LA-UR-97- 3371

Approved for public release;
distribution is unlimited

Title: Microbial Acquisition of Iron from Ferric Iron
Bearing Minerals

Author(s): L. E. Hersman, LS-7
G. Sposito, UC-Berkeley

Submitted to: DOE Office of Scientific and Technical Information

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Los Alamos
NATIONAL LABORATORY

Los Alamos National Laboratory, an affirmative action/equal opportunity employer, is operated by the University of California for the U.S. Department of Energy under contract W-7405-ENG-36. By acceptance of this article, the publisher recognizes that the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or to allow others to do so, for U.S. Government purposes. Los Alamos National Laboratory requests that the publisher identify this article as work performed under the auspices of the U.S. Department of Energy. Los Alamos National Laboratory strongly supports academic freedom and a researcher's right to publish; as an institution, however, the Laboratory does not endorse the viewpoint of a publication or guarantee its technical correctness.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

**Portions of this document may be illegible
in electronic image products. Images are
produced from the best available original
document.**

Microbial Acquisition of Iron from Ferric Iron Bearing Minerals

Larry E. Hersman*
Los Alamos National Laboratory

Garrison Sposito*
University of California-Berkeley

Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). Iron is a universal requirement for all life forms. Although the fourth most abundant element in the geosphere, iron is virtually insoluble at physiological pH in oxidizing environments, existing mainly as very insoluble oxides and hydroxides. Currently it is not understood how iron is solubilized and made available for biological use. This research project addressed this topic by conducting a series of experiments that utilized techniques from both soil microbiology and mineral surface geochemistry. Microbiological analysis consisted of the examination of metabolic and physiological responses to mineral iron supplements. At the same time mineral surfaces were examined for structural changes brought about by microbially mediated dissolution. The results of these experiments demonstrated that 1) bacterial siderophores were able to promote the dissolution of iron oxides, 2) that strict aerobic microorganisms may use anaerobic processes to promote iron oxide dissolution, and 3) that it is possible to image the surface of iron oxides undergoing microbial dissolution.

Background and Research Objectives

Iron is nearly a universal requirement for all life forms, where it is used for DNA synthesis, electron transfer proteins, hemoglobin, and nitrogen fixation. Although iron is abundant in the environment, iron suffers from an extreme insolubility in an aerobic environment at physiological pH (solubility $< 10^{-9}$; Schwertmann, 1991). This insolubility of iron at first would appear to present a paradox to organisms akin to the sailor's lament, "water water everywhere but not a drop to drink." In other words, living systems have developed an essential requirement for an unavailable element. Hence it is not surprising

*Co-Principal Investigators, E-mail: hersman@lanl.gov and gsposito@nature.berkeley.edu

that aerobic and facultative anaerobic microorganisms have developed a high affinity system for Fe (III) acquisition. The iron uptake pathway is known to consist of two components, namely, siderophores and the siderophore transport apparatus.

Siderophores are low-molecular-mass, ferric-specific ligands that are induced at low iron concentrations for the purpose of biological assimilation of Fe(III). Over 200 siderophores have been isolated and several score have been fully characterized to date, most being classified chemically as either catechols or hydroxamates. Siderophores have a very strong binding affinity for Fe(III), with complex formation constants in the range of 10^{30} or higher. An effective ligand for Fe(III) chelation has a molecular mass of 500–1000 daltons; however, such a molecular size exceeds the free diffusion limit of the outer membrane of microorganisms. In order to compensate, microorganisms have specialized surface proteins (receptors) and associated internal enzymes and proteins that transport the siderophore-Fe(III) complex to the sites of physiological utilization (Inouye, 1979).

Siderophores and the siderophore transport apparatus are areas of active scientific research. Indeed, much is known about siderophore structure, the coordination chemistry of the siderophore-Fe(III) complex, membrane receptors, molecular genetics and regulation, and iron storage. Ironically, for all that is known, the initial acquisition of Fe(III) in an aerobic environment is still a mystery. For years it has been assumed that siderophores are directly responsible for the dissolution of ferric iron from iron bearing minerals, but there is no documentation of this assumption in the literature. Few studies have investigated the acquisition of iron in an aerobic environment at physiological pH; as a consequence, there is almost no quantitative literature on this topic. Therefore, the purpose of this research was to elucidate the mechanism(s) that are used by microorganisms to solubilize mineral iron in natural environments.

The specific objectives of the proposed research were:

- A. To determine the rate of iron dissolution by selected microorganisms for representative ferric minerals,
- B. To determine the physiological and metabolic responses of microorganisms to iron deprivation in the presence of ferric minerals,
- C. To determine the effect that microorganisms have on the surfaces of ferric minerals, and
- D. Using the above information, to determine the mechanism(s) used by microorganisms to obtain ferric iron from minerals.

Importance to LANL's Science and Technology Base and National R&D Needs

The subject of the research is directly related to research activities both at Los Alamos National Laboratory (LANL) and at the University of California-Berkeley (UCB), and is an outgrowth of collaboration between the authors. The research is applicable immediately to work being done on the Yucca Mountain Project (YMP). The ability of microorganisms to solubilize ferric iron oxides in minerals is related to their ability to solubilize plutonium oxides because there are remarkable similarities between Pu(IV) and Fe(III). These include the similar ionic radius/charge ratio for Fe(III) and Pu(IV) (4.6 and 4.2, respectively), and their formation of highly insoluble hydroxides. Information gained from the research on iron oxide solubilization could be of immediate use in experimental design of Pu binding experiments, and to develop bounding calculations regarding siderophore-promoted Pu transport in subsurface environments. Our work can contribute substantially to this objective by providing essential, basic information about the surface reactions of Fe minerals in the presence of microorganisms.

Scientific Approach and Accomplishments

Microorganisms could use one of several mechanisms, or combinations of mechanisms, for obtaining ferric iron from the environment. Firstly, siderophores could be used to destabilize the iron-oxygen covalent bonds of a mineral through ligand promoted dissolution. "Ligands shift the electron density towards the central metal ion (in this case iron), thus enabling the detachment of the central metal ion into solution" (Stumm, 1992). Secondly, microorganisms could use the central metal ion as a terminal electron acceptor for oxidative phosphorylation. Here electrons generated as part of catabolic energy production would be transferred through cytochromes and other electron transport molecules to ferric oxides. As with ligand-promoted dissolution, the addition of electrons to the central metal ion promotes detachment. Finally, through general metabolic activities, microorganisms could alter the immediate surface pH and redox environment, thereby altering the solubility of the metal ion. In this scenario, direct microbial contact with the surface may be required. Our research was directed towards investigating the above mechanisms and to determine which one, individually or in combination with each other, is used to acquire iron. The approach was to perform a series of experiments, which when analyzed together provided a more complete view of microbial mediated mineral dissolution.

Guided by these goals, we have conducted a series of experiments to examine the uptake of Fe by an aerobic bacteria from Fe oxides, and to examine the effects of the microorganisms on the surface of Fe oxides. The microorganism used for these experiments was a soil bacterium isolated from surface soil samples collected at the Nevada Test Site. Fatty acid analysis performed by Dr. Myron Sasser of Microbial ID, Inc. (Newark, DE), determined this bacterium to be most similar to *Pseudomonas stutzeri*. This organism tested positive for catalase, oxidase, nitrate reduction (assimilatory); but tested negative for fermentation, and dissimilatory nitrate (unlike *P. stutzeri*), Fe, and sulfate reduction. Therefore, this organism is an obligate aerobe, not capable of using Fe as a terminal electron acceptor for oxidative phosphorylation.

Growth on FeEDTA. We first examined the growth of the *Pseudomonas* sp. as a function of Fe (FeEDTA) concentration. FeEDTA was added to 125 ml Teflon™ flasks containing 20 ml of succinate medium, yielding final Fe concentrations of 0, 0.08 μM , 0.4 μM , 2.0 μM , and 10 μM . The flasks were incubated aerobically at room temperature (22°C) for 6 days while being agitated at 50 rpm. Growth was measured by absorbance at 600 nm wavelength (Fig. 1).

Growth on Hematite. A simple way to demonstrate that iron is being obtained from a mineral is to monitor microbial growth. As stated earlier, iron is an essential nutrient for growth; therefore, if it is limiting, microorganisms should respond with limited growth. If, under iron limitation, iron is supplied in the form of a mineral and one observes increased microbial growth, then one can assume that the microorganism is obtaining iron from the mineral.

Synthetic Fe(III) oxide (hematite) was prepared according to Schwertmann and Cornell's (1991) recipe for hematite. This synthetic hematite consisted of hexagonal plates up to 1.5×10^{-6} m in size, and had a surface area of approximately $29 \text{ m}^2\cdot\text{g}^{-1}$. The hematite was examined using Scanning Electron Microscopy (SEM) equipped with Energy Dispersive Spectroscopy (EDS). EDS analyses confirmed that the mineral was pure, but SEM analysis revealed that some goethite was also present. This process commonly produces goethite (Schwertmann, personal communication); therefore for simplicity this combination of hydrous Fe(III) oxides (goethite and hematite) will be referred to as hematite for the remainder of this paper.

Hematite was added to 125 ml Teflon™ flasks containing 20 ml of succinate medium to a concentration of either 0.0, 6.7×10^{-6} , 1.8×10^{-5} , 3.9×10^{-5} , or 1.7×10^{-4} ($\text{g}\cdot\text{ml}^{-1}$). Prior to conducting the experiment the control flasks were premixed with

hematite for 6 days, then the hematite was removed by filtration. All the flasks were inoculated and incubated at 22°C for 6 days at 50 rpm.

The FeEDTA flasks contained the same concentration (10 μM FeEDTA) as the previous experiment. (Prior to conducting the experiment the control flasks were premixed with hematite for 6 days, then the hematite was removed by filtration). The flasks were inoculated and incubated at 22°C for 6 days at 50 rpm. The results presented in Fig. 2 show clearly that the bacteria responded to increasing amounts of hematite. As with the FeEDTA growth experiment, the low growth on the lower hematite concentrations can be interpreted as iron limitation. The lack of growth in the control flasks demonstrated the iron was not being removed from the hematite by the succinate medium, otherwise iron would have been leached from the hematite in the 6 day preconditioning period and would have been available for microbial growth. These results clearly demonstrate that this aerobic bacteria was able to obtain iron from hematite to support growth. Based on comparisons to growth on FeEDTA, the area-base dissolution rate of Fe was approximately $10^{-10} \text{ mol m}^{-2} \text{ h}^{-1}$.

Siderophore/Hematite. Hersman et al. (1995) have demonstrated that the siderophore produced by the *Pseudomonas* sp. is capable of promoting hematite dissolution. Briefly, the siderophore from the *Pseudomonas* sp. was purified as described by Hersman et al. (1993). Triplicate batch dissolution experiments were conducted in acid washed 250 ml Teflon™ flasks containing 50 ml H_2O (ionic strength = 10^{-3} M NaNO_3). This experiment was performed at pH 3 to allow for the comparison to published results of other ligand promoted dissolution experiments performed at pH 3 (Zinder et al., 1986). The hematite and siderophore concentrations were $4.32 \times 10^{-4} \text{ g}\cdot\text{ml}^{-1}$ and $2.85 \times 10^{-5} \text{ M}$, respectively. The experiments were performed in the dark under positive ultra-pure N_2 pressure ($2.5 \text{ ml}\cdot\text{s}^{-1}$) at 21°C, while being agitated at 100 rpm. Consecutive sampling of the stirred hematite suspensions over time periods up to 25 h was effected by syringe, filtered (0.2 micron), and measured for total soluble Fe using a Perkin-Elmer ICP-AA at 259 nm wavelength. At 2.5 h the concentration of soluble iron increased to greater than $3.0 \mu\text{M Fe/liter}$, then slowly increased to $6 \mu\text{M Fe/liter}$ at 24 h (Fig. 3). Clearly, the siderophore enhanced Fe dissolution. Typical linear plots (Stumm and Sulzberger, 1992) gave an excellent fit to a regression equation ($R^2 > 0.992$). The slope of this equation corresponded to area-based dissolution rates of $10^{-8} \text{ mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$.

Iron Reduction. We examined the extracellular products of the bacteria for the capability of reducing Fe(III), as supplied as FeEDTA or hematite. Bathophenanthrolinedisulfonic acid (4,7-diphenyl-1, 10-phenanthrolinedisulfonic acid [BPS]), a chelator of ferrous iron, is often used to determine Fe(II) concentrations in various samples and is used to determine iron reduction in biological systems (Cowart et. al., 1993). In this experiment, BPS was used to investigate the production, and the size, of extracellular Fe reductants by the *Pseudomonas* sp. Sixty-five ml of the succinate medium (with 10 μ M FeEDTA supplement) was inoculated, and incubated at 30°C until the absorbance equaled 0.8 (middle log growth, Fig. 1) at 600 nm wavelength. The medium was filter sterilized (0.2 micron), and 15 ml was saved and is referred to as "0.2 micron" (Table 1). To the remaining 50 ml, 5 ml was removed and boiled (approx. 93°C at 2625-m elevation) for 30 min (labeled "Boiled"). The remaining 45 ml of filtrate that was passed through a 3000 molecular weight cutoff sieve of which 15 ml was saved and labeled "3000 mw filtrate." The final 30 ml was placed above a 500 mw cutoff sieve. Fifteen ml was passed through the sieve (labeled "500 mw filtrate") and the remaining 15 ml that was retained by the 500 mw sieve was saved and labeled "500 mw concentrate." To 2 ml of each of the above samples 2.0 μ M of FeEDTA and 0.5 μ M of nicotinamide adenine dinucleotide (NADH) was added. Following a 30-min incubation at 22°C in the dark, 6.6 mM of BPS was added and this solution was incubated in the dark at 22°C for 30 min.

In a second experiment, we investigated the reduction of Fe in hematite by the filtrate. In this experiment (referred to as "hematite"), 1.7×10^{-3} g of hematite (instead of FeEDTA) and 5.0 μ M of NADH were added to 10 ml of "0.2 micron" filtrate and incubated in the dark at 22°C for 30 min. Two milliliters were removed and filtered (0.2 micron). To this filtrate 6.6 mM of BPS were added and the solution was incubated in the dark for 30 min.

Both of the reduction experiments (FeEDTA and hematite) were performed in triplicate. Absorbance (535 nm) was measured against a control (deferrated, uninoculated succinate medium plus either FeEDTA or hematite, NADH, and BPS at concentrations described above) in the reference beam of a dual-beam Perkin-Elmer Lambda 3B UV-Vis spectrophotometer.

The results of the Fe reduction assays are presented in Table 1. The concentration of reduced Fe for the "0.2 micron" filtrate was 4.2 μ M, indicating that the bacteria produced an extracellular compound that causes iron reduction. The "boiled" treatment concentration was 2.8 μ M, which suggests that the compound was not completely

denatured by boiling. The concentration of the "3000 mw filtrate" was 4.4 μM , virtually the same as the concentration of the "0.2 micron" filtrate, indicating that the Fe reduction compound(s) had a molecular weight smaller than 3000 daltons, as all of the reduction activity passed through the sieve. However, the Fe concentration in the "500 mw filtrate" was less than half (1.8) that of the "3000 mw filtrate" (4.4), while the Fe concentration in the "500 mw concentrate" was 7.3 μM , nearly twice the "3000 mw filtrate." This indicates that the reduction activity was retained by the 500 mw cutoff sieve. Therefore, the size of the compound(s) causing the reduction was between 500 and 3000 daltons.

Table 1. Average and standard deviation values for μM of Fe(III) reduced to Fe(II). Fe(II) concentration was determined using $A = alc$; where A = absorbance (535 nm), a = molar absorptivity of the BPS/Fe(II) complex @ 535 nm = 22,140 liters $\text{eq}^{-1} \text{cm}^{-1}$, l = length of the light path (1.0 cm), and c = concentration.

	Treatment					
	0.2 micron	Boiled	3000 mw filtrate	500 mw filtrate	500 mw Conc	Hematite
Average	4.2	2.8	4.4	1.8	7.3	2.0
Std. Dev.	0.36	0.41	0.77	0.26	0.99	0.14

Heat stability is not a common characteristic among proteins (there are some notable exceptions, such as RNAases), but rather a general characteristic of peptides. This information is consistent with what is known regarding the siderophore produced by this microorganism. The siderophore has a molecular weight of 1150 daltons and contains several amino acid residues with peptide linkages (IR data not shown), which would give it heat stability.

Finally, there did appear to be some reduction of the Fe in hematite, as indicated by the presence of 2.0 μM of Fe(II) in the "hematite" experiment.

High Performance Liquid Chromatograph (HPLC). Twenty nine milligrams of hematite was added to each of 10 flasks containing succinate medium that had been extracted with 8-hydroxyquinoline. An additional 10 flasks containing unextracted succinate medium served as a negative control. All 30 flasks were then inoculated with 1 ml of a 12 h culture of the *Pseudomonas* sp., and incubated at 22°C while shaking at 50 rpm.

On days 1, 2, 4, 7, and 11 the extracellular metabolites in two flasks of the hematite and two flasks of the control medium were analyzed using HPLC. Suspended solids (cells

and hematite) were removed by centrifugation (4000 G, 1 h, 4°C) and filtration (0.2 micron). The supernatant (750 ml) was reduced in volume to 10 ml using roto-evaporation. The 10-ml concentrate was lyophilized, weighed, and 40 mg was resuspended in 1.0 ml of deionized H₂O. This solution was injected into a Waters HPLC equipped with a Waters C-4 Delta Pak column. The samples were eluted at 2.0 ml·min⁻¹, using a gradient of 99.9% H₂O/0.1% trifluoroacetic acid (TFA) to 99.9% acetonitrile/0.1% TFA over 200 min and monitored at 230 nm. The results of these experiments are presented in Figs. 4 and 5. Each of these fractions was analyzed for Fe using a Perkin-Elmer ICP-AA. Although these results were averaged for peak area, this analysis was qualitative due to the difficulty in obtaining a quantitative weight for the material in the HPLC fraction. The results of this analysis are presented in Fig. 6.

For comparison, under similar experimental conditions, this bacterium was grown on FeEDTA as an Fe source (10 µM). These cultures were analyzed after 7 days of incubation, and the results are presented in Fig. 7.

The data presented in Figs. 4 and 5 illustrate the differences in the metabolic responses of the *Pseudomonas* sp. to hematite and a culture without added Fe. Several peaks were present in the hematite experiment (Fig. 4), but were absent in the control (Fig. 5). Apparently, when Fe was only available as hematite, this resulted in the induction of several metabolic products. This becomes more apparent when one compares growth on hematite to growth on FeEDTA. In the presence of FeEDTA few metabolic products appeared after 7 days of incubation, which was ample time for such products to be induced. The optical densities of the hematite and FeEDTA were comparable (1.0 abs, 600 nm) after 7 days of growth; therefore, differences in metabolic response can be attributed to Fe source and not to a difference in cell density.

For the hematite experiment the response of the bacteria increased with time, with days 7 and 11 having the largest peak heights. In previous experiments we have demonstrated that peaks in the 55 to 73 min range are siderophores (Hersman et al., 1993). Interestingly, ICP-AA analysis of these peaks showed an intermediate amount of Fe (Fig. 6). Peaks in the 37 and 55 min range are unidentified, yet these peaks contained the most Fe (Fig. 6). Peaks in the 5 and 20 min ranges did not contain significant amounts of Fe.

AFM and TMAFM. A Nanoscope III atomic-force microscope (AFM) manufactured by Digital Instruments was used in these experiments. Design and operation of the AFM in contact mode in air and aqueous solutions has been described in detail elsewhere (Hochella et al., 1990; Johnsson et al., 1991; Maurice et al., 1995). For in-situ

experiments, integrated Si_3N_4 tips were used. For ex-situ TMAFM experiments, integrated Si tips were used.

Methods for culturing bacteria with hematite have been described earlier, while methods for preparing reacted samples (bacterial promoted dissolution of hematite) evolved over the course of these experiments. Following the method of Lindahl and Bakken (1994), Nycodenz (Gibco BRL) was used to separate the bacteria from the hematite surfaces. These extracts were syringe filtered through Nucleopore polycarbonate (pc) membrane filters, in the method described by Maurice et al. (1995). The extraction procedure was modified whereby samples were imaged prior to extraction, following simple extraction in dionized water with centrifugation, and following Nycodenz extraction with additional dionized water washing to remove the Nycodenz. It was found that the dionized water/centrifugation process did not adequately separate the bacteria from the particles. Either imaging particles with no extraction or with Nycodenz extraction followed by water washing appeared to be viable procedures. The former allowed imaging of particles containing bacterial remains, while the latter allowed imaging of the actual particle surfaces: however, as only a few such surfaces have as yet been imaged, further evaluation of the potential artifacts is needed.

When dissolution features were observed, dissolution tended to be limited to a few particles and the dissolution appeared to be very general. The dissolution features did not resemble those types associated with the more classical ligand or reductive features typically seen by Maurice (personal communication). Rather, the dissolution promoted by the *Pseudomonas* sp. is a nonspecific destruction of vast areas of the hematite particle(s) (Fig. 8).

Finally, it is important to point out that we were able to image bacteria in situ, attached to the surfaces of the hematite. This ability will allow us to monitor specific locations of the surface of the hematite for preferential attachment of bacteria, and to further monitor the attached bacteria for development and enlargement of pits.

Summary of Technical Progress

From the data presented in this section one can conclude that:

1. The *Pseudomonas* sp., a strict aerobe, was able to obtain Fe from hematite.
2. The siderophore produced by this microorganism had a significant effect on the dissolution of hematite, comparable to that of common organic acids (oxalate or ascorbate) at much higher concentrations.

3. An extracellular compound(s) produced by the *Pseudomonas* sp. is capable of reducing the iron contained in hematite.
4. The compound(s) responsible for reductase activity has a molecular weight between 500 and 3000 daltons and is relatively heat stable.
5. There were significant differences among the metabolic responses of the *Pseudomonas* sp. to different sources of Fe. Most notable was the response of the bacterium to hematite. Hematite appeared to induce several metabolites, including siderophores. As measured by ICP-AA, the siderophores did not contain the majority of the Fe; but rather the Fe was concentrated in intermediate peaks that eluted at 37 and 55 min.
6. Few particles show dissolution features (using AFM), but those that do have profound surface disturbances.
7. Results of the AFM work clearly emphasize the importance of sample preparation, including selection of starting materials, and of minimizing the introduction of contaminants onto the surfaces being examined.
8. The use of controls is important for AFM image interpretation. Control samples must be put through the full routine of procedures used for the experimental samples, because artifacts can originate at any step along the preparation pathway.
9. Given the heterogeneity of the samples' surface structure, composition, microtopography, reactivity, and the likelihood of artifacts, samples must be imaged in numerous locations and under a wide variety of conditions. Although AFM imaging of such complex systems is difficult and time-consuming, a painstaking approach is needed.

In conclusion, these studies have demonstrated that microorganisms use more than one mechanism to promote the dissolution of Fe oxides. These studies have contributed to our basic understanding of dissolution processes. This research will continue under other auspices beginning in FY97. The following are publications, invited papers, and abstracts from this project.

Publications

- Hersman, L., P. Maurice, and G. Sposito. 1996. Iron Acquisition from Hydrous Fe(III) Oxides by an Aerobic *Pseudomonas* sp. *Chem. Geology*. In press.
- Maurice, P., L. Hersman, and G. Sposito. 1996. Application of Atomic-force Microscopy to Studies of Organic and Microbial Interactions with Hydrous Fe(III) Oxides. *Chem. Geology*. In press.
- Forsythe, J., P.A. Maurice, L. Hersman, and G. Sposito. 1996. Imaging the results of bio-geochemical reactions with tapping mode atomic force microscopy. *Goldschmidt Geochemistry Conference*, in press.
- Hersman, L., T. Lloyd, and G. Sposito. 1995. Siderophore-Promoted Dissolution of Hematite. *Geochim. Cosmochim. Acta*. 59(16):3327-3330.
- Story, S.P. and L.E. Hersman. 1996. Iron acquisition and growth kinetics of an aerobic *Pseudomonas* sp. on iron oxide minerals: Is bacterial attachment necessary to obtain iron under iron limiting conditions. In preparation for *Geomicrobiology J.*

Invited Papers

- Hersman, L., M. Harrington, M. Martinez, S. Story, P. Maurice, and J. Forsythe. 1996. Dissolution of hematite by an aerobic *Pseudomonas* sp. *Gordon Conference. Environmental Sciences: Water*. 23-29 June. New Hampton, NH.
- Hersman, L., P. Maurice, and G. Sposito. 1995. Iron acquisition from hematite by an aerobic *Pseudomonas* sp. *Ann. Meeting American Chemical Society*. 2-6 April. Anaheim, CA.
- Maurice, P.A., J. Forsythe, G. Sposito, and L. Hersman. 1995. Applications of atomic-force microscopy to studies of organic and microbial interactions with hydrous iron oxides. *Invited paper, Ann. Meeting American Chemical Society*. 2-6 April, Anaheim, CA.
- Hersman, L., T. Lloyd, and G. Sposito. 1994. Microbial enhanced mineral dissolution. *Ann. Meeting American Chemical Society*. 13-17 March, San Diego, CA.

Abstracts

- Hersman, L., T. Lloyd, and G. Sposito. 1994. Iron Uptake by Microorganisms from Iron Oxides. *Abs. Ann. Meeting ASM.*, 319.

References

- Cowart, R.E., F.L. Singleton, and J.S. Hind. 1993. *Anal. Biochem.* 211:151-155.
- Hersman, L.E., P.D. Palmer, and D.E. Hobart. 1993. *Mat. Res. Soc. Symp. Proc.* 34:765-770.
- Hersman, L., T. Lloyd, and G. Sposito. 1994. Iron Uptake by Microorganisms from Iron Oxides. *Abs. Ann. Meeting ASM.*, 319.
- Hochella, M.F., Jr., C.M. Eggleston, V.B. Elings, and M.S. Thompson. *American Mineralogist.* 75:723-730.
- Inouye, M. 1979. *Bacterial Outer Membranes.* J. Wiley and Sons, New York.
- Johnsson, P.A., C. M. Eggleston, and M.F. Hochella, Jr. 1991. Imaging molecular-scale structure and microtopography of hematite with the atomic force microscope. *American Mineralogist.* 76:1442-1445.
- Lindahl, V. and L.R. Bakken. 1995. *FEMS Microbiol. Ecol.* 16: 135-142.
- Maurice, P.A., M.F. Hochella., Jr., G. Sposito, and U. Schwertmann. 1995. *Clays and Clay Minerals.* 43:60-81.
- Schwertmann, U. 1991. *Plant and Soil*, 129:1-25.
- Schwertmann, U. and R.M. Cornell. 1991. *Iron Oxides in the Laboratory: Preparation and Characterization.* V.C.H. Weinheim.
- Stumm, W. 1992. *Chemistry of the Solid-Water Interface.*, J. Wiley and Sons, New York.
- Stumm W. and G. Furrer. 1987. In *Aquatic Surface Chem.* J. Wiley and Sons, New York. pp. 197-219.
- Stumm W. and B. Sulzberger. 1992. *Geochim. Cosmochim. Acta.* 56:3233-3257.
- Zinder, B., G. Furrer, and W. Stumm. 1986. *Geochim. Cosmochim. Acta.* 50:1861-1869.

Figure 1. Growth versus Fe concentration.

Figure 2. Growth versus hematite concentration (g/ml).

Figure 3. Siderophore dissolution of hematite.

Figure 3. Siderophore dissolution of hematite.

Figure 4. Metabolic response of *Pseudomonas* sp. to hematite as an Fe source, as measured by HPLC.

Figure 5. Metabolic response of *Pseudomonas* sp. in an Fe-free medium, as measured by HPLC.

Figure 6. ICP-AA measurements of Fe (ppm) for each of the major HPLC fractions of a 7-day-old culture of a *Pseudomonas* sp. (hematite as an Fe source). Control measurements were made at 3, 47, and 74 min. (as indicated by the arrows) and were 0.189, 0.376, and 0.089 ppm, respectively.

Figure 7. HPLC chromatograms of extracellular metabolites (FeEDTA - upper, hematinic - lower) on a reverse phase analytical column.

Figure 8. AFM image of microbially promoted hematite dissolution.