associated with iron hydroxide and oxy-hydroxide impurities usually in the form of Fe^{3+} phases adsorbed onto the mineral surface. In all cases, the presence of iron affects the colour and the physical properties of the mineral, and so lowers the industrial value and limits their application. Due to the environmental, economic and operational disadvantages associated with conventional physical and chemical leaching processes, this work considers a microbial leaching process in a batch experiment. The iron removal of Fe(III)-bearing impurities from these samples was investigated using iron-reducing bacteria *Bacillus spp*. from reservoir Ružín. The flasks were incubated under static conditions for 15 and 30 days at 24 °C. The experimental results showed that these bacteria are able to remove 9.29 % of Fe occurring in kaolin in amorphous form of oxyhydroxides (K-I and K-R samples) and approximately 12 % of Fe from bentonite (B-K and B-JP samples) after 30 days of bioleaching process. The purpose of this work was to investigate the phenomenon of microbial iron reduction in industrial minerals and materials.

Keywords: bioleaching, kaolin, bentonite, iron minerals

1 Introduction

Non metallics are important and valuable industrial raw materials widely used in paper, glass, ceramics, cosmetics, paint, porcelain, potteries, and rubber production. They are also used in waste treatment, agriculture, pigments, nanocomposites as a coating, and filler materials [1].

Kaolin is an economically important raw material often used in wide variety of ceramic applications, from high quality tableware and sanitaryware to electrical porcelain, tiles and glasses. There are some less common uses including glass fibre, white cement and refractory insulation bricks. Bentonite are a kind of wide-spread and low-cost natural minerals and are characterized by many specific properties, e.g. high ion exchange capacity, compliance to water dispersion, excellent theological and sorption properties, strong swelling in polar liquids, and high tixotropy. These properties determine its commercial applications in a wide range of markets, including foundry, drilling, civil engineering, agriculture, ceramics etc [2].

The quality and commercial value of these minerals largely depends on their overall purity, texture and colour properties such as brightness, whiteness, tint and opacity [3].

Crystal size and shape of the mineral particle, and their bulk chemical composition, are, among other properties of considerable importance in industrial application, in addition to their gloss, rheological and abrasion properties. These minerals are generally not pure, often associated with iron hydroxides impurities usually in form of Fe^{3+} that are adsorbed on the mineral surface covering the entire mineral mass or admixed as a separate iron bearing phase. The impurities are generally in the form of hematite, magnetite, goethite, lepidocrocite, and/or ferrihydrite coating and binding strongly on the surface of individual grains or as discrete fine particles covering the entire clay mineral. The presence of such associated impurities remarkably lowers their industrial value and limits their area of application [4]. Therefore to achieve and maximize the required industrial quality and value specification of kaolin and bentonite, removal of associated iron impurities becomes absolutely imperative. The current conventional practice for the removal of iron impurities both as surface coating and as discrete fine form, involves physical and chemical methods such as froth flotation, magnetic separation, and selective flocculation [5]. However, these methods are known to have substantial technological, economic and environmental disadvantages. Because of the environmental disadvantages associated with the conventional physical and chemical refinement processes, recently, microbial leaching of iron has been receiving much attention as an alternative for refining Fe(III)-

containing minerals. Microbial Fe(III) reduction has over the decades been established as an important process that catalysis the oxidation of organic matter coupled to Fe(III) reduction. Therefore, the ability to remove ferric iron impurities in kaolin and bentonite through other means, such as application of metabolic activities of dissimilatory iron-reducing microorganisms and other fermentative organisms as the agent of iron bioleaching may potentially reduce cost, allow lower grade minerals to be recovered and hence improve the efficiency of mineral processing [6]. The purpose of this work was to investigate the phenomenon of microbial iron reduction in industrial minerals and materials.

2 Material and methods

2.1 Experimental samples

Natural samples of kaolin and bentonite were investigated. First sample of rich Fe kaolin was taken from the Horná Bříza deposit (denoted as K-I) and second kaolin sample with lower Fe content was from the Rudník deposit (denoted as K-R). Samples of Fe rich bentonite was collected from the Kadaň deposit (denoted as B-K) and bentonite with lower Fe content was from the Jelšový potok deposit (denoted as B-JP). Individual samples were different by form of Fe binding and with different content of iron. Their chemical composition is shown in Table 1.

2.2 Bacteria and media

Bacteria were isolated from the sediment of the river Hornád locality near the Ružín dam, loaded with toxic metals from the metallurgical industry. The strains were purified by heating at 80 °C for 15 min to kill vegetative cells. The isolate was identified with the BBL Crystal Identification System (Becton, Dickinson & Co., Franklin Lakes, NJ). For identification, the isolate was cultivated on Columbia agar plates according with the instructions of the manufacturer. The strains of *Bacillus sp*. were grown in Nutrient broth No.2 (Imuna, Slovak Republic) at 28 °C for 18 hours. Cells were centrifuged at 4000 rpm for 15 min, washed twice with saline solution (0.9 % NaCl). Medium contained (per liter) N and P fertilizers (DAM 390, Pekavit), chelating agent 0.59 g Na_2EDTA, 0.5 g $MgSO_4$, 0.14 g $CaCl_2$, 0.075 g NaCl, 0.35 g KCl, 2 g Na_2SO_4, 0.05 g yeast extract, 20 g glucose and 0.01 g nystatine as a fungal growth inhibitor; pH 7.0 (adjusted with NaOH).

2.3 Bioleaching of mineral samples

Bioleaching of the 10 g kaolin and bentonite samples was carried out in 250 ml flasks containing 100 ml of medium inoculated with a mixture of *Bacillus sp*. bacteria. The flasks were incubated under static anaerobic conditions at 24 °C for 15 and 30 days of bioleaching process. Bioleaching test involved two changes of 100 ml medium. Bioleaching process includes production of organic acids into leachates during the fermentation process when glucose was used as a source of carbon, because pH at the beginning of laboratory test was 7 and after 30 days was lowered to 4 in every sample. The results were compared with the abiotic control experiments. The abiotic controls were incubated under similar conditions but did not include an inoculum, only medium with glucose.

2.4 Chemical analyses

Concentration of metals in liquid phase were evaluated by standard analytical method – atomic absorption spectrometry on a VARIAN spectrometer AA - 30 apparatus (Varian, Australia) after dissolution of the samples by standard procedure.

3 Results and discussion

The release of Fe was used as indicator of mineral dissolution and beneficiation of kaolin and bentonite quality in this study. K-I sample of kaolin raw material composed of 8.91 wt.% iron (Table 1), which was found especially in the form of Fe oxyhydroxides. The presence of Fe oxyhydroxides was identified by visible brown-red colour of this material. The second kaolin sample K-R differed from K-I sample in lower content of iron (Table 1). K-R sample contained 1.10 wt.% of Fe, however, this material was of white colour. White colour suggests that a substantial part of iron in the sample is bound in the aluminosilicate lattice. Bentonite samples also had significantly different content of iron. B-K sample of bentonite composed of 15.2 wt.% iron in the form of Fe oxyhydroxides which confirmed its red colour. B-JP sample of bentonite contain only 2.99 wt.% iron and had a white colour (Table 1).

Table 1. Chemical composition of kaolin and bentonite samples

Element	Na [%]	Mg [%]	Al [%]	Si [%]	K [%]	Ca [%]	Ti [%]	Fe [%]
Kaolin-Horná Bříza (K-I)	0.26	1.37	2.4	56.4	3.64	0.29	1.18	8.91
Kaolin-Rudník (K-R)	0.36	0.83	23.2	66.2	3.33	0.17	0.47	1.10
Bentonite-Kadaň (B-K)	0.27	2.06	18.6	53.2	0.98	2.91	5.11	15.2
Bentonite-Jelšový potok (B-JP)	0.54	4.3	20.9	60.5	0.83	1.98	0.30	2.99

Chemical analyses of the solid phases showed that reductive dissolution of iron from kaolin and bentonite increased due to the heterotrophic bioleaching. The concentration of Fe in the kaolin sample K-I decreased by 2.57 % in the sample K-I 15D after 15 days and by 9.29 % in the sample K-I 30D after 30 days of bioleaching process and maximal concentration of dissolved iron in leachates was 82805 mg/l (Figure 1). The removal of iron from the second kaolin sample K-R with lower iron content was by 1.51 % after 15 days and by 7.54 % after 30 days of bioleaching process. Maximal concentration of dissolved iron in K-R sample was 5802 mg/l (Figure 1). In the bioleaching process of bentonite samples, the iron removal was lower from sample B-K with higher iron content. The concentration of iron in sample B-K after 30 days of bioleaching was decreased only by 5.33 % and maximal concentration of iron in leachates was 81077 mg/l (Figure 2). It can be linked with presence of fungi in sample B-K 30D which can inhibit activity of *Bacillus spp*. in the reductive iron dissolution because loss of Fe in sample B-K15D after 15 days was 4.20 %. The bioleaching of sample B-JP with lower iron content showed better iron removal than in case of sample B-K after 30 days of bioleaching process. The concentration of iron was decreased by 7.58 % in the sample B-JP 15D and by 12.02 % in the sample B-JP 30D, the maximal concentration of dissolved iron was 21039 mg/l after 30 days (Figure 2).

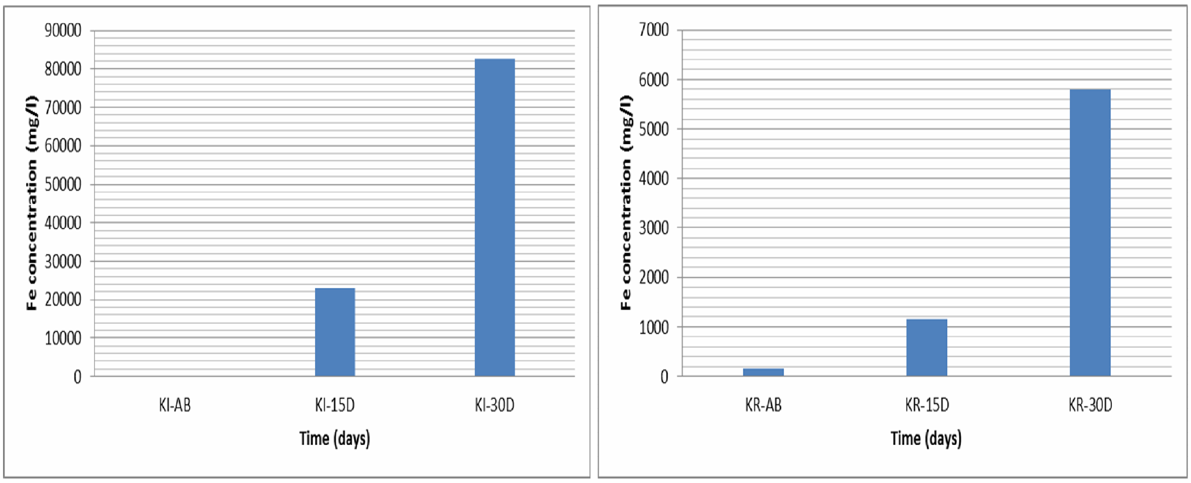

Fig. 1. Concentration of dissolved iron in kaolin samples after 15 (15D) and 30 days (30D) of bioleaching and their comparison with abiotic control (AB)

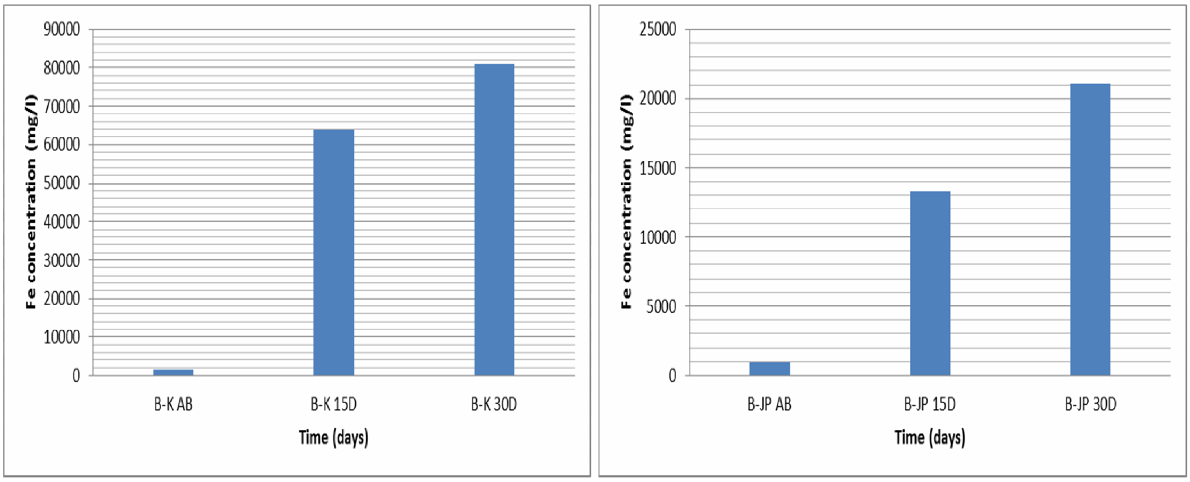

Fig. 2. Concentration of dissolved iron in bentonite samples after 15 (15D) and 30 days (30D) of bioleaching and their comparison with abiotic control (AB)

4 Conclusions

Bacterial leaching is a suitable way for the improvement of qualitative properties of raw materials as kaolin and bentonite which have a significant value for their utilization in different areas. Bacteria of *Bacillus spp.* can decrease the content of Fe, which often contaminates kaolin and bentonite. An enrichment of the kaolin samples by fine-grained fraction is also an accompanying positive fact of bacterial leaching. This process is time consuming from the technological view but very advantageous with the respect to ecology and economical costs. The process of conversion the insoluble iron compound to a water soluble form can remove Fe from the non-metallic materials in different intensities which can influence their properties. A leaching process can break Fe binding and transform Fe particles to the active form, which participates in surface reactions forming complexes via Fe ions and which could be used for the improvement of adsorption properties of clay materials.

Acknowledgements

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NEW BIO-HYDROMETALLURGICAL APPLICATION TO RECOVER ZINC AND MANGANESE FROM ALKALINE SPENT BATTERIES

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Abstract

The consumption of batteries has continually accelerating trend. Disposal of spent batteries represent severe environmental problem due to the metallic content, being considered as hazardous waste. Reutilization and recycling of spent batteries are appearing in order to minimize waste as well as to preserve raw materials.

The present experimental work involves the new bio-hydrometallurgical application, considering the integration of physical-chemical and biological-chemical methods for Mn–Zn recovery from spent alkaline batteries. The main aims of the research were to recover selectively MnO_2 and Zn from bioleaching solutions containing Mn and Zn by electrolysis, with the scope to apply electrochemical technologies for Zn and Mn recovery from spent batteries and to verify the possibility of spent batteries processing by bioleaching with *Acidithiobacillus ferrooxidans* together with the molasses coming, as waste materials, from agro-food industry.

Average content of Zn in alkaline batteries was about 22 %. The average Mn content was similar (21 %). Average content of K was 4.9 %, while Ni 0.65 % and Fe 0.6 %.

Experimental results have demonstrated the technical feasibility of the application of bio-hydrometallurgical processes - integrated by electrochemical technologies - for Zn and Mn recovery from exhaust batteries.

On the basis of the experiments outlined, a preliminary flow-sheet for the recycling of alkaline batteries has been realized.

Keywords: bio-hydrometallurgy, heavy metals, spent batteries, electrowinning, zinc, manganese

1 Introduction

The consumption of batteries has continually accelerating trend. Disposal of spent batteries represent severe environmental problem due to the metallic content, being considered as hazardous waste. Reutilization and recycling of spent batteries are appearing in order to minimize waste as well as to preserve raw materials [1].

According to European Union Battery Directive Extended Impact Assessment [2] each year approximately 800 000 tones of automotive batteries, 190 000 tones of industrial batteries and 160 000 tones of portable batteries are placed on the community market. The total weight of portable batteries sold in the East and West Europe in 2003 was about 164 000 tones, of which 50 197 and 99 138 tones were zinc–carbon and alkaline batteries, respectively (30.5 % and 60.3 % of the total annual sales). The collection rate in average is currently 13.6 % for the EU whereas the target is 25 % in 2012.

Battery is a collection of two or more electrochemical cells which represent a source of direct current. The inside of battery contains an anode, a cathode, an electrolyte and separators [3]. In an alkaline battery, the anode is made of zinc powder (which allows more surface area for increased rate of reaction therefore increased electron flow) and the cathode is composed of carbon and manganese dioxide (MnO_2). Acquisition of electric energy is based on chemical reaction between zinc and carbon in the presence of alkaline electrolyte (potassium hydroxide) [4]. Electrochemical reactions are:

- anodic: $Zn + 2OH^- \rightarrow ZnO + H_2O + 2e^-$
- cathodic: $2MnO_2 + H_2O + 2e^- \rightarrow Mn_2O_3 + 2\ OH^-$

Zinc and manganese represent the greatest percentage of metals in alkaline and zinc-carbon batteries. The amounts of these majority elements in alkaline and zinc-carbon as well as mixture of alkaline and zinc-carbon batteries according chosen authors describes Table 1. The minority elements are carbon (3.5 - 3.7 wt.%), potassium (3.5 - 7.5 wt.%), iron (0.1 - 0.5 wt.%), lead (0.005 – 0.03 wt.%) and nickel (0.3 - 0.5 wt.%).

Table 1. Amount of zinc and manganese in alkaline and zinc-carbon batteries

Battery	Alkaline	Zinc-carbon	Mixture of alkaline and zinc-carbon
	(wt. %)		
Mn	36.53	26.30	33.59
Zn	17.05	28.3	15.46
Ref.	[5]	[6]	[7]

After exhausting of chemical energy batteries become hazardous waste, but contains useful parts particularly Zn and Mn in the different forms. The spent zinc-carbon batteries contain Zn, MnO_2, as well as ZnO and Mn_2O_3 produced from discharging reaction [8]. Therefore, it is zinc and manganese that are of interest when it comes to the recycling of these batteries.

General ways of their recycling can be divided into three groups: physical, pyrometallurgical and hydrometallurgical treatment.

In physical treatment the same methods are used as are commonly used in mineral and ore processing with the aim to separate interesting metals or to create fractions rich in metals. Usually it is first step also in pyrometallurgical or electrolytic treatment. But pyrometallurgical processes need a lot of energy because the temperature during treatment is often more than 800 – 1000 °C [9]. When hydrometallurgical processes are used, metals are leached into solution and then recovered by precipitation or electrolysis. Zinc as amphoteric element may be leached in sulphuric acid but also in sodium hydroxide. The advantage of acidic leaching is relatively high efficiency; but part of the manganese is also leached. When hydroxide is used efficiency of Zn leaching is lower, only up to 40 %, but other metals stay insoluble. So it is possible to remove Zn selectively [10]. Very interesting possibility although not very studied in present time is the possibility offered by biohydrometallurgical processes using living bacteria to recover metals [11].

Bioleaching is environmentally-friendly, economical and cost-effective. The activity of different groups of microorganisms, alkalophilic or acidophilic mainly mesophilic, moderate and extremely acidophilic bacteria are used in bioleaching. These microbes aid in dissolving metals by secreting inorganic and organic acids into aqueous media. Among major groups of bacteria most commonly used are acidophilic and chemolithotrophic microbial consortia of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans, Leptospirillum ferrooxidans* and heterotrophs for example *Sulfolobus sp.* In addition, microscopic fungi such as *Penicillium sp.* and *Aspergillus niger* are examples of some eukaryotic microorganisms used in bioleaching during metal recovery from industrial waste [12]. Acidophilic and chemolithotrophic bacteria produce enzymes, oxidize Fe^{2+} to Fe^{3+}, produce H_2SO_4, oxidize film of elemental sulphur formed on the leaching material surface and create the environment for the metal dissolution. Bacteria *Acidithiobacillus* are most used for the bioleaching of the spent batteries. These bacteria are chemoautotrophic and strictly aerobic. They gain energy by oxidation of reduced sulphur compounds. Some species oxidize ferrous ions or use sulphides to gain energy, some species oxidize hydrogen. As the carbon source they utilize CO_2. The growth optimum is 30 – 35 °C for mesophilic species and 45 °C for moderately thermophilic species. They are tolerant to acidity. Optimum pH is lower than 4 [13]. One of the first studies on microbiological leaching of metals from electronic waste was carried out in presence of mixed bacteria of *A. ferrooxidans* and *A. thiooxidans* [14]. Authors observed toxic influence of waste on microorganisms. Gradual adaptation of bacteria to the environment and the addition of acidifying agent improved the efficiency of the process. Research on bioleaching of metals from spent batteries by using acidophilic and chemolithotrophic bacterial strains were also carried out by some authors [15, 16].

The present experimental work involves the new bio-hydrometallurgical application, considering the integration of physical-chemical and biological-chemical methods for Mn–Zn recovery from spent alkaline batteries. The main aims of the research were: to develop the preliminary experimental work to recover selectively MnO_2 and Zn from bioleaching solutions containing Mn and Zn by electrolysis, with the scope to apply electrochemical technologies for Zn and Mn recovery from spent batteries; to verify the possibility of spent batteries processing by bioleaching with *Acidithiobacillus ferrooxidans* together with the molasses coming, as waste materials, from agro-food industry [17].

2 Material and methods

2.1 Physical preparation and characterization

By manual dismantling, products of spent alkaline batteries (Fig. 1), such as plastic films, ferrous scraps and paper pieces were separated, while mixture of the cathodic (manganese oxides) and the anodic (zinc oxides and electrolytic solution) materials, under form of powder, was extracted [18].

The powder was dried ground, using a ball mill, sieved and analyzed by X-ray diffraction (XRD), utilizing an automatic diffractometer Bruker mod. D8 Advance (data not shown here). Quantitative chemical analysis was conducted by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Average content of Zn in alkaline batteries was about 22 %. The average Mn content was similar (21 %). Average content in K was 4.9 %, while Ni 0.65 % and Fe 0.6 %.

Iron has been precipitated by an alkaline reagent, while potassium has been removed by washing with distilled water. Fig. 2 shows the equipment used for physical treatment (sampling, magnetic separation, milling).

Fig. 1. Sample of exhaust alkaline batteries

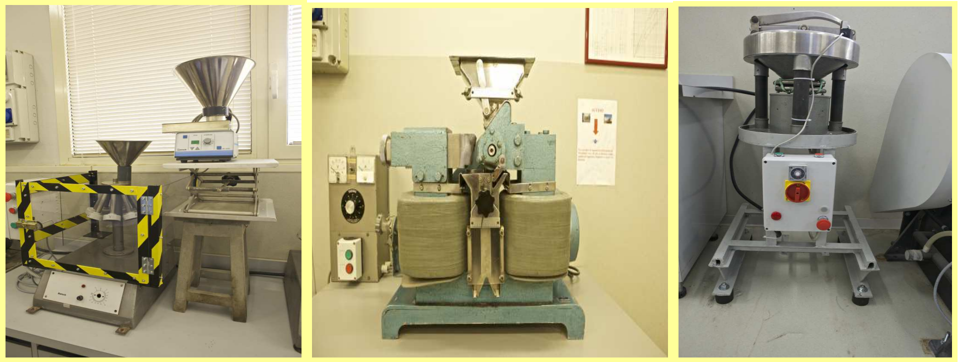

Fig. 2. Equipment used for physical treatment (sampling, magnetic separation, milling)

2.2 Bioleaching

A pure bacterial culture of *Acidithiobacillus ferrooxidans* originated from acid mine drainage coming from Smolník, Central Slovakia, has been utilized. Inoculation and growth and cultivation of the bacteria have been carried out [19, 20]. Bacteria adaptation to the powder of alkaline batteries was done by adding 5 g powder per 1L of 9K medium. Adaptation process took one month at t = 30 °C. Before the start of the bioleaching experiments, the adapted bacteria were transferred into the fresh 9K medium and cultivated for 3 days, after adding of 10g/L powder of alkaline batteries. Then 5g/L molasses were added as reducing agents. The bioleaching experiments were carried out at two different levels of temperatures (22 °C and 35 °C) [21]. Kinetic of the bioleaching process was studied by analysis of the liquid samples – collected during the bioleaching process - for 29 days. The Zn and Mn concentration in each sample was determined by Atomic Absorption Spectrometry (Varian AA20+).

2.3 Electrowinning

To develop the electrowinning study, synthetic solutions, constituted from a manganese and zinc sulphate, together with the dissolved metals presents in the real solutions from bioleaching, have been prepared, by reagents of high analytical degree of purity [22].

A cylindrical glass laboratory cell of 200 mL in volume has been used (Fig. 3), equipped by a thermostatic water jacket connected with thermostatic bath, magnetic system for stirring and three electrodes [23].

The cell was connected to a potentiostat-galvanostat (AMEL mod. 555B), equipped with an instrument system to automatically control the process parameters at constant potential conditions (Fig. 3). By analysing the residual solution with ICP-MS, the purity of the MnO_2 and Zn deposition was monitored, while the deposit was analysed by XRD.

Electrowinning experiments were carried out in potentiostatic operations at constant anodic potential and stirring conditions. The kinetics of MnO_2 and Zn deposition were studied by selected voltages [24].

The voltage between working and reference electrodes was automatically monitored. The corresponding voltages (V) between working and counter electrodes (cell voltages) were measured with differential electrometer.

Average experimental cell voltages were utilised to calculate the energy consumption during the tests. The current flowing through the electrolytic cell was automatically integrated. To calculate the current efficiency and the average energy consumption, experimental data were utilized [25].

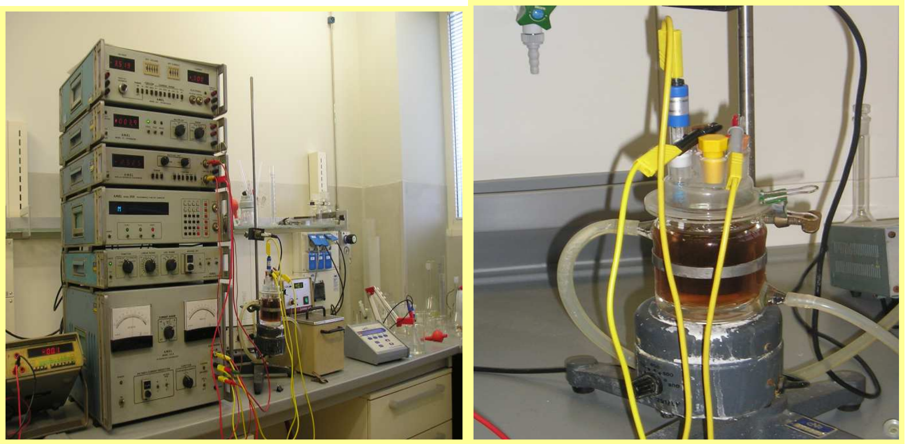

Fig. 3. Equipment used for chemical treatment-electrowinning, with the electrolytic cell

3 Results and discussion

The bioleaching experiment was carried out to study the influence of temperature and molasses on Mn and Zn dissolution [26]. Zinc concentration in the solution increased in the first 14 days of bioleaching when the highest amount of Zn was dissolved. In general, a higher metal leaching efficiency by *A. ferrooxidans* is reached in the 35 – 38 °C temperature range [27]. However, based on our results (Tab. 2) a higher Zn bioleaching efficiency was reached at a lower temperature (room temperature). The addition of molasses as a reducing agent did not enhance Zn dissolution.

The Mn bioleaching process rates were the similar to those in Zn bioleaching. Manganese dissolution was the highest during the first 14 days of bioleaching. Mn bioleaching efficiency at a low temperature (22 °C, room temperature) and in the absence of molasses was found to be 30 %. The addition of molasses did not increased Mn bioleaching efficiency significantly (Tab. 2).

Based on the results (Tab. 2) it may be assumed that a higher temperature can slightly enhance Mn dissolution from spent alkaline batteries.

Table 2. Efficiency of alkaline batteries bioleaching after 14 days

Conditions	Zn	Mn
	%	
22 °C	63	45
22 °C + reducing agent	45	41
35 °C	53	51.5
35 °C + reducing agent	45	41

Main observations consequent to the experimental texts for electrolytic selective recovery of MnO_2 from synthetic solution containing Zn, have been the following:

- On the basis of data achieved during the preliminary electrodeposition texts, the experiments should be conducted at temperature of 95 °C, at constant potential, at fixed value of 1.8 V (Anode - SCE) and corresponding cell potential (anode - cathode) of 2.6-2.8 V.
- After 120 min of electrolysis 97.55 % of MnO_2 has been recovered, with average faradic yield of about 49 %, while the energetic consumption was of 3.48 kWh/kg of MnO_2 recovered.
- Zinc remains quantitatively in solution, after MnO_2 anodic deposition.
- By Zn electrolysis of a solution containing only Zn sulphate, conducted at temperature of 40 °C, constant potential, fixed value of 1.4 V (cathode - SCE) and corresponding cell potential (anode - cathode) of 3.6 V, about 99 % of metallic cathodic deposition of Zn was achieved, with a faradic current efficiency of 72.89 %, in 2 hours of electrolysis and an energetic consumption 4.08 kWh/kg of Zn recovered.
- Selective recovery of MnO_2 and Zn from synthetic solution containing Mn and Zn, both in concentration of 10 g dm^{-3} has been achieved. Best electrowinning results of the experiments have been shown in Tab. 3. MnO_2 and Zn deposit at high grade of purity (99 %) has been attained; this fact was demonstrated from the results of the analyses conducted by diffractometric techniques.

Table 3. Main electrowinning results of the solution containing Mn and Zn

Species	Recovery	η	E
	%		(kWh/kg)
MnO_2	97.55	49.00	3.48
Zn	98.90	72.89	4.08

η = faradic current yield, E= energetic consumption

These laboratory results are very encouraging, considering that it is an innovative process that permits to recover purified metals. The further work, including the optimization of parameters and operating conditions and the subsequent scale-up in continuous applications, will permit to increase the process yields and to determine the economical sustainability of the application.

According to the experimental results, the following preliminary flow-sheet has been proposed (Fig.4).

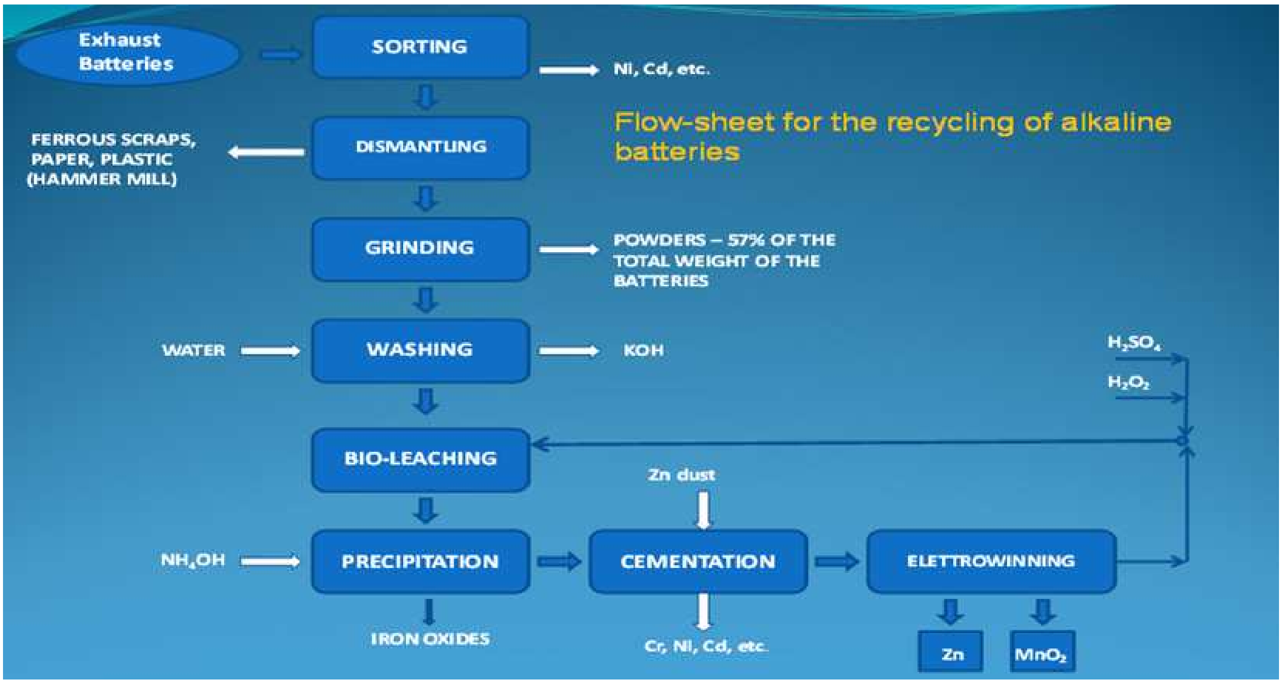

Fig. 4. Flow-sheet for the recycling of alkaline batteries

4 Conclusions

The obtained results have demonstrated the technical feasibility of the application of bio-hydrometallurgical processes - integrated by electrochemical technologies - for Zn and Mn recovery from exhaust batteries.

Bioleaching of spent batteries is one of the possibilities how in economical and environmentally friendly way recover metals from batteries. As there is only very little knowledge about alkaline batteries bioleaching our aim was to verify the possibility to use bacteria in this process. In according to our results, bioleaching - integrated by electrochemical technologies - can be successfully used in the process of spent alkaline batteries treatment. Influence of temperature on Zn and Mn bioleaching can help in selective recovery of mentioned metals from batteries.

A quantitative recovery (about 98 %) of purified manganese (MnO_2) and zinc dissolved in solution has been achieved at the end of the electrodeposition step.

Acknowledgements

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FUNGAL DESORPTION OF ANTIMONY FROM MANGANESE OXIDES

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Abstract

In this paper fungal contribution on antimony mobility was evaluated during 14-day *Aspergillus niger* cultivation in presence of manganese oxides. Manganese oxides biotransformation process was analysed by X-ray powder diffraction analysis (XRD), and fungal antimony bioaccumulation and biovolatilization was confirmed and determined by flame atomic absorption spectrometry (FAAS). XRD analysis determined manganese oxides as hausmannite which was dissolved during cultivation and transformed to manganese oxalate - lindbergite. Due to rapid manganese biomineralization, almost 99 % of antimony was desorbed from manganese mineral surface. Antimony mobilization was further enhanced by fungal biovolatilization and relative small amount of antimony remained in fungal biomass (8-17 %). This concludes that enhanced activity of microscopic filamentous fungi significantly increases mobility of antimony in the environment.

Keywords: filamentous fungi, bioleaching, antimony

1 Introduction

Manganese oxides are highly reactive mineral phases [1] and one of the strongest oxidants in the natural environment. Thus, they take part in various redox and sorption reactions in wide range of pH [2]. For their strong ability to scavenge elements and compounds, manganese oxides are recognized as important natural geochemical barrier [3]. This also includes efficient immobilization of antimony [4]. Despite its low distribution, industrial application of antimony is widespread, thus posing serious problem for the environment, especially in the vicinity of mining and smelting areas [5].

One of the most significant environmental factors affecting antimony and manganese geochemistry is microbial activity. Especially in case of bioleaching, various risks emerge from enhanced activity of autochthonous microbial consortia in contaminated areas and should be elucidated in more details. Both bacteria and fungi have been recognized as the important manganese and antimony transformation agents [6]. However, filamentous fungal bioleaching is advantageous over bacteria because of (1) the higher leaching rate, (2) ability to grow under wide pH range and (3) resistance to high toxic metal concentrations exposure [7]. Therefore, enhanced fungal metabolic activity can cause mineral phases deterioration and enhance the mobility of various hazardous metals in the environment.

Although microbial formation of manganese oxides have gained substantial attention, especially as a fundamental factor in potentially toxic metals and metalloids geochemistry [8], little is known about the fungal contribution to the manganese oxides solubilization and transformation [9] and its impact on environmental fate of inorganic pollutants. Therefore, the main objective of this study was to evaluate fungal manganese oxide (hausmannite) biodeterioration and bioleaching of potentially toxic element (antimony), as well as to elucidate effects of biologically induced mineral transformation on antimony mobility, bioavailability, accumulation and volatilization by fungus.

2 Material and methods

2.1 Fungal strain

Aspergillus niger strain was isolated from mercury contaminated soil at Slovakia. Strains are deposited in fungal collection of the Department of Mycology and Physiology, Institute of Botany at Slovak Academy of Sciences.

2.2 Manganese oxide synthesis

Manganese oxides used in this study was prepared by alkaline (40 g NaOH) precipitation of $MnSO_4$ (111.5 g $MnSO_4.6H_2O$) in 1 liter of deionized water under laboratory conditions, respectively. After 5 hour heating under reflux, the freshly prepared precipitate was filtered, washed with distilled water and dried at 80 °C. Precipitate was then oven heated at 95 °C for 1 hour prior to use and analysis. Sample X-ray characteristics were established by X-ray powder diffraction (XRD) analyses on difractometer BRUKER D8 Advance in Bragg-Brentano geometry (theta-2theta). The XRD patterns were collected using Cu $K\alpha 1$ (λ $K\alpha 1$=1.5406 Å) radiation in the 15 - 65 2θ range with 0.01 step size and a counting time of 1 s per step.

2.3 Bioleaching experiment

Series of 50 mL culture Sabouraud Dextrose Broth media (HiMedia, India) autoclaved at 121 °C for 15 min were supplemented with 0.1 g Mn_3O_4. Except of control experiments, all treatments were supplemented with 5 mL of 98 mg l^{-1} or 528 mg l^{-1} potassium antimony tartrate concentrations and stirred at 130 rpm (Unimax 2010, Heidolph, Germany) for 2 hours and subsequently inoculated with fungal spores followed by 14-day incubation at 25 °C in the dark. Thereafter, fungal biomass was separated from the culture media, washed with distilled water and dried at 25 °C. Prior analytical determination of antimony, the resultant insoluble residue in culture medium was collected by filtering the medium through 0.45 µm MCE membrane filter, analyzed by XRD and then dissolved in 5 ml of concentrated HNO_3. Dry biomass was weighted, and pH of filtered culture medium was determined (HI 8424; Hanna, Italy) before analysis of antimony. Control experiments were carried out without either antimony or synthesized phase following the same protocol as described previously. All treatments were in triplicate.

2.4 Analysis of antimony in culture medium, biomass and insoluble residue

Total biomass antimony was analyzed following microwave digestion (Multiwave 3000, Anton Paar, Austria) in 8 ml of concentrated HNO_3. Total amount of antimony was analyzed by flame atomic absorption spectrometry (F-AAS) with the Perkin-Elmer Atomic Absorption Spectrometer model 1100 (USA) [10-13].

Analytical procedure accuracy was tested by analyses of certified reference materials (CRM) of plants NCS DC 73349 (Bush Branches and. Leaves) and NCS DC 73350 (Poplar Leaves); both from the China National Analysis Centre for Iron and Steel, Beijing, China.

3 Results and discussion

X-Ray diffraction patterns in Fig. 1 detected our laboratory prepared manganese oxide as hausmannite $[Mn^{2+}(Mn^{3+})_2O_4]$. Hausmannite was transformed completely during 14-day cultivation of *A. niger* into new biomineral, while no changes in XRD patterns were detected in fungal-free control. Filamentous fungi naturally produce great amount of oxalic acid [14]. In our case, this organic acid served as suitable substrate for manganese biomineralization. Our newly formed manganese containing biomineral was identified by XRD (Fig. 1) as oxalate monohydrate - lindbergite $[Mn(C_2O_4).H_2O)]$.

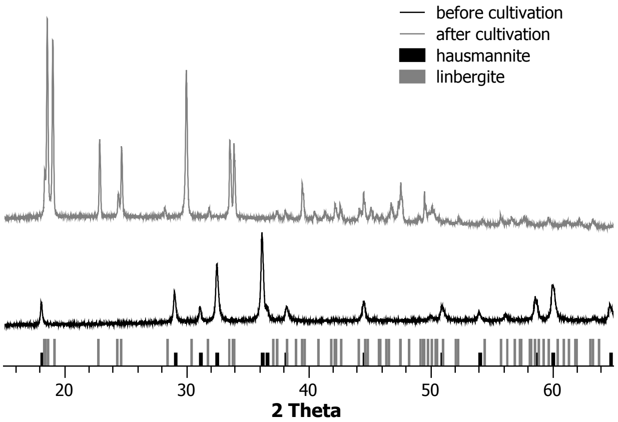

Fig. 1. XRD patterns of synthetically prepared and biotransformed manganese mineral phases hausmannite $[Mn^{2+}(Mn^{3+})_2O_4]$ and lindbergite $[Mn(C_2O_4).H_2O)]$

From geochemical point of view, this unique and rapid biotransformation with significant contribution of filamentous fungi as geoactive agent [15] can affect not only environmental fate of manganese, but also the fate of some nutritious elements and pollutants scavenged by manganese oxides [16]. This also includes antimony which was pre-adsorbed onto hausmannite surface in our experiments.

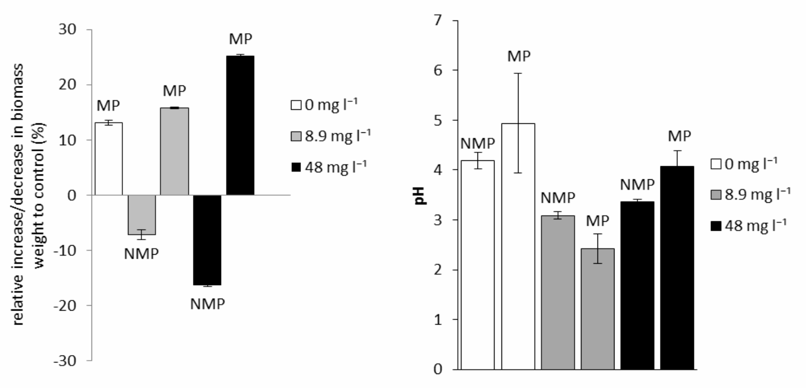

Fig. 2. Changes in pH values and values of relative biomass dry weight increase or decrease compared to antimony(III)- and hausmannite-free control after 14-day cultivation period in mineral phase treatments (MP) and treatments without mineral phase (NMP). Values in legend indicate initial antimony concentrations in culture media.

It was suggested that highly insoluble mycogenic metal oxalates are formed to enhance fungal resistance against metal toxicity [17]. Therefore we consider manganese solubilization and biomineralization an efficient fungal mechanism to regulate concentration of bioavailable manganese in the culture medium. Furthermore, the uptake and distribution of manganese is critical for proper function of various manganese-requiring enzymes, including free radical detoxifying enzymes [18]. Fig. 1 highlights that the biomass dry weight in hausmannite presence was significantly higher compared to control, even in antimony(III) treatments. This finding is explained by (1) excellent sorption properties of applied mineral phase restricting antimony bioavailability in culture media during initial growth phases; and its role as fungal growth enhancing nutrient [19], and (2) enrichment of culture media with tartaric antimony(III) salt. Tartrate can be utilized by *A. niger* as carbon source and thus enhancing fungal growth with significantly higher biomass yield compared to control as reported by Littera et al. [20].

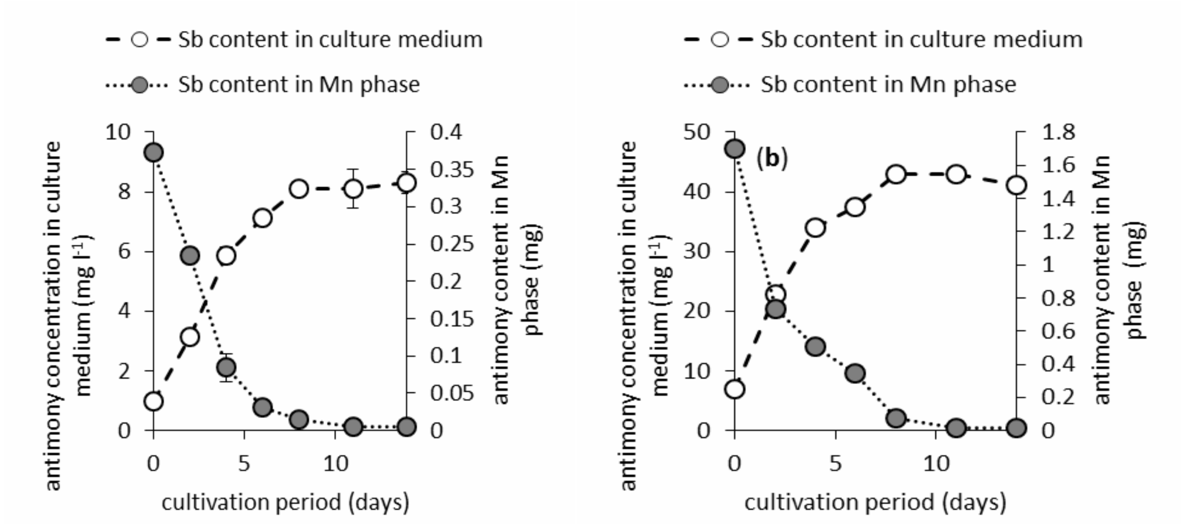

Fig. 3. Changes antimony content in culture medium and manganese mineral phase during 14-day static *A. niger* cultivation. Treatments varied in initial antimony concentration – (a) 8.9 mg l^{-1} (b) 48 mg l^{-1}. Manganese mineral phase was biologically transformed during cultivation with initial hausmannite to final lindbergite mineral. This resulted in antimony bioleaching and its negligible immobilization in new biogenic mineral phase after *A. niger* cultivation.

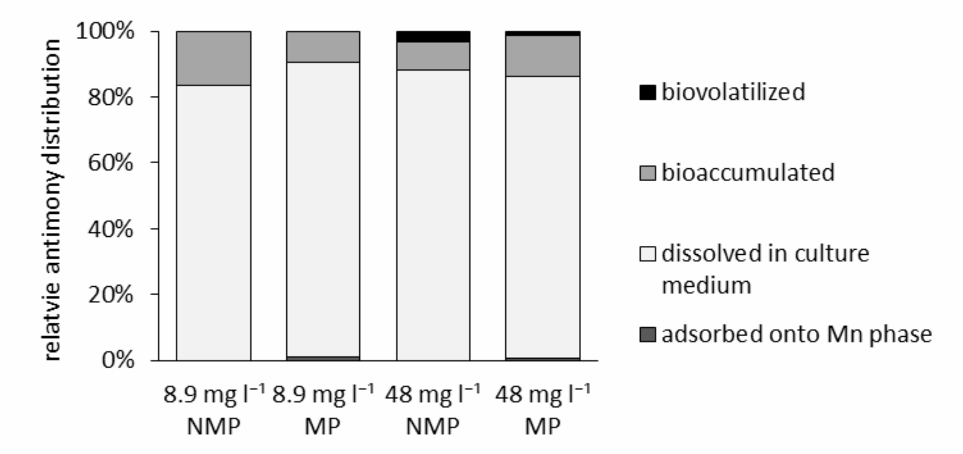

Fig. 4. Relative antimony distribution in biomass, culture medium and mineral phase with indicated volatilization efficiency after 14-day cultivation period of *A. niger*. Labels in bars indicate total amounts of dissolved, adsorbed, bioaccumulated and biovolatilized antimony. Antimony adsorption onto mineral phases was negligible with up to 1 % efficiency. Distribution of antimony in treatments with mineral phase (MP) was relatively similar compared to controls without mineral phases (NMP).

Fig. 2 also depicts culture medium pH decrease in all treatments including control during 14-day cultivation period. Naturally, we would expect that media acidification reflected metabolic activity of fungus, including organic acid production and biomass synthesis [21, 22]. However, it is more likely that antimony prolonged one of the early fungal growth phases resulting in higher culture media pH of antimony-free treatments on the 14th cultivation day.

Table 1. Calculated Langmuir isotherm parameters with estimated errors for antimony(III) sorption onto manganese phases

manganese phase	**Langmuir constant K_L (L.mg^{-1})**	**maximum sorption capacity for antimony S_{max} (mg g^{-1})**	**r^2**
hausmannite	0.049±0.011	40.2±2.2	0.98
lindbergite	0.067±0.058	3.7±0.5	0.82

Nevertheless, hausmannite solubilization and transformation was triggered by culture media pH decrease and fungal organic acid production [23]. This also resulted in biologically induced antimony leaching from hausmannite surface. While at the beginning of cultivation almost 85 % of total Sb was immobilized on the hausmannite surface, at the end of cultivation the amount of antimony detected in insoluble residue was negligible (Fig. 3). This is most likely due to hausmannite biotransformation to lindbergite. We hypothesize that during this process some of fungal exometabolites (e.g. oxalate, pyruvate) reduced manganese(III) to soluble manganese(II) in conjunction with dissolved oxygen depletion during fungal incubation [23]. This led to hausmannite solubilization and continual antimony release from mineral surface and its subsequent bioaccumulation [24]. Oxalate then complexed with dissolved manganese and formed new mycogenic mineral lindbergite. However, our results indicate that this mineral had significantly lower efficiency to scavenge dissolved antimony (Table 1).

This phenomenon has significant impact on understanding of antimony geochemistry in soils. We know that in soil fairly large amount of antimony is likely to be sorbed onto oxides/oxohydroxides, including manganese oxides [25]. Therefore, antimony mobility in soil is considered low [26]. However, our results indicate that in case of enhanced fungal activity, antimony can be easily remobilized from manganese oxide surfaces due to (1) manganese reductive or acidic solubilization and (2) manganese biomineralization in form of mycogenic minerals with low sorption capacity for antimony. Besides, antimony mobility in the environment is further enhanced by its unique microbial transformation into its volatile derivatives [27, 28]. It is clear from Fig. 4 that bioleached antimony was available for fungal bioaccumulation and biovolatilization. Furthermore, our results show that there is no significant difference in antimony distribution in hausmannite treatments compared to controls with no mineral phases. This is despite initial antimony up to 85 % efficient adsorption onto hausmannite.

This finding highlights environmental significance of manganese bioleaching and biomineralization by fungi in the environment, where manganese oxides are important sink of toxic metals [29]. Our results indicates that antimony is easily leached at fungal presence from mineral surfaces, accumulated and transformed into volatile derivatives by biovolatilization, such as in case of arsenic [30].

4 Conclusions

Manganese oxides play an important role in geochemical cycle of various elements. Because of their extensive occurrence and high sorption capacity, manganese oxides are recognized as scavengers and reservoir of potentially toxic elements. Our results highlighted that increased fungal activity and acidic metabolite production led to hausmannite (manganese oxide mineral) solubilization and transformation into mycogenic manganese oxalate. This was identified by XRD as lindbergite. This phenomenon has affected antimony(III) mobility in cultivation system. While at the beginning of cultivation almost 85 % of antimony was adsorbed onto mineral phase, after hausmannite solubilization and lindbergite biomineralization only negligible amount of antimony was immobilized in mineral phase. At fungal presence, antimony mobility was further enhanced by its unique microbial transformation into its volatile derivatives. These findings provide a deeper insight and understanding of both manganese and antimony geochemistry and their fate in the environment. It also gives a basic background for further studies on potentially toxic elements mobility at contaminated sites treated by bioremediation techniques, including bioaugmentation.

Acknowledgements

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BIOLEACHING OF METALS FROM ELECTRONIC WASTE USING COLUMN REACTOR

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Abstract

Article presents results of bioleaching experiments of Cu and selected associated metals (Zn, Al, Pb) from electronic waste in the form of a printed circuit boards by column method, using *Acidithiobacillus ferrooxidans* bacteria. The influence of the basic parameters such as pH and oxidation-reduction potential (ORP) on the extraction of metals from the solid phase into solution was taken into account. Bioleaching was conducted in two separate tests using column reactor filled with: i) waste material shredded into the fraction < 0.2 mm, ii) waste material shredded into the fraction <5 mm. Within 45 days 36.5 % of copper, 50 % of Zn and Al of 3.4 % was recovered from PCB fraction < 0.5 mm. For e-waste shredded into grain size < 0.2 mm results were slightly better: 77.1 % of Cu, 49.8 % of Zn and 14.6 % of Al.

Keywords: e-waste, bioleaching, column reactor, copper, *A. ferrooxidans*

1 Introduction

Periodical leaching is commonly used method in research of metal extraction from waste material using microorganisms. Few reports in the literature (concerning polymetallic postconsumer waste) focused on conducting the experiments in dynamic conditions using column method [1]. Typical column tests are carrying out to simulate the long-lasting physical, chemical and biological processes occurring in industrial condition (or natural) on the leached heaps or landfills of waste coming from treatments of minerals or metal ores. Undoubtedly, an advantage of continuous methods is the possibility to conduct the experiment with the participation of significantly higher levels of solid and liquid (leaching) phase, in the same time the process enables also to choose better the physicochemical parameters that influence on the kinetics of the metal dissolution process, by this on its effectivity.

In the experiment the column reactor was used to extract metals from shredded e-waste in the form of Printed Circuit Boards (PCB). The main aim of the work was to estimate the kinetics of copper ion oxidation and other accompanying metals such as zinc, aluminium, lead using *Acidithiobacillus ferrooxidans* bacteria in the controlled conditions of leaching solution flow in the closed circuit of column reactor. It was considered the influence of the basic parameters such as pH and oxidation-reduction potential ORP on the extraction of Cu, Zn, Al and Pb from the solid phase into solution. Experiment of biological leaching was carried out in two independent variants applying electronic waste PCB shredded into the fraction: i) < 0.2 mm; ii) < 5 mm.

2 Material and methods

The material under investigation was electronic waste in the form of printed circuit board (PCB) coming from spent cell phones. Material was shredded by means of cutting mill into the fraction of particles lower than 0.2 mm and cut into pieces with size lower than 5 mm. The content of metals in samples was determined by atomic adsorption spectrometry (SOLAAR M6-UNICAM) – Table 1 shows the results of the analysis.

Table 1. The content of the basic metals in PCB

Metal	Cu	Fe	Zn	Pb	Al
Content %	39.55	0.62	0.23	0.67	0.55

Acidithiobacillus ferrooxidans bacteria strain was used in the research – this bacteria was cultured in the Silverman/Lundgren culture medium 9K (S/L), with following composition (g/dm^3): $(NH_4)_2SO_4$ - 3.0; KCl - 0.1; K_2HPO_4 - 0.5; $MgSO_4 \cdot 7H_2O$ - 0.5; $Ca(NO_3)_2$ - 0.01; $FeSO_4 \cdot 7H_2O$ - 44.2; initial concentration

Fe(II) – 9.0 g/dm^3, initial pH – 2.5. The leaching solution (9K) with volume of 2.7 dm^3, inoculated with 300 cm^3 of bacteria, was used in experiments.

Experiments were carried out in the column reactor made from plexiglas in the form of pipe with 1000 mm length and 40 mm diameter – see Fig. 1. Column was fulfilled with 100 g of cut PCB material (I variant) and 50 g of shredded PCB material (II variant). Column was supplied with the leaching solution from the bottom using the peristaltic pump with the flow 10 dm^3/h. Solution was received at the top of the column and directed to the overflow tank with the capacity of 5 dm^3. The research was carried out in the ambient temperature (20–22 °C) without aeration of the leaching solution. pH and oxidation-reduction potential (ORP) was measured in the overflow tank. Measurements were conducted at pH $\leq$ 2.0 and corrected the deviation by addition of H_2SO_4. The concentration changes of the solution components were analyzed by taking the solution samples from the overflow tank. The content of copper and other accompanying metals such as Zn, Al, Pb was measured using AAS method.

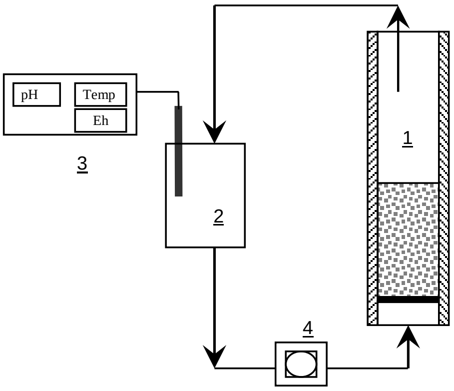

Fig. 1. The scheme of measuring apparatus: 1 – reaction column with leaching material, 2 – overflow tank, 3 – control of physic-chemical parameters, 4 – peristaltic pump

3 Results and discussion

Fig. 2 presents the change of pH during bioleaching of electronic waste packed in the column for two variants (I – fraction < 0.2 mm and II – fraction < 5 mm). To ensure the appropriate condition of growth and activity of microorganisms pH of solution was corrected by sulphuric acid maintaining the level value at pH 2.0. pH of the leaching solution circulating in the column fulfilled with the shredded waste (fraction < 0.2 mm) was corrected several times. The effect of acidification of the system was observed from the 27th day of the process. The course of pH changes in time is the confirmation of microbiological mechanism of oxidation the ions Fe^{2+} to Fe^{3+}, under which the solution vaccinated by microorganisms are gradually acidified. In the column with bed in the form of fraction < 5 mm, after correction of the pH solution (pH 2.0) in the initial phase of the process, the increase of pH to 3.1 was observed. The increase of pH may be associated with the presence of alkaline components [2] in the leaching material and accompanying this delayed growth of bacteria – this is reflected in the ORP changes registered during the experiment.

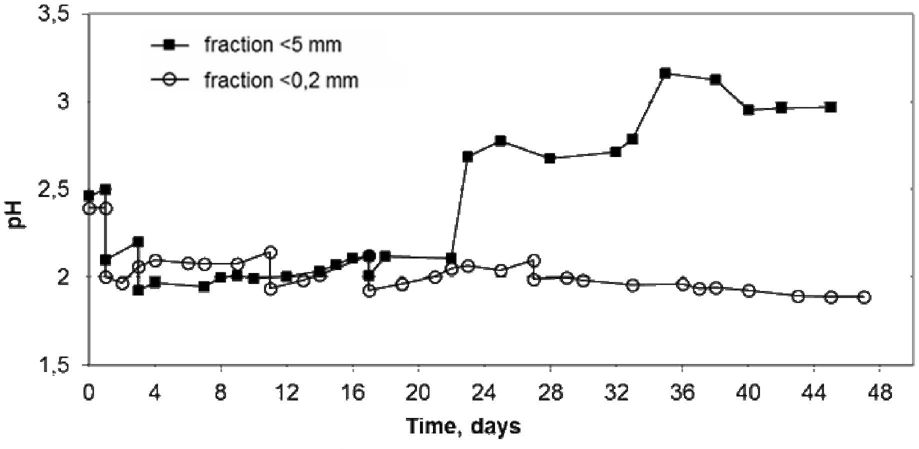

Fig. 2. Changes of pH during e-waste bioleaching process

Fig. 3 summarizes the changes of ORP registered in the time of PCB bioleaching. In the bioleaching with fraction < 5 mm the value of ORP after initial keeping on the constant level (350 mV) was, as the reaction was going on, gradually reduced to the level of 298 mV – in the same indicating that there is Fe(III) oxidizer usage in the process of waste dissolution. During the next days the value of ORP oscillated in the range 302-318 mV. Probably the extremal environment condition (high value of pH) influenced negatively on the microorganisms causing reduction of biomass growth and the limitation of oxidizing abilities. Oxidation of ions of iron (II) by *At. ferrooxidans* rapidly going down when pH is higher than 2.5 [3, 4].

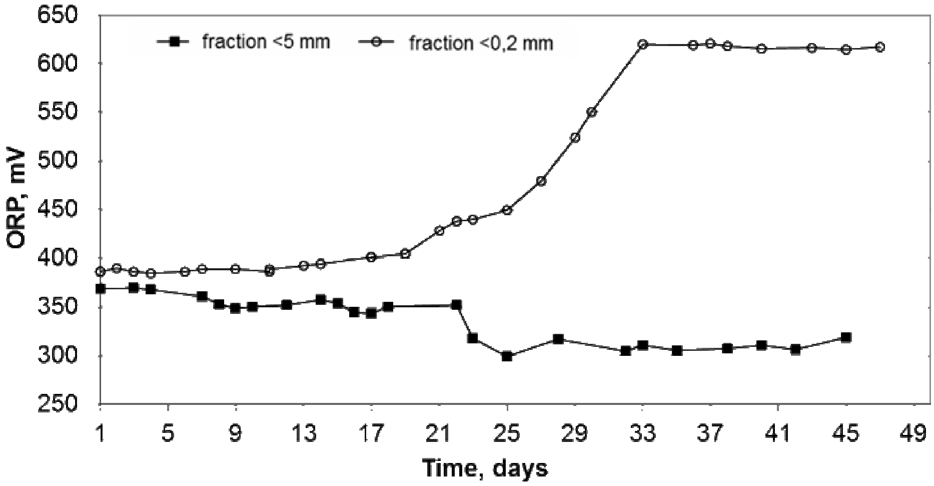

Fig. 3. Values of ORP recorded during e-waste bioleaching process

In bioleaching of shredded material the constant increase of ORP was observed from initial value 385 mV till 405 mV in the 19th day of the experiment. As the reaction was going on in the next 14 days the oxidation-reduction potential rapidly increased to the value 620 mV. The increase of ORP together with low pH during leaching is the indicator of significant growth of microorganisms and shows the course of the reaction by the biological way.

Fig. 4 and Fig. 5 show the comparison of metals bioleaching efficiency from electronic waste during 45 days of bioleaching respectively for fraction < 0.2 mm and fraction < 5 mm. In the process of PCB leaching with particle size < 5 mm the efficiency of copper and zinc extraction was increasing with time. During 45 days the recovery of copper was at the level 36.9 % and there was also 50 % degree of zinc extraction to the solution. Aluminium concentration in the solution was kept on the constant level from the 18 day (about 3.5 % aluminium recovery). Lead, which in the initial period (from 25th day) went into the solution in the amount of 0.5 %, during the next days its concentration in solution was decreased – it probably went to the precipitate.

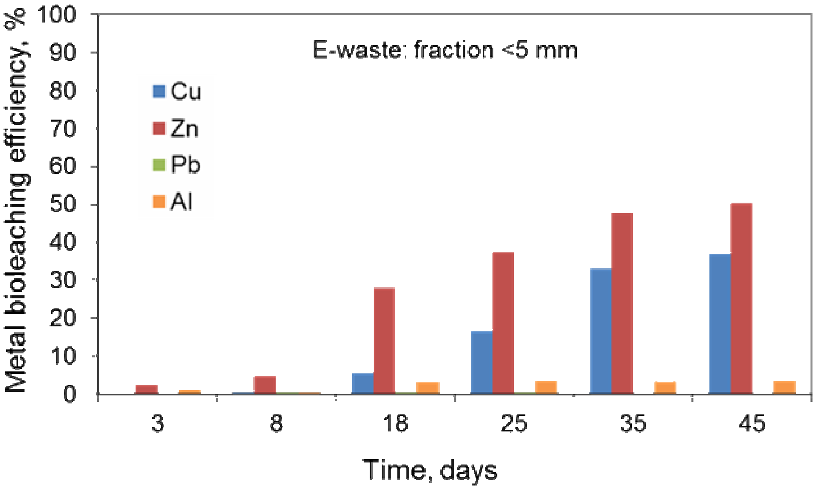

Fig. 4. Efficiency of metals bioleaching from PCB: fraction < 5mm

In the column filled with the pulverized fraction of electronic material (< 0.2 mm) the degree of metal extraction from solid phase into solution was constantly increasing with time. The efficiency of bioleaching was as follows: Cu 77.1 %, Zn 49.8 % and Al 14.6 %. In the solution there was no lead, which went to the precipitate. The similar observation was noted in tests of bioleaching of the electronic waste using periodical method [5].

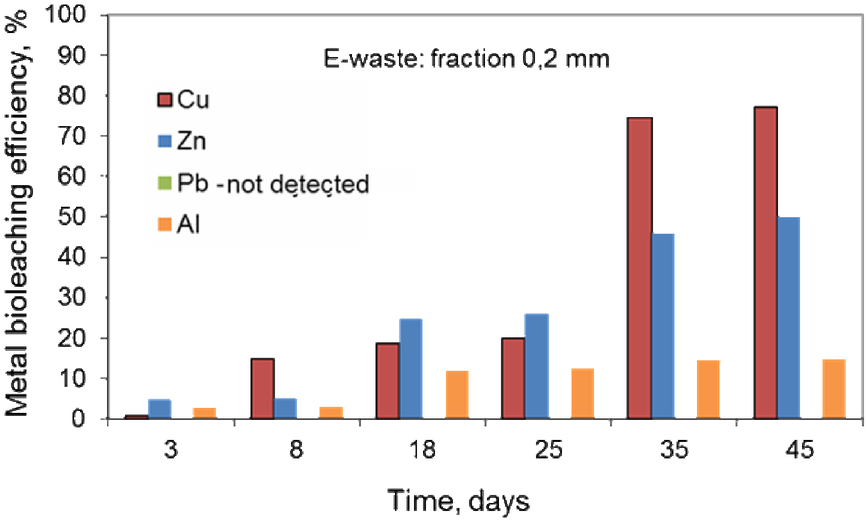

Fig. 5. Efficiency of metals bioleaching from PCB: fraction < 0.2 mm

4 Conclusions

Based on the bioleaching of metals from PCB using column reactor it was stated that for the fraction < 5 mm the process of bioleaching oxidation was limited. Most likely the rapidly increase of pH caused stopping the metabolic activity of bacteria. In the condition of limited growth of microorganisms the following results of metals recovery were obtained: 36.9 % of copper, 50 % of zinc and 3.4 % of aluminium. Registered during the experiment decreasing values of pH solution and accompanying this increase of oxidation-reduction potential confirm the course of bioleaching reaction from pulverized fraction (< 0.2 mm) on biological way. During 45 days the following efficiency of metals extraction into solution was obtained: 77.1 % of Cu, 49.8 % of Zn and 14.6 % of Al.

Acknowledgements

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Assoc. prof. Ing. Mária Kušnierová, PhD.

She was born on 9. 10. 1946 in Košice. After graduating from high school she continued her studies at the Faculty of Mining of Technical University in Košice in the field of mineral processing. She successfully completed her studies as a mining engineer in 1971.

In the same year she started a working study stay at the Institute of the Rock Properties of Slovak Academy of Sciences (later renamed to Mining Institute of SAS, currently the Institute of Geotechnics of SAS). During this stay she focused on the study of magnetic separation and flocculation. After finishing her study stay in 1974, she was employed at the research workplace of governmental company Geological research named „Applied technology of raw minerals“.

In the period of years 1974-1975 her research work was focused on developing of the processing and exploitation of the sillimanite corundum mineralization in the locality of the stream Kapka to produce high temperature resistant mullite materials. She found the unique fixation of the NH_4^+ in the structure of mica and consequently prepared the proposal of original technology for the comprehensive exploitation of the raw materials. In 1975-1977 and after her maternity leave in years 1979-1981 she focused her work on the field of basic technological research of crystalline and amorphous silicate mineral phases. She proposed the original technology of processing and comprehensive exploitation of these materials. For this technology she obtained two patents.

In 1982-1986 she worked in research focusing on study of the occurrence of rare trace elements and precious metals in sulphide materials of Slovakia in order to study the possibility of their complex processing. During this period she attended external scientific postgraduate study in the field of mineral processing at the Mining Institute of SAS in Košice. In 1986 she successfully completed postgraduate study by defence of her dissertation titled “Mineral processing utilization for the abrasive solids preparation from the primary and secondary raw materials”.

The results highlighted the insufficient valorisation of raw materials and the need for development of new technologies for their modification using the biological-chemical methods. She found conditions for this research at the Mining Institute of SAS in the department of prof. RNDr. Klára Tkáčová, DrSc. During this time a new research group had been created by Dr. h. c. prof. Ing. František Špaldon, DrSc. in this department, with the aim to research the biological-chemical methods for bioleaching of sulphidic concentrates.

In 1991 she was awarded by the Slovak Academy of Sciences with qualification degree IIa - Senior Researcher and obtained the financial support from Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (VEGA) for the research project. These facts were the basis for the establishment of a new department – Department of Mineral Biotechnology. She was founder and head of the department over next 15 years until 2006.

During this time she established 5 specific laboratories for the biotechnological research in the area of mineral raw materials and waste processing - biohydrometallurgy.

She dealt with the activities focused on monitoring the occurrence of chemolithotrophic bacteria *Acidithiobacillus ferrooxidans* in sulphide deposits in Slovakia; and later on building a collection of bacterial cultures used in the bacterial leaching of minerals. Later, she focused on the basic technological research in the field of physiological adaptations of bacteria strains on technologically real parameters in bio-oxidation processes to study the effect of biodegradation processes to extraction of precious metals from refractory Au and Cu sulphide concentrates. After 1995, she focused on research of indigenous microflora of deposited industrial waste and the possibility of their use in environmental technologies together with sulphate-reducing bacteria.

Since 1998, her significant research was also focused on the possibilities of processing and comprehensive utilization of energetic waste and waste from aluminum production. She proposed a number of non-waste treatment technologies, processing, and their complex utilization. She was awarded a patent SR on the one of these technologies.

In 2000 she habilitated in Czech Republic, in the field of mineral processing – mineralurgy and environmental technologies at the VŠB - Technical University of Ostrava, Faculty of Mining and Geology.

Assoc. prof. Ing. Mária Kušnierová, PhD. was a principal investigator of 5 Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (VEGA) projects and 5 bilateral projects; the scientific co-worker of 6 VEGA projects, 5 bilateral projects, 4 Slovak Research and Development Agency (SRDA) projects and other projects (VTP 95-513-III-07, USA-Slovak project ID 031-95, ŠO 51/03R0600/03R06042, ŠP 26/0280C00/0280C01, ITMS 26220220051 etc.).

Her publication activity is also significant. She participated mainly as an author, but also as a co-author. She compiled 5 foreign monographs and 170 original scientific papers published in journals registered in the international databases and collections of scientific conferences at home and abroad. She compiled 14 scientific research works and 15 final research reports and studies. She is the author of 3 patents. It is necessary to mention her extensive work as an opponent of expert studies for various committees and number of master, diploma and doctoral thesis, habilitation works etc.

In 1990 she initiated the organization of the 1st international scientific conference Biohydrometalurgy I. (Košice) and later she also guaranteed the 2nd and the 3rd conference on Biohydrometallurgy. She was also the professional guarantor for many years of domestic international conference Wastes (Spišská Nová Ves). She was a member of the scientific committee for 3 years of domestic international conference Biotechnology and Metals (Košice) and periodically organized foreign international conferences New Trends in Mineral Processing, Environment and Mineral Processing (Ostrava, Czech Republic) on the base of the cooperation with prof. Ing. Peter Fečko, CSc. form VŠB - Technical University of Ostrava, Faculty of Mining and Geology. She participated as a member of the scientific committee in an international conference Waste Recycling and in years 2007 and 2010 she was one of main organizers - Waste Recycling XI and XIV (Košice).

In years 1995- 2000 she worked as an external lecturer at the Faculty of Mining, Ecology, Process Control and Geotechnology of the Technical University in Košice. For the needs of teaching she compiled syllabus of the subject “Microorganisms in industry” for study field of mineral processing and environmental engineering at the Department of Mineralurgy and Environmental Technologies. Since 1984 she worked as an external lecturer in the same field at the VŠB - Technical University of Ostrava, Faculty of Mining and Geology. She was a supervisor of 4 PhD students in the field of mineral biotechnology (Ing. Iveta Štyriaková, PhD., Ing. Alena Luptáková, PhD., Ing. Mária Praščáková, PhD. and Ing. Andrea Šlesárová, PhD.), 1 PhD student of Institute of Geotechnics SAS (Ing. Oľga Šestinová, PhD.), 5 PhD students of Environmental institute of VŠB-TU Ostrava and consultant/assistant supervisor of 10 PhD. students.

Assoc. prof. Ing. Mária Kušnierová, PhD. was a member of the Scientific Board of IGT SAS since 2006, deputy director of IG SAS (2002-2004), member of Board of scientists, later Congress of scientists (2000-2004), member of the Commission for the control of intellectual property of the SAS (2000-2008), member of the Commission No. 6 of the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (VEGA, 2002-2006), member of the Council for doctoral studies of VŠB-TU Ostrava since 2004 until now, member of the editorial board of reviewed journal Brown coal since 2010. She was a member of the following scientific organizations – Slovak Mining Society, The Minerals, Metals and Materials Society (USA), Czechoslovak Society for Microbiology and Czechoslovak Chemical Society.

On behalf of the Institute of Geotechnics SAS, we would like to express our thanks to Assoc. prof. Ing. Mária Kušnierová, PhD., for her significant contribution to the field of biohydrometallurgy in Slovakia.

We wish you good health and still many pleasant years of life,

Your colleagues, co-workers and friends.

Slovak Academy of Sciences
Institute of Geotechnics

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

Subjects of activity of the Institute of Geotechnics SAS:

1. Basic research of processes in the field of rock cutting, rock mechanics and underground constructions, the transport of energy and mass in the rock disintegration processes; basic research of solid dispersions origin patterns and their properties modifications by physical, mechanical, chemical and biotechnological processes; qualitative and quantitative evaluation of phase interactions at the disperse systems origin and at their spreading in working and living environment.
2. Application of theoretical knowledge from presented areas for detailing the top technologies principles in the following fields: rock cutting, 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 remediation.
3. Advisory and expertise services related to main activities
4. Scientific education in terms of generally valid legislation,
5. Publication of the scientific-research activities using the periodic and non-periodic press. The publishing of periodic and non-periodic press follows the regulations of the SAS Presidium.

The Institute of Geotechnics is divided into five research departments:

- Department of Physical and Physico-Chemical Mineral Processing Methods
- Department of Destructional and Constructional Geotechnics
- Department of Environment and Hygiene in Mining
- Department of Mineral Biotechnology
- Department of Mechanochemistry

Department of Mineral Biotechnology

Research program in the field of mineral biotechnology at the Institute of Geotechnics SAS was founded by Dr.h.c. prof. Ing. František Špaldon, DrSc. in 1985. In 1992, a detached Department of Mineral Biotechnology 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 of MVDr. Daniel Kupka, PhD.

Research in the department is focused on the biogeochemistry, geomicrobiology and environmental biotechnology. Research in **biogeochemistry** and **geomicrobiology** is focused on the investigation of biogeochemical processes in the upper part of the Earth geosphere in connection with mineral weathering and global cycle of the elements, microbial interactions with inorganic and organic components of the rock environment in the mineral transformation processes in mineral deposits and mine and industrial waste repositories, paragenesis of secondary mineral structures, biodegradation of natural and synthetic materials, biodiversity of various natural matrices (soil, water, sediments).

Research in the field of **environmental biotechnology** is focused on 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 anthropogenic 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.

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

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

Pavol Jozef Šafárik University in Košice

Faculty of Science

The Pavol Jozef Šafárik University in Košice belongs to the significant and recognised teaching and educational facilities not only in the Slovak Republic but in the developed Europe as well. The University belongs to the institutions, which present a long tradition of scientific research, which follows the mission of the university and which belongs to its priorities.

The Faculty of Science of the Pavol Jozef Šafárik University in Košice is a significant scientific institution, which offers courses in undergraduate, graduate and post-graduate level of studies in the field of natural sciences, mathematics, computer science and teacher studies of select academic courses. The Faculty, amid natural science oriented faculties, is long considered as one of the top scientific, research and teaching institutions in the Slovak Republic (it has been accredited in 8 out of the 16 scientific fields of accreditation at the Pavol Jozef Šafárik University). The achieved results in all of the fields of natural science: biology, physics, chemistry, computer science and mathematics, which are taught either as one major or two major courses in teaching, a wealth of publications, international connections and cooperation with people in the field elevate its position among scientific institutions not just at home but in Europe and in the world as well. The faculty’s main role in scientific research activities is the support of successful grant schemes, strengthening of the interdisciplinary and multidisciplinary character of science and research and the expansion of international cooperation, mainly by taking part in European scientific and scientific and technological projects.

Institute of Biology and Ecology

Institute of Biology and Ecology is an institution with a complex approach to the solution of broad range of biological and ecological problems of the current science using the newest molecular methods. Highly educated researchers and up-to-date equipment provide education in main study branches – Biology and Ecology. Institute is part of the Faculty of Science and consist of 8 Departments:

- Department of Botany
- Department of Cell Biology
- Department of Animal Physiology
- Department of Genetics
- Department of Microbiology
- Department of Zoology
- Laboratory of Molecular–biological Diagnostics
- Division of Didactics of Biology

Department of Microbiology

Department of Microbiology covers several scientific topics from the field of clinical, environmental and applied microbiology. The main emphasis in the field of environmental and applied microbiology is on the metal-microbe interactions study on population as well as individual levels. It deals with a characterisation of microorganisms from extreme conditions (including high metal contamination), with a study of metal resistance and cross metal and antibiotic resistance with the aim to shed light on mechanisms which could lead to the development of bioremediation technologies.

Knowledge obtained from the research is spread in educational process within several study courses such as Microbiology and Virology, Environmental Microbiology, Applied Microbiology, Bioinformatics, Genetic Engineering, Laboratory Diagnostics in Microbiology and Environmental Biotechnology.

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