

NUTRIENTS ENHANCING THE BACTERIAL IRON DISSOLUTION IN THE PROCESSING OF FELDSPAR RAW MATERIALS

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*Naturally occurring iron oxides are often coated on silicate grains or are impregnated in the matrix in silicates of industrial importance. These Fe-rich impurities can be removed from industrial minerals such as granite through bioleaching. Heterotrophic bioleaching may substantially reduce the need of aggressive chemical bleaches treat industrial silicate minerals. This process involves a siliceous matrix, which is why silicate heterotrophic bacteria of the genus *Bacillus* are of potential use. These organisms are noted for their ability to reduce ferric iron coupled with dissolution. Solution phase assays were used to monitor iron reduction activity by a *Bacillus* sp. during the bioleaching of natural feldspar raw materials under various experimental conditions. The rate of reductive iron dissolution was dependent on the presence of yeast extract, nitrate and sulphate in the medium. Quinone stimulated the Fe(III) reduction in feldspars raw materials in cell suspensions. The dissolution of iron was enhanced in the presence of technical-grade sucrose and molasses. The amendment of the medium with river water and indigenous bacteria increased the bacterial reduction of iron. The Fe content in granite samples treated by bioleaching decreased by about 60 %. The process needs controlled conditions for the bacterial iron reduction and is dependent on the mineralogical composition of non-metallic ores.*

INTRODUCTION

Industrial silicate-based minerals such as kaolins, feldspars and quartziferous sands often contain impurities that lower their economic value and hinder their application in the ceramic and paper industries. The main impurities are Fe(III)-oxides because they lower the degree of whiteness, an important factor related to the quality of the product [1].

Red and yellow pigmentations in many clay deposits are due to Fe(III)-oxides such as hematite (α -Fe₂O₃; red), maghemite (Fe₃O₄; reddish brown), goethite (α -FeOOH; brownish yellow), lepidocrocite (γ -FeOOH; orange), and ferrihydrite (Fe₅HO₈·4H₂O; brownish red). These phases occur as coatings on individual grains or as discrete fine particles throughout the clay mass. Concentrations as low as 0.4% Fe(III) may be sufficient to impart colour to the clay mass [2].

Chemical treatment methods are based on the leaching with mineral acids and treatment with reducing agents such as Na-dithionite and Al-sulphate, sulphur dioxide and Al-powder, or sulphur dioxide and Zn-powder. These bleaching methods are usually suitable for achieving a high degree of iron removal but they are expensive, have complex operating conditions, and are

environmentally hazardous [3]. In the last ten years there has been interest in finding alternative chemical processes for iron removal that would be efficient and environmentally acceptable [1].

De Castro and Ehrlich [4] demonstrated that a *Bacillus* sp. reduced enzymatically Fe(III). Iron reduction by the *Bacillus* sp. required glucose in the medium. Iron was not reduced in the absence of glucose or in sterile glucose-containing media. Furthermore, Fe(III) was not reduced when uninoculated medium was acidified, indicating that low pH during the incubation does not facilitate the abiotic reduction of Fe(III).

Humic substances can shuttle electrons between the humic-reducing microorganisms and Fe(III) oxides [5]. The bioavailability of Fe(III) is increased in environments that are rich in organic compounds including humic substances. Humic substances are formed from incomplete degradation of complex plant polymers such as lignin and can chelate a variety of metals including Fe. In addition, microorganisms secrete metabolites such as carboxylic acids and siderophores that chelate ferric iron and make it accessible for biological reduction and cellular uptake [6]. The reduction of Fe(III) for incorporation into biomolecules is assimilatory iron reduction. In contrast, the dissimilatory iron reduction is

coupled with electron transport and energy transduction. In the assimilatory pathway, chelated Fe^{3+} is reduced by a ferric reductase either before or after transport into the cell. Ferric iron assimilatory reductases have been described from numerous bacteria including *Bacillus* spp. This enzyme system is not influenced by the concentration of iron in the culture medium [7].

The finding that bacteria can donate electrons to humic acids has important implications for the removal of iron impurities from non-metallic minerals. However, it is not known whether comparable iron removal can result from the biological reduction of Fe(III)-oxides or whether humic acid analogues such as AQDS (anthraquinone-2, 6-disulfonate, quinone) can enhance the extent of the reduction of Fe oxides by assimilatory iron-reducing *Bacillus* spp.

Bioleaching processes using heterotrophic microorganisms have received little attention although heterotrophs in the genera *Bacillus* and *Pseudomonas* have been found effective in the bioleaching of non-sulfidic minerals [8]. Some bioleaching studies have used synthetic solid phases and iron oxides for better control over the mineral structure in initial iron reduction experiments [9]. Naturally occurring silicates contain oxidic iron minerals as coatings on grains or impregnations in the matrix. The extent of iron removal from industrial silicate minerals depends on the mineralogy and distribution of iron in silicate rocks. For this reason, bioleaching studies with industrial minerals for their beneficiation have examined the kinetics of iron dissolution from the siliceous matrix [1]. In the present work, iron reduction was monitored as a measure of bacterial activity in the bioleaching of feldspar raw materials. Our objectives were: (1) to remit on bacterial kind of *Bacillus* spp. can be used for bioleaching, (2) to study the effect of media composition with and without AQDS on the extent of bacterial reduction of iron impurities from feldspars, and (3) to determine the carbon sources suitable for bioleaching in practice. Changes in the ability of the bacteria to reduce ferric iron coupled with dissolution were characterized by comparison of chemical analyses of leach solutions during the bioleaching experiments. Changes in iron removal from feldspars was used to assess the effectiveness of the heterotrophic bioleaching process. Experimental conditions for the iron reduction activity were varied as part of an effort to optimize bioprocesses for treating feldspar raw materials. The reproducibility of the results was dependent on the mineralogical composition of silicates.

EXPERIMENTAL

Iron solubilization from a granitic (feldspar) sample was investigated with *Bacillus* spp. The sample was Rudník-Poproč-granite designated as RZ and contained iron-bearing minerals (mica, anatase, smectite, ferrihydrite and goethite), which decreased the quality of the feldspar for industrial use. Chemical characteristics of the sample are listed in Table 1. The granite sample was dried at 22°C, pulverized in a achate ball mill, and sieved to a particle size of <0.7 mm.

The bioleaching experiments were carried out in 300 ml Erlenmeyer flasks containing 10g powdered feldspar and 100 ml liquid medium. Three mineral salts solutions and river water were tested as liquid media (Table 2). The media were designed to examine individual (A, OA, MM) and heterogeneous (natural surface water) supplements. Fe(III) reduction in feldspar raw materials was tested in these media with and without 100mg/l AQDS. AQDS is a humic acid analogue and it was of interest to test whether it can enhance the extent of the bioleaching of Fe minerals from natural silicates by heterotrophic iron-reducers. Results of stimulation of bacterial iron extraction represented the means for duplicate model experiments.

The carbon sources used in this work were glucose, sucrose, galactose, technical-grade sucrose and molasses (all at 20 g/l). Anand et al. [10] and Deo [11] observed that 2% sucrose was the optimum concentration for growth of *Bacillus* spp. and for Ca and Fe removal from the ore. The culture flasks were separately inoculated with 1ml of 10^7 cells of *Bacillus* spp. and with *Bacillus cereus*, *B. megaterium*, and *B. pumilus*, which were originally isolated from the Horná Prievrana kaolin deposit in Lučenecká Kotlina, Slovakia and with indigenous *Bacillus cereus* and *B. megaterium* isolated from natural water of the Rudník deposit. The strains were purified by heat treatment at 80°C for 15 min followed by streak plating on nutrient agar cultures. The isolates were identified with the BBL Crystal Identification System (Becton, Dickinson & Co., Franklin Lakes, NJ). For identification, the isolates were cultivated on Columbia agar plates per manufacturer's instructions. The flasks were incubated under static conditions at 25°C. Appropriate abiotic controls were included in the experiments. The presence of vegetative bacterial cells in culture flasks and their morphology were regularly examined by light microscopy after Gram staining.

Table 1. Chemical composition (wt.%) of RZ before and after leaching by *Bacillus* spp.

Sample	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	TiO ₂	CaO	MgO	Na ₂ O	K ₂ O	MnO
Untreated	77.8	13.1	0.29	0.09	0.35	0.20	6.57	0.84	0.03
Bioleached	77.0	12.9	0.12	0.09	0.25	0.26	7.41	0.82	0.01

Prior to experimental use, these bacterial strains were grown in Nutrient broth No.2 (Imuna, Šarišské Michalany) at 28°C for 18 hours. Nutrient broth No. 2 (pH 7) contained 5 g/l each of meat extract and peptone 5g/, and 2.5g/l NaCl. 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 liquid medium (Table 2).

Table 2. Liquid media used in the bioleaching experiments.

Components	Medium (g/l)		
	A	OA	MM
K ₂ HPO ₄	1.0	2.0	2.5
NaCl	0.075	0.075	
Urea	0.2	0.2	0.2
MgSO ₄ ·7H ₂ O		0.5	0.5
(NH ₄) ₂ ·SO ₄		1	2
Yeast extract		0.15	0.15
Na ₂ SO ₄ ·10H ₂ O			0.5
KCl			2.5
Ca(NO ₃) ₂ ·4H ₂ O			0.5
Glucose	20	20	20

After incubation, cells were harvested by centrifugation at 6000 rpm for 15 min. Dissolved Fe²⁺ and Fe³⁺ were measured colorimetrically using the *o*-phenanthroline method [12, 13]. Redox potential was measured with a platinum microelectrode (GPRT 1400 A, Greisinger, Germany). Solid residues were air dried and analyzed by X-ray diffraction using a Philips X'Pert SW-binary diffractometer with CuK α radiation (40 kV, 50 mA), equipped with an automatic divergence slit, sample spinner, and a graphite secondary monochromator. Data were collected for 2 to 60 °2 θ with a step width of 0.05° and a counting time of 30 sec per 0.05°.

RESULTS AND DISCUSSION

Effect of medium on iron dissolution

The dissolution of iron from the granite sample as a function of time is presented in Figure 1. Iron dissolution was enhanced by yeast extract and increasing the concentration of K₂HPO₄, MgSO₄, (NH₄)₂·SO₄, but decreasing the concentration of Na₂SO₄, KCl and Ca(NO₃)₂ in the medium. The components and their concentration in culture medium for the leaching experiment were selected based on the previously described Ashby [14] and Bromfield [15] media. The time to reach high iron concentration was shortened from 14 days to 6 days using medium OA. Medium MM with nitrate or sulphate did not have an effect on the bacterial iron reduction and dissolution of iron. The iron dissolution with the MM medium was the lowest.

In this work, the bioleaching tests were performed with iron-reducing *Bacillus* spp. in the presence of 100 mg/l AQDS at 25°C, pH 6.5 and at 10% (w/v) pulp density. As a humic acid analogue AQDS may promote the reduction of crystalline Fe(III) oxides. AQDS was reported to stimulate the reduction of three single-phase oxides (hematite, goethite, and hydrous ferric oxide) by dissimilatory Fe(III) reducing *Shewanella putrefaciens* [16]. AQDS enhances microbial respiration and electron delivery to the oxide, and contains a quinone group that is known to be present in humic substances [17, 18].

The addition of AQDS stimulated the extent of Fe(III) reduction from the feldspar raw materials by the *Bacillus* sp. test culture (Figure 1). The concentration of Fe in solution increased from 200 mg/l to 280 mg/l after 9 days of incubation when medium A and OA were supplemented with 100 mg AQDS/l. Previous experiments have shown that anaerobic conditions were formed within one day of incubation because of microbial respiration and the initial positive redox potential value (350 mV) was reduced to negative values (-110 mV to -380 mV) [19].

AQDS enhanced the Fe(III) reduction and Fe dissolution under non-controlled anaerobic conditions. Similarly, Lovley et al. [5] observed enhanced dissolution of Fe by iron-reducing *Geobacter metallireducens* with highly purified soil humic acids. In the present study, humic acids extracted from coal without purification did not stimulate bacterial activity of *Bacillus* spp. (data not shown). Thus AQDS is a supplement that enhances the dissolution of Fe from silicate lattice.

Effect of carbon on iron dissolution

The effect of carbon source was tested in the OA media inoculated with *B. cereus* by the addition of glucose, sucrose, galactose, technical-grade sucrose and molasses. The results (Figure 2) showed that the extent of iron dissolution was higher in the presence technical-grade sucrose and molasses after 6 days of bioleaching than in the presence glucose and sucrose or approached to rate of iron dissolution during addition glucose in experiments showed in Figure 1. The reduction of iron in the presence of glucose and sucrose was higher than in the presence technical-grade sucrose and molasses during the first few days of incubation but the concentration of dissolved iron remained approximately constant. The effect of galactose was similar to that of food-sugar and molasses but after 11 days bioleaching. Molasses can be used as cheap bulk carbon source to enhance biomass growth and production of leaching agents.

Molasses is the liquor remaining after crystallization sucrose from sugar beet juice. The composition of molasses is variable, depending on the quality of sugar beet and processing technology, and varies in the following ranges: 76-84% solids (including sucrose,

46-51%); 1.0-2.2% reducing substances; 0.8-1.2% raffinose; 0.2-1.0% inverted sugar; 1.2% volatile acids; 4-8% pigments; and 6-10% ash [20]. It is a relatively inexpensive carbon source used for various industrial fermentations. Molasses contain also other nutrients that may account for the enhancement of iron dissolution in this study. The admixture of pigments in molasses colored the feldspars, but the discoloration could be removed by the addition of 0.05 % NaClO following the bioleaching step. Sodium hypochlorite as continually added from 0.01 to 0.05% concentrations to solution and discoloration of feldspars sample could be visually observed.

Iron dissolution and reduction were tested with three *Bacillus* cultures in media amended with technical-grade sucrose (Figure 3). In the absence of added AQDS, the removal of iron in the three test cultures was similar. In the presence of AQDS, the bioleaching by *B. cereus* clearly produced the highest yield of iron dissolution and reduction. Approximately 265 mg Fe²⁺/l was solubilized in 17 days.

Indigenous bacteria were isolated from river and well water samples, but these isolates were not as efficient as *B. cereus* in solubilizing Fe (Figure 4). The addition of river water without or with the bacterial isolates enhanced iron dissolution and reduction of *B.*

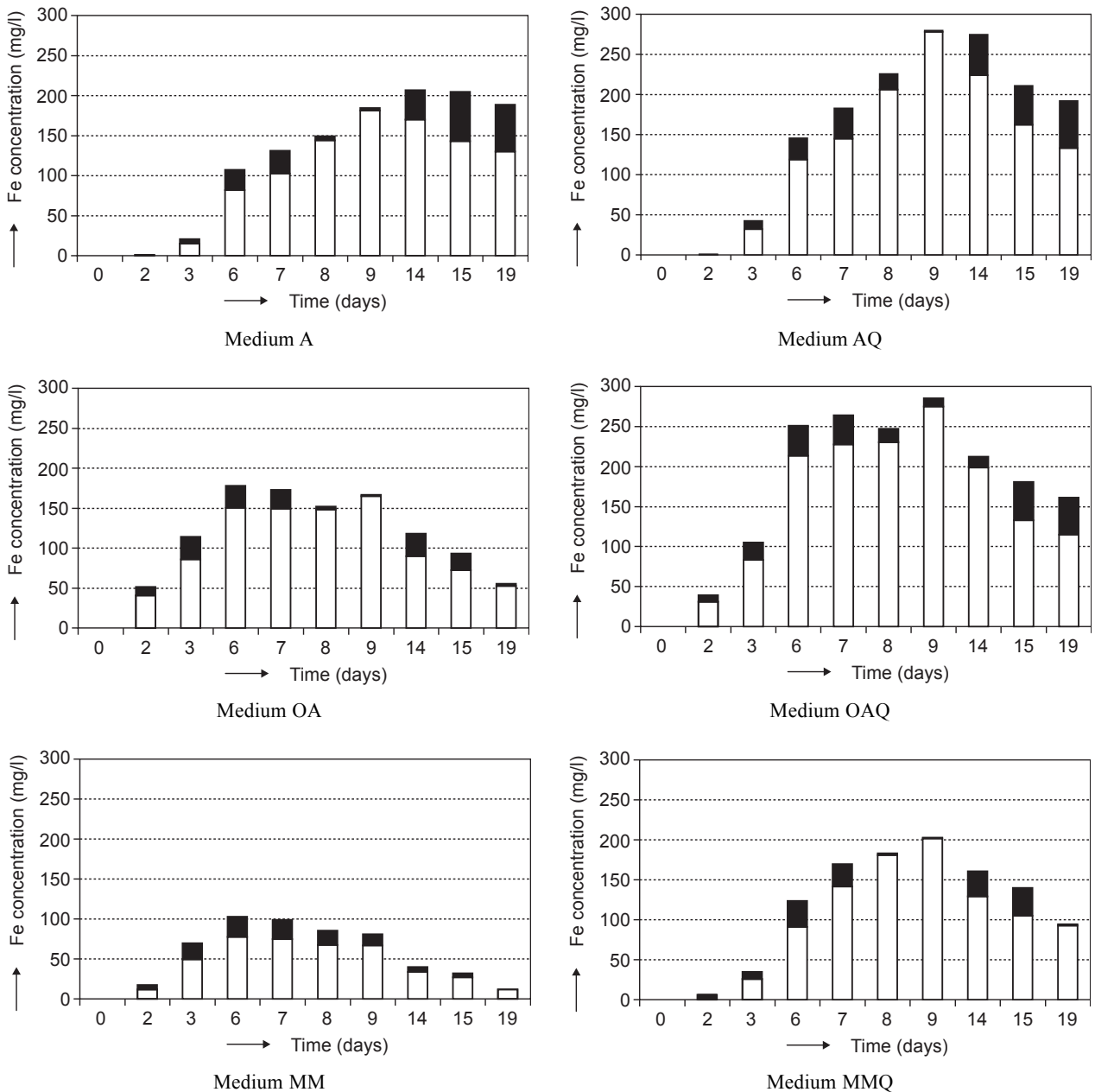


Figure 1. Dissolution of Fe³⁺ (■) and Fe²⁺ (□) from sample RZ in static *B. cereus* cultures in medium A, OA, and MM without and with 100 mg AQDS/l (AQ, OAQ, MMQ).

cereus, with a final yielding of 318 mg Fe²⁺/l (Figure 5). Surface water may be suitable in bioleaching processes in industrial scale without sterilization.

Iron dissolution and reduction in *Bacillus* suspensions increased with time. Iron reduction ceased when the pH decreased to 4 and the redox potential gradually increased from the initial level of -110 mV. Iron oxidation increases the redox potential and results in the precipitation of fine-grained iron oxides. The oxidation and

precipitation may be prevented by using a discontinuous process whereby the medium is replaced after each exponential cycle of bacterial iron reduction and dissolution. In the abiotic controls, the relative releasing of iron was very low than of those in the inoculated samples. Thus, the release of iron in the *B. cereus* cultures was many times higher in comparison with the abiotic control.

The data showed that the most suitable raw material for the bioleaching process with molasses (0.3 g molasses per kg rock sample) was the Rudnik feldspar (RZ). This RZ sample contained plagioclase and quartz as the main mineral phases as well as smectite and micas, which are Fe-containing silicates. The presence of Fe and Mn oxides in the sample was confirmed by EDX analysis. Fe extraction (Figure 6) continued to increase after 25 days. Bioleaching decreased the Fe-content by 60% after 95 days (Table 1) of contact time in medium A with the absence of AQDS [19].

In the manufacture of ceramics, feldspar is the second most important ingredient after clay minerals. Feldspars are used as fluxing agents, to form a glassy phase at low temperatures, and as a source of alkalis and alumina in glazes. Feldspar ores in Slovakia are mainly

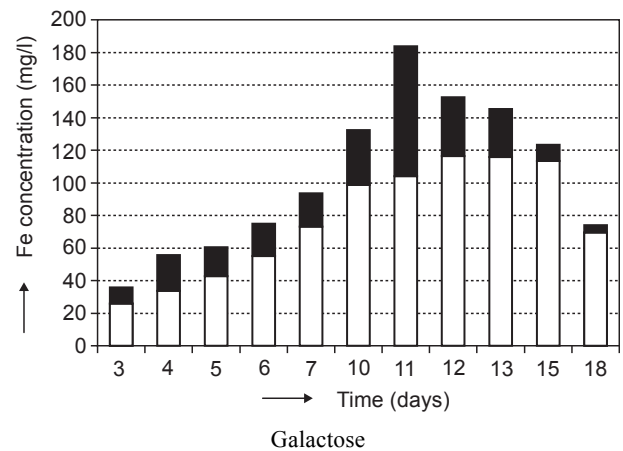
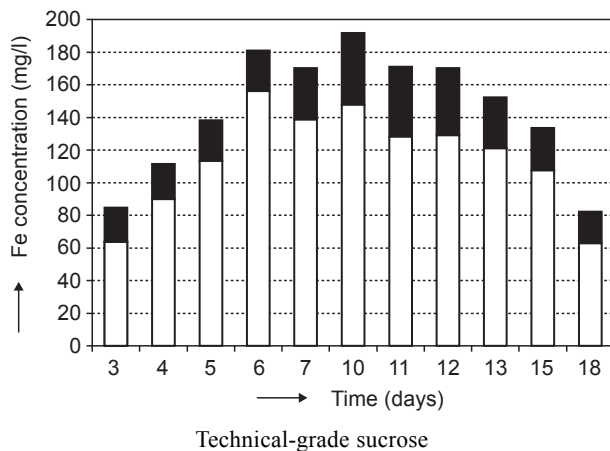
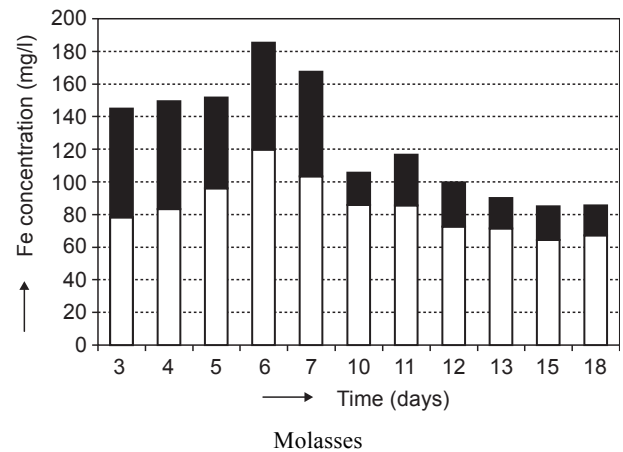
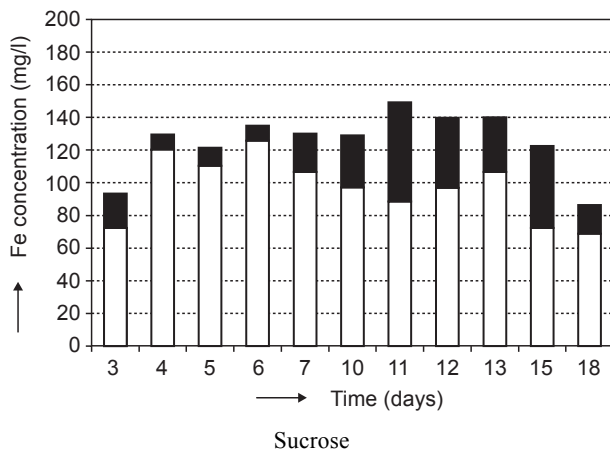
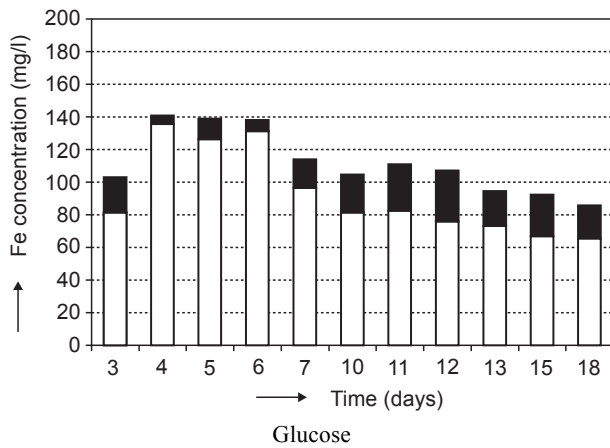


Figure 2. Dissolution of Fe³⁺ (■) and Fe²⁺ (□) from sample RZ in static *B. cereus* cultures in medium OA amended with glucose, sucrose, molasses, crystalline food-sugar, and galactose.

of albite, of which a substantial proportion contains only iron as a main impurity, with relatively low levels of titanium-bearing minerals. Iron-bearing minerals can be easily removed by magnetic separation, but ultra-fine iron particles are difficult to treat by conventional mineral processing methods. Thus bioleaching is an attractive alternative for effective removal of iron minerals.

The use of heterotrophs in metal extraction from non-sulphidic ore has not received much attention for several reasons. The circumneutral pH range at which these organisms grow along with the use of carbon

sources such as sugars readily allows for contamination. Sterilization is costly and neither feasible nor practical as it presents a technical problem for large-scale operations [21]. The removal of Fe(III) impurities could be performed by indigenous microorganisms but is greatly enhanced by iron-reducing *B. cereus*. In this study, the refinement of feldspar raw materials was accomplished with isolates of indigenous microorganisms and a *Bacillus* spp. inoculum. The white color of the bacterially treated feldspar raw material RZ confirmed the improved quality obtained in this biological treatment. *Bacillus* spp. production of organics by fermentation [22], or reductive dissolution of Fe mineral phases from non-metallics [23] greatly accelerates destruction of

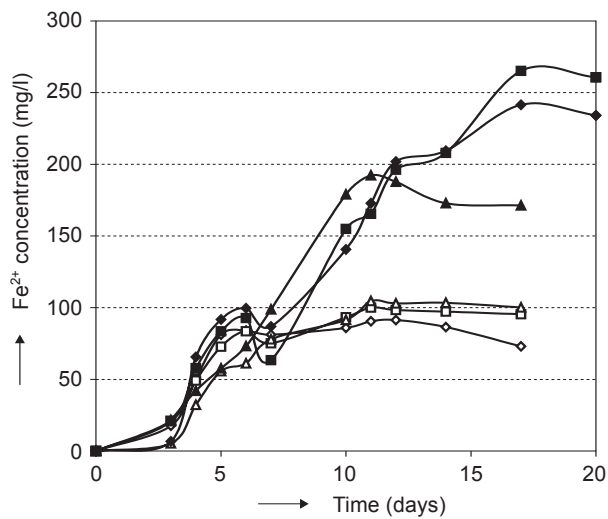


Figure 3. Iron dissolution and reduction in suspensions inoculated with *B. cereus* (□), *B. megaterium* (◇), or *B. pumilus* (△) in medium A amended with technical-grade sucrose (open symbols) and medium A amended with 100 mg AQDS/l (closed symbols).

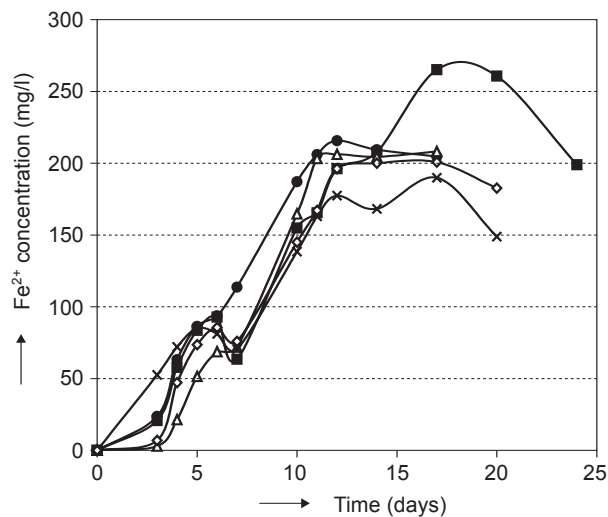


Figure 4. Iron dissolution and reduction in suspensions inoculated with *B. cereus* (■) and with indigenous bacteria isolated river water (△ - *B. cereus*, ◇ - *B. megaterium*) or well water (× - *B. megaterium*, ● - *B. cereus*). Medium A with sugar was amended with 100 mg AQDS/l.

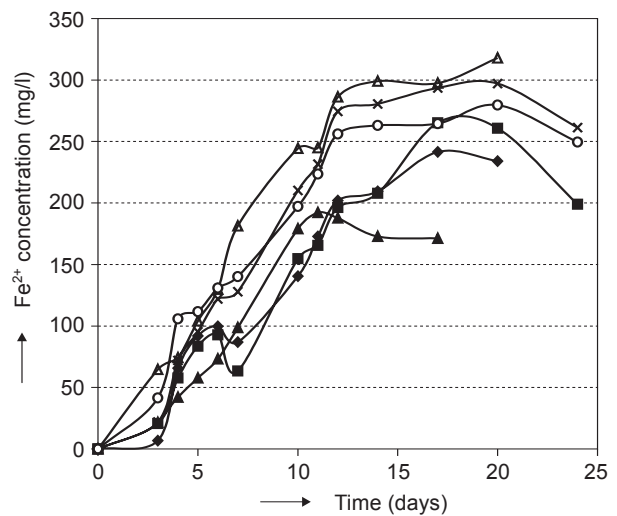


Figure 5. Iron dissolution and reduction in suspensions inoculated with *B. cereus* (■), *B. megaterium* (◆), or *B. pumilus* (▲) in medium A with 100 mg AQDS/l, and *B. cereus* in river water (△), well water (○) or pond water (×) amended with 100 mg AQDS/l.

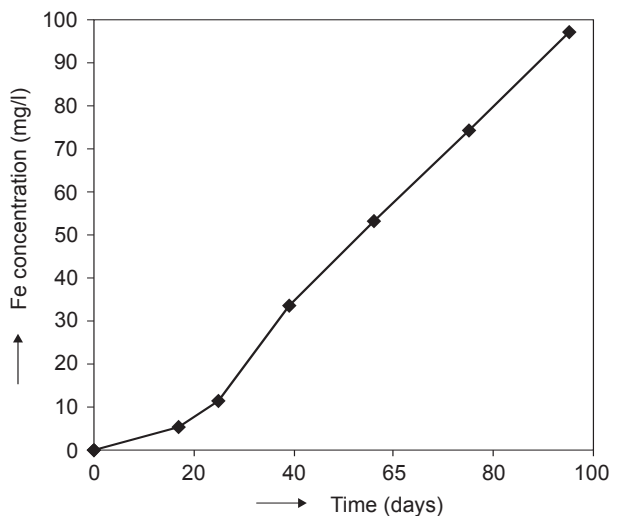


Figure 6. Changes in Fe concentrations over time during the bioleaching of sample RZ.

mica [24, 25] and smectite [19] or silicate matrix that is impregnated with iron minerals [26]. This is why these bacteria are silicate heterotrophic bacteria ubiquitous in environments with silicate minerals (soil, sediments). Thus, it is likely that heterotrophic bacteria of *Bacillus* strains are also ubiquitous in non-metallic deposit [27], they are non-pathogenic and are facultative anaerobic bacteria enabling an easy manipulation during non-metallic treatment, increased rapidly in the numbers of cell during non-metallic treatment and targetly produced organic acid in silicate structures. In contrast two anaerobic organisms *Shewanella putrefaciens* and *Geobacter metallireducens* that can respire on ferric iron and reduce iron in stoichiometric (100%) proportion to carbon oxidized as these can use ferric iron as sole electron acceptor but produce green-house gases. These are the most important from bioremediation point of view and especially take part in simple bioleaching process during quality improvement of sample on non-metallic deposits (in situ).

The removal of Fe(III) impurities could be performed by indigenous microorganisms but is greatly enhanced by iron-reducing *B. cereus*. In this study, the

refinement of feldspar raw materials was accomplished with isolates of indigenous microorganisms and a *Bacillus* spp. inoculum. The white color of the bacterially treated feldspar raw material RZ confirmed the improved quality obtained in this biological treatment. After the bioleaching, a new solid phase, weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), was enriched in the fine-grained fraction (Figure 7). Weddellite is Ca-oxalate precipitate and was formed Ca-plagioclase during the leaching action by oxalic acid produced in the *Bacillus* cultures. The chemical content of CaO decreased by 28 % (Table 1). Mandal and Banerjee [28] reported that several fungi, *Aspergillus niger* especially, and their culture filtrates could leach iron from China clay samples. Oxalic acid was found to be the main organic acid component of the culture filtrate and is known to chelate Fe efficiently. However, the use of fungi involves two steps: (1) metabolite (oxalic acid) production followed by (2) contact with the mineral for the leaching [21]. In contrast, the bioleaching of silicate minerals by heterotrophic iron-reducing bacteria does not require separate steps in the process.

CONCLUSION

This study has shown that molasses, sugars and AQDS enhance the biological dissolution of iron from feldspar by *Bacillus* spp. The Fe content in the feldspar samples by the biological leaching decreased as much as 60%. Molasses can be used as bulk carbon source to enhance biomass growth and production of leaching agents after removing of pigments by 0.05% NaClO. The addition of AQDS may enhance the rate of the iron reduction, a major kinetic obstacle. The feasibility of the bioleaching treatment has to be tested specifically to each type of silicate raw materials.

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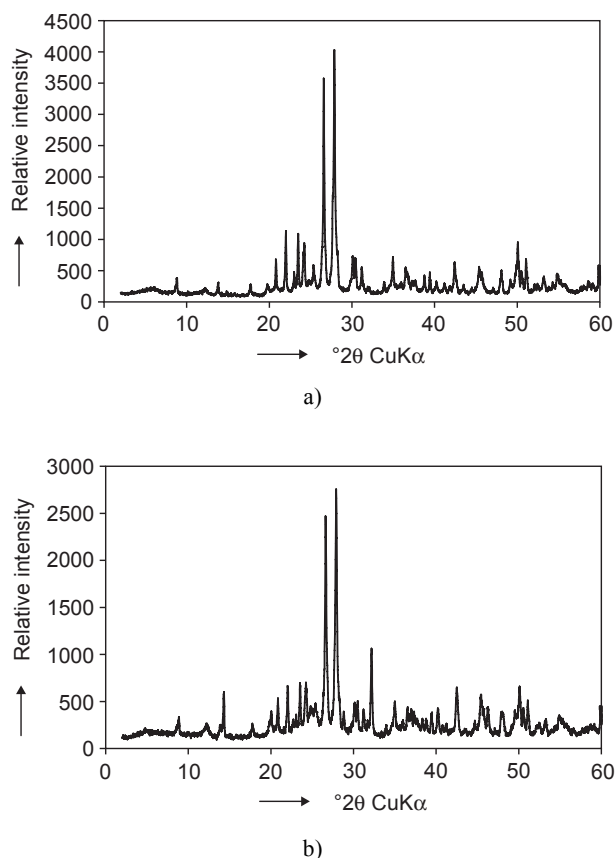


Figure 7. X-ray diffractogram of sample RZ before (A) and after (B) the bioleaching. Abbreviations: S = smectite, M = Mica, P = Na,Ca-plagioclase, Q = quartz, A = anatase, W = weddellite, F = K-feldspar.

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Prírodné oxidy železa pokrývajú povrch silikátových zŕn, alebo sú impregnované v silikátovom matrixe významných priemyselných minerálov. Prostredníctvom biolúhovania je možné odstrániť jemnodisperzné železité nečistoty z minerálov ako napr. granitov. Heterotrófne baktérie rodu *Bacillus* rástli v prítomnosti živcovej suroviny a boli schopné extrahovať Fe. Množstvo rozpusteného a odstráneného Fe záviselo od podmienok biolúhovania, pričom pozitívny priamy kontakt minerálnej fázy, baktérií a nutričných látok v médiu je ovplyvnený obsahom kvasničného extraktu, dusičnanov a síranov v médiu. V závislosti na dosiahnutých výsledkoch bakteriálnej extrakcie Fe z pevnej fázy, aj prídavkom quinonu je možné ovplyvniť koncentráciu a rýchlosť bakteriálnej redukcie a disolúcie Fe. Vhodným organickým zdrojom počas bakteriálneho lúhovania je taktiež melasa alebo technický cukor, pričom extrakcia Fe do roztoku je podobná, alebo aj vyššia, ako pri použití glukózy,

sacharózy alebo galaktózy. Jednoduchý porovnávací laboratórny biolúhovací experiment pre zníženie Fe zo živcovej suroviny heterotrófnymi baktériami bol uskutočnený pre nariadenie podmienok potenciálneho použitia v priemyselných merítkach. Rýchle formovanie anaeróbných podmienok v uzatvorenom systéme média umožňuje jednoduchú manipuláciu s lúhovacím roztokom. Použitie povrchovej vody z rieky, s potvrdenou prítomnosťou autochtónnych baktérií, neovplyvnilo negatívne dosiahnuté výsledky bakteriálnej extrakcie Fe aktívnym kmeňom *Bacillus cereus*. Získané 60% zníženie Fe z granitov ložiska Rudník naznačuje dostatočné skvalitnenie suroviny s možnosťou skrátenia biolúhovacieho času pri dodržiavaní životných potrieb aktívneho kmeňa rodu *Bacillus*. Baktérie rodu *Bacillus patria* k najrozšírenejším pôdnym mikroorganizmom, ktoré sa veľmi rýchle rozmnožujú, intenzívne produkujú rôzne metabolity, najmä rôzne organické kyseliny. Ich izolácia a kultivácia nie je zložitá, sú flexibilné a prístupné umelým zásahom, ktoré môžu meniť ich vlastnosti a rýchlosť disolúcie Fe minerálov. V prírode sa zúčastňujú procesu transformácie silikátových minerálov, anorganických a organických materiálov a tvorby ílových minerálov, čo dáva predpoklady pre ich praktické využitie v predúprave nerudných nerastných surovín. Mnohé silikátové suroviny, ktoré sa tradične priemyselne využívajú, vykazujú nevhodnosť pre prípravu špeciálnych produktov v určitých oblastiach výrobných sféry, obsahujú mnohé nežiaduce prímеси, ktoré znižujú ich kvalitatívne vlastnosti. Využitie biotechnológií pri úprave silikátových surovín predstavuje novú alternatívnu cestu efektívneho spracovania nerastných zdrojov.