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

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 Banská 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 vrste izolata bakterija (Bacillus megaterium 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 prikuljenih 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 profil, pulverizirani i granulirani 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 megaterium bl1 i Bacillus mycoides bl2. Zato je eksperiment bio-luženja mješavinom te dvije vrste bacila trajao 35 dana.

Ključne riječi: koncentrati sulfida, koncentrati silikata, 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. Tešić and Todorović [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 biobenefication 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
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 1D 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 10^9 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.

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

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 1. Composition of modified Ashby salt medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20,0</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>0.1</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mineralogical composition</th>
<th>weight / %</th>
<th>Chemical composition</th>
<th>weight / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>20,0</td>
<td>SiO₂</td>
<td>20,00</td>
</tr>
<tr>
<td>Pyrite</td>
<td>42,5</td>
<td>Zn</td>
<td>3,62</td>
</tr>
<tr>
<td>Sfalerite</td>
<td>5,4</td>
<td>Cu</td>
<td>0,57</td>
</tr>
<tr>
<td>Galenite</td>
<td>3,5</td>
<td>Pb</td>
<td>3,00</td>
</tr>
<tr>
<td>Chalcopyrite</td>
<td>1,7</td>
<td>S</td>
<td>25,47</td>
</tr>
<tr>
<td>Gold</td>
<td>traces</td>
<td>Fe</td>
<td>26,53</td>
</tr>
<tr>
<td>Size fraction</td>
<td>10-100µm</td>
<td>Au</td>
<td>120 g/t</td>
</tr>
</tbody>
</table>

In FC was less of Mn than 0.1 % and therefore this percentage is not included in the Table 2.

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

<table>
<thead>
<tr>
<th>Mineralogical composition</th>
<th>weight / %</th>
<th>Chemical composition</th>
<th>weight / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>50 ... 65</td>
<td>SiO₂</td>
<td>73,49</td>
</tr>
<tr>
<td>Feldspar</td>
<td>2 ... 5</td>
<td>Zn</td>
<td>0,15</td>
</tr>
<tr>
<td>Pyrite</td>
<td>0,3</td>
<td>Cu</td>
<td>0,034</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>0,08</td>
<td>Pb</td>
<td>0,069</td>
</tr>
<tr>
<td>Galena</td>
<td>0,2</td>
<td>S</td>
<td>0,26</td>
</tr>
<tr>
<td>Rhodonite</td>
<td>0,5 ... 1</td>
<td>Fe</td>
<td>2,01</td>
</tr>
<tr>
<td>Gold</td>
<td>traces</td>
<td>Al</td>
<td>1,14</td>
</tr>
<tr>
<td>Size fraction</td>
<td>10-70 µm</td>
<td>Au</td>
<td>2,6 ppm</td>
</tr>
</tbody>
</table>

Bioleaching of mineral samples

Bioleaching of all 7 polished sections as well as of 10 g-pulverulent 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.

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Table 4. Mineralogical and chemical composition of gravitati-
onal concentrate (GC) before bioleaching
Tablica 4. Mineraloški i kemijijski sastav gravitacijskog koncentra-
ta (GC) prije podvrgavanja biološkom ljuženju

<table>
<thead>
<tr>
<th>Mineralogical composition</th>
<th>weight / %</th>
<th>Chemical composition</th>
<th>weight / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>60 ... 45</td>
<td>SiO₂</td>
<td>71,12</td>
</tr>
<tr>
<td>Feldspar</td>
<td>2 ... 6</td>
<td>Zn</td>
<td>0,22</td>
</tr>
<tr>
<td>Pyrite</td>
<td>6,3</td>
<td>Cu</td>
<td>0,064</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>0,3</td>
<td>Pb</td>
<td>1,64</td>
</tr>
<tr>
<td>Galenite</td>
<td>1,9</td>
<td>S</td>
<td>3,82</td>
</tr>
<tr>
<td>Chalcopyrite</td>
<td>0,2</td>
<td>Fe</td>
<td>3,65</td>
</tr>
<tr>
<td>Rhodонite</td>
<td>0,1 ... 0,5</td>
<td>Al</td>
<td>1,36</td>
</tr>
<tr>
<td>Gold</td>
<td>traces</td>
<td>Mn</td>
<td>0,47</td>
</tr>
<tr>
<td>Size fraction μm</td>
<td>100...1000</td>
<td>Au</td>
<td>59,3 g/t</td>
</tr>
</tbody>
</table>

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 Baci-
lus spp. isolates was prolonged to 35 days under the same
conditions. The Ashby medium was changed under asept-
ic conditions at 7- day intervals. Abiotic controls were set
up under the same conditions.

The concentrations of elements extracted from pulver-
ized and granulated samples by leaching were continually
determined in all leachates by atomic absorption spectrom-
etry 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 (b11) form-
ed ovoid colonies and was identified by BBL system as
Bacillus megaterium. The second strain (b12) formed rhiz-
oid colonies of different size on agar plates. It was identi-
fied 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 de-
scribed 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 min-
erals 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 floated in the min-
ing factory at Banska Hodruša and the concentrates were
obtained from this factory. Moreover, the polished sec-
tions 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 b11 and Bacillus mycoides b12
strains (data not shown). Therefore, the 35 days’ bioleach-
ing experiment was carried out with a mixture of these
two Bacillus spp. strains which displayed the highest ac-
tivity in elements extraction after first 7 days in compari-
on with individual strains.

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

![Graph showing the progress of the extraction of detectable elements from flotation concentrate (FC) with a higher sulphidic concentration during bioleaching.](image-url)

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 sulphidic ma-
trix 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 re-
moved 33 % of Si, 75 % of Pb, 76 % of Zn, 76 % of Cu,
9 % of Al, 3 % of Fe, and 82 % of Mn after 35 days.

![Graph showing the progress of the extraction of detectable elements from flotation concentrate (FC) with a lower sulphidic concentration during bioleaching.](image-url)
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

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**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 pyrite 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%
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**


