I. ŠTYRIAKOVÁ, I. ŠTYRIAK, T. SASVARI

ISSN 0543-5846
METABK 43 (4) 293-297 (2004)
UDC - UDK 622.75:541.48:66.098=111

EXTRACTION OF ELEMENTS FROM SULPHIDE AND SILICATE CONCENTRATES BY SELECTED BACILLUS ISOLATES

Received - Primljeno: 2003-04-12
Accepted - Prihvaćeno: 2003-08-30
Preliminary Note - Prethodno priopćenje

Using two *Bacillus* spp. isolates (*Bacillus megaterium* bl1 and *Bacillus mycoides* bl2) mineral dissolution of sulphides and silicates was investigated by AAS (atomic absorption spectrophotometry) and microscopic techniques in order to determine bacterial effects on the extraction of elements from samples and morphological changes of mineral surfaces. These bacteria were isolated from soil samples collected from a mine dump near Banska Štiavnica (Slovakia) and their extraction activity was studied in pure and mixed cultures. The samples were in the forms of polished sections, pulverized and granulated mineral products (flotation concentrate - FC, post- flotation waste - FW, gravitational concentrate - GC). The extraction of most elements (Al, Si, Zn, Cu, Au) from these samples was the best after 7 days of cultivation with a mixture of the *Bacillus megaterium* bl1 and *Bacillus mycoides* bl2 strains. Therefore, a 35 days bioleaching experiment was carried out with a mixture of these two *Bacillus* spp. strains.

Key words: *sulphidic concentrates, silicate concentrates, Bacillus isolates, elements extraction*

Ekstrakcija elemenata iz sulfidnih i silikatnih koncentrata odabranom izolatom bakterija. Uporabom dvije vrsta izolata bakterija (*Bacillus megaterijum* bl1 i *Bacillus mycoides* bl2) istraživana je mineralna rastopina sulfida i silikata tehnikom AAS i mikroskopskim tehnikama radi utvrđivanja bakterijskog učinka na ekstrakciju elemenata iz uzoraka i morfološke promjene na površinama minerala. Te bakterije su izolirane iz uzoraka tla prikupljenih na odlagalištu jednog rudnika u blizini Banske Štiavnice (Slovačka) a njihovo ekstrakcijsko djelovanje je proučavano u čistim i mješovitim kulturama. Uzorci su bili razni uglačani profili, pulverizirani i granulrani mineralni proizvodi (flotacijski koncentrat - FC, post-flotacijski otpad - FW, gravitacijski koncentrat- GC). Ekstrakcija većine elemenata (Al, Si, Zn, Cu, Au) bila je najbolja nakon 7 dana kultiviranja mješavinom *Bacillus megaterijum* bl1 i *Bacillus mycoides* bl2. Zato je eksperiment bio-luženja mješavinom te dvije vrste bacila trajao 35 dana.

Ključne riječi: *koncentrati sulfida, koncentrati slikata, izolati bakterija, ekstrakcija*

INTRODUCTION

Bacteria of the genus *Bacillus* are ubiquitous and common as soil microorganisms that should be one of the subjects of increasing research interest in near future. *Bacillus* spp. play an important role in silicate biodegradation during the process of rock disintegration [1, 2]. The results of such activity involve both geochemical and structural changes in silicate minerals and rocks. Tešič and Todorovič [3] have proposed that so-called "silicate bacteria" should belong to the *Bacillus circulans* group. The mechanism of microbial destruction of silicates and aluminosilicates by these bacteria is not understood yet. However, it is known, for example, that their activity leads to a decrease in Si content of bauxites of lower quality [4], and to the extraction of Al, Ti, U, Au and other elements from silicates and aluminosilicates [5]. The biobeneficiation of bauxite by means of *Paenibacillus (Bacillus) polymyxa* which was able significantly to remove calcium and iron from bauxite ore, was reported by Anand et al. [6]. A possible industrial use of these bacteria is considered also in solid mineral-waste biodegradation as well as in biosorption of heavy metals from solutions especially in the wastewater cleaning of toxic metals, if their bioaccumulation properties could be used [7].

The purpose of our study was to investigate the ability of two selected *Bacillus* spp. isolates to destroy some silicates and sulphidic minerals and to release metal cations

I. Štyriaková, Institute of Geotechnics of the Slovak Academy of Sciences, Košice, Slovakia, I. Štyriak, Institute of Animal Physiology of Slovak Academy of Sciences, Košice, Slovakia, T. Sasvari, BERG Faculty Technical University of Košice, Košice, Slovakia

from their structure with the aim of a possible solid silicate-sulphide waste detoxification and biodegradation.

MATERIALS AND METHODS

Bacteria and media

Two strains of *Bacillus* spp. were selected out of more than twenty *Bacillus* isolates from soil samples taken on a 15th-century-slag-heap in "Lúky pod Tanádom" near Banská Štiavnica after heating them at 80 °C for 15 min to kill the non-sporeforming species. Individual bacterial strains were obtained by colony reisolation on Nutrient agar No. 2 (Imuna, Šarišské Michaľany) plates and identified as *Bacillus* spp. strains on the basis of their cell morphology and some biochemical characteristics. Moreover, two selected *Bacillus* strains were identified by means of BBL CRYSTAL ID panel (Becton-Dickinson, USA). For experiments, these bacterial strains were grown in Nutrient broth No. 2 (Imuna) at 28 °C for 18 hours. Bacterial cells were harvested by centrifugation at 4000 rpm (15 min), subsequently washed twice with saline solution (0,9 % NaCl) and added in a concentration of 10^{10} cells per ml to modified Ashby liquid medium [8] with individual mineral samples, as described below. The composition of modified Ashby medium is shown in Table 1.

Table 1. **Composition of modified Ashby' salt medium**

Tablica 1. **Sastav modificiranog Ashbyjevog slanog medija**

Component	Amount / (g/l)
Glucose	20,0
$(NH_4)_2SO_4$	0,1
K_2HPO_4	1,0
$MgSO_4 \cdot 7H_2O$	0,5
NaCl	0,2
pH	7,0

Bacterial extraction activity was studied with pure and mixed cultures. The presence of vegetative bacterial cells in Erlenmayer flasks during bioleaching was regularly detected by light microscopy after Gram staining, and the spores were stained by malachite green.

Table 2. **Mineralogical and chemical composition of flotation concentrate (FC) before bioleaching**

Tablica 2. **Mineraloški i kemijski satav flotacijskog koncentrata (FC) prije izlaganja utjecaju biološke lužine**

Mineralogical composition	weight / %	Chemical composition	weight / %
Quartz	20,0	SiO_2	20,00
Pyrite	42,5	Zn	3,62
Sfalerite	5,4	Cu	0,57
Galenite	3,5	Pb	3,00
Chalcopyrite	1,7	S	25,47
Gold	traces	Fe	26,53
Size fraction	10-100μm	Au	120 g/t
In FC was less of Mn than 0,1 % and therefore this percentage is not included in the Table 2.			

Mineral samples

The influence of selected bacterial isolates on the structure of minerals was studied. The mineral samples used in these experiments were in the form of polished sections as well as pulverized and granulated products after ore dressing at the Hodruša deposit.

The polished sections were of an allotriomorphic and granulated structure of mineral aggregates. Quartz and aluminosilicates dominated the polished sections. There were also traces of sphalerite, galenite, rhodonite, pyrite and gold aggregates in those polished sections. The pulverized and granulated samples of the Hodruša deposit products, including the flotation concentrate (FC), the post-flotation waste (FW) and the gravitational concentrate (GC), were also examined.

The mineralogical and chemical composition of these samples is given in Tables 2., 3. and 4.

Table 3. **Mineralogical and chemical composition of post-flotation waste (FW) before bioleaching**

Tablica 3. **Mineraloški i kemijski sastav post-flotacijskog otpada (FW) prije izlaganja biološkoj lužini**

Mineralogical composition	weight / %	Chemical composition	weight / %
Quartz	50 ... 65	SiO_2	73,49
Feldspar	2 ... 5	Zn	0,15
Pyrite	0,3	Cu	0,034
Sphalerite	0,08	Pb	0,069
Galena	0,2	S	0,26
Rhodonite	0,5 ... 1	Fe	2,01
Gold	traces	Al	1,14
		Mn	0,3
Size fraction	10-70 μm	Au	2,6 ppm

Bioleaching of mineral samples

Bioleaching of all 7 polished sections as well as of 10 g-pulveru1ent samples of FC, GC and FW were carried out in 300 ml Erlenmeyer flasks containing 100 ml modified Ashby medium inoculated by individual bacterial strains of *Bacillus* spp. or by a mixture of them. Cells were resuspended in saline (0,9 % NaCl) to a concentration of approximately 10^9 cells/ml. The microbial cultures were added to these flasks in the active logarithmic phase of growth under aseptic conditions. The culture flasks with the polished section were incubated at 28 °C on a shaker with amplitude of 30 mm and 240 rpm for 7 days. Then the Ashby medium was changed under aseptic conditions

Table 4. **Mineralogical and chemical composition of gravitational concentrate (GC) before bio1eaching**
Tablica 4. **Mineraloški i kemijski sastav gravitacijskog koncentrata (GC) prije podvrgavanja biološkom luženju**

Mineralogical composition	weight / %	Chemical composition	weight / %
Quartz	60 ... 45	SiO_2	71,12
Feldspar	2 ... 6	Zn	0,22
Pyrite	6,3	Cu	0,064
Sphalerite	0,3	Pb	1,64
Galenite	1,9	S	3,82
Chalkopiryte	0,2	Fe	3,65
Rhodonite	0,1 ... 0,5	Al	1,36
Gold	traces	Mn	0,47
Size fraction μm	100...1000	Au	59,3 g/t
The chemical composition of mineral samples is expressed in % with exception of Au.			

and the individual leachates were investigated by AAS. The bioleaching experiment with a mixture of both *Bacillus* spp. isolates was prolonged to 35 days under the same conditions. The Ashby medium was changed under aseptic conditions at 7- day intervals. Abiotic controls were set up under the same conditions.

The concentrations of elements extracted from pulverized and granulated samples by leaching were continually determined in all leachates by atomic absorption spectrometry on a AA 30 VARIAN spectrometer during 35 days of bioleaching.

RESULTS

Bacterial identification and morphology

Two out of more than twenty *Bacillus* spp. isolates were selected after repeated single-colony isolation on Nutrient agar No. 2. They were differentiated on the basis of their different colony morphology. One of the strains (bI1) formed ovoid colonies and was identified by BBL system as *Bacillus megaterium.* The second strain (bI2) formed rhizoid colonies of different size on agar plates. It was identified as *Bacillus mycoides.* All strains were propagated in Nutrient Broth No. 2, washed in saline solution and added individually as well as in a mixture to the modified Ashby medium in Erlenmeyer flasks with mineral samples as described above.

The regular examination of bacterial morphology showed that the size of these bacterial cells decreased and after 7 days endospores appeared. The dissolution of minerals from FC, FW, and GC samples was stopped after 7 days of bacterial leaching, and therefore culture medium was regularly changed during 35 days of bioleaching at 7-day intervals.

Bioleaching of mineral samples

Ore raw material was pulvered and flotated in the mining factory at Banská Hodruša and the concentrates were obtained from this factory. Moreover, the polished sections were prepared from these materials. The extraction of most elements (Al, Si, Zn, Cu, Au) from these samples was the best after 7 days of cultivation with a mixture of the *Bacillus megaterium* bI1 and *Bacillus mycoides* bI2 strains (data not shown). Therefore, the 35 days' bioleaching experiment was carried out with a mixture of these two *Bacillus* spp. strains which disp1ayed the highest activity in elements extraction after first 7 days in comparison with individual strains.

Figure 1. shows the progress of the extraction of detectable elements (Si, Pb, Zn, Cu) from the FC substrates with a higher sulphidic concentration. The mixture of *Bacillus* isolates extracted 39 % of Si, 60 % of Pb, 43 % of

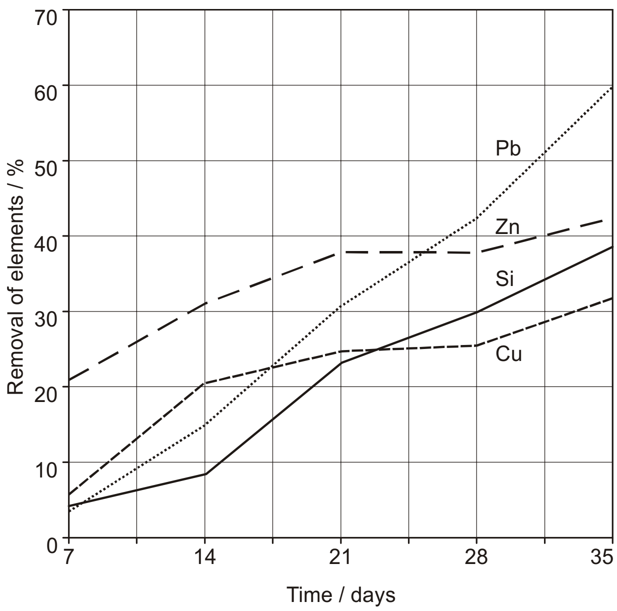

Figure 1. **The progress of the extraction of detectable elements from the flotation concentrate (FC) with a higher sulphidic concentration during bioleaching**
Slika 1. **Proces ekstrakcije odjeljivih elementa iz flotacijskog koncentrata (FC) s većom koncentracijom sulfida tijekom biološkog luženja**

Zn, 24 % of Fe, 13 % of Ag, and 32 % of cu from FC after 35 days of bioleaching. However, Au was not removed by these strains because it is disseminated in su1phidic matrix which was typical only by superficial corrosion after bacterial leaching.

Figures 2.a and 2.b show the progress of elements (Si, Pb, Zn, Cu, Al, Mn) extraction from the FW substrates with the lowest sulphidic concentration. There was removed 33 % of Si, 75 % of Pb, 76 % ofZn, 76 % of Cu, 9 % of Al, 3 % of Fe, and 82 % of Mn after 35 days.

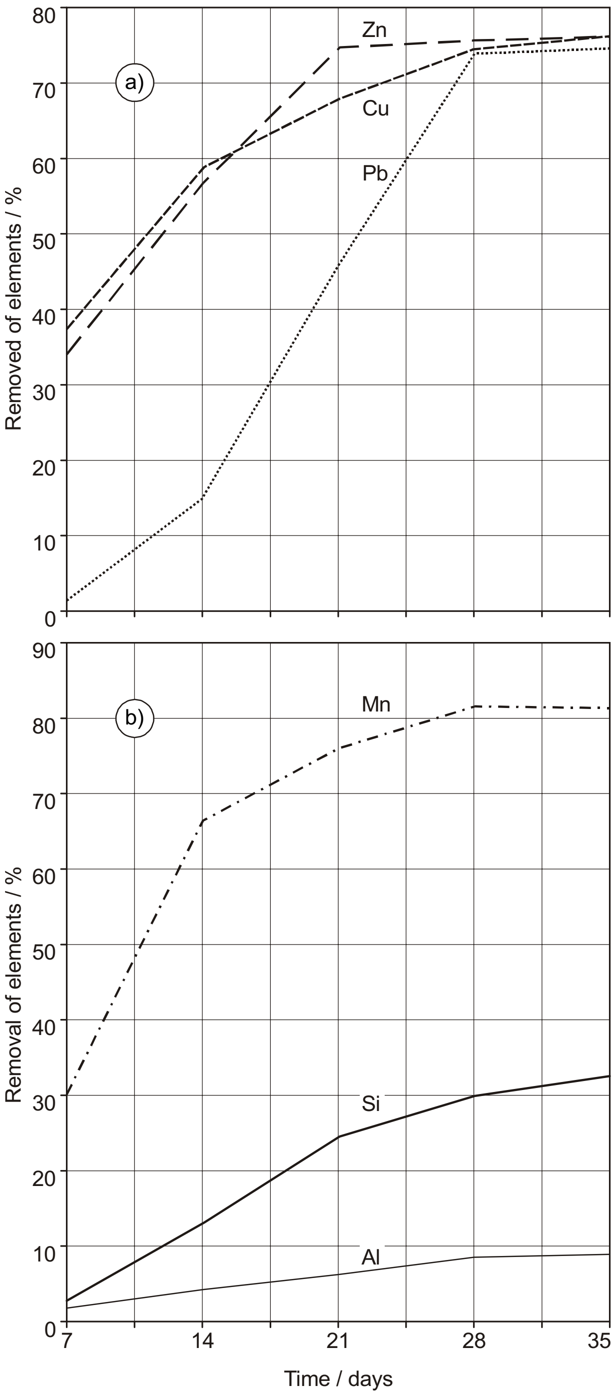

Figure 2.a,b **The progress of elements (Si, Pb, Zn, Cu, Al, Mn) extraction from the FW substrates with the lowest sulphidic concentration during 35 days**
Slika 2. a,b **Razvoj elemenata (Si, Pb, Zn, Cu, Al, Mn) ekstrakcijom iz supstrata FW najnižom sulfidnom koncentracijom tijekom 35 dana**

Figures 3.a and 3.b show elements extraction from the GC substrates where quartz and pyrit were the main constituents of this sample. The gold was finely disseminated in quartz found in GC. The particle size was in the range of 10 to 20 μm. These isolates were able to release 30 % of Si, 59 % of Pb, 35 % of Zn, 56 % of Cu, 78 % of Mn, 37 % of Al, 30 % of Ag, and 30 % of Au from GC during 35 days. These *Bacillus* isolates removed Au only from quartz matrix because of targeted profound corrosion of silicates in the form of etch pits in regions of the fine dissemination of gold [9] and in regions of pyrite dissemination.

By the investigation of morphological changes of the surfaces of silicates and sulphides in the form of polished

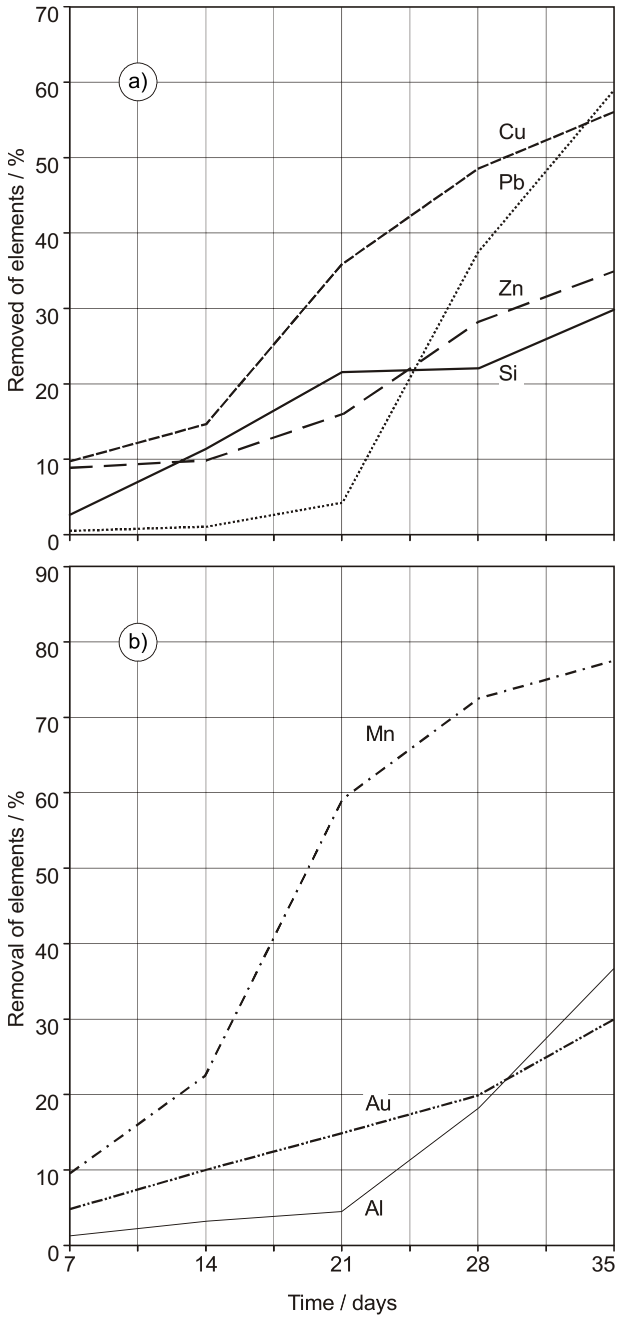

Figure 3.a,b **The extraction of detectable elements from the GC substrates where quartz and pyrit were the main constituents**
Slika 3. a,b **Ekstrakcijaodjeljivih elemenata iz više supstrata GC u kojima su kvarc i pirit glavni konstituenti**

sections, there was possible to observe an effect of heterotrophic bacteria and their metabolites. The morphological changes of sulphidic mineral surfaces and elements extraction confirm the consideration that sulphidic minerals are destroyed by organic acids only superficially (the grains of sulphides were only face-corroded) and that is why a significant extraction of non-Fe metals and low Au extraction (too low for analyses) from flotation concentrate (FC) with predominance of sulphidic minerals was observed. Flotation concentrate (FC) contains the highest proportion of sulphidic minerals and only 20 % of quartz. Small particles of gold (10 to 20 μm) are singly impregnated in quartz and in carbonates as well as in the form of inclusions in the grains of polymetallic sulphides.

Quartz was the main mineral in gravitational concentrate, the accompanying minerals were pyrite and several aluminosilicates. Intensive destruction of quartz matrix in the locality of impregnated sulphides and gold particles was observed on the polished sections. Due to this destruction, 30 % of gold and 30 % of Ag from gravitational concentrate (GC) was recovered.

A process of biological removal of elements from FC, GC and FW was the result of microbial surface attachment and of organic acids production by strains of the genus *Bacillus,* however, these bacteria synthesized also polysaccharides during bioleaching.

Thus, it is possible that polysaccharides were also implicated in the biodestruction and the formation of authigenic minerals as for example acanthite [9].

DISCUSSION

Many physico-chemical processes could be used for the destruction of ores and minerals as well as for removal of undesirable mineral constituents from ores. However, all of them are energy and cost intensive, less flexible, and often pose environmental problems.

The mining industry in Slovakia is at present in a difficult period. Ores and non-ore raw materials with low contents of metals and too complicated mineral structure require non-traditional methods of treatment, which could not only be cheaper but also more acceptable for the environment. Although biological methods of mineral- and ore-dissolution have not even been well understood, they could be really cheaper and much more suitable for the environment. Some years ago, only the autotrophic thiobacilli had been considered for metals recovery and detoxification of industrial waste products as well as for biotechnological use, especially in the bioleaching of sulphides. Nowadays new methods are being developed for the extraction of valuable metals from oxide and silicate minerals and ores using heterotrophic microorganisms. It is known that bacilli which solubilize ores by reduction may also promote selective leaching. An example would be *Bacillus* GJ33 which selectively leaches Mn, Co, Ni and to some extent Cu from marine ferromanganese nodules without significantly solubilizing the iron in the nodules [10].

A great advantage of bacilli is also their ability to form endospores. The endospores are resistant structures which allow bacilli to survive under unfavourable conditions and subsequently under suitable conditions to transform their spores to vegetative cells and to continue their life cycle.

Microbial processes can be used for either the decreasing of non-Fe metals content in sulphidic concentrates or the solubilization of a metal value in silicate concentrates. The perspective application of biotechnology in these areas will utilize native microbial species under controlled conditions to enhance their effectiveness. A long term goal will also be the genetic modification of the useful microbes to increase their utility. Bioleaching of valuable metals from industrial waste products could not only contribute to an increase in the supply of raw materials in the future but should also be useful for the detoxification of industrial waste products, thus overcoming some of our environmental pollution problems.

Although the present commercial application of microbial leaching is located outside Slovakia, the biotechnology has the potential to become an indispensable part of the mineral industry including industrial waste biodegradation also in Slovakia.

REFERENCES

[1] G. I. Karavaiko, Z. A. Frutsko, E. O. Melnikova, Z. A. Avakyan, Y. I. Ostroushko: Role of microorganisms in spodumene degradation, Microbio1ogy-USSR, 49 (1980) 547 - 549.

[2] G. I. Karavaiko, N. P. Belkanova, V. A. Eroshchev-Shak, Z. A. Avakyan: Role of microorganisms and some physico-chemica1 factors of the medium in quartz destruction, Microbio1ogy-USSR, 53 (1984) 795 - 800.

[3] Z. P. Tešić, M. S. Todorović: Prilog ispitivaniju “Silicatnih Bakterija”, Zemljište i biljka (Beograd) 1 (1952) 3 - 18 (in Serbian).

[4] V. I. Groudeva, S. N. Groudev: Bauxite dressing by means *of Bacillus circulans,* In: Travaux ICSOBA Congress, Zagreb (1983), 257 - 263.

[5] S. N. Groudev: Workshop on the aluminosilicate mineral biodegradation: a synopsys, 5th European congress on biotechnology, July 8 - 13, 1990, Copenhagen, Denmark.

[6] P. Anand, J. M. Modak, K. A. Natarajan: Biobeneficiation of bauxite using *Bacillus polymyxa:* calcium and iron removal, Int. J. Miner. Process., 48 (1996) 51 - 60.

[7] M. D. Mu1len, D. C. Wolf, F. G. Ferris, T. J. Beveridge, C. A. Fleming, G. W. Bailey: Bacterial sorption of heavy metals, Appl. Environ. Microbiol., 55 (1989) 3143 - 3149.

[8] E. S. Shelobolina, Z. A. Avakyan, E. S. Bulygina, T. P. Turova, A. M. Lysenko, G. A. Osipov, G. I. Karavaiko: Description of a new species of mucilaginous bacteria, *Bacillus edaphicus* sp. nov., and confirmation of the taxonomic status of *Bacilus mucilaginosus* by Avakyan et al. (1986) based on data from phenotypic and genotypic analysis, Microbio1ogy-USSR, 66 (1997) 681 - 687.

[9] I. Štyriaková, I. Štyriak, M. Kušnierová: The release of sulphidic minerals from aluminosilicates by *Bacillus* strains, In: Proc. Biohydrometal1urgy and the environment toward the mining of the 21ST century, Process Metallurgy 9A, Madrid (1999), 587 - 596.

[10] H. L. Ehrlich, Bacterial Leaching of manganese ores, In: Biogeochemistry of Ancient and Modern Environments (Eds.: Trudinger, P. A., Walter, M. R. and Ralph, B. J.), Australian Acad. of Sci., Canberra (1980), 609 - 614.