THE RELEASE OF IRON-BEARING MINERALS AND DISSOLUTION OF FELDSPARS BY HETEROTROPHIC BACTERIA OF BACILLUS SPECIES

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Dissolution of aluminosilicates and Fe-oxyhydroxide minerals from granitic eluvium using bacteria of Bacillus genus was monitored with solution chemistry, granulometric and X-ray analyses as well as microscopic techniques to determine the effects of these bacteria on crystal surface and releasing mechanism of K, Si, Fe, and Al from minerals. Feldspars, quartz and micas are dominant minerals in granitic eluvium (GE). Oxyhydroxides of Fe are found in the intergranular spaces of minerals, contaminating and making most feldspar raw materials unsuitable for commercial applications. Bacteria of Bacillus spp. decrease pH of leaching medium by production of organic acids. These organic acids are directed by glycocalyx of adherent bacteria to specific sites on mineral surface (e.g. to crystal defects). The impregnated iron minerals are released by bacterial destruction of intergranular and cleavage spaces of silicate grains. This bacterial activity results in the release of Fe, Si, and K from feldspar and Fe oxyhydroxides. However, pH of leaching medium is adjusted to neutral value in regular intervals during bioleaching to maintain bacterial activity. The decrease of fine-grained fraction is the result of bacterial destruction of GE. Despite the impoverishment of the distribution of fine-grained fraction, there was observed the increase of the surface area of feldspar grains from the value 3.65 m²/g to value 4.82 m²/g. This fact confirms the activity of bacteria of Bacillus genus together with hydrolysis in point corrosion of mineral grains. After 120 days of bioleaching, 31 % Fe extraction from granitic eluvium was observed. It was also possible to accelerate this process by using 0.1 M oxalic acid after 1 month's bacterial pretreatment. Moreover, the bacterial pretreatment facilitated the access of oxalic acid to Fe-bearing minerals and showed a possibility to use the oxalic acid in lower concentration. This fact is important especially from the view of producing less-agressive effluent to the environment and decreasing of costs in subsequent recyclation of oxalic acid.

INTRODUCTION

Mica and feldspars are the essential constituents of rocks which were weathered into secondary minerals, according to the environmental conditions. In temperate regions, feldspars (especially K-feldspars) are often considered as relatively stable, and phyllosilicates (mica or chlorite) are the main source of clay minerals, such as vermiculite or smectite. In humid tropical climates, both micas and feldspars are completely transformed into kaolinite and/or gibbsite, and iron oxides [1]. Hydrolysis is the process on which governs weathering. If intense leaching of silicates by water occurs, it may lead to dissolution of the structure, desilicification and precipitation of new secondary minerals (generally 1:1 clay minerals or oxides and hydroxides). This process is the main source of clays in tropical areas. Feldspars and micas experimentally weathered by water show pits in their structure in addition to the formation of hydroxides of Al and Fe [2]. Weathering is inextricably bound to biological processes, for organisms inhabit a wide range of nichens in surface and subsurface environments and influence various mineral transformation reactions [3, 4].

Experimental studies of the effect of microorganisms on mineral weathering rates and reactions are commonly performed in either closed (batch) or 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 containing mineral samples and reaction rates are calculated from effluent concentration and flow rate. Mineral weathering rates determined *in vitro* typically exceed those derived from field-based measu-

rements by one to three orders of magnitude, because these controlled laboratory experiments can not simulate the more complicated water-rock interactions in nature environments [5, 6]. While difficult to predict the absolute magnitude of a reaction, one may detect effects of microbial metabolites on weathering reactions [7].

Several strains of bacteria released by this way the cations from biotite (Si, Fe, Al), plagioclase, and feldspar (Si, Al) much more than abiotic procedures [8].

Microbial production of organics by fermentation, or reductive dissolution of Fe - Mn mineral phases can greatly accelerate weathering rates of aluminosilicate minerals [9, 10 11].

The red and yellow pigmentations noticed in many clay deposits are mainly due to the associated oxides, hydroxides, and oxyhydroxides of ferric iron such as hematite (red), maghemite (reddish brown), goethite (brownish yellow), lepidocrocite (orange), and ferrihydrite (brownish red). These oxides and hydroxides occur either as coatings on individual grains or as discrete fine particles throughout the clay mass. Quantities as low as 0.4 % of ferric iron may be sufficient enough to impart colour to the deposit. Removal of these associated impurities improves the quality of the material. The beneficiated clay finds use in paper coating, paper filling and as extenders in paints and polymers [12].

Considerable efforts have been devoted to the problem of removing ferric contaminants by physical [13] and chemical means. High intensity magnetic separation is a standard method used, removing substantial quantities of iron and titanium as mineral impurities, consequently improving the brightness of silicates. The chemical methods consist of leaching with mineral acids, and treatment with reducers, such as sodium dithionite plus aluminium sulphate, sulphur dioxide plus aluminium powder, and sulphur dioxide plus zink powder. These bleaching methods are usually suitable for achieving a higher degree of iron removal but they are more expensive, have complex operating conditions, and are environmentally hazardous [14]. Groudev [15] demonstrated that iron could be removed from quartz sand by using microbial leaching. His paper also reported microbial removal of iron from clays, and improvement of kaolin and ceramic properties via the action of metabolic products.

The ability of heterotrophic microorganisms to leach iron from oxide minerals was used previously for the removal of this element from kaolin of Horná Prievrana deposit [16].

In this work, the role of bacteria of *Bacillus spp*. in the leaching process and extraction of elements from rock containing feldspars is presented. The laboratory experiments performed indicate the feasibility of new biotechnological ways of processing of non-metallic raw materials.

EXPERIMENTAL

Granitic eluvium

Granitic eluvium (GE) is derived from muscovite-biotitic granodiorite and was sampled in Turčianske Teplice region near Žiar mountain (Slovakia). It is composed of quartz (55 - 60 %), feldspar (40 - 35 %) and mica (10 - 20 %). Among feldspars, plagioclases (oligoclase, andensine) dominate in comparison with K-feldspars (orthoclase, microcline). The presence of Fe oxyhydroxides was identified by visible brown-red colour of this material. Two mica species of white (muscovite) and black (biotite) colour are included in the sample of granitic eluvium. The iron-bearing minerals decrease the quality of this raw material. Its chemical characteristics are shown in table 1.

Table 1. The chemical composition of granitic eluvium samples.

	chemical composition (wt. %)			
sample	GE	1.GEBL	2.GEBL	GEBLCH
SiO_2	73.25	64.14	53.47	68.52
Al_2O_3	11.51	11.56	11.73	11.70
Fe_2O_3	1.44	1.23	0.99	0.79
K_2O	2.48	2.39	2.15	2.40

Legend: granitic eluvium (GE) from Turčianske Teplice (Slovakia), granitic eluvium after 40 days bioleaching (1.GEBL), granitic eluvium after 120 days bioleaching (2.GEBL) and granitic eluvium after 1 month bioleaching and 2 days chemical leaching by oxalic acid (GEBLCH)

Bacteria and media

Two bacterial strains (*Bacillus cereus* and *Bacillus pumilus*) were isolated from a kaolin quarry in Horná Prievrana. They were identified by means of the Becton-Dickinson microbiology system (Becton Dickinson, Cockeysville, USA). For the species identification, the strains were cultivated on Columbia agar plates according to recommendation of the system producer. Prior to experimental use, these bacterial strains were grown in Nutrient broth No.2 (Imuna, Šarišské Michaľany) at 28°C for 18 hours. 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^{10} cells per ml to modified Bromfield liquid medium [17]. Bioleaching of the samples was carried out in 3000 ml Erlenmeyer flasks containing 200 g of GE in 2000 ml of modified Bromfield medium (NaH_2PO_4 - 0.5 g/l, $MgSO_4 . 7H_2O$ - 0.5 g/l, $(NH_4)_2 SO_4$ - 1.0 g/l, NaCl - 0.2 g/l, molasses - 30 g/kg GE) inoculated with a mixture of both *Bacillus cereus* and *Bacillus pumilus* strains. The flasks were incubated

statically for 1 and 3 months at 28°C. The abiotic controls were cultivated under the same conditions. After incubation, the culture solutions were separated from the biomass by means of membrane filtration. The presence of vegetative bacterial cells in Erlenmeyer flasks and their morphology were regularly examined by light microscopy after Gram staining.

Chemical analyses

Quantitative changes of samples (solid and liquid phases) investigated from view of element composition stability were evaluated by standard analytical method - atomic absorption spectrometry on a VARIAN spectrometer AA - 30 apparatus (Varian, Australia) after dissolution of the samples according to the Czechoslovak norm ČSN 720101 (Basic analysis of silicates. Decompositions).

Granulometric analysis

The particle size distribution of feldspar sample was measured by the laser radiation scattering on a Laser - Particle - Sizer Analysette 22 (Fritsch, Idar - Oberstein, Germany).

X-ray diffraction analysis

The structural destruction of bacterially leached samples was determined by X-ray diffraction analysis on a diffractometer SIEMENS D500 (Siemens, Germany) by using the following regime: radiation CuK_α, 40 kV, 30 mA, time constant 1 s, limit of measurement 10 impulses/s, rate of detector 0.02°/min.

Scanning electron microscopy

The morphological changes in the surfaces of individual minerals were investigated by SEM (scanning electron microscopy) and the changes of chemical composition by energy-dispersion microanalysis (EDS). All mineral samples were coated with carbon and subsequently examined in a scanning electron microscope Tesla BS 340.

Specific surface area examination

The surface area was determined by nitrogen sorption method (BET) at liquid nitrogen temperature, using a Micromeritics Gemini 2360 Surface Area Analyzer (USA).

RESULTS AND DISCUSSION

The release of major structural elements, dissolved K, Si, Fe and Al from granitic eluvium, was used as an overall indicator of mineral dissolution of aluminosilicates and iron minerals during these experiments (figure 1). Previous experimental weathering studies have shown no systematic variation in cation leaching with changes in solution chemistry [18]. In the present dissolution experiments with *Bacillus* strains, K, Fe, Si, and Al release to solution was greatly increased due to the production organic acids which decreased solution *pH*. This *pH* decrease was continually neutralized during bacterial leaching. Although organic acids were not measured in this experiment, a previous study confirmed that these bacterial strains produce several organic acids such as acetic, butyric, pyruvic, lactic, and formic acid during bioleaching of aluminosilicate samples [19].

It is well known that many organic compounds produced by microorganisms, such as acetate, citrate and oxalate [20] can increase mineral dissolution rates in laboratory experiments [21, 18]. Carboxylic acid groups which were shown to promote dissolution of silicates [22] are also common in extracellular organic material. Moreover, some microorganisms in soil environment contain enzymes that function in ways analogous to chitinase and cellulases, i.e. they specifically break down mineral structures and extract elements required for metabolism or structural purposes (e.g., "mineralases") [7]. This may be especially important for ions such as Fe^{3+} and Al^{3+} which are expected to be rather insoluble [23, 24].

The acids production and microbial colonization of the mineral surfaces (figure 2) greatly accelerated the release of mineral elements to solution from feldspar sample. K, Si and Fe concentrations in leachates were approximately 10 - 20 times higher during bioleaching of rocks (GE) with high content of feldspars (contaminated with Fe oxyhydroxides) than during leaching of the same sample in abiotic control. Al extraction was characteristic by low effectivity. Higher K and Si extraction (figure 1) was observed at GE bioleaching because of a lower resistance of feldspars to activity of heterotrophic bacteria of *Bacillus spp.* However, this effect was not constant over time. Dissolved Fe and Al were undetectable in the abiotic control samples (data not shown) because of their too low concentration. The chemical analyses of liquid phase served only as a qualitative control for the confirmation of the release of mineral elements to leachates caused by bacterial activity during bioleaching. The quantitative evaluation of the extraction of individual mineral elements is possible only by analyzing of solid phases because both divalent and trivalent cations (such as iron) are often competently sorptive on cell membranes of bacteria [25].

Feldspars are dissolved incongruently at neutral *pH*, nonstructural ions (Na, Ca, K) are preferentially leached from the surface, and Si is preferentially released with respect to Al. As acidity increases, Al/Si release increases too [18] leaving a residual material enriched in Si but depleted in other cations [26]. Low dissolution of Al in comparison with Si is possible to explain by the fact, that bioleaching by *Bacillus spp* occurs at neutral and gentle acidic *pH*. Moreover, these bacteria do not produce oxalic acid as described in our previous publication [19]. It is known that feldspars with higher concentrations of Al are dissolved faster than those with lower Al concentration in presence of some organic acids, mainly oxalic acid. Oxalic acid is known to mediate biochemical weathering of many framework silicates, mainly plagioclase feldspars [18]. In some abiotic dissolution experiments at neutral *pH*, biotite weathering is predominantly incongruent. The mechanism involves loss of interlayer cations, frequently charge balanced by oxidation of Fe^{2+} to Fe^{3+}, and inheritance of the 2:1 layer [27, 28]. Acidification can change this process, resulting in a switch to congruent dissolution with an associated dramatic increase in Fe release to solution and an overall acceleration of degradation of the aluminosilicate structure [29].

In the present experiments, a dramatic increase in Fe, Si, and K release was observed due to destruction of feldspar and Fe oxyhydroxides in granitic eluvium. Granitic rocks are often a source material for kaolinitic sands in the nature. Their destruction by bacteria of *Bacillus spp*. under laboratory conditions suggests that this bacterial genus, ubiquitous in clay soils, plays an important role in bio-chemical destruction of feldspars as well as in the circle and transformation of iron-bearing minerals.

Berthelin and Belgy [30] reported the complete removal of K and Ti from biotite in a granitic sand by community of bacteria and fungi after a 22 week perfusion experiment, resulting in brittle, white micaceous particles.

Bobos and Gomes [31] investigated K-feldspars weathering at the kaolin deposit of Sao Vicente de Pereira (Portugal). Their analyses indicated that the weathering products were typical by high content of Al and small contents of Si, Fe, and K. Moreover, altered K-feldspar grains exhibit "porous" surfaces. This fact indicates that the K-feldspar has been rapidly dissolved or corroded by solutions with an acid pH, whereas no secondary mineral phases were produced [31].

X-ray patterns (figure 3) of GE sample show higher quartz/feldspars ratio after bacterial leaching. The investigation of feldspar grains by SEM before (figure 4) and after (figure 5) bacterial leaching confirmed the destruction of many grains caused by corrosive enantiomorphic pits as shown in detail on figure 6. Also, no secondary mineral phases were recognized on feldspar surfaces by EDS or by X-ray analyses.

Similar morphological changes in the surfaces of feldspars were observed by SEM after bioleaching of the polished sections. The etch pits in feldspars represent the typical demonstration of bacterial activity of *Bacillus spp*. and they morphologically differ from superficial chemical dissolution [19].

The impoverishment by fine-grained fraction is the result of bacterial destruction (Figure 7). The amount of finest-grained fraction (from 0.9 to 7.5 μm) was in GE sample decreased, and on the other hand, the distribution of fraction with particle size from 9.0 to 103.0 μm was increased. Despite the decrease of distribution of fine-grained fraction, there was observed the increase of the surface area of feldspar grains from the value 3.65 m²/g to value 4.82 m²/g. This fact confirms the activity of bacteria of *Bacillus* genus in point corrosion of mineral grains.

Feldspars in GE sample (table 1) contain 1,44 wt.% iron. Iron was found especially in the form of Fe oxyhydroxides. The presence of iron minerals was identified also by visible brown-red colour of this material. The releasing of Fe was characteristic by the change of visible brown-red colour of these non-metallic materials to brown-white. As can be seen from the results presented in table 1, the *Bacillus* strains used in this study markedly decreased Si content and partially decreased also content of potassium what resulted to a weak enrichment of Al content. After 40 days of bioleaching, only 15 % Fe extraction was observed in GE while 31 % extraction was detected after 120 days of bacterial leaching of GE.

The release of Fe from GE suggests the removal of the cementing iron oxides from the silicate surface. This partial Si and Fe removal can also improve the access of organic acids to iron-bearing minerals and enable the increase of the feldspar raw material quality by subsequent leaching with less concentrated organic acids. That is why bacterial destruction of intergranular spaces of silicate grains, often impregnated with iron minerals, should be used as pretreatment of raw material before chemical treatment of the sample by oxalic acid.

After 1 month's biological pretreatment of GE and 2 days of chemical treatment by 0.1M oxalic acid, there is possible to reach 46 % removal of Fe without an additional destroying of feldspars during chemical leaching (table 1).

The mechanism of the reductive dissolution of iron oxides by organic reagents has been investigated by several workers [32, 33, 34].

It was observed that oxalic and citric acids were much more effective for bleaching of kaolin than other organic acids such as acetic, lactic and ascorbic [14]. Another important observation was the influence of temperature on the bleaching process. The use of high temperatures, rather than raising the concentration, proved to be more effective in enhancing the bleaching process of kaolin when commercial oxalic acid was used [14].
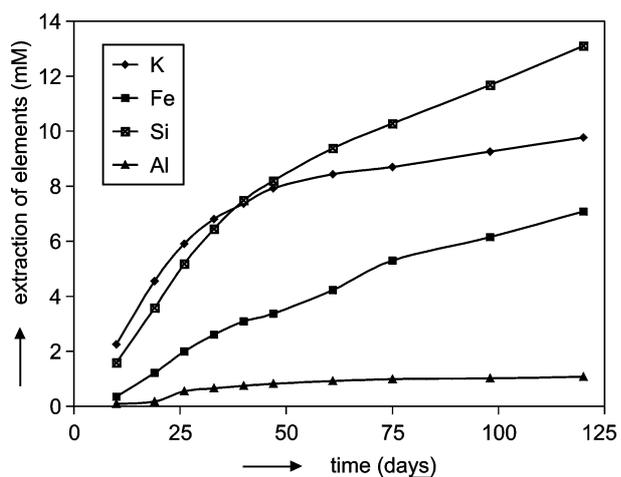
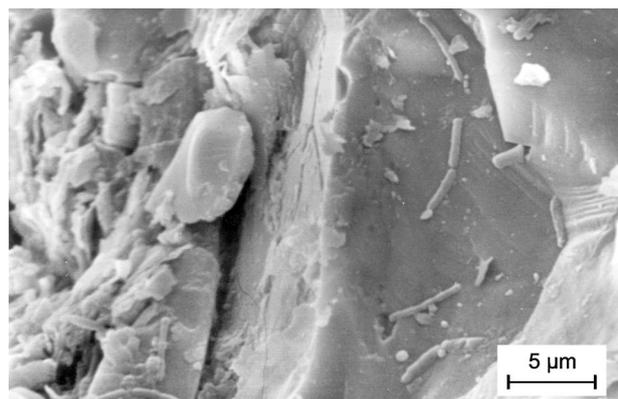Figure 1. The bioextraction of elements from granitic eluvium (GE).

Figure 2. SEM micrograph of bacterial cells of *Bacillus spp.* on feldspar surface.
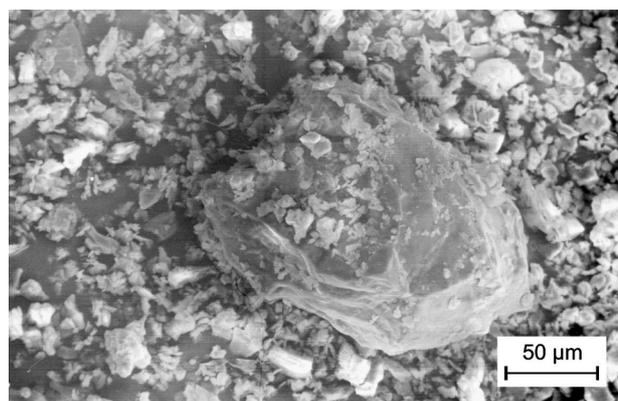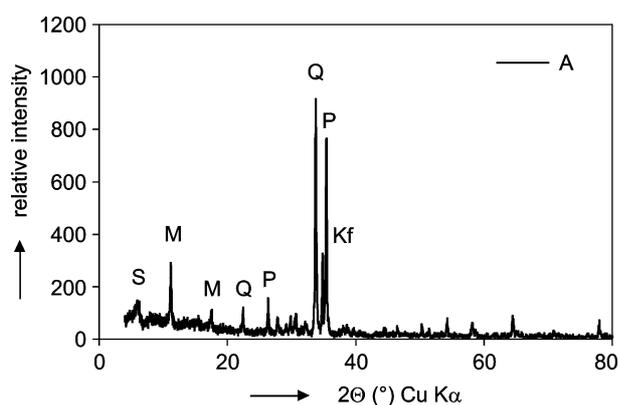

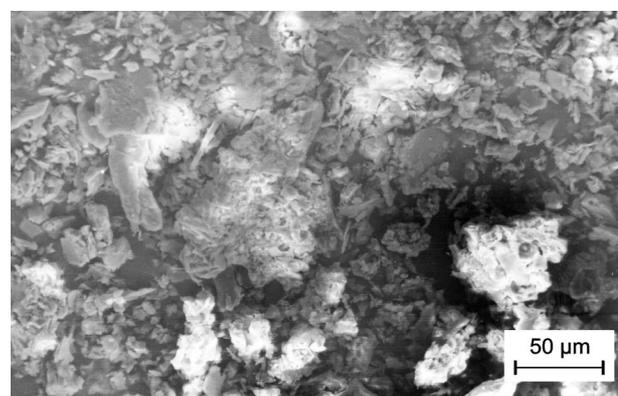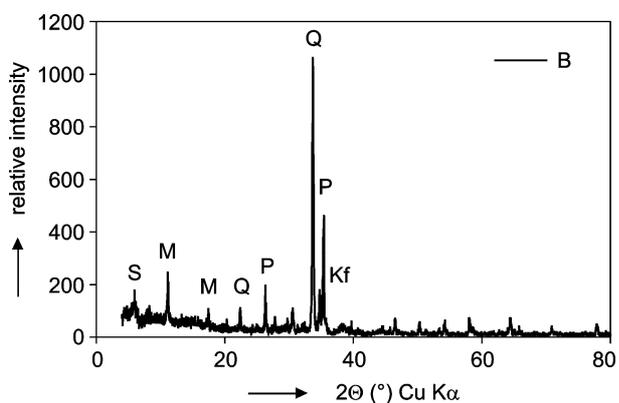Figure 4. SEM micrograph of feldspar grains before bioleaching.

Figure 3. The mineral composition of granitic eluvium (GE) according to X-ray diffraction pattern (Q - quartz, P - plagioclases, Kf - potassium feldspars, M - mica, S - smectite); a) before bioleaching, b) the higher quartz/feldspars ratio after bioleaching.

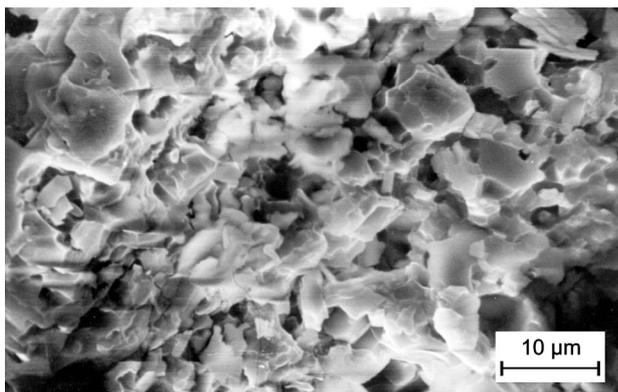Figure 5. SEM micrograph of feldspar grains after bioleaching.

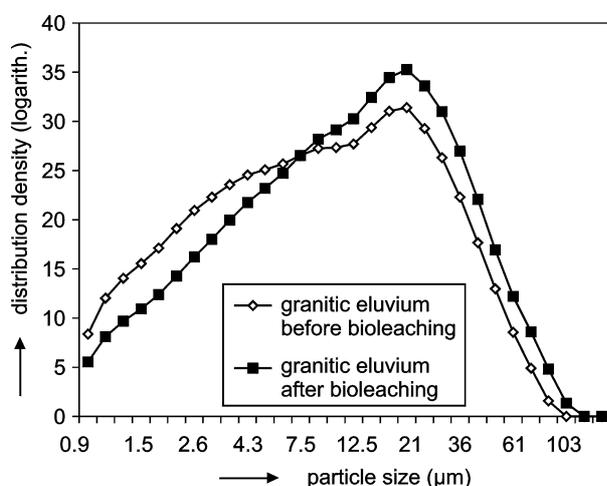Figure 6. SEM micrograph of pits on feldspar surface after bioleaching.

Figure 7. The impoverishment by fine-grained fraction in GE sample as a result of bacterial leaching. A decrease of the finest-grained fraction from 0.9 to 7.5 μm and the increase of distribution of the fraction with particle size from 9.0 to 103.0 mm was observed after bioleaching.

In our experiments, the slight increase in the specific surface area and the exposition of intergranular spaces of silicate grains (often including iron minerals) increased the effect of 0.1M oxalic acid. The increase of iron removal in these experiments was similar to the results of Mesquita [14] with 0.25M oxalic acid. The corresponding removal of iron oxides was obtained also in the leaching tests with 0.05M oxalic acid at 100°C for 90 min or with 0.15M oxalic acid at 27°C for 8 days [12].

The bioleaching pretreatment showed a possibility to use less concentrated oxalic acid and therefore to produce less-agressive effluent to the environment or a decrease of costs for recyclation of soluble complexes (e.g. trisoxalatoiron(III) anions) formed during leaching by oxalic acid. It is also possible to accelerate bioleaching process by continual monitoring of sugars consumption from molasses, which was used as the energy source during samples bioleaching.

CONCLUSION

The interaction of bacteria of *Bacillus spp.* under static laboratory condition resulted in the high release of Fe, K, and Si from feldspars as non-metallic phase and in the significant colour changes. Our results indicate that non-metallic raw materials of lower quality (contaminated by iron-bearing minerals) should be pretreated by bacteria because they are able to expose the intergranular and cleavage spaces in which iron phase occurs. After bacterial pretreatment, the higher effect of the treatment with oxalic acid was observed.

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UVOĽŇOVANIE ŽELEZITÝCH MINERÁLOV A ROZPÚŠŤANIE ŽIVCOV HETEROTRÓFNYMI BAKTÉRIAMI DRUHU BACILLUS

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Priemyselné suroviny ako kaolín, kremenný piesok, živce sú často kontaminované nežiadúcimi prímesami, napr. oxidmi železa, ktoré znižujú ich hodnotu. V súčasnosti sa pri skvalitňovaní nerudných surovín využívajú fyzikálne (plavenie a magnetická separácia), fyzikálnochemické (flotácia) a chemické (lúhovanie anorganickými kyselinami) procesy. Žiadny z týchto procesov nebol celkom úspešný pre univerzálnu aplikáciu, nakoľko tieto procesy sú často drahé a hlavne vedú k vzniku nežiadúcich komponentov, ktoré sú typicky spracovávané neutralizáciou, precipitáciou a recykláciou vody. Využitie biotechnológií pri úprave silikátových surovín predstavuje novú alternatívnu cestu efektívneho spracovania nerastných zdrojov.

Rôzne varianty podmienok bakteriálneho lúhovania ako aj zefektívňovania úpravy živcovej suroviny následným chemickým dolúhovaním prostredníctvom organickej kyseliny boli testované v našich experimentoch.

Výsledky experimentov potvrdili schopnosť baktérií rodu *Bacillus* korodovať K-živce v granitickom elúviu s následnou extrakciou intragranulárne a na štiepnych trhlinách viazaného Fe ako nežiadúcej prímesi v živcovej surovine. V dôsledku deštrukcie aluminosilikátových zŕn došlo čiastočne k zníženiu podielu najjemnejšej zrnitostnej frakcie od 0,9 do 7,5 μm a k zvýšeniu povrchovej plochy jednotlivých zŕn z hodnoty 3,65 m^2/g na hodnotu 4,82 m^2/g, čo je spôsobené tvorbou korozívnych enantiomorfných dier na povrchu aluminosilikátových zŕn.

Extrakcia Fe z granitického elúvia je pravdepodobne spojená s biologickou deštrukciou tmeliacich oxidov Fe z povrchu aluminosilikátových zŕn. Bakteriálna jednomesačná predúprava suroviny a následná chemická úprava suroviny s 0,1M kyselinou oxálovou umožnila odstrániť 46 % Fe z granitického elúvia.

Biologická predúprava vytvára reálnu možnosť využitia menej koncentrovanej kyseliny oxálovej pre zvýšenie efektívnosti odstraňovania Fe a tým zníženia nákladov pri recyklácií rozpustných komplexov, ktoré sa formujú po chemickom lúhovaní organickými kyselinami.