Weathering of phlogopite by *Bacillus cereus* and *Acidithiobacillus ferrooxidans*

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Abstract: The purpose of the study was to assess the weathering of finely ground phlogopite, a trioctahedral mica, by placing it in contact with heterotrophic (*Bacillus cereus*) and acidophilic (*Acidithiobacillus ferrooxidans*) cultures. X-ray diffraction analyses of the phlogopite sample before and after 24 weeks of contact in *B. cereus* cultures revealed a decrease in the characteristic peak intensities of phlogopite, indicating destruction of individual structural planes of the mica. No new solid phase products or interlayer structures were detected in *B. cereus* cultures. *A. ferrooxidans* cultures enhanced the chemical dissolution of the mineral and formed partially weathered interlayer structures, where interlayer K was expelled and coupled with the precipitation of K-jarosite.

Key words: *Acidithiobacillus ferrooxidans*, *Bacillus cereus*, mica, phlogopite, weathering

Introduction

Micas are common accessory minerals in rocks, sediments, and soils. Mica weathering has been frequently documented in environmental situations, in soils especially, that involve organic acid production and metal sequestration (Silverman 1979; Robert and Berthelin 1986). Leyval and Berthelin (1991) showed that rhizosphere microorganisms associated with pine roots extracted K from phlogopite, and this was attributed to organic acid-mediated dissolution. Mycorrhizae are involved in the weathering process in the plant root environment (Paris et al. 1995). Frankel (1977) reported microbial penetration into the interlamellar spaces of weathered biotite, but it was not clear whether the microorganisms were directly involved in the weathering process. Berthelin and Belgy (1979) demonstrated the complete removal of K and Ti from biotite by a microbial consortium in a granitic sand, which resulted in brittle, white micaceous

particles. The susceptibility to microbial weathering varies with the mica and its chemical composition. In soils, weathering of silicates serves as one of the natural mechanisms to replenish K as a plant nutrient (Hinsinger et al. 1992; Mengel and Uhlenbecker 1993; Mengel and Rahmatullah 1994). Many heterotrophic bacteria, including various *Bacillus* spp., have been implicated with the weathering of silicates in soils.

Mica is an undesirable mineral in the processing of clays such as kaolin because it causes discoloration of kaolin-based products and increases the abrasiveness and scratching by kaolin slurries used in paper production. Discoloration is due to Fe impurities, such as hematite, that are often associated with mica particles in raw materials. Heterotrophic bacteria, including ferric iron reducers, have been tested for removal of Fe impurities in kaolin (Štyriakova and Štyriak 2000; Lee et al. 2002). The role of ferric-iron reducing bacteria in the heterotrophic leaching is not clear because Fe can be dissolved from kaolinite with organic acids (Ambikadevi and Lalithambika 2000). Some organic acids (e.g., oxalic acid) act as ligands for Fe-complexation in the heterotrophic bioleaching process, thereby enhancing Fe dissolution (Berthelin 1988; Stucki et al. 1992). Fe(III) may also be reduced biologically and abiotically because of anaerobic conditions and use of substrates such as reducing sugars in heterotrophic leaching systems (Berthelin 1988; Stucki et al. 1992).

Exposure of micas to ambient conditions in sulfide ore deposits enhances weathering and may lead to expandable silicate phases (Bhatti et al. 1994), which cause compaction and may change the channeling of rainwater and leach solutions. Thus, heap leaching systems for sulfidic ore materials may be compromised by excessive levels of micas that are readily susceptible to weathering. Acidophilic bacteria such as *Acidithiobacillus* spp. are common in heap leaching system and in exposed sulfide ore deposits (Hallberg and Johnson 2001; Rawlings 2002). Iron-

oxidizing *Acidithiobacillus ferrooxidans* and other acidophiles oxidize sulfide minerals to soluble end products as well as ferrous iron to ferric iron, which hydrolyses and often precipitates as Fe(III)-hydroxysulfates in acid environments (Ahonen and Tuovinen 1994; Suzuki 2001).

The purpose of this work was to assess the weathering of phlogopite in the presence of heterotrophs (*Bacillus cereus*) and iron- and sulfur-oxidizing bacteria (*A. ferrooxidans*). The *Bacillus*-phlogopite system was intended to represent the effects of heterotrophic bioleaching in soils, and the *Acidithiobacillus*-phlogopite system was designed to assess weathering in situations where acid bioleaching processes are prevalent.

Materials and methods

Phlogopite sample

The chemical and mineralogical characterization of the phlogopite sample used in this study has been previously published (Bigham et al. 2001). Almost 85% of the octahedral positions of the mica were occupied by Mg, thereby distinguishing it from biotite. The sample was ground and sieved to a -200 mesh particle size fraction. X-ray diffraction (XRD) analysis revealed no mineralogical impurities in the phlogopite sample.

Bioleaching of phlogopite with *B. cereus*

Bioleaching experiments were carried out in culture flasks containing 5 g phlogopite and 100 ml medium containing (per liter) 0.5 g NaH_2PO_4, 1.0 g $(NH_4)_2 SO_4$, 0.2 g NaCl, and 20 g glucose). The flasks were inoculated with a culture of *Bacillus cereus* (strain ISHP-1) isolated from the Horná Prievrana kaolin deposit in Lučenecká Kotlina, Slovakia. The cultures were incubated in shake

flasks or under static conditions. In general, static conditions were preferred for *B. cereus* because of acid production at low dissolved O_2 concentrations.

The cultures were incubated for a total of 24 weeks at 28°C. Cultures were neutralized at 3 to 5 day intervals with NaOH. The liquid medium was changed aseptically three times (every six weeks) during the time course. Upon replacement of culture media, the culture solutions were separated from the biomass by means of 0.9 μm pore size membrane filtration.

Changes in the chemical composition of leach solutions were measured with a model 30 Varian atomic absorption spectrometer (Varian Techtron Pty. Ltd., Mulgrave, Vic., Australia). The particle size distribution was measured by laser radiation scattering on a model 22 Laser-Particle-Sizer Analysette (Fritsch, Idar-Oberstein, Germany). The mean particle diameter (d_m 0.9-175 μm) was calculated from granulometric data.

The structural destruction of bacterially leached samples was determined by X-ray diffraction analysis of powdered samples using FeKα radiation and a DRON 3.0 diffractometer equipped with a wide-range GUR-5 goniometer and a monochromator (Tekhsnabexport, Moscow, Russia). Samples were scanned in increments of 0.02 °2θ with a 0.5-sec counting time.

Bioleaching of phlogopite with *A. ferrooxidans*

A. ferrooxidans (strain TFI-35) was routinely grown in a mineral salts solution containing 120 mM $FeSO_4$ as the energy source. For leaching experiments, 100 ml cultures in shake flasks (150 rev/min) received 2.5 g phlogopite at pH 2. Leaching experiments were also carried out in media where $FeSO_4$ was replaced by tetrathionate (10 mM $K_2S_4O_6$). The cultures reached stationary phase within a couple days, but the time course of phlogopite leaching was extended to 3 months in an effort to maximize phlogopite transformation.

At the termination of the time course, the chemical composition of leach solutions was analyzed by ICP. Solid residues were air dried and gently ground with an agate mortar and pestle, followed by XRD analysis of topfill powder mounts using CuKα radiation and a vertical wide-range goniometer equipped with a diffracted beam monochromator and a theta-compensating slit. Samples were scanned in increments of 0.05 °2θ with a 4-sec counting time.

Results and discussion

Weathering of phlogopite in *B. cereus* cultures

The *Bacillus* test culture was active at circumneutral pH values under static incubation conditions. Growth was accompanied by extracellular polysaccharide formation only under aerobic conditions. Under static incubation conditions, initially positive redox potential values decreased within a couple days to negative values, suggesting the presence of anaerobic or microaerophilic conditions and the formation of incompletely oxidized metabolites. Without pH adjustment, the growth was strongly acid-producing under static incubation conditions and the media decreased to pH 4 within two days of incubation in the presence of phlogopite. Under aerobic conditions, acid production was not as intense. The decrease to pH 4 induced endospore formation in the test cultures. For this study, the acid production in the culture flasks was regularly neutralized with NaOH at 3 to 5 day intervals. After every six weeks, 80 ml of the liquid medium was replaced with fresh medium while the solids were retained by filtration (0.9 μm pore size). The spent medium (leachate) was sampled for metal analysis.

The chemical analyses of the leachates from the three exchanges of medium are shown on as cumulative dissolution curves in Fig. 1. Mineral dissolution experiments showed an increase in the release of K, Mg, Si, Al, and Fe from phlogopite in the inoculated cultures. In the abiotic

controls, the relative releases were less than 5% of those in the inoculated samples. Thus, the release of these elements in the *B. cereus* cultures was many times higher in comparison with the abiotic control.

Previous literature on heterotrophic bioleaching of silicate minerals clearly shows that the dissolution is enhanced by organic acid production (Barker et al. 1997; Valsami-Jones and McEldowney 2000). Organic acid production was extensive in the *B. cereus* cultures used in the present work, necessitating intermittent neutralization to prevent the formation of prohibitively low pH values. Thus it is concluded that phlogopite destruction in this work was due to organic acids produced by *B. cereus*; these include acetic acid as the main metabolite and lesser amounts of lactic, pyruvic, butyric and formic acid analyzed in a previous study (Štyriakova et al. 1999).

No new mineral phases after laboratory bacterial leaching of the phlogopite sample were detected by XRD analysis of leach residues; however, decreased intensity of the diffraction peaks of phlogopite (Fig. 2) was associated with grain size reduction. There were no changes in the relative peak ratios of the major peaks. Granulometric analysis of the phlogopite before and after bioleaching confirmed the particle size reduction (Fig. 3). After bioleaching, the fine particle size fraction (1.8-30 μm) increased while the 36-175 μm size fraction decreased.

Weathering of phlogopite in *A. ferrooxidans* cultures

In contrast to heterotrophic *Bacillus* spp., iron- and sulfur-oxidizing acidophiles such as *A. ferrooxidans* use CO_2 as the sole source of cellular C and excrete only negligible amounts of organic acid metabolites. These bacteria were grown in acid media, at pH ~2, where phlogopite is subject to proton attack. In *A. ferrooxidans* cultures growing with tetrathionate, phlogopite dissolution was confirmed by elemental analysis of the leachate after 90 d contact time (Fig. 4, Table 1). Al, Si, Fe, and Ca in the leach solution were clearly derived from phlogopite

dissolution as these elements were not in the mineral salts formulation. The level of K was elevated because tetrathionate was used as a K-salt. The final concentration of Mg was higher than that initially in the mineral salts solution. No new mineral phases were detected in phlogopite residues of tetrathionate grown cultures (Fig. 4).

A. ferrooxidans cultures grown with ferrous sulfate produced jarosite precipitates, accompanied with structural alteration of the phlogopite to a mixed layer structure composed of vermiculite and phlogopite (Fig. 5). This interstratified phase was formed by replacement of the interlayer K with Mg and other solvated ions (Bigham et al. 2001). Interlayer K, once released into solution, was available not only as a nutrient ion for bacteria but also to promote the formation of K-jarosite, $KFe_3(SO_4)_2(OH)_6$. In this mineral salts medium, K-jarosite is the predominant Fe(III)-precipitate as long as K is available for the reaction

$$3Fe^{3+} + K^+ + 2SO_4^{2-} + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6\downarrow + 6H^+$$

Chemical analysis of the leach solutions after 90 d contact time (Fig. 4, Table 1) was consistent with solubilization of major elements from the solid phase. The level of K was below the concentration used in the mineral salts solution, in keeping with its precipitation as K-jarosite. The initial Fe concentration, 6 100 mg/l, was reduced to <4 000 mg/l because of jarosite precipitation. The levels of dissolved elements in spent Fe^{2+}-grown *A. ferrooxidans* cultures were higher than those in tetrathionate cultures (Table 1). These data indicate that the extent of phlogopite dissolution was enhanced by formation of the interstratified structure.

Conclusions

The results of this study highlight two different mechanisms of mica transformation. First, the solubilization of phlogopite may be mediated by proton attack with organic acids produced by heterotrophs. The acid attack may be enhanced by the complexation properties of carboxylic

acids (Valsami-Jones and McEldowney 2000), although the role of complex formation in phlogopite dissolution is unclear. Second, a solid phase transformation of phlogopite (10 Å unit layer) to an interstratified structure with mixed phlogopite and vermiculite (14.3 Å unit layer) layers occurred when there was expulsive replacement of interlayer K with solvated ions. Complete replacement of the interlayer K in phlogopite would lead to the formation of vermiculite or more highly expandable smectite.

Acknowledgements

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Legend to Figures 1-4

Fig. 1. Release of major elements from phlogopite dissolution in *B. cereus* culture over a 24-week period.

Fig. 2. X-ray diffraction patterns of phlogopite before bioleaching and after 24 weeks of contact in *B. cereus* cultures. The vertical bar shows the intensity scale for both samples. The samples were normalized by the mass for x-ray diffraction analysis. Line spacings are given in Ångstroms.

Fig. 3. Granulometric analysis of phlogopite before bioleaching and after 24 weeks of contact in the abiotic control and in *B. cereus* cultures.

Fig. 4. Changes in the concentration of dissolved K and Mg in *A. ferrooxidans* cultures that were grown with ferrous iron or tetrathionate.

Fig. 5. X-ray diffraction pattern of phlogopite after 3 months contact in *A. ferrooxidans* cultures grown either with tetrathionate or ferrous sulfate. Line spacings are given in Ångstroms. J, jarosite. The vertical bars show the intensity scale.

Table 1. Leach solution analysis of *A. ferrooxidans* cultures after 3 months of contact with 2.5% phlogopite.

Sample	pH	Concentration (mg/l)					
		Si	Al	Fe	K	Mg	Ca
Fe^{2+}-culture	2.0	490	1 070	3 980	61	1 550	240
$S_2O_4^{2-}$-culture	2.3	240	450	112	2 623	1 097	220

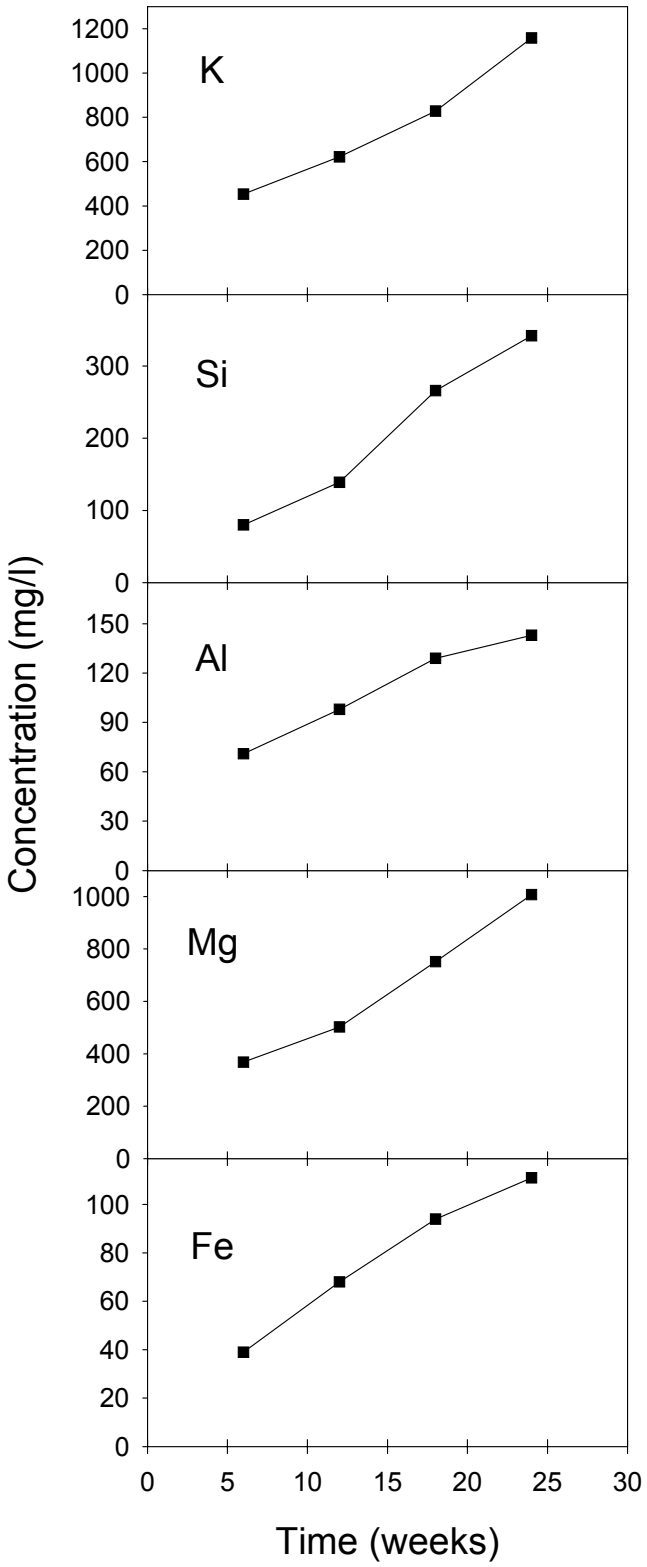

Figure 1

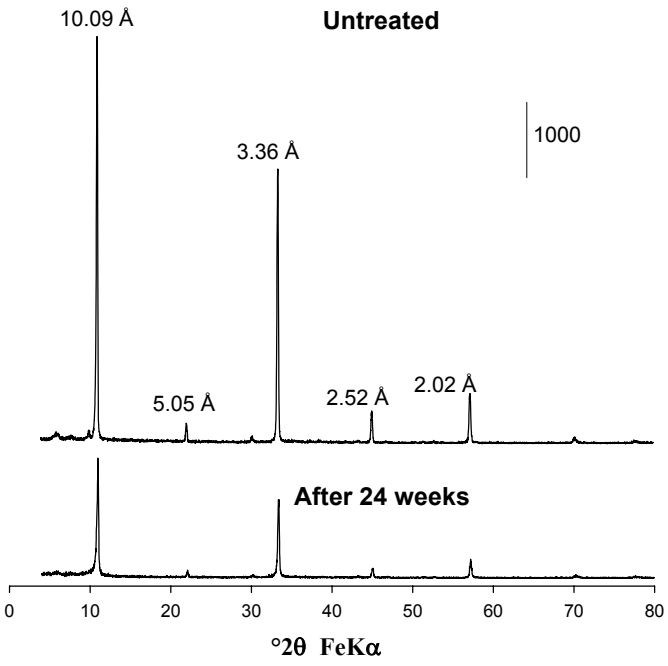
10.09 Å
Untreated
1000
3.36 Å
5.05 Å
2.52 Å
2.02 Å
After 24 weeks
0
10
20
30
40
50
60
70
80
°2θ FeKα

Figure 2

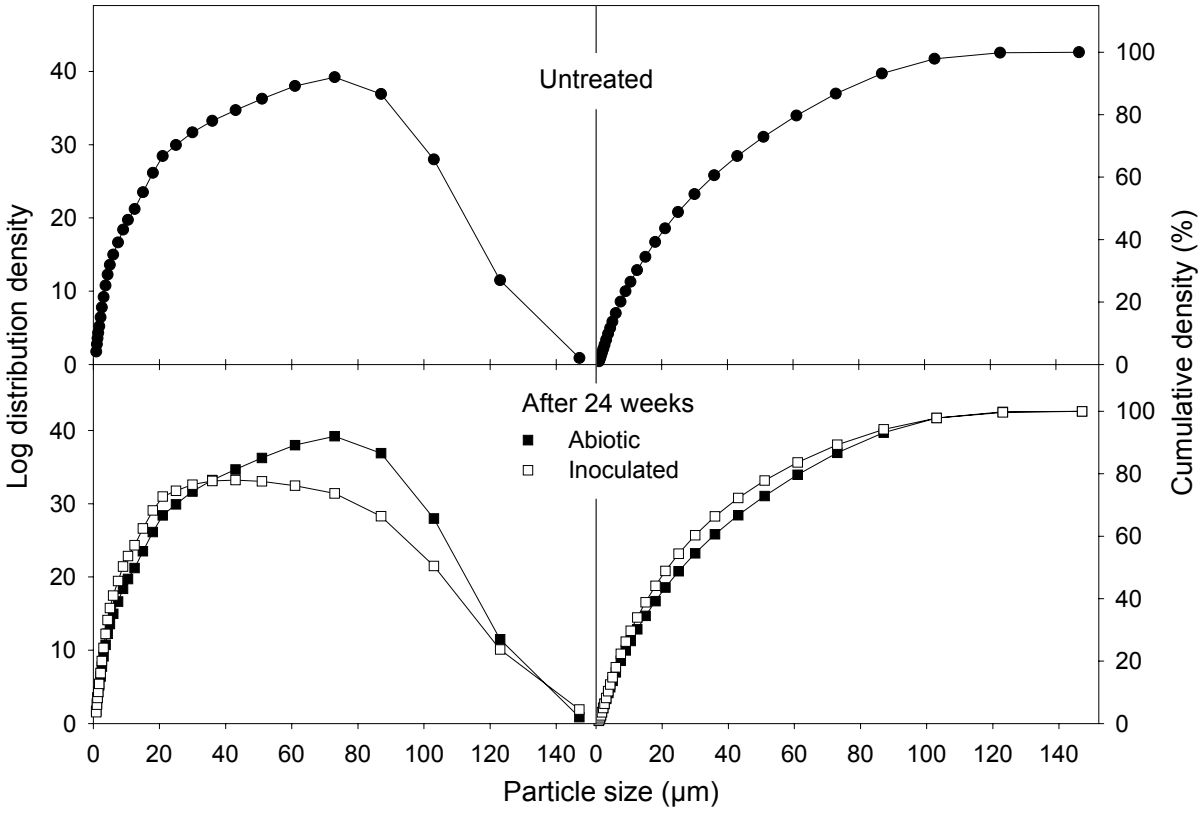
Untreated
After 24 weeks
Abiotic
Inoculated
Log distribution density
Cumulative density (%)
Particle size (µm)
0
10
20
30
40
0
20
40
60
80
100
120
140

Figure 3

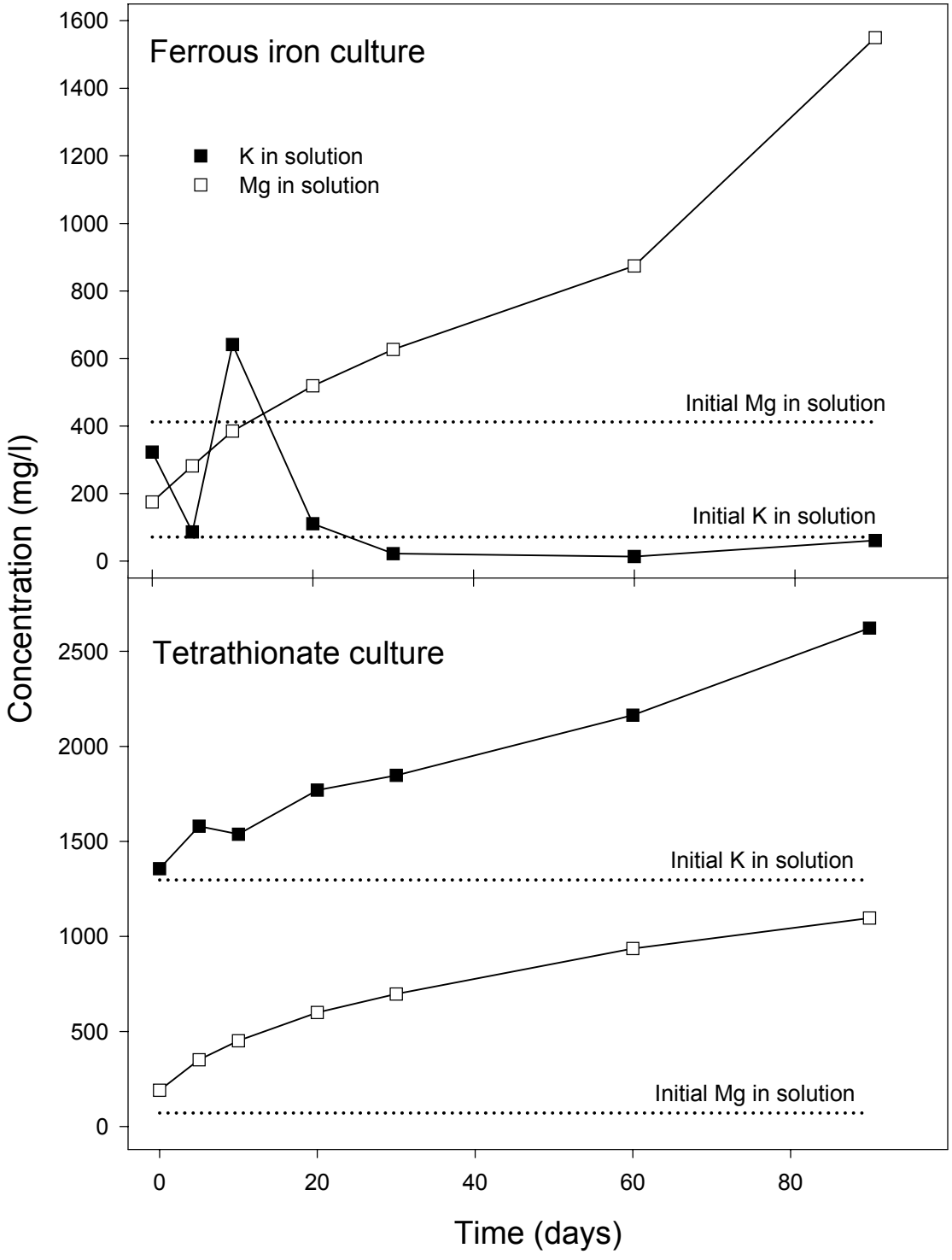
Ferrous iron culture
K in solution
Mg in solution
Initial Mg in solution
Initial K in solution
Tetrathionate culture
Initial K in solution
Initial Mg in solution
Concentration (mg/l)
Time (days)
0
200
400
600
800
1000
1200
1400
1600
500
1500
2000
2500
0
20
40
60
80

Figure 4

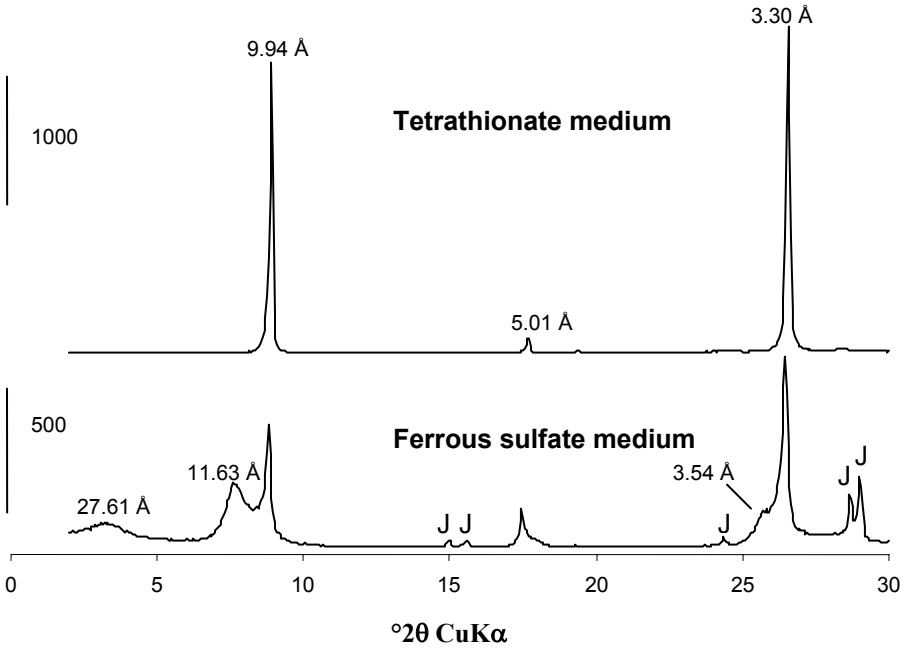
3.30 Å
9.94 Å
Tetrathionate medium
1000
5.01 Å
500
Ferrous sulfate medium
11.63 Å
3.54 Å
27.61 Å
J J
J
J J
0 5 10 15 20 25 30
°2θ CuKα

Figure 5