Fe oxidizing bacteria and the weathering of Fe silicate minerals

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Abstract

This study was designed to determine whether chemolithotrophic bacteria that derive metabolic energy from iron oxidation can be sustained by the flux of ferrous iron released by dissolution of fayalite (Fe_2SiO_4) and to evaluate the impact of metabolic products on the dissolution kinetics. When fayalite was dissolved abiotically, almost all dissolved iron remained as Fe^{2+} over the duration of the experiment. However, in the presence of *Thiobacillus ferrooxidans* aqueous iron was oxidized to Fe^{3+}. Dead cells did not induce significant ferrous iron oxidation and had minimal effect on the reaction rate. These results confirm that *T. ferrooxidans* was active in our experiments and can be sustained by fayalite dissolution. In the presence of *T. ferrooxidans* the total Fe accumulated in solution was only 5 to 50% of that in abiotic experiments. Silicon was not strongly incorporated into secondary minerals. Thus, Si release rates can be used to evaluate fayalite dissolution rates. The data indicate inhibition of fayalite dissolution in the presence of microorganisms. TEM and SEM analyses confirm that fayalite in the biotic experiments was much less extensively reacted than in the organism-free experiments. However, some precipitation of secondary iron oxyhydroxide minerals, especially in proximity to cells, leads to solutions with different stoichiometry than that of fayalite. The iron oxyhydroxides occur as aggregates of few nanometer diameter particles. These are unlikely to suppress the reaction rate via diffusion inhibition. Consequently, abiotic experiments were conducted to test the hypothesis that the ferric iron byproduct of microbial metabolism was responsible for the decreased dissolution rates in biological experiments. Addition of aqueous Fe^{3+} inhibited both silica and iron release by

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approximately 30% compared to the abiotic control, but added Fe^{2+} and Mg^{2+} had no effect. The similarity between the extent of inhibition due to addition of very low concentrations of Fe^{3+} and the rate suppression observed in biological experiments suggests that Fe^{3+} adsorption to surfaces sites is the primary cause for lower rate of accumulation of iron in solution in biological experiments. We propose biological inhibition of fayalite dissolution is due to binding of ferric iron to ferrous iron sites on the fayalite surface. This may generate a laihunite-like ($\sim Fe^{++}_{0.8}\, Fe^{+++}_{0.8} SiO_4$) surface layer that is fundamentally less reactive than fayalite.

Introduction

Weathering reactions control water chemistry and water rock interaction, exert long-term control on both atmospheric and oceanic compositions, and result in the creation of soils. Weathering of ferromagnesian silicates involves the destruction and reformation of metal-oxygen bonds (Casey et al., 1993). Consequently the reactivity of the mineral will depend on the metal-oxygen bond strength as well as the degree of polymerization of the silicate anion. Fe rich silicate minerals tend to weather very rapidly (see Goldich, 1938; Westrich et al., 1993 and Hoch et al., 1996) because the Fe(II)-O bond is very reactive. Weathering of Fe-silicate minerals can be complicated by Fe redox chemistry because oxidation of Fe at the mineral surface can catalyze mineral alteration rates (White and Yee, 1985).

Microbes utilize Fe for a variety of purposes. Firstly, most organisms utilize trace amounts of iron in a variety of proteins. However, in most oxygenated environments, iron is commonly biologically 'unavailable' as insoluble hydroxides or silicates. Microorganisms have devised mechanisms to solubilize Fe from Fe bearing minerals (see

Barker et al., 1997 and Hersman et al.1996 for reviews). Secondly, in anoxic environments microbes can use ferric iron oxyhydroxide phases as alternate electron acceptors (see Ehrlich et al. 1996 for review). Microbial Fe reduction can greatly increase release of soluble Fe and Si and other metals to solution (Welch 1996; Grantham et al., 1997). Thirdly, Fe oxidizing lithotrophic microbes have the potential to utilize reduced Fe as an electron donor in energy generation. This aspect of microbial iron biogeochemistry has received considerable attention when environments are highly acidic. However, the role of Fe-oxidizing microbes in near neutral pH solutions associated with weathered rock has received little attention.

Indirect evidence for Fe oxidizing microbes has been observed on oceanic basalts (Thorseth et al., 1995; Fisk et al., 1998). Chemolithotrophic iron oxidizers have been detected in soils and ground water in microenvironments where pO_2 is low or at oxic/anoxic interfaces (Emerson and Moyer, 1997; Emerson et al., 1999). Based on thermodynamic calculations, Jakosky and Shock (1998) predict that substantial populations of Fe oxidizing microorganism could be supported from the alteration of Fe silicate minerals, approximately 2 g of biota from the alteration of 1 mole of fayalite. However the thermodynamic argument fails to consider whether the rate of the mineral alteration reaction is sufficient to sustain a community of Fe oxidizing microorganisms.

Although Fe oxidizing chemolithotroph microorganisms may have limited importance in silicate mineral alteration now compared to the effect of heterotrophic organisms and plants, it is likely that they have been very important in earlier geologic periods. Phylogenetic analyses of small subunit ribosomal RNA sequences indicate that earliest organisms for which we have genetic signatures were autotrophs (Barns and

Nierzwicki, 1997) (organisms capable of converting CO_2 to organic carbon). Furthermore, metabolic gene sequence analyses suggest that many autotrophic pathways evolved billions of years ago (Bult et al., 1996). Although the relative importance of inorganic energy sources has changed over geologic time with the evolution of higher life forms, energy associated with reduced metals (especially Fe) in silicate minerals may have been, and remain, quite significant.

The purpose of these experiments was to determine whether Fe oxidizing microorganisms could live using Fe released by dissolution of Fe silicates as an energy source, and if so, the impact of this metabolism on the kinetics of Fe-silicate mineral weathering.

Experimental methods

Minerals

Natural fayalite was obtained from the American Museum of Natural History (AMNH 10928) and from the Smithsonian (R 3516). Sample R 3516 is from Cheyenne County, Colorado. Few millimeter-wide crystal aggregates were crushed, sieved, and characterized by X-ray diffraction and high-resolution transmission electron microscopy (HRTEM). The 25-75μm size fraction was used for the experiments. Synthetic fayalite was provided by Henry R. Westrich (Sandia National Laboratories). Synthetic fayalite had a particle size of 25-75 μm and a surface area of 0.125 m^2/g and was used as received, without any sample pretreatment.

Microbes

A lithotrophic iron oxidizing bacterium, *Thiobacillus ferrooxidans*, was used in the biotic experiments. *T. ferrooxidans* was cultured in a 9K media (Silverman and Lundgren 1959a as cited in Ehrlich, 1996) on an orbital shaker at 25 °C at 130 rpm for several weeks. The 9K media consists of 3.0 g $(NH_4)SO_4$, 0.1 g KCl, 0.5 g K_2HPO, 0.5 g $MgSO_4 \bullet 7H_2O$, 0.01 g $Ca(NO_3)_2$, 44.22 g $FeSO_4 \bullet 7H_2O$, and 1 L autoclaved, distilled water. The bacteria were separated from their culture media by a series of filtrations. The culture media with bacteria was initially filtered using a paper filter (approximately 10 μm pore size) to separate out the iron hydroxide precipitates from the media and bacteria. The filtrate was collected and then filtered again using an autoclaved 0.22 μm polycarbonate filter to collect the bacterial cells. This filter was flushed with several milliliters of distilled water to remove residual dissolved Fe from the bacterial cells. The filter with bacterial cells was placed in a polycarbonate centrifuge tube with a few ml of sterile nanopure water. The sample was then vortexed and sonicated to remove cells from the filter. This solution was then examined by light and epifluorescence microscopy for bacterial cells and for iron precipitates. Trace quantities of iron precipitates were detected in the cell slurry. The cell solution was stored in the refrigerator until the bacteria could be added to the experiments, usually within several hours. All media and solids were cleaned and autoclaved to minimize possible contamination by other microorganisms.

Biological Experiments

Fayalite dissolution experiments were carried out in batch reactors. 100 mg of fayalite was added to 50 ml of solution in acid-washed polycarbonate flasks. The

solutions used for all dissolution experiments consisted of dilute sulfuric acid, 1 mM sodium bicarbonate, 1 mM ammonium sulfate, 100 μM potassium phosphate, and filtered nanopure water. This media was chosen since it would provide trace inorganic nutrients required by the lithotrophic microorganisms without severely complicating the solution chemistry for the dissolution experiments. Solution pH was adjusted by changing the molarity of sulfuric acid, either 1 or 10 mM, and then titrated with either dilute HCl or NaOH before experiments.

The experiments done at an initial pH of 2, 3, and 4, consisted of three different treatments: an abiotic treatment with fayalite and solution, a biological treatment with approximately 2-3 ml of the cell solution, fayalite and media, and a no fayalite control, which consisted of 2-3 ml of the cell solution in 50 ml of media. There were approximately 10^7 cells/ml in the bulk solution in the biological and no fayalite treatment. Cell numbers remained relatively constant throughout the course of the experiment. The no fayalite control was done to determine the amount of Fe and Si associated with the bacterial cells. There were three replicates for both the abiotic and the biological treatment, though the no fayalite treatment was not replicated. Flasks were placed on an orbital shaker at 120 rpm to keep solutions aerated and well mixed during the experiments. 5 ml aliquots were taken periodically from each bottle for about 2 weeks using a sterile syringe and then filtered with a 0.22 μm acrodisc syringe filter to remove the bacteria and mineral particles. 5 ml of fresh solution was then added to the flasks to keep the volume constant.

Experiments were also completed at pH 2 using dead cell controls to distinguish whether the cells were passively forming iron oxides or actively metabolizing the iron.

Cells were deenergized using 1 mM NaN_3 (sodium azide from Fisher Chemical Co.) (Mera et al., 1992). For this set of experiments, there were 5 treatments, three of which are the same from the previous experiments: abiotic, live cells and no fayalite. Two sets of flasks contained sodium azide, an abiotic + azide treatment to determine if Na_3N affected the dissolution reaction, and cells + azide treatment to determine the effect of dead (or nonmetabolic) cells on fayalite dissolution. The experiments were done similarly to the previous ones, except fresh solution was not added after sampling.

Abiotic Experiments

Since *T. ferrooxidans* had a profound effect on the fayalite dissolution reaction, a second set of abiotic experiments was done to determine the effect of solution redox chemistry on fayalite dissolution. All abiotic redox experiments were carried out at an initial solution pH of approximately 2, under conditions very similar to the abiotic treatments in the previous experiments

One experiment tested the effect of solution oxygen content on fayalite dissolution. The oxic vs. anoxic fayalite dissolution experiments consisted of two treatments with two replicates each. The solution was prepared in the same manner as for the pH 2 fayalite dissolution experiments. 100 ml of solution and 100 mg of natural fayalite was added to each reactor. In the oxic treatment, oxygen was bubbled through the solution for approximately 10 minutes initially, and every day after a sample was taken. Similarly, nitrogen was bubbled through the solution in order to achieve anoxic conditions. Bottles were not constantly agitated but were swirled gently by hand before samples were taken. A 5 ml aliquot was taken periodically from each bottle for 10 days.

Samples were filtered with a 0.22 μm acrodisc filter. Reactors were not replenished with fresh media.

A second set of abiotic fayalite dissolution experiments was done to determine the effect of solution iron speciation on fayalite dissolution. The Fe^{2+} vs. Fe^{3+} experiments consisted of four different treatments with two replicates each: 10 mM H_2SO_4, (as a control), 10 mM H_2SO_4 plus 1 mM Mg^{2+}, 10 mM H_2SO_4 plus Fe^{2+}, and 10 mM H_2SO_4 plus Fe^{3+}. 100 ml of solution and 100 mg of synthetic fayalite was added to polycarbonate bottles and placed on a shaker table for eight days. Samples were taken periodically using a syringe and filtered with a 0.22 μm filter.

A third set of abiotic fayalite dissolution experiments was performed to determine the effect that varying amounts Fe^{3+} in solution had on fayalite dissolution. The experiment consisted of reacting fayalite in media containing 100 μM Fe^{3+}, 500 μM Fe^{3+}, 1 mM Fe^{3+}, and 5 mM Fe^{3+}. The duplicated batch experiments consisted of 100 mg of fayalite reacted in 100 ml of solution in polycarbonate flasks. Samples were collected periodically for 10 days using a syringe and filtered with a 0.22 μm filter. Fresh media was not added after sampling.

Analytical

Analysis for Fe^{2+} and total Fe was done using the ferrozine method (Stookey, 1970). Absorbance was measured using a Perkin Elmer UV/VIS spectrophotometer. Dissolved silica was measured using a Technicon AutoAnalyzer II with the molybdate blue method. Solution pH was determined with a Denver Instruments pH meter.

At the end of the dissolution experiments, a fraction of the mineral sample was removed from the reactor and mounted on a SEM stub with carbon tape. Mineral

samples were allowed to air dry. Samples were sputter coated with either platinum or gold and examined with a LEO 982 high resolution scanning electron microscope. For some experiments, mineral samples were prepared for TEM analysis. Mineral grains were gently crushed using a mortar and pestle, suspended in deionized water, and placed on a formvar coated copper grid. Samples were examined using a Philips CM200 ultratwin transmission electron microscope operated at 200 kV.

Results

The rates of iron and silica release were measured in both abiotic and biologic experiments. Rates depended on solution pH and on the presence or absence of microbial populations.

pH 2 Experiments

Abiotic and biologically mediated fayalite dissolution experiments at pH 2 were conducted with both natural (Figure 1) and synthetic fayalite (Figure 2). In the abiotic experiments, Fe release to solution was rapid in the first few day of the experiment and then leveled off and eventually decreased with time (Fig 1a). Approximately half of the fayalite has reacted in the first 4 days of the experiment. The initial Fe release rate (before Fe concentration becomes relatively constant) is ~1.3 x 10^{-11} moles Fe^{2+} cm^{2-} s^{1-}. Essentially all of the iron in solution in the abiotic experiments was as Fe^{2+}. The Si release curve is very similar to Fe, though Si concentrations are approximately half those of Fe, reflecting net stoichiometric dissolution (Figure 1b).

Iron release rates in the presence of microorganisms were slower, and concentrations were significantly lower than in the abiotic solutions (approximately 11

mM versus 2 mM at the end of the experiment). In the biological experiments, reduced iron concentrations were considerably lower than total iron concentrations (Figure 1a). Silica concentration versus time for these experiments is plotted in Figure 1b. Silica released into solution was half that of iron, indicating approximately stoichiometric fayalite dissolution in both the biological and abiotic experiments. Total Fe and Si release in the abiotic experiments was approximately 5 times greater than for the biological experiments. The Fe and Si released in the fayalite-free control was either undetectable or negligible compared to the mineral dissolution experiments.

The experiment was repeated using a synthetic fayalite. Results of the abiotic treatment were similar to the experiments with natural fayalite except concentrations were lower, approximately 40% less than the experiments with natural fayalite. Initial iron release rates, however, were comparable to those of the natural fayalite ~1.6×10^{-11} moles Fe^{2+} cm^{2-} s^{1-}. In the abiotic solution, the rate of iron release was very rapid in the first couple of days of the experiment and then Fe concentration was approximately constant with time (Figure 2a). Approximately 35% of the fayalite had reacted within the first two days of the experiments. Dissolved Fe was again almost entirely as reduced iron, Fe^{+3} was undetectable within the analytical precision of our method. Si release from the synthetic fayalite followed Fe release, overall dissolution was approximately stoichiometric. However, in the biologic experiments, Fe and Si release rates and concentrations were extremely low, approximately 25 to 40 times lower than in the abiotic experiments (Figure 2) and approximately an order of magnitude lower than the biotic experiments with natural fayalite. Furthermore, the reaction was apparently nonstoichiometric, with Si being preferentially released compared to Fe. Final Si

concentrations were comparable, approximately 150 μM, which corresponds to 2% of the fayalite dissolved. Over the course of the experiment, bulk solution pH increased dramatically from and initial value of 2 to pH 3.6 in the abiotic experiments. In the biological experiments however, pH remained relatively constant at pH 2 to 2.3.

In the experiments to test the effect of dead cells, the results of the control, live cells, and fayalite-free treatments were nearly identical to the previous experiment done at pH 2 with synthetic fayalite Figure (2 and 3). Total and reduced iron released from fayalite in the azide and dead cell experiments was only approximately 10% lower than the abiotic control. Silica release in the azide and dead cell experiments was indistinguishable from the control. These results indicate that neither azide nor dead cells had a significant effect on the dissolution reaction.

Solution saturation state was calculated using PHREEQC (Parkhurst 1995). Within the first two days of the experiments, solutions became supersaturated by up to several orders of magnitude with respect to goethite, hematite, quartz and chalcedony in both the abiotic in biologic samples of the natural and synthetic fayalite experiments. Saturation index for conditions at the ends of the experiments are given in Table 1.

Figure 4 shows typical high-resolution SEM images of natural fayalite reacted at pH 2 for eight days. There is evidence of extensive reaction in both sets of experiments. It is evident from the SEM images that minerals in the abiotic experiments are much more extensively reacted than minerals in the biological experiments. Figure 4a shows a very extensively reacted natural fayalite grain from the abiotic experiments. Deep etch channels and pits formed on the mineral surfaces. Although these etch channels are somewhat irregular, their orientation is generally normal to **c**. The extent and pattern of

etching varies substantially for different surfaces. The (001) surface appears to be relatively unreacted. Figure 4b shows another very rough etched surface, with holes and channels forming parallel to (001). In the biotic experiments, the etching is dominated by periodic parallel planar channels formed parallel to (001) (Figure 4c). Based on SEM observations these etch channels appear to be typically on the order of 10's to 100 nm wide and spaced approximately 1 μm apart. The micron-sized ovals are bacteria on the fayalite surface. Figure 4d is a higher magnification image of the mineral surface showing dividing cells. Piles of material surround the cells on the mineral surface.

Although it was difficult to obtain accurate cell counts, the total number of cells in suspension did not appear to change significantly (< a factor of 2) throughout the experiment. Based on estimates from the SEM observations and total cell counts, approximately 10 to 50% of the cells are attached to mineral surfaces.

pH 3 Experiments

At pH 3, iron and silica release into solution is about 3 orders of magnitude lower than for the experiment at pH 2 with natural fayalite. This corresponds to approximately 0.1% of the solid material reacting during the course of the experiments. Figure 5 shows Fe and Si concentrations plotted against time. In the abiotic treatment most of the iron released into solution is Fe^{2+}, as for all the experiments done at pH 2.

In the biotic experiments with *T. ferrooxidans* most of the iron in solution was ferric iron. The dissolution reaction was not stoichiometric in either the biotic or abiotic treatment. Si accumulates in solution preferentially compared to Fe. The total dissolved Fe/Si ratio was 0.5 (a 4x concentration of Si compared to Fe, relative to fayalite) in the

biotic experiments compared to 1.0 in the abiotic experiment. Fayalite dissolution rate at pH 3, based on Si release, is $6.2*10^{-15}$ mol/cm^2 in the abiotic experiment. Overall, the presence of microorganisms inhibited the release of silica by about a factor of two compared to the abiotic experiments. The fayalite-free control showed relatively little dissolved iron or silica in solution for both the abiotic and biologic samples.

Solution pH remained relatively constant with time for both the abiotic and biologic experiments, increasing from approximately 3.0 to 3.1 throughout the course of the experiment. The solutions also became supersaturated with goethite and hematite by the end of the experiment (Table 1).

pH 4 Experiments

At pH 4, iron and silica release from synthetic fayalite dissolution was approximately 3 orders of magnitude lower than for the experiments at pH 2. The fayalite dissolution rate, based on Si release, is $4.6*10^{-15}$ mol/cm^{2}s. The amount of iron released into solution was significantly greater in the abiotic experiments compared to the biologic ones (Figure 6a), as for the experiments at pH 2 and 3. In contrast with pH 2 and 3 experiments, solutions in pH 4 experiments were dominated by Fe^{+2} in both the abiotic and biologic experiments. However, measured Fe concentrations are extremely low in the biological experiments, very close to the detection limit for the analytical method. The plot of silica concentration versus time (Figure 6b) shows that silica accumulates in solution preferentially over iron, especially for the biologic experiments (where iron is barely detectable). However, silica concentrations in the abiotic experiments were still about a factor of 2 higher than for the biologic experiments. The fayalite-free control

showed no detectable iron or silica in solution. The pH remained relatively constant (4.0 to 4.3) for both the biologic and abiotic samples, with solutions supersaturated with respect to goethite and hematite only in the biologic experiments (Table 1).

Effect of solution redox conditions on fayalite dissolution

Anoxic vs. Oxic Experiments

Abiotic experiments were done at pH 2 to determine if dissolved oxygen concentration affected the fayalite dissolution rate. Figure 7 shows iron released into solution for both oxygenated and anoxic solutions. Results were very similar to the natural fayalite abiotic experiments at pH 2. Dissolved Fe increased with time for the first few days of the experiment and then was approximately constant. All iron in both the oxic and anoxic experiments was in the reduced state, and there was no significant difference between Fe release rate or Fe concentration in the two experiments.

Fe^{2+} and Mg^{2+} Experiments

Experiments were done at pH 2 to determine the effect of Fe^{2+} and Mg^{2+} on fayalite dissolution. Figure 8 shows average total iron released into solution over the duration of the experiments. As in the previous abiotic experiments, all dissolved iron is Fe^{2+}. Neither Fe^{2+} nor Mg^{2+} in solution had an effect on fayalite dissolution compared to the sulfuric acid control.

Fe^{3+} Addition Experiments

Addition of Fe^{3+} greatly inhibited iron release from the fayalite. Total Fe release to solution from natural fayalite in the Fe^{+3} treatment was approximately 30% of the control at the end of the experiment.

In order to determine the effect of increasing amounts of Fe^{3+} in solution, dissolution experiments of synthetic fayalite were performed at pH 2 using 100 μM Fe, 500 μM Fe, 1 mM Fe, and 5 mM Fe in solution, along with a control without any added Fe to solution for comparison (Figure 9). Fe and Si concentrations released into solution in the control experiments were very similar to the control experiments done at pH 2. (the reaction was stoichiometric, and all the dissolved iron was reduced, and approximately 40% of the fayalite dissolved by the end of the experiment). Addition of 100 μM Fe^{+3} to solution decreases Fe and Si release by almost 1/3 compared to the controls. The reaction was not stoichiometric. The dissolved total Fe was approximately 1000 μM lower than concentration predicted from the stoichiometric dissolution of fayalite. This is approximately equivalent to the precipitation of a goethite layer 150 nm thick over the mineral surface.

The addition of 500 and 1000 μM Fe^{+3} to solution decreased net Fe and Si release from fayalite by 60 and 90% compared to the abiotic control. The reaction was approximately stoichiometric, the measured Fe in solution was slightly lower than added Fe^{3+} plus Fe predicted from Si release from fayalite dissolution. This difference between measured and predicted iron could be attributable to goethite layer that was only a few tens nanometers thick over the initial mineral surface area.

Net Si release from fayalite in the experiments with 5 mM Fe^{+3} is only 3% of Si in the control at the end of the experiment. Approximately 80 to 90% of the added iron precipitates during the experiment, this could correspond to a goethite layer 600 to 700 nm thick over the mineral surface.

TEM and AEM characterization of reaction products

HRTEM images and energy-dispersive X-ray analyses (analytical electron microscopy; AEM) show that the nanocrystalline (few nanometer in diameter) particles of iron oxyhydroxides are produced in biological dissolution experiments. Interplanar spacings in the product indicate a mixture of ferrihydrite and goethite. Little Si was detected in secondary minerals. Particles were typically found in flocculated aggregates, not as tightly adhering layers on altered fayalite surfaces.

DISCUSSION

The result of our experiments demonstrate that fayalite dissolution at low pH is able to sustain an active population of the iron oxidizing bacterium *Thiobacillus ferrooxidans*. Metabolic activity is evidenced by the production of almost the maximum concentration of ferric iron in solution, persistence of populations over the duration of experiments, production of precipitates in proximity to cells, and presence of dividing cells on the fayalite surface. Our results also show that the fayalite dissolution rate increases with increasing acidity, implying a larger microbial population can be sustained at low pH. However, the activity of the iron oxidizing bacterium suppresses the rates of fayalite dissolution at low pH. Based on the results of abiotic, ferric iron-supplemented controls, this is attributed to oxidation of Fe^{+2} to Fe^{+3}.

Abiotic rates

In all of our abiotic batch dissolution experiments, the release of Fe and Si to solution is nonlinear with time. This nonlinear release of mineral components to solution is a very typical feature of batch dissolution experiments and it reflects the changes in

experimental conditions over time. The very rapid initial dissolution rates are due primarily to proton consumption and preferential dissolution of more reactive material. The slower release rates reflect conditions where solution pH is higher, and solutions are supersaturated with respect to several possible secondary phases and chemical affinity approaches zero. Rates also decrease with time because 30 to 50% of the available material reacts within the first few days of the dissolution experiments. Abiotic fayalite dissolution rates can be calculated from the initial stage of the reaction where Si and Fe are rapidly released to solution and no precipitation occurs.

The abiotic dissolution rate of fayalite determined from the increase in Fe or Si in solution ranged from approximately $1*10^{-11}$ mol/cm^{2}s at initial pH 2 to $5*10^{-15}$ mol/cm^{2}s at initial pH 4. These rates are comparable to other experimentally determined dissolution rates for Fe-silicate minerals. For example, Westrich et al. (1993) calculated a fayalite dissolution rate of $6*10^{-11}$ mol/cm^{2}s at pH 2, slightly faster than our rates. In our pH 2 experiments, acidity decreased with time as the mineral dissolved, therefore the rate is for an average pH of approximately 2.4. Wogelius and Walther (1992) measured fayalite (Fo_6) dissolution rates in flow through reactors of $5*10^{-12}$ to $7*10^{-14}$ mol/cm^{2}s from pH 2 to pH 5. Experimentally determined mineral dissolution rates typically vary by as much as on order of magnitude depending on experimental conditions, i.e. batch or flow through reactors, mineral:solution ratio, mineral composition, mineral preparation, pretreatment etc, so our experimental rates are consistent with others. However, mineral dissolution experiments done where all conditions are held constant and only one parameter is varied tend to be very reproducible (see for example Figure 2 and 3), and differences between experiments, either in terms of rate of release of mineral components

or concentrations in solution, can be attributed to the affect of that variable (i.e. changing solution pH, the presence of microbes, dissolved O_2 content etc).

Effect of *Thiobacillus ferrooxidans* on fayalite dissolution

Fayalite dissolution experiments conducted in the presence of iron oxidizing microbes showed a suppression of both iron and silica release into solution compared to abiotic controls. SEM images of the reacted minerals at pH 2 also confirm the geochemical results, which show mineral grains from the abiotic experiments are much more reacted than those from the biologic experiments. The apparent inhibition of the reaction could be due to several factors.

A decrease in mineral dissolution rates over time has been attributed to the formation of a diffusion inhibiting leached layer or secondary precipitates on the mineral surface (Schott and Berner, 1983; 1985; Chou and Wollast,1984).

The formation of an iron rich product on the fayalite surface is consistent with the solution chemistry, which shows a preferential accumulation of Si compared to Fe in solution in the biological experiments, and that solutions are apparently supersaturated with respect to several possible secondary Fe phases (Table 1). In the biotic experiments at pH2, total Fe concentration is at the end of the dissolution experiment is approximately 150 μM less than what would have been predicted from Si concentration for stoichiometric fayalite dissolution. If all this iron precipitated as goethite, it would form a 15 nm thick layer over the fayalite surface (assuming a fayalite surface area of 1000 cm^2/g and no precipitation in solution or the reaction vessel). However, there is no evidence that the goethite has a form that could inhibit diffusion of ions to or from the mineral surface. TEM (also see Welch and Banfield in prep.) and SEM images show no

sign of an extensive leached layer or precipitated layer on the mineral surface. In fact, the particulate form of the goethite product suggests that this product does not significantly modify the reactivity of the fayalite surface. Furthermore, surface reaction rates, and therefore dissolution rates, are so much slower than diffusion rates. Thus, we conclude that a diffusion-inhibiting layer composed of iron oxyhydroxide minerals does not explain the suppression of fayalite dissolution rates in the presence of iron-oxidizing microorganisms.

In the experiments at higher pH, 3 and 4, there is an approximately 50% inhibition of the fayalite dissolution rate compared the organism-free experiments. It is again unlikely that this is due to formation of a diffusion inhibited barrier on the surfaces, especially considering the total amount of material reacted in these experiments is less that one unit cell thick over the mineral surface.

Another explanation for the suppression of fayalite dissolution rates in the presence of microorganisms or ferric iron is that Fe^{+3} absorbs to, and chemically passivates, the mineral surface. The rates of solvent exchange around the Fe^{+3} ion are about 3 orders of magnitude slower than rates surrounding Fe^{+2}, therefore, the reactivity of the Fe^{+3}-O bond should be orders of magnitude slower than the Fe^{+2}-O bond (Casey et al., 1993). Furthermore, it is clear that ferric iron-rich olivine is far less reactive that unoxidized olivine. Naturally weathered olivines are highly reactive, except in regions containing planar defects of laihunite, an olivine with Fe^{+3} substituted primarily in the M2 octahedral sites and M1 vacancies (i.e., $[\,]_x(Fe^{+2})_{2-3x}(Fe^{+3})_{2x}SiO_4$) (Banfield et al., 1990, 1992; Casey et al., 1993).

Because etching, and thus surface area, is primarily on (001) (also see Welch and Banfield, in prep.), passivation is most effective if ferric iron binds to these surfaces. It is notable that (001) contains the octahedral planes onto which vacancies and ferric iron order in laihunite. Thus, we propose that surface passivation of fayalite during dissolution in the presence of ferric iron is due to exchange of ferric for ferrous iron in M2 sites, charge balanced by low occupancy of adjacent M1 sites. In other words, we propose that fayalite dissolution is dramatically inhibited by formation of laihunite-like layers (perhaps a few atomic layers deep) on (001) surfaces (Figure 10).

Effect of solution redox chemistry on fayalite dissolution

It is apparent from our experiments that solution redox chemistry can dramatically affect rates and mechanisms of Fe-silicate mineral weathering. Our experiments clearly demonstrate that Fe^{+3} in solution, either produced by *T. ferrooxidans* or added to solution abiotically inhibited fayalite dissolution under mildly acidic conditions. This effect increased with increasing Fe^{+3} in solution. The presence of dissolved oxygen, which can abiotically oxidize Fe^{+2} to Fe^{+3} had no detectable affect on the reaction. However, under the experimental conditions the abiotic oxidation rate of Fe^{+2} is extremely slow, approximately 0.1 μmol/l/day (Nordstrom and Southam 1997), therefore Fe^{+3} was not detected during the duration of the abiotic experiments.

Others however, have reported a decrease in Fe silicate reaction rates under oxic conditions due to the oxidation of Fe^{+2}. For example, Wogelius and Walther (1992) note a decrease in fayalite dissolution rates over time in their flow through reactors, which they attribute to the slow oxidation of reduced iron and precipitation of ferric hydroxides

on the mineral surface. Siever and Woodford (1979) saw an increase in dissolution of many Fe containing rocks and minerals, fayalite, hypersthene, basalt, and obsidian under slightly acidic anoxic conditions compared to air saturated or O_2 saturated solutions. Total amount of material released to solution was up to an order of magnitude greater under anoxic conditions than for comparable experiments in equilibrium with air or O_2, presumably due to the precipitation of iron phases on mineral surfaces.

In contrast to this however, several researchers report an increase in dissolution/weathering rate of Fe silicates, due either to oxygen or another oxidizing agent. Hoch et al. (1996) saw a significantly higher flux of Si, Ca, and Mg from augite in their 1.5 ppm O_2 experiments at near neutral pH compared to their 0.6 ppm O_2 experiments, whereas dissolved O_2 content had no apparent effect on diopside dissolution. Diopside dissolution rates were lower than augite rates. They attribute the increase in reaction rate with increasing dissolved O_2 and faster rates in Fe-rich minerals to the oxidative dissolution of Fe from the mineral. In a similar study, White and Yee (1985) measured an enhancement of augite dissolution in the presence of dissolved O_2 and Fe^{+3}. They attributed this increase in reaction rate to an electron transfer between Fe^{+3} in solution and Fe^{+2} on the mineral surface. The resulting formation of a surface Fe^{+3} leads to a charge imbalance, and an increase in the flux of other ions to solution.

Microbial growth from Fe silicate minerals

Both SEM and epifluorescence microscopic examination of the mineral surfaces and of solution show evidence of dividing cells in our experiments. The almost total oxidation of Fe^{+2} to Fe^{+3} in solution and the formation of iron hydroxide precipitates

Thus, we predict that rates will not be strongly affected by iron oxidation, in contrast to the process at low pH, where ferric iron has a significant residence time in solution.

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

surrounding the cells indicates that at least some of the cells were metabolically active during the experiment. If mineral dissolution rates and microbial iron oxidation rates are known then its possible to estimate sustainable numbers of iron oxidizing microorganisms (Banfield et al., in revision). Based on an iron oxidation rate of $\sim 2*10^{-17}$ mol Fe^{+2}/cell•s (Edwards et al., 2000?) and a fayalite dissolution rate of $\sim 10^{-10}$ to 10^{-11} mol /cm^2•s then we predict that approximately 10^8 to 10^9cells can be sustained by fayalite dissolution at pH 2 in our experiments. This is consistent with the numbers of cells detected.

Conclusion

Results of this study show that, under some conditions, iron silicate dissolution reactions can sustain microbial populations. Although the conditions utilized in our experiments were chosen because they allowed use of an easily culturable, acidophilic iron oxidizing bacterium, the findings should be more broadly relevant. Neutrophilic iron oxidizing microbes are common in natural microaerophilic environments, such as those expected at Fe-silicate surfaces early in chemical weathering of rocks (these were not utilized in this study because they are difficult to grow in the laboratory). At near-neutral pH the solubility of ferric iron is so low that, once formed, it precipitates very close to the oxidation site (e.g., on the cell surface; see Banfield et al. in press). Under these conditions, it is likely that ferric iron suppression of Fe-silicate dissolution will not occur.

Table 1

Saturation index (SI) with respect to several possible secondary phases for conditions at the end of the dissolution experiments. SI calculated using PHREEQC (Parkhurst, 1995)

	goethite	*hematite*	*quartz*	$SiO_{2\,(a)}$
Biological pH 2	1.41	4.83	.18	-1.09
Biological pH 3	2.76	7.53	-1.12	-2.38
Biological pH 4	3.67	9.35	-1.41	-2.68
Abiotic pH 2	3.00	8.00	1.6	0.33
Abiotic pH 3	2.09	6.18	-0.90	-2.17
Abiotic pH 4	---	---	-0.98	-2.24

Figure captions

Figure 1 (a) Total (open symbols) and reduced Fe (filled symbols) concentration for natural fayalite dissolution at initial pH 2 for the controls (■), microbial (●) and no fayalite (▲) treatments. (b) Si concentration from dissolving natural fayalite at initial pH 2 for the controls (■), microbial (●) and no fayalite (▲) treatments.

Figure 2 (a) Total (open symbols) and reduced Fe (filled symbols) concentration for synthetic fayalite dissolution at initial pH 2 for the controls (■), microbial (●) and no

fayalite (▲) treatments. (b) Si concentration from dissolving synthetic fayalite at initial pH 2 for the controls (■), microbial (◐) and no fayalite (▲) treatments.

Figure 3 (a) Reduced, (b) total and (c) Si concentrations from dissolving fayalite in dead cell experiments. Treatments are abiotic (■), azide addition (❑) dead cells with azide (○) live cells (●) and no fayalite (▲). The abiotic, live cell, and no fayalite treatments are nearly identical to those in Figure 2.

Figure 4 Scanning electron microscope images of natural fayalite reacted in abiotic and biological experiments at initial pH 2. (a, b) Fayalite reacted in abiotic experiments. The surfaces are characterized by very extensive etching and deep holes and channels forming normal to c*. (c) Fayalite reacted in with T. ferrooxidans. Etch texture is characterized by narrow planar etch channels normal to c*. The micron-sized ovals on the mineral surface are T. ferrooxidans cells. (d) Higher magnification image of cells on the fayalite surface. Cells are surrounded by extracellular polymers and iron oxyhydroxide precipitates. Note the dividing cell and flagella.

Figure 5 pH 3 (a) Total (open symbols) and reduced Fe (filled symbols) concentration for natural fayalite dissolution at initial pH 3 for the controls (■), microbial (●) and no fayalite (▲) treatments. (b) Si concentration from dissolving natural fayalite at initial pH 3 for the controls (■), microbial (●) and no fayalite (▲) treatments.

Figure 6 (a) Total (open symbols) and reduced Fe (filled symbols) concentration for natural fayalite dissolution at initial pH 4 for the controls (■), microbial (●) and no fayalite (▲) treatments. Iron concentration in the biological and no fayalite treatments are at the detection limit for the method (b) Si concentration from dissolving natural fayalite at initial pH 2 for the controls (■), microbial (●) and no fayalite (▲) treatments.

Figure 7 Reduced and total Fe is solution from synthetic fayalite dissolution under oxic (■),and anoxic (●)conditions.

Figure 8 Total iron release from fayalite dissolution at initial pH 2 in 1 mM solutions of Fe^{2+} (■), Mg^{2+}(◆), Fe^{+3}(▲), and a control (●).

Figure 9 (a) reduced iron (b) total iron and (c) Si from fayalite dissolution at initial pH 2 in a control (■), and with 100 μM (◆), 500 μM (●), 1 mM (▲), and 5 mM (*) Fe^{3+} added to solution.

Figure 10 Diagram of fayalite surface depicting Fe+3 adsorption and the formation of a laihunite-like layer.

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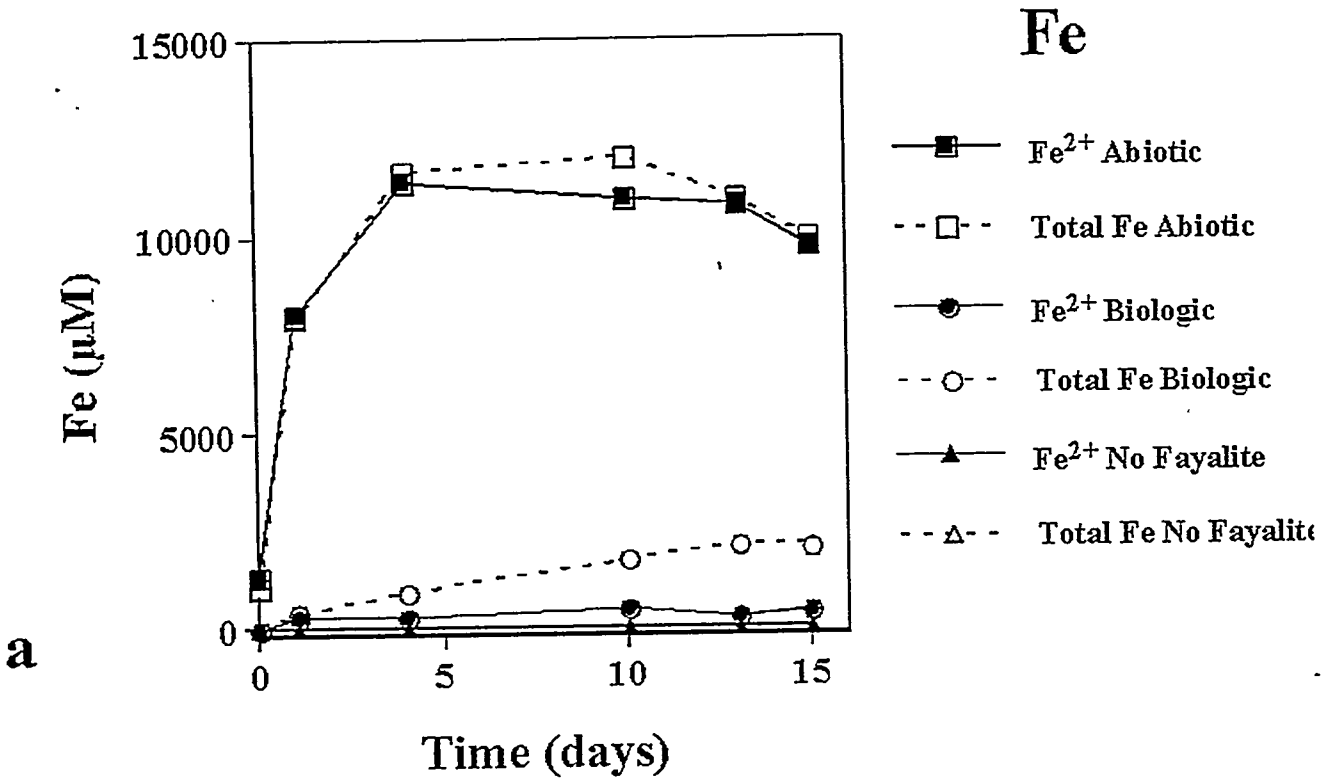
Figure 1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5000
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Fe (µM)
0
5
10
15
Time (days)
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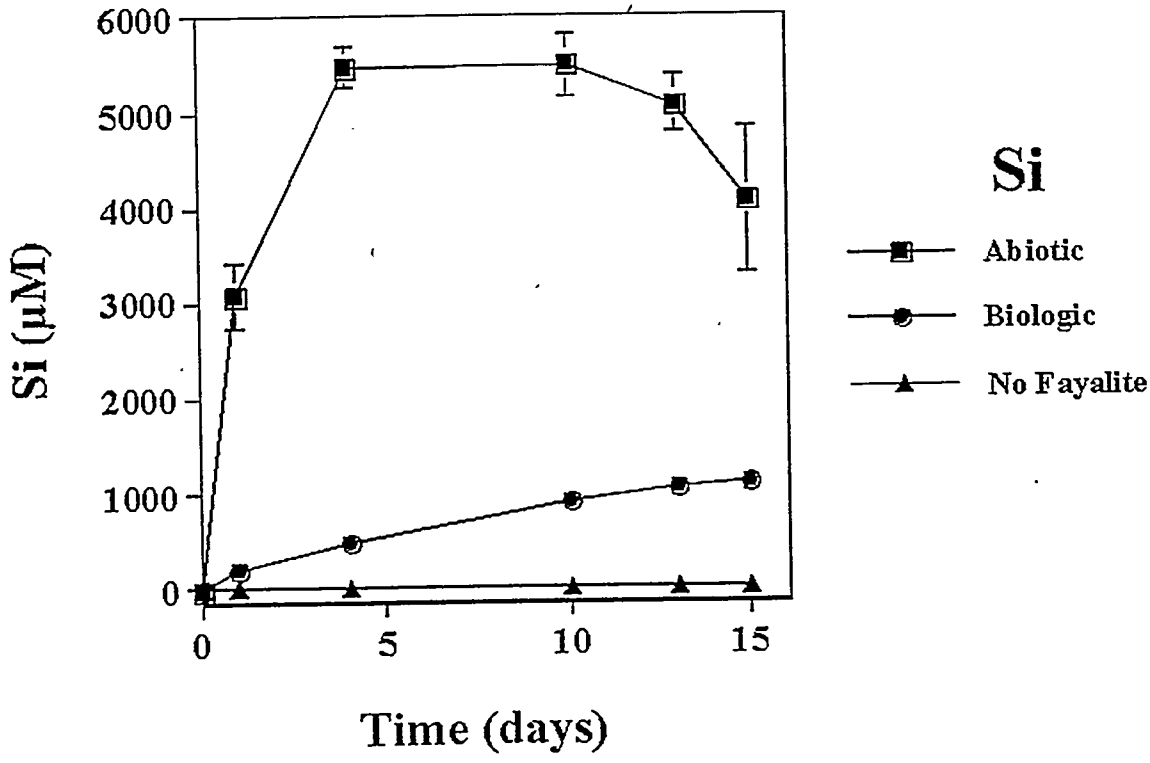
Total Fe Abiotic
Fe2+ Biologic
Total Fe Biologic
Fe2+ No Fayalite
Total Fe No Fayalite

a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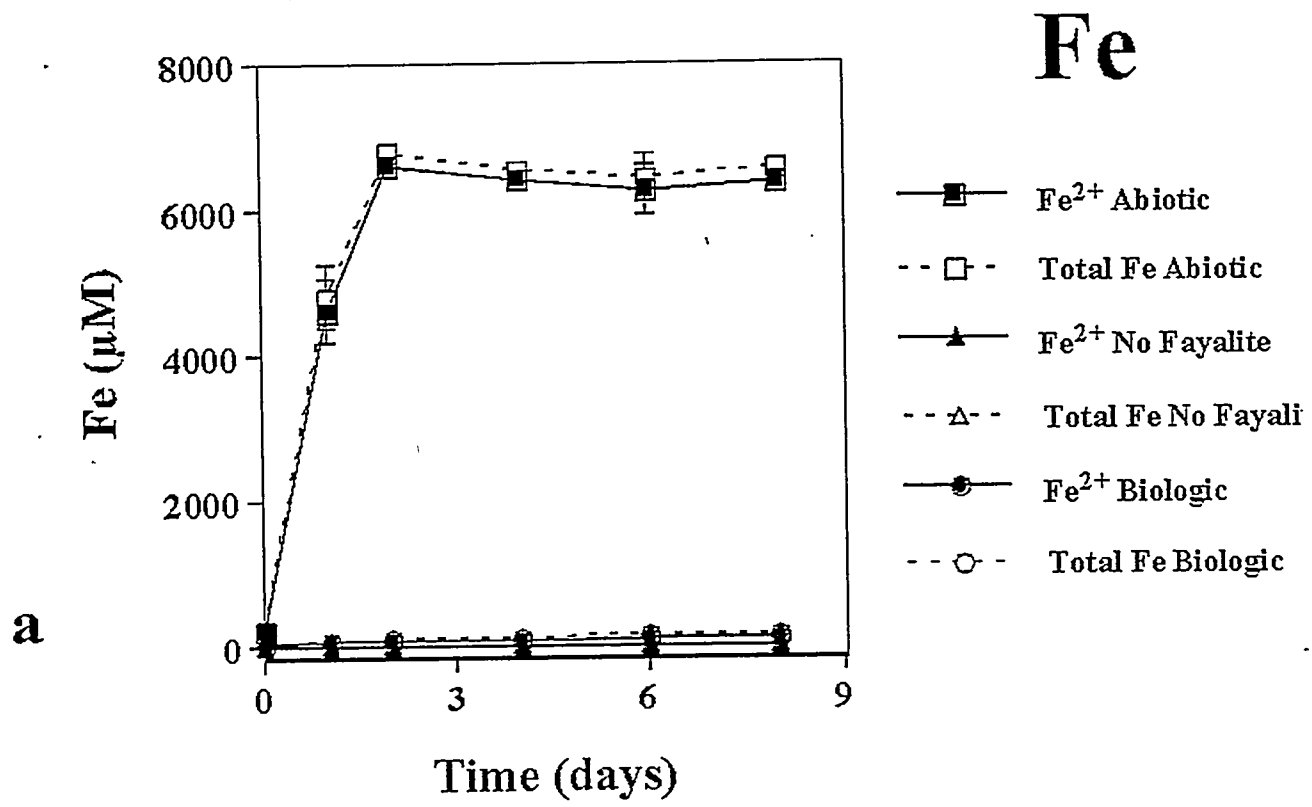
Abiotic
Biologic
No Fayalite

b

Figure 2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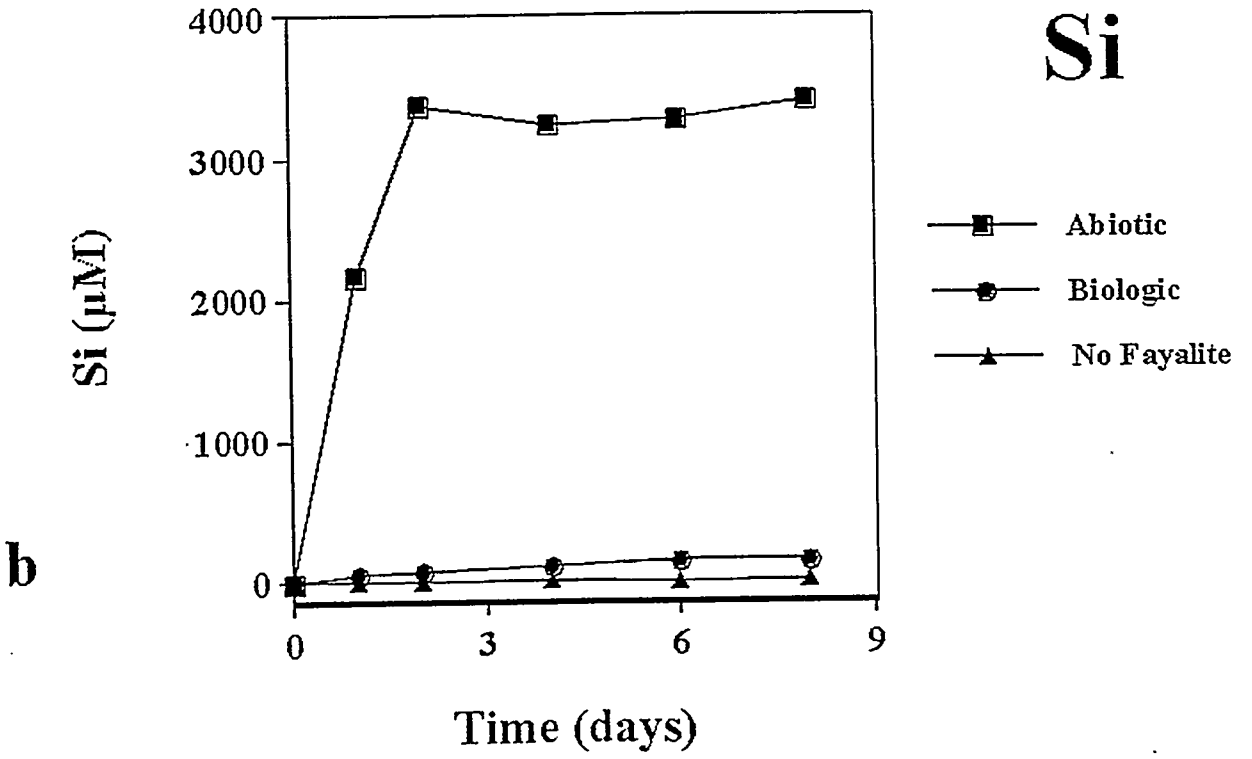
Total Fe No Fayali
Fe2+ Biologic
Total Fe Biologic

a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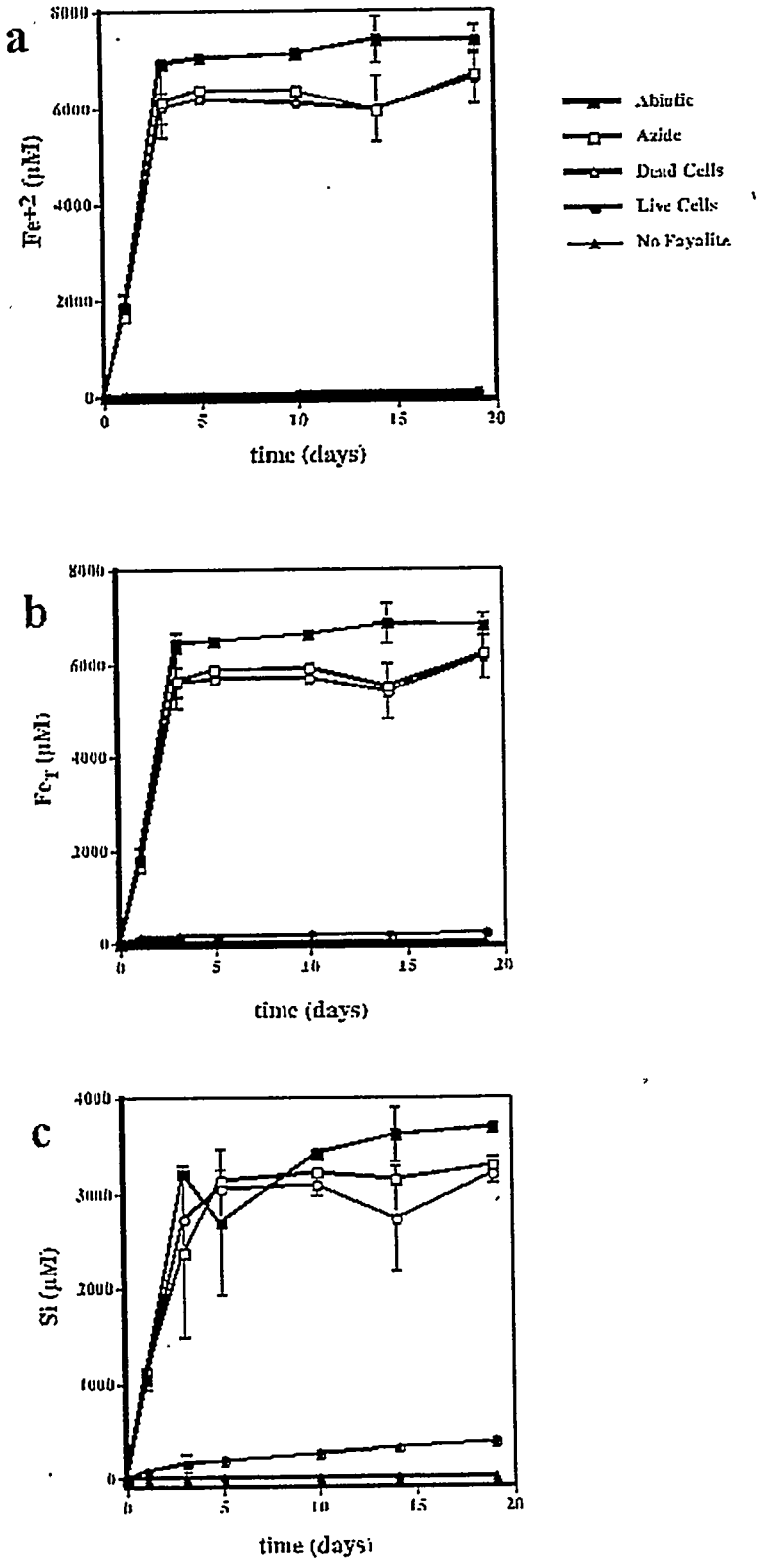
3
6
9
Time (days)
Abiotic
Biologic
No Fayalite

b

Figure 3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b
c
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Fe_T (μM)
Si (μM)
time (days)
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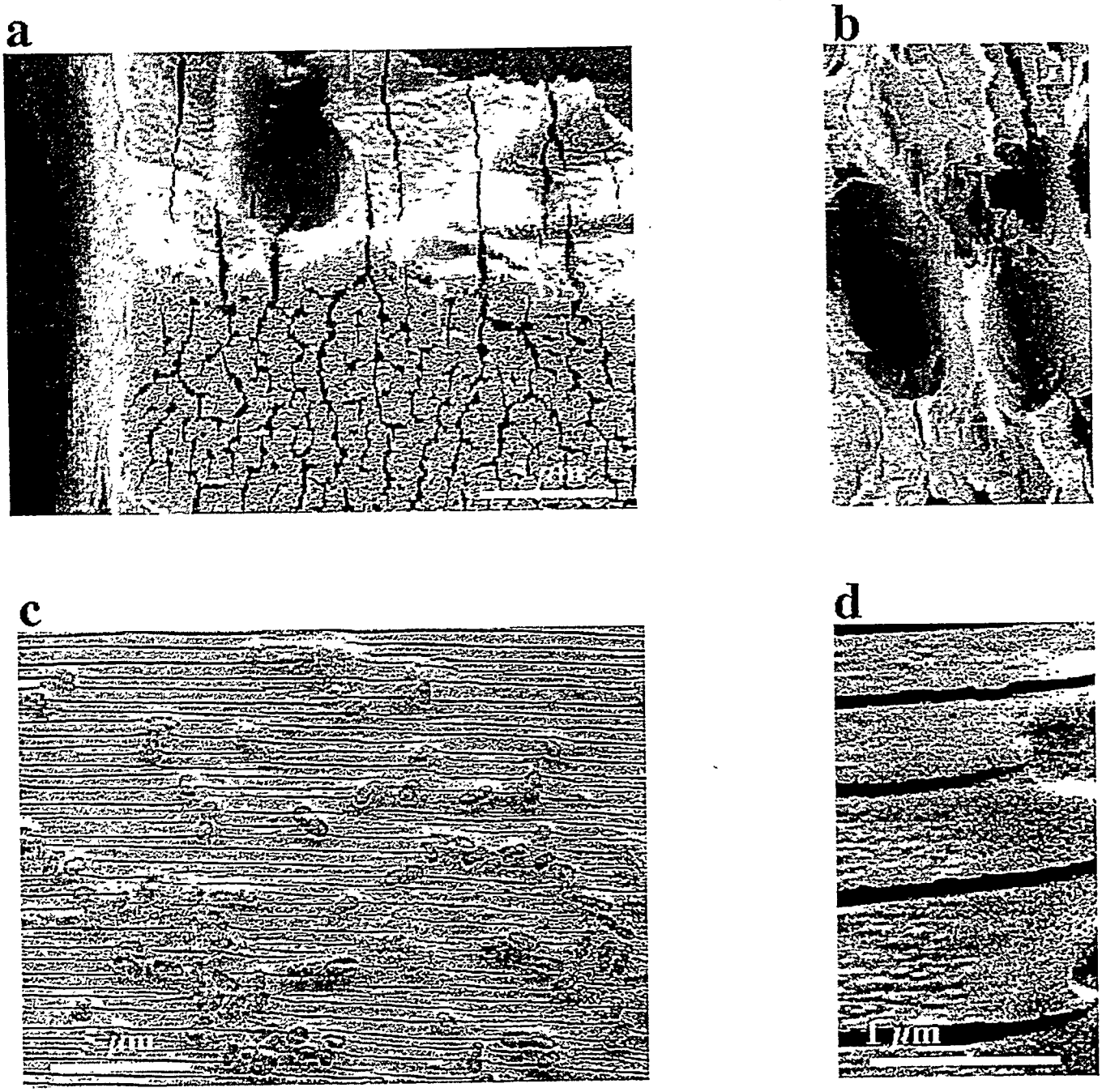
Azide
Dead Cells
Live Cells
No Fayalite

Figure 4



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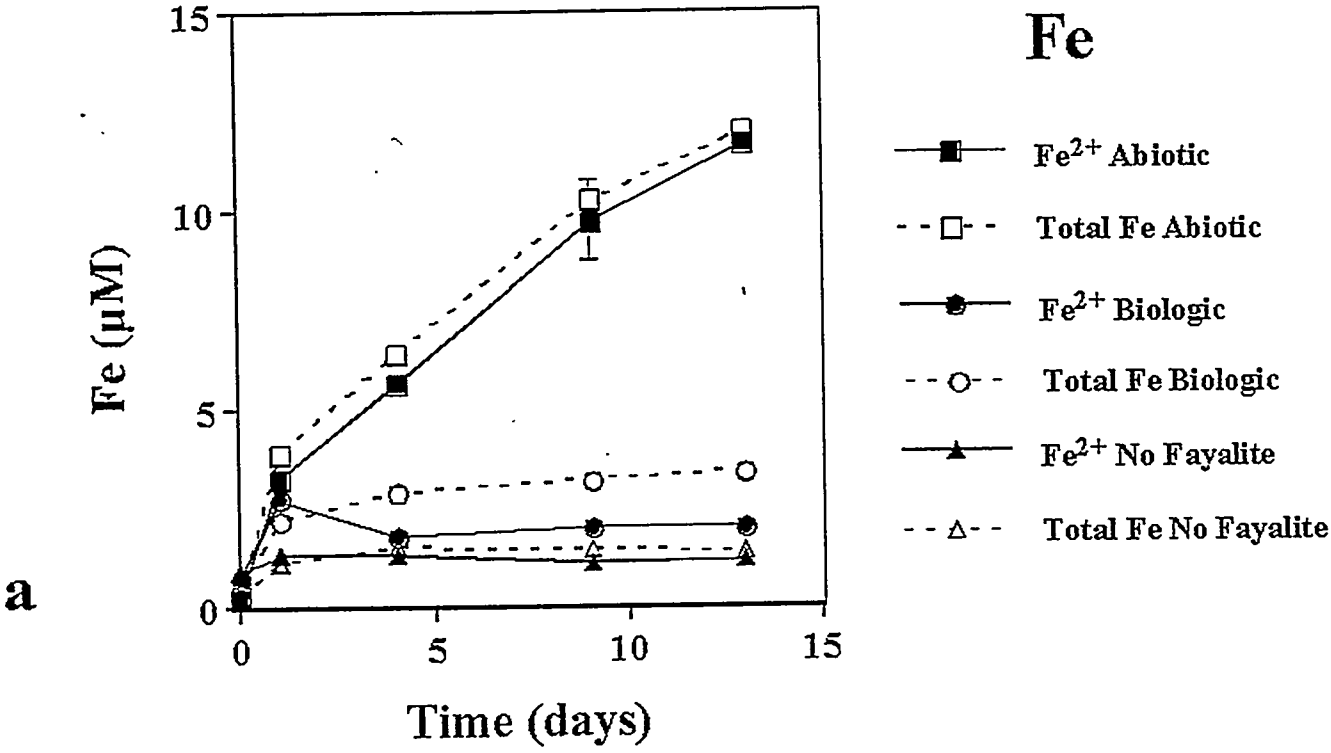
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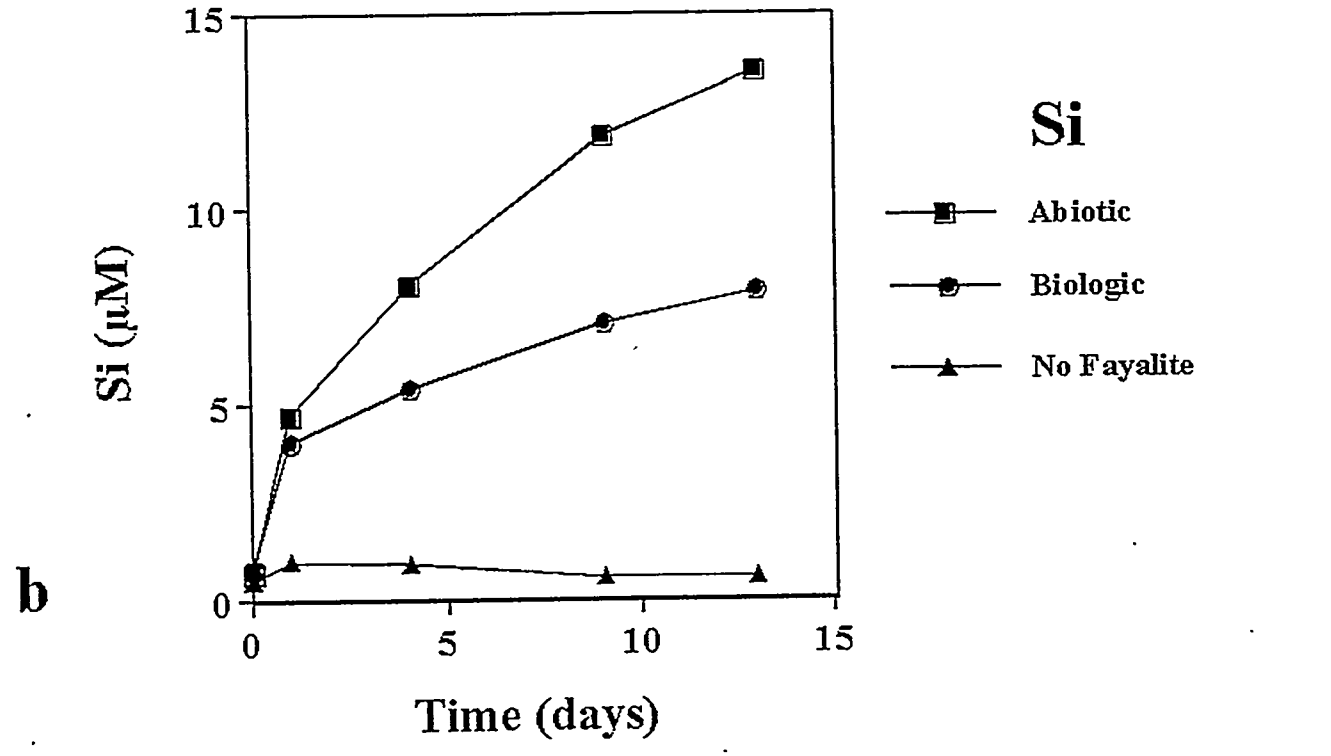
10
15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Biologic
No Fayalite
Si (µM)
Time (days)
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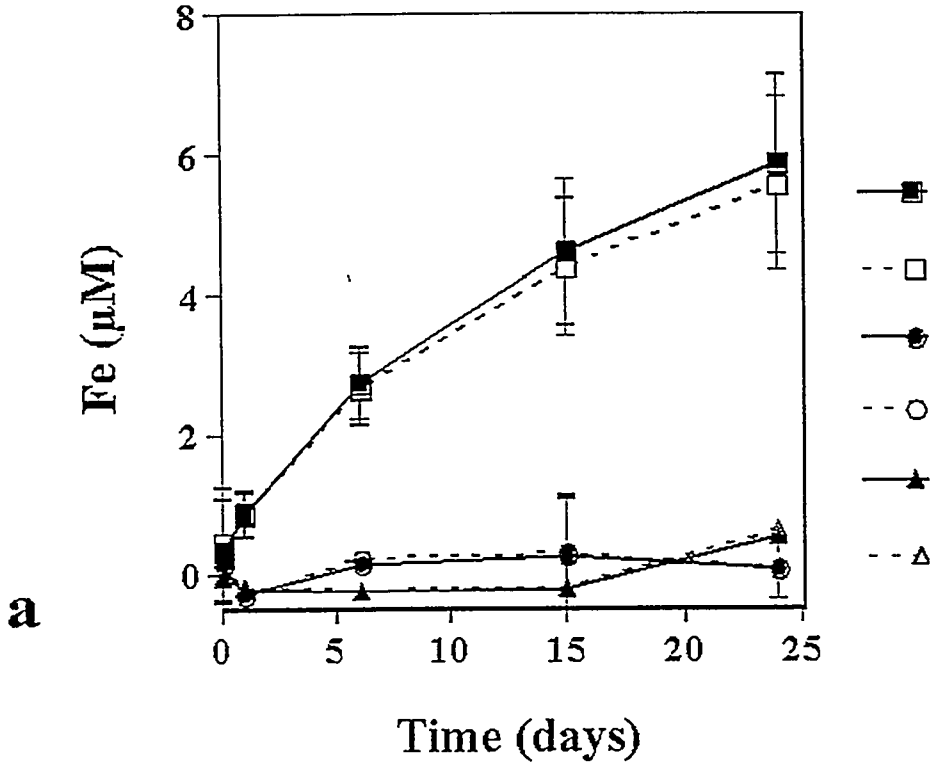
Figure 6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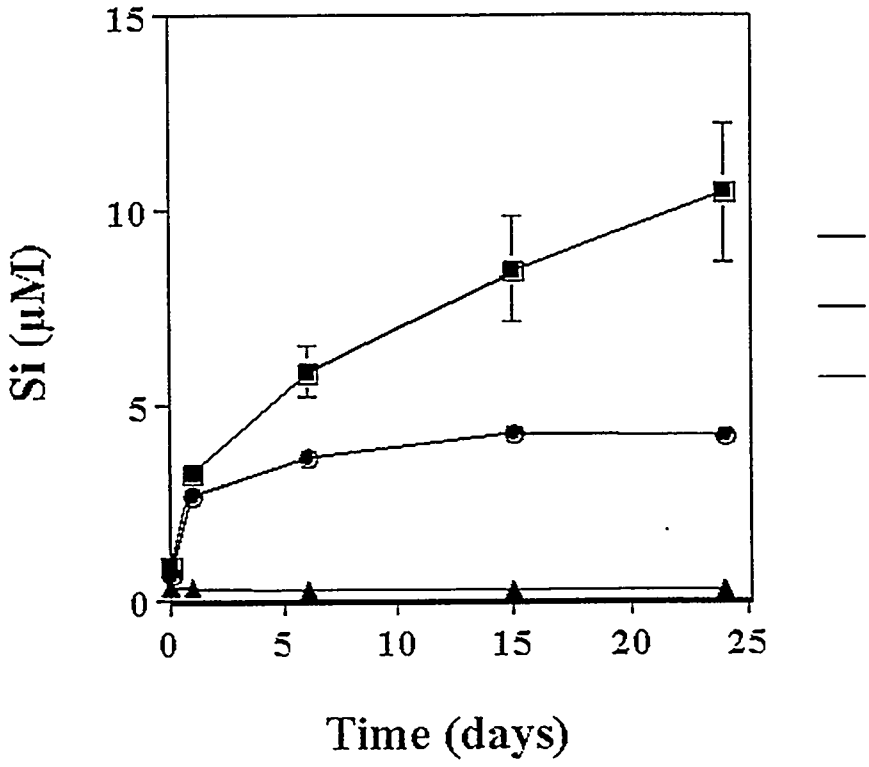
25
Time (days)

a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Si (µM)
0
5
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15
20
25
Time (days)

b

Figure 7

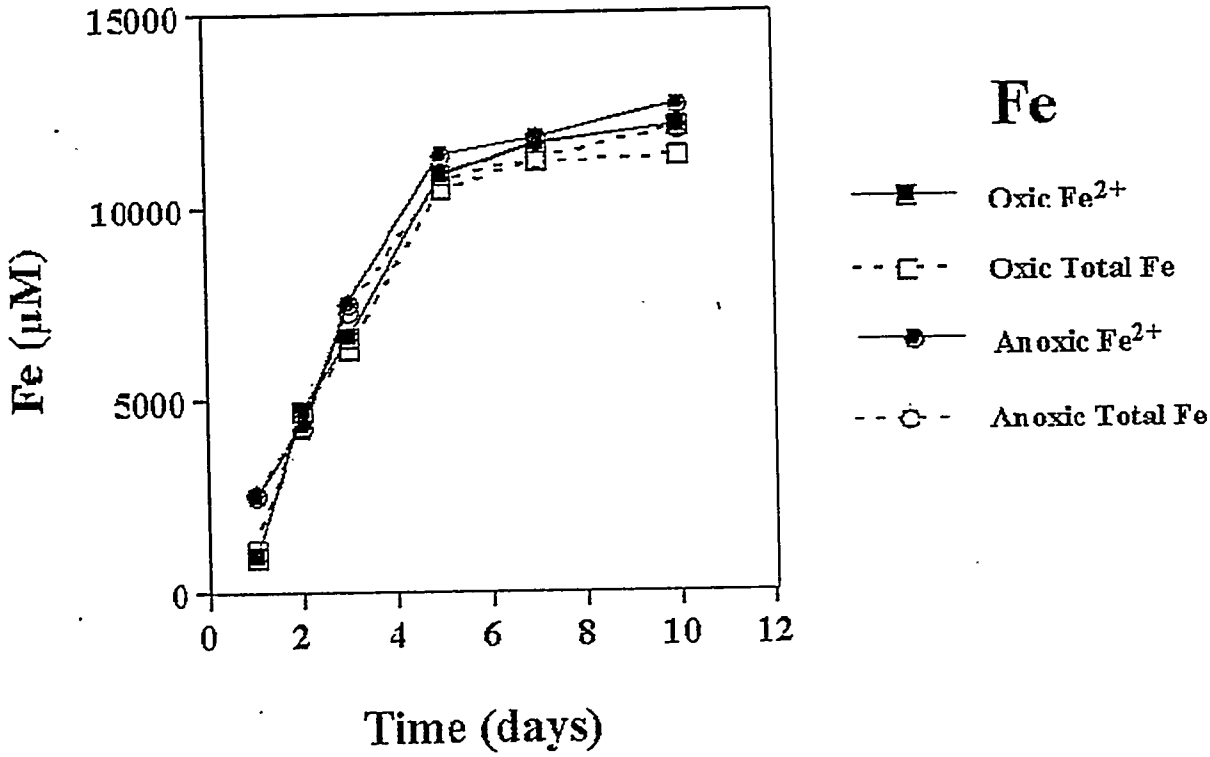


Figure 8

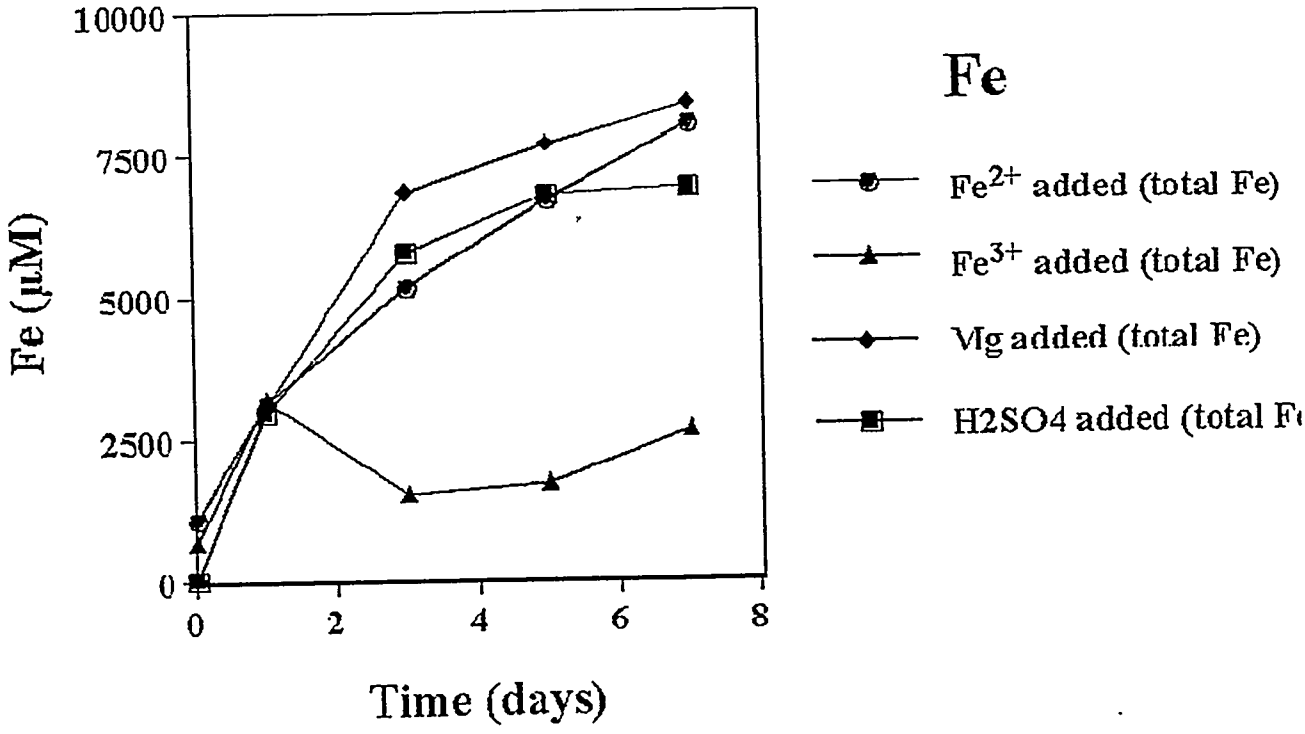
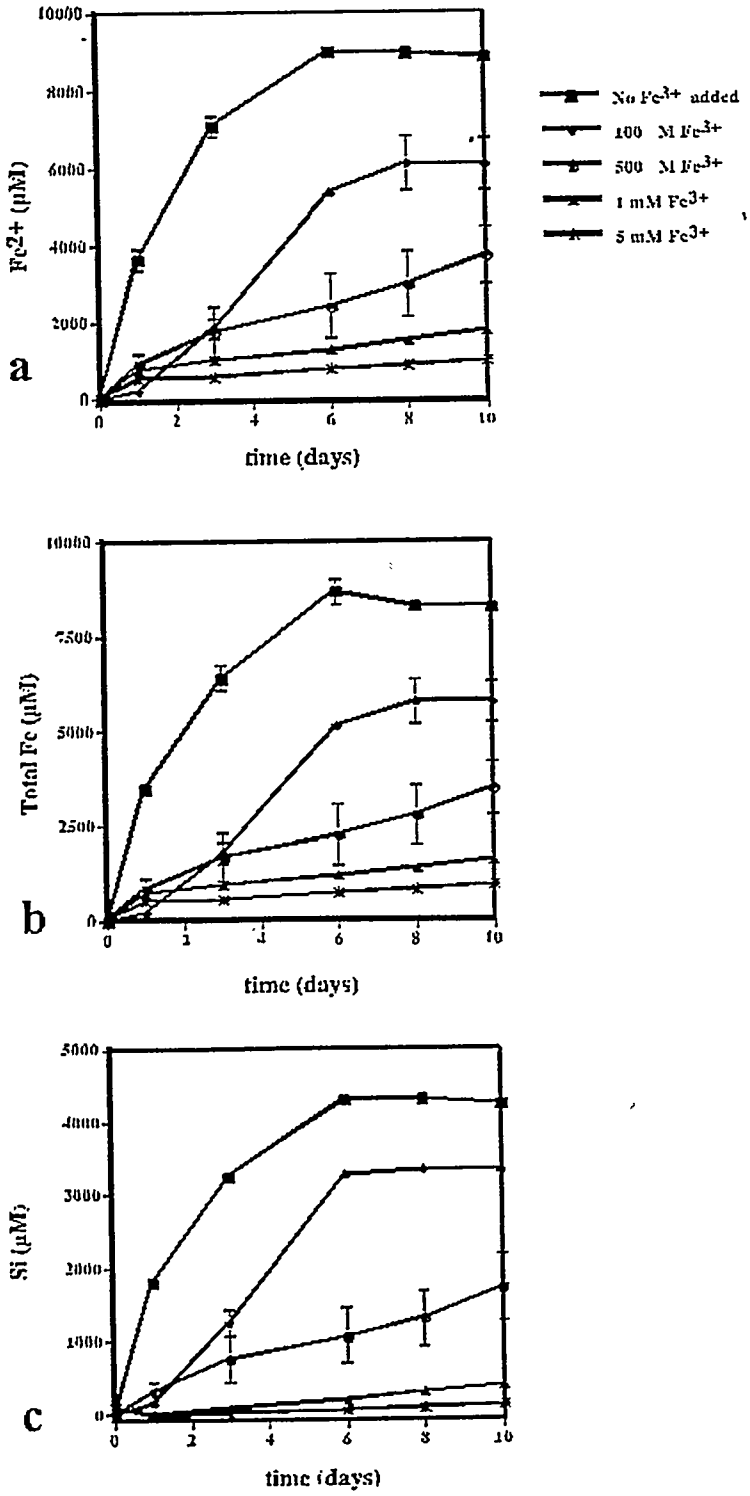


Figure 9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time (days)
No Fe^{3+} added
100 M Fe^{3+}
500 M Fe^{3+}
1 mM Fe^{3+}
5 mM Fe^{3+}
b
Total Fe (μM)
time (days)
c
Si (μM)
time (days)