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# ***Final Report***

**DOE Project DE-FG02-99ER15002**

## **Microbial Community Acquisition of Nutrients from Mineral Surfaces**

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Microbial Community Acquisition of Nutrients from Mineral Surfaces

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**PRINCIPAL OBJECTIVES:**

Minerals and microbes undergo complex interactions in nature that impact broad aspects of near-surface Earth chemistry. Our primary objective in this project was to gain insight into how microbial species and communities acquire critical but tightly held nutrients residing on or within minerals common in rocks and soils, and to quantitatively study related microbe-mineral interactions including cell adhesion, electron transfer, and siderophore-mineral interaction processes.

## **PROJECT DESCRIPTION:**

The overall purpose of this project was to search for and delineate fundamental physiological strategies and/or environmental conditions by which microbial species interact with minerals and mediate release of strongly bound nutrients/contaminants from mineral surfaces. The underlying hypothesis is that microbes employ specific physiological strategies and/or take advantage of specific environmental circumstances that result in release of chemical components tightly bound to mineral surfaces. This was studied in various ways, including 1) using a model nutrient-mineral system of orthophosphate sorbed to goethite in the presence of a soil derived microbial consortium, 2) using biological force microscopy (BFM) techniques to characterize interactions of metal reducing bacteria with iron oxyhydroxide minerals, and 3) using chemical force microscopy (CFM) to study the interaction of bacteria-produced siderophores on iron oxyhydroxide minerals.

## **RESULTS AND CONCLUSIONS:**

The principal results and conclusions of this three-year study are given in the bulleted list below. Peer-reviewed publications from this project are listed in the section following this one. Full details of our work can be found within these publications.

- We ran long-term (several weeks to two months) chemostat experiments using a soil inoculum and synthetic goethite with sorbed orthophosphate (Tadanier, 2002). In these runs, we determined that the consortium utilized about 80% of the sorbed phosphate during the four-day hydraulic retention time in the chemostat. A low molecular-weight organic acid produced by one or more members of the consortia was identified as either citric or gluconic acid. Goethite was also reductively dissolving, and it aggregated at low glucose concentrations in the chemostat bioreactor. Methods were optimized to allow direct extraction of DNA from microorganisms in our soil inoculum and chemostat effluent. The dominant player in our chemostat runs was identified as *Burkeholderia cepacia*. This study demonstrated the ability of a soil consortium of microorganisms to liberate a critical, tightly bound nutrient from mineral surfaces, allowing several species to thrive under the conditions of our experiments (Tadanier et al., 2002).

- As a major portion of this study just described above, we focused on the ability of various surface complexation models to adequately describe nutrient adsorption and mineral surface charging phenomena. Our results demonstrate that commonly used surface complexation models are unable to adequately describe orthophosphate-goethite adsorption behavior over a wide range of surface coverage nor adequately describe surface charging behavior. We therefore implemented the thermodynamically, electrostatically, and crystallographically more robust Charge-Distribution Multi-Site Complexation model in FITEQL 4.0, which allowed both the adsorption and surface charge data to be satisfactorily modeled for the first time (Tadanier and Eick, 2002).
- We used BFM to study the nanomechanical properties of biomolecules that bridge the interface between *Shewanella* and goethite when they are in contact. The force curves collected by BFM suggests the presence of an outer membrane protein produced by the bacterium that may play a role in mediating electron transfer to the mineral. The results of these studies provide direct evidence that microorganisms actively recognize minerals and produce or localize biomolecules at the interface with the mineral (Lower et al., 2000b, 2001, 2002)
- We also successfully isolated the siderophore azotobactin (1.3 kDa) from *Azotobacter vinelandii* (a nitrogen-fixing soil aerobe), and selectively attached it to hydrazide-terminated AFM tips using protein-coupling techniques. Using this CFM approach, we have shown that azotobactin has a much greater affinity for iron oxyhydroxide mineral surfaces compared to isostructural aluminum minerals (Kendall and Hochella, 2003). This result supports our working hypothesis that siderophore-mediated acquisition of iron involves direct interaction between the siderophore molecule and the mineral surface in aqueous Fe-limited environments. Molecular modeling of this system suggested specific siderophore-mineral conformations that most likely dominate this processes (Kendall et al., in prep.).

#### KEYWORDS:

Biogeochemistry  
 Mineral-Microbe Interactions  
 Goethite  
 Orthophosphate  
 Siderophore-Mineral Interactions  
 Biological force microscopy (BFM)  
 Chemical force microscopy (CFM)  
 CD-MUSIC model

## **PUBLICATIONS:**

Note: All publications listed below are in peer-reviewed books, journals, and proceedings. Each publication (except the abstracts listed at the end) specifically acknowledges DOE, under grant number DE-FG02-99ER15002, for financial support.

### ***Book Chapters:***

Tadanier C.J., Little J.C., Berry D.F., and Hochella M.F., Jr. (2002) Energetics of Microbial Nutrient Acquisition from Mineral Surfaces. In *Geochemical Society Special Publication – David A. Crerar Honorary Volume* (eds. R. Hollmann and S.A. Wood), Geochemical Society, St. Louis, MO, 339-364.

### ***Articles:***

Hochella M.F., Jr. (2002) Nanoscience and technology: The next revolution in the Earth sciences. *Earth and Planetary Science Letters*, 203, 593-605.

Hochella M.F., Jr. (2002) Sustaining Earth: Thoughts on the present and future roles of mineralogy in environmental science. *Mineralogical Magazine*, 66, 627-652.

Hochella M.F., Jr. (2002) There's plenty of room at the bottom: Nanoscience in geochemistry. *Geochimica et Cosmochimica Acta*, 66, 735-743.

Kendall T.A. and Hochella M.F., Jr. (in press) The measurement and interpretation of molecular level forces of interaction between the siderophore azotobactin and mineral surfaces. *Geochimica et Cosmochimica Acta*.

Kendall T.A., Becker U., and Hochella M.F., Jr. (in final stages of preparation) Molecular modeling of the interactions between the siderophore azotobactin and mineral surfaces. To be submitted to *Geochimica et Cosmochimica Acta* in June, 2003.

Lower S., Tadanier C., Hochella M.F., Jr. (2000a) Dynamics of the mineral-microbe interface: Potential applications of force microscopy to studies of mineral dissolution. *Geomicrobiology Journal*, 18, 63-76.

Lower S., Tadanier C., Hochella M.F., Jr. (2000b) Measuring interfacial and adhesion forces between bacteria and mineral surfaces with bacterial force microscopy. *Geochimica et Cosmochimica Acta*, 64, 3133-3139.

- Lower S.K., Hochella M.F., Jr., Banfield J., and Rosso K.M. (2002) Nanogeoscience: From the movement of electrons to lithospheric plates. EOS Transactions, American Geophysical Union, 83, 53-56.
- Lower S.K., Hochella M.H., Jr., and Beveridge T.J. (2001) Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and  $\alpha$ -FeOOH. Science, 292, 1360-1363.
- Tadanier C.J., Berry D.F., and Knocke W.R. (2000) Dissolved organic matter apparent molecular weight distribution and number-average apparent molecular weight by batch ultrafiltration. Environmental Science and Technology, 34, 2348-2353.
- Tadanier C.J., Berry D.F., and Knocke W.R. (2003) Modeling uncoupled solute transport in natural organic matter size fractionation by ultrafiltration. Journal of Environmental Engineering, 129, 33-43.
- Tadanier C.J. and Eick M. (2002) Formulating the charge-distribution multisite surface complexation model using FITEQL. Soil Science Society of America Journal, 66, 1505-1517.

#### ***Abstracts:***

- Cail T.L. and Hochella M.F., Jr. (2002) Experimentally derived collision efficiencies of microparticles using atomic force microscopy: Toward a better understanding of particle transport in porous media. Fall AGU, San Francisco, CA.
- Cail T.L. and Hochella M.F., Jr. (2003) Experimentally derived sticking efficiency of microspheres using atomic force microscopy: Toward a better understanding of particle transport in porous media. 2003 American Chemical Society National Meeting, Orlando, Florida.
- Hochella M.F., Jr. (2001) Contribution of Microscopy to the Environmental Sciences: From Atoms to Bacteria. Proceedings of the Microscopical Society of Canada, June, 2001. Fredericton, New Brunswick, Canada.
- Hochella M.F., Jr. (2001) There's plenty of room at the bottom: The geochemistry version (Presidential Address). 11<sup>th</sup> Annual Goldschmidt Conference Program (May, 2001), Hot Springs, VA.



- Hochella M.F., Jr. (2002) Nanoscience and technology: The next revolution in the Earth sciences. Spring AGU abstract.
- Hochella M.F., Jr. (2002) Sustaining Earth: Thoughts on the present and future roles of mineralogy in environmental science. IMA 2002, Edinburgh, Scotland.
- Kendall T. and Hochella M.F., Jr. (2002) Probing siderophore-mineral interaction using force measurements. 2002 Goldschmidt Conference, Davos, Switzerland.
- Kendall T. and Hochella M.F., Jr. (2002) Using chemical force microscopy to understand the interaction between complex organic molecules and mineral surfaces. 2002 GSA Annual Meeting, Denver, CO.
- Kendall T. and Hochella M.F., Jr. (2001) Investigating the mechanism of siderophore Fe release from mineral surfaces using chemical force and confocal laser scanning microscopy. 11<sup>th</sup> Annual Goldschmidt Conference Program (May, 2001), Hot Springs, VA.
- Kendall T. and Hochella M.F., Jr. (2002) Probing the biomolecule-mineral interface: Molecular biogeochemical techniques in force microscopy. Fall AGU, San Francisco, CA.
- Lower S. and Hochella M.F., Jr. (2001) Nanoforce and nanomechanical measurements between living microorganisms and mineral surfaces: Evidence of specific biomolecule-mineral recognition. 11<sup>th</sup> Annual Goldschmidt Conference Program (May, 2001), Hot Springs, VA.
- Lower S. K. and Hochella M. F., Jr. (2000) Mineral-microbe interactions quantitatively measured in energy and distance nanospace. American Geophysical Union Fall Meeting Program, San Francisco, CA.
- Lower S. K. and Hochella M. F., Jr. (2000) Probing the dynamic nanoscale world at the interface between microorganisms and minerals. 10<sup>th</sup> Annual Goldschmidt Conference Program (September, 2000), Oxford, UK.
- Lower S., Tadanier C., Hochella M.F., Jr., Berry D.F., and Potts M. (1999) The bacterial-mineral interface: Probing nanoscale forces with bacterial force microscopy. Geological Society of America Abstracts with Programs 1999, Denver, CO.
- Tadanier C. and Hochella M.F., Jr. (2000) An Integrated Biogeochemical Approach for Studying the Bioavailability of Microbial Nutrients Sorbed on Mineral Surfaces. 10<sup>th</sup> Annual Goldschmidt Conference Program (September, 2000), Oxford, UK.

Tadanier C. J. and Eick M. J. (2000) Surface Complexation Modeling of Pi-Goethite Adsorption Behavior: Integrating Adsorption Edge and Surface Charging Data. 10<sup>th</sup> Annual Goldschmidt Conference Program (September, 2000), Oxford, UK.

Tadanier C. J. and Lower S. K. (2001) Modeling Nanoforce Measurements Between Viable Microorganisms and Mineral Surfaces: Influence of Environment on Interfacial Phenomena. 11<sup>th</sup> Annual Goldschmidt Conference Program (May, 2001), Hot Springs, VA.