

The role of biostimulation in iron bioleaching and purification of quartz sands

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The biostimulation for iron mineral dissolution and reduction with chelators and organic source were investigated on ferrihydrite and quartz sands contaminated with iron minerals. The finding that bacteria can donate electrons to chelators has important implications for the removal of iron impurities from industrial minerals such as quartz sands. The effect of media composition with chelators (EDTA, NTA, AQDS) on the extent of bacterial dissolution of iron mineral was studied under laboratory condition and selected a chelator Na₂EDTA was studied under in-situ conditions. A stronger stimulation and continuous Fe dissolution by the form of EDTA favors its in-situ use. Ultra-fine iron particles coating and impregnating quartz particles are difficult to treat by conventional mineral processing methods. Biological leaching appears to be the alternative for the effective removal of iron minerals. The in-situ removal of Fe-phases from quartz sands via bioleaching was optimized with regard to the rate of iron reduction and dissolution by biostimulation with Na₂EDTA.

Key words: quartz sands, bioleaching, *Bacillus* sp., iron reducing heterotrophic bacteria

Introduction

Red and yellow pigmentations in many industrial minerals are due to Fe(III)-oxides such as hematite, maghemite, goethite, lepidocrocite, and ferrihydrite (Ambikadevi and Lalithambika, 2000). Raw materials of quartz sand for the production of clear glass requires removal of iron, which is typically present as discrete iron oxide particles or coatings on the surfaces of quartz particles.

Iron is an essential element for all biological organisms and bacteria typically regulate their iron metabolism in response to iron availability (Andrews et al., 2003). Microorganisms can affect the mobility of iron as well as its accumulation. Both oxidative and reductive reactions of iron brought about by microorganisms play important roles in the iron cycle. Iron can even serve as an energy source for some bacteria (Barns and Nierzwicki-Bauer, 1997). Important group of microorganisms is aerobic heterotrophic bacteria producing Fe(III)-specific chelating agents called siderophores. De Castro and Ehrlich (1970) demonstrated that a *Bacillus* sp. reduced enzymatically Fe(III). Iron reduction by the *Bacillus* sp. required glucose in the medium. It is widely accepted that biological mechanisms are often involved case in the mobilization of iron in natural systems. Microbial communities include strains with novel metabolic properties potentially useful to industries or in biotechnology (Fredrickson et al., 1991). These Fe-rich impurities can be removed from industrial minerals through bioleaching that is why this mechanism can use in iron removal by bioleaching for quality improvement of quartz sands.

Heterotrophic bioleaching may substantially reduce the need of aggressive chemicals for silicate minerals treatment. This process involves a siliceous matrix, which is why silicate heterotrophic bacteria of the genus *Bacillus* are of potential use. The rate of reductive iron dissolution is dependent on the presence of organic source, yeast extract, nitrate and sulphate in the medium (Štyriaková et al., 2007) and a variety of additional factors, including pH, oxide surface area, and phase identity of the iron oxides, ferrous iron biomineralization, presence of electron shuttles such as humic substance, and organic ligands that enhance iron oxide dissolution or Fe(II) solubilization also appear to exert some control on the rate and extent of reduction of these minerals (Lovley, 1991; Roden, and Urrutia, 1999; Fredrickson et al., 1998; Zachara et al., 2001; Arnold, 1988). A study showed that cell contact is not a requisite for reduction of solid Fe(III) oxides (Caccavo et al., 1992), if the electrons are not directly transferred from the iron reducing bacteria to the Fe(III) oxides, the electron transfer could be accomplished by diffusion of electron mediators or of free or complexed Fe(III) (Gaspard et al., 1998).

Iron-reducing bacteria was stimulated by the addition of the Fe chelator, nitriliacetic acid (NTA) which was used to increase the bioavailability of Fe in the clay and to intensify natural processes (Shelobolina, et al., 2005). 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 (Schröder et al., 2003). 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. Other chelator of

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ethylenediaminetetraacetic acid (EDTA) is the most commonly used chelate because of its strong chelating ability for different metals, although extraction efficiency depends on many factors such as the lability of metals in silicate matrix and pH.

The objective of this study was to examine the influence of the chelators on the iron mineral dissolution from poorly crystalline Fe oxyhydroxide and quartz sands by the iron-reducing bacterium. Laboratory studies with chelators have been carried out investigating conditions of biological processes for the quality improvement of quartz sands. The development of bioleaching media with content of stimulators was aimed at the increase rate of removal with the highest iron concentration.

Materials and methods

Amorphous iron oxyhydroxide was formed by neutralizing a 0,4 M solution of FeCl₃ to a pH of 7 with NaOH (Lovley and Phillips, 1986). The laboratory bioleaching experiments were carried out in conical flasks containing 10 g powdered samples of iron mineral and 100 ml liquid medium containing NaH₂PO₄ – 0.5g.l⁻¹, MgSO₄ · 7H₂O – 0.5g.l⁻¹, (NH₄)₂ SO₄ – 1.0g.l⁻¹, NaCl – 0.2g.l⁻¹, glucose – 20g.l⁻¹. Fe (III) reduction in samples was tested in media with and without 2 mM EDTA (ethylenediaminetetraacetate), 2 mM NTA (nitrilotriacetic acid) and 2 mM AQDS (anthraquinone-2, 6-disulfonate, quinone). Appropriate abiotic controls with chelators (undetected data not shown) and biotic control without chelators were included in the experiments.

The quartz sands (Q, Tab. 1) were from the locality Šaštín (Slovakia). 13 tons of Q were treated in fixed-basin container (volume 16.3 m³) filled with a leaching solution consisting of 7000 l fresh surface water supplemented with (per liter) 0.5 g NaH₂PO₄, 1.0 g (NH₄)₂SO₄ and 0.2 g NaCl. Moreover 20 g of technical-grade glucose A60 per liter media was added into the container, respectively. The no sterile medium W was inoculated with 1 m³ of mixture of *Bacillus cereus*, *Bacillus megaterium* and autochthonous iron reducing bacteria. The *in-situ* stimulation of ferric iron dissolution by the two *Bacillus* isolates or iron-reducing bacteria was tested in quartz sand suspension in the presence of 2 mM Na₂EDTA. The container was incubated under static ambient conditions for 3 months. The liquid phase was replaced four times with 7000 liters of fresh medium. The spent media (leachates) were sampled for iron analysis.

Quantitative changes in the solid phase were measured with a Model 30 Varian atomic absorption spectrometer. Dissolved Fe²⁺ and Fe³⁺ were measured spectrophotometrically using the *o*-phenanthroline method (Herrera et al., 1989 and Stucky et al., 1981). Total iron concentration was measured spectrophotometrically using FerroVer Iron Reagent Powder Pillow (HACH, Germany) during *in-situ* bioleaching condition.

Tab. 1. Chemical composition of untreated samples of quartz sands (Q).

component [%]	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	TiO ₂	CaO	MgO	Na ₂ O	K ₂ O
Q	92.7	3.95	0.32	0.06	0.16	0.15	0.93	1.32

Results and discussion

Microorganisms secrete metabolites such as carboxylic acids and siderophores that chelate ferric iron and make it accessible for biological reduction and cellular uptake (Guerinot, 1994). The finding that bacteria can donate electrons to chelators has important implications for the removal of iron impurities from non-metallic minerals such as quartz sands. However, it is not known whether comparable iron removal can result from the biological reduction of Fe oxides or whether chelators analogues such as EDTA, NTA and AQDS can enhance the extent of the dissolution of Fe oxides by iron-reducing *Bacillus* spp. From the laboratory batch chelator - bacteria extraction studies on iron oxides follow that prior to the addition of different forms of chelators EDTA (Fig. 1, A), NTA (Fig. 1, B) and AQDS (Fig. 1, C) the concentration of dissolved iron was much higher than biotic control without chelators (Fig. 1, D) or abiotic control with chelators with undetected iron concentration. The dissolution of Fe during bioleaching of natural iron minerals with these chelators is presented in Fig. 1. This indicated that bacteria more rapidly solubilized iron minerals in presence of chelators. However, the addition of EDTA and NTA caused that the totally dissolved iron was greater than in case of AQDS. However, NTA activated partially slower dissolution of Fe from samples (8 days) than EDTA (6 days). Changes in iron removal from iron mineral samples during laboratory bioleaching were used to assess the chelator effectiveness of the heterotrophic bioleaching process. Chelators make the compound complex with Fe more soluble in bioleaching medium and reduce the rate of precipitation of secondary iron minerals. The addition of chelators may also enhance the iron concentration and major kinetic obstacle during *in-situ* bioleaching.

Na₂EDTA used as an extracting agent presents three important advantages: a) pH of the final solution is usually alkaline and facilitates the development of further mechanisms for iron retention, b) Na₂EDTA is

slowly biodegraded in groundwater and c) Na₂EDTA possesses a high complexation capacity (Moutsatsou et al., 2006). The effect of media composition with selected chelator Na₂EDTA was studied under *in-situ* condition on the extent of bacterial dissolution of iron mineral from quartz sands.

Phylogenetically diverse iron-reducing bacteria have been reported to conserve energy from Fe(III) reduction coupled to oxidation of organic acids, sugars, and aromatic hydrocarbons (Lovley et al., 1986, Anderson et al., 1998)

The effect of carbon source was tested in a media inoculated with *B. cereus* by the addition of glucose, sucrose, galactose, technical-grade sucrose and molasses. The results showed that the extent of iron dissolution was higher in the presence of technical-grade sucrose and molasses after 6 days of bioleaching than in the presence of glucose and sucrose or approached to the rate of iron dissolution during addition of glucose in experiments. The reduction of iron in the presence of glucose and sucrose was higher than in the presence of 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 of bioleaching. Molasses can be used as a cheap bulk carbon source to enhance biomass growth and production of leaching agents (Štyriaková et al., 2007).

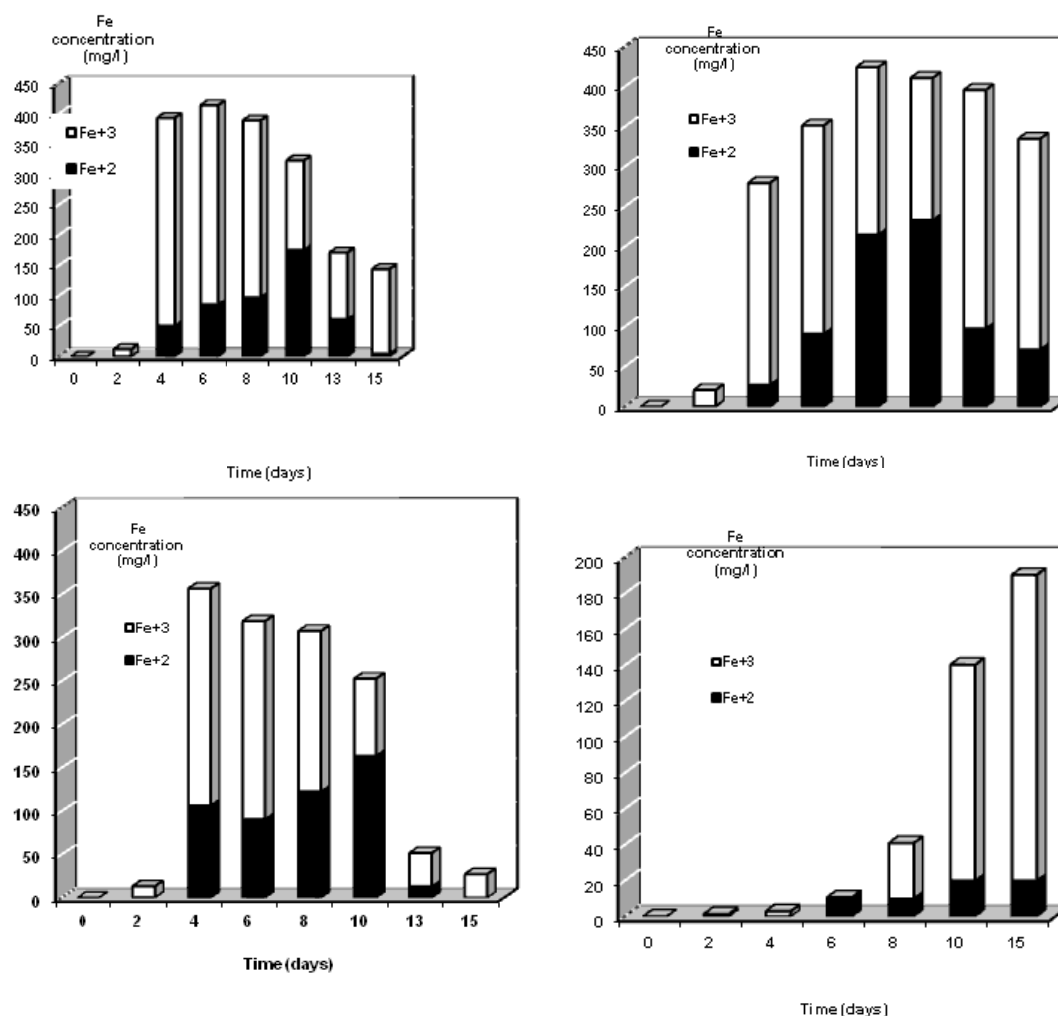


Fig. 1. Dissolution of Fe from ferrihydrite by iron-reducing heterotrophic strains of *Bacillus* spp. stimulated by chelators (EDTA- A, NTA - B and AQDS - C) compared without chelators - D during laboratory leaching.

Bacterial reduction may be important in increasing of the iron oxides availability where there is abundant available carbon source. Iron bioleaching can be carried out "*in situ*" also by biostimulation with adding a sugar (glucose) as a carbon nutrient (Tab. 2). Iron heterotrophic bacteria use glucose, a monosaccharide or simple sugar, because many bacteria possess the enzymes required for the degradation and oxidation of this sugar. The bacteria utilize sugars and cause production of organic acids and gas that is why decreased the pH media below 4. The glucose was added for metabolic acid production resulting from bacterial utilization of the carbohydrate present in medium. Dissolution of Fe is going during 4 steps of glucose consumption of discontinue *in-situ* bioleaching of quartz sands. Glucose was used as the bulk carbon source to enhance the biomass growth and production of organic acids as *in-situ* leaching agents. However,

iron concentration in medium was different after consumption of some glucose amount. All of glucose was consumed during first step of bioleaching at the beginning of bioleaching process caused by bacterial reproduction but iron concentration was lower than in the second step of bioleaching with higher iron removing and lower glucose consumption. The producing of organic acid within the steps of bioleaching of quartz sands was typical for bacterial growth in the presence of no sterile bioleaching conditions, as the pH decrease was neutralized at each replacement of the medium.

In previous studies with Al-silicates and *Bacillus* isolates, oxalic, acetic, lactic, pyruvic, butyric, and formic acid were detected in spent media (Štyriaková et al., 1999, 2006). The dissolution of iron impurities from quartz sands was strongly influenced by the presence of organic acids through both their effect on pH and their effect as chelators of Fe ions (Fe^{3+} , Fe^{2+}) in solution. Acidic formation condition caused to the increase of the amount of Fe^{2+} and Fe^{3+} in solution. The heterotrophic bacteria, used in this *in-situ* study are Fe^{3+} -reducing bacteria acting by means of secreted metabolites (organic acids) on the dissolution of iron which is subsequently chelated by EDTA to Fe^{3+} ions and finally, enzymatic reduction of Fe^{3+} was going on Fe^{2+} . However, the rate of dissolution drastically increased when the medium was acidic and also had low redox potential (-150 mV). Eh decreased intensely in the medium containing glucose. This was caused more bacterial growth and the microbial consumption of dissolved oxygen in the medium.

Tab. 2. Iron concentration and glucose consumption during *in-situ* steps of quartz sands bioleaching (BL1 – the first bioleaching, BL2 – the second bioleaching, BL3 – the third bioleaching, BL4 – the fourth bioleaching).

Bioleaching steps	BL1	BL2	BL3	BL4
Bioleaching time [days]	38	15	25	27
Max. iron concentration [mg.l^{-1}]	92	216	278	88
Glucose consumption [mg.l^{-1}]	20,431	4,143	7,302	12,288
Decrease pH 7	3.89	3.98	3.90	3.93

Conclusions

The rate of dissolution and reduction of iron from minerals was influenced by various stimulators in form of chelators and carbon source which caused Eh and pH changes. The removal of iron from quartz sands was higher in the medium supplemented with glucose under formation of anaerobic condition at *in-situ* temperature changes. Glucose could be suggested as the best carbon source to support the bacterial growth, formation anaerobic condition and organic acid production as leaching agents of iron impurities. The rate of bacterial iron dissolution increased when the medium was acidic and contained chelators.

Bioleaching is a process combining of the production of organic acids of an iron reducing heterotrophic bacteria with the chelating acceleration of iron dissolution. The bioavailability of Fe in the synthetic ferrihydrite was also more rapid extracted during adding of chelators.

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References

- Ambikadevi, V.R., Lalithambika, M.: Effect of organic acids on ferric iron removal from iron-stained kaolinite. *Appl. Clay Sci.*, 16, 2000, 133-145.
- Anderson, R.T., Ronney-Varga, J.N., Gaw, C.V., Lovley, D.R.: Anaerobic benzene oxidation in the Fe(III) reduction zone of petroleum-contaminated aquifers. *Environ. Sci. Tech.*, 32, 1998, 1222-1229.
- Andrews, S.C., Robinson, K.A., Quinones F.R.: Bacterial iron homeostasis. *FEMS Microbiology Reviews*, 788, 2003, 1-23.
- Arnold, R.G., Dichristina, T.J., Hoffmann, M.R.: Reductive dissolution of Fe(III) oxides by *Pseudomonas*. *Biotech. Bioeng.*, 32, 1988, 1081-1096.
- Barns, S.M., Nierzwicki-Bauer, S.A.: Microbial diversity in ocean, surface and subsurface environments. *In: Geomicrobiology: Interaction between microbes and minerals*, 35, 1997, 35-79.
- Caccavo, F., Blakemore, R.P., Lovley, D.R.: A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay estuary, New Hampshire. *Appl. Environ. Microbiol.*, 58, 1992, 3211-3216.

- De Castro, A.F., Ehrlich, H.L.: Reduction of iron oxide minerals by a marine *Bacillus*. *J. Microbiol. Serol.* 36, 1970, 317-327.
- Fredrickson, J.K., Brockman, K.J., Workman, D.J., Li, S.W., Stevens, T.O.: Isolation and characterization of a subsurface bacterium capable of growth on toluene, naphthalene, and other aromatic compounds. *Appl. Environ. Microbiol.*, 57, 1991, 796-803.
- Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Dong, H., Onstott, T.C., Hinman, N.W., Li, S.M.: Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by groundwater bacterium. *Geochim. Cosmochim. Acta*, 62, 1998, 3239-3257.
- Gaspard, S., Vazquez, F., Holliger, Ch.: Localization and Solubilization of the Iron(III) Reductase of *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.*, 64, 1998, 3188-3194.
- Guerinot, M.L.: Microbial iron transport. *Annu. Rev. Microbiol.*, 48, 1994, 743-772.
- Herrera, L., Ruiz, P., Aguillon, J.C., Fehrman, A: A new spectrometric method for the determination of ferrous iron in the presence of ferric iron, *J. Chem. Technol. Biotechnol.* 89, 1989, 171-181.
- Lovley, D.R., Baedecker, M.J., Lonergan, D.J., Cozzarelli, I.M., Phillips, E.J.P., Siegel, D.I.: Oxidation of aromatic contaminants coupled to microbial iron reduction. *Nature*, 339, 1989, 297-300.
- Lovley, D.R.: Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.*, 55, 1991, 259-287.
- Moutsatsou, A., Gregou, M., Matsas, D., Protonotarios, V.: Washing as a remediation technology applicable in soils heavily polluted by mining – metallurgical activities. *Chemosphere* 63, 2006, 1632-1640.
- Shelobolina, E.S., Pickering, S.M., Lovley, D.R.: Fe-cycle bacteria from industrial clay mined in Georgia, USA. *Clays and Clay Minerals*, 53, 2005, 580-586.
- Schröder, I., Johnson, E., De Vries, E.S. : Microbial ferric iron reductases. *FEMS Microbiol. Rev.* 27, 2003, 427-447.
- Stucky, J.W., Anderson, W.L.: The quantitative assay of minerals for Fe²⁺ and Fe³⁺ using 1,10-phenanthroline: I. Sources of variability, *Soil Sci. Soc. Am. J.* 45, 1981, 633-637.
- Štyriaková, I., Štyriak, I., Kušnierová, M.: The release of sulphidic minerals from aluminosilicates by *Bacillus* strains. In: Amils, R., Ballester, A. (eds.): *Biohydrometallurgy and the Environment toward the Mining of the 21st Century*. Elsevier, Amsterdam, 1999, 587-596.
- Štyriaková, I., Štyriak, I., Malachovský, P., Lovás, M.: Biological, chemical and electromagnetic treatment of three types of feldspar raw materials. *Minerals Engineering* 19, 2006, 348-354.
- Štyriaková, I., Štyriak, I., Malachovský, P.: Nutrients enhancing the bacterial iron dissolution in the processing of feldspar raw materials. *Ceramics – Silikáty*, 51, 2007, 202-209.
- Roden, E.E., Urrutia, M.M.: Ferrous iron removal promotes microbial reduction of crystalline iron(III) oxides. *Environ. Sci. Technol.*, 33, 1999, 1847-1853.
- Zachara, J.M., Fredrickson, J.K., Smith, S.C., Gassman, P.L.: Solubilization of Fe(III) oxide-bound trace metals by a dissimilatory Fe(III) reducing bacterium. *Geochim. Cosmochim. Acta*, 65, 2001, 75-93.