

## Electron Microscopic Characterization of Mineral-Microbe Interactions

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Microbes are capable of reducing structural Fe(III) in minerals and promoting the formation of amorphous or crystalline solid phases as byproducts. The extent and rate of bioreduction depends on a number of factors, including mineral surface area, sorption capacity of mineral surfaces for Fe<sup>2+</sup>, the presence or absence of chelating agents (i.e., AQDS and humic acids), complexing agents, electron donor or acceptor, and type of microorganisms [1]. Microbial reduction of Fe(III) in minerals often results in mineral dissolution and neof ormation of biogenic minerals [2]. Few attempts have been made to show the dynamic nature of microbial-mediated mineral reactions, especially at the micro- and nano-scale. Here we present evidence for formation of non-stoichiometric mineral phases as a result of microbial reduction of Fe(III) of magnetite. These mineral phases are important in geological processes such as metal sorption, nutrient cycling, and contaminant degradation. More importantly, if these non-stoichiometric minerals can be used as a characteristic of biologically induced solid phases, they may be used as a biosignature for the search for evidence of ancient terrestrial and extraterrestrial life.

Magnetite was produced from microbial reduction of hydrous ferric oxide (HFO). *Shewanella putrefaciens* CN32 cells were incubated with lactate as the electron donor and Fe(III) in magnetite as the sole electron acceptor in bicarbonate buffer. Controls consisted of solutions with anaerobic bicarbonate buffer in place of CN32 cell suspensions. The extent of microbial reduction of Fe(III) in the magnetite structure was monitored by measuring Fe(II) production with 1,10-phenantroline assay. Mineral products were characterized by X-ray diffraction (XRD). Transmission electron microscopy (TEM) characterization was performed to observe cell-mineral associations and to study the mineral transformation processes at the micro- to nano-scale. The procedure consisted of fixation, dehydration, and sectioning with a Reichert Ultracut E ultramicrotome as described by Dong et al. (2003). Electron energy loss spectra (EELS) and lattice-fringe images were obtained with a JEOL JEM-2010 transmission electron microscope operating at 200 keV with a LaB6 filament [3].

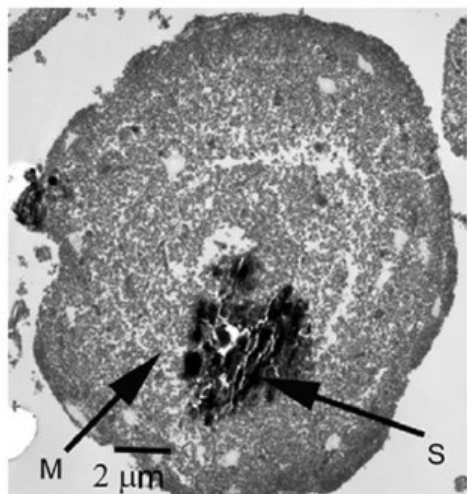
The bioproducted Fe(II) concentration increased with time, and at the end of the incubation experiment, the Fe(II) concentration was 1.5 to 1.7-fold greater in the bioreduced sample than in the uninoculated control. Initial reduction proceeded via intimate association between bacterial cells and nanometer sized magnetite crystals, where cells were coated by magnetite crystals. Magnetite crystals at the close contact with cells show non-typical selected area electron diffraction pattern, where the strongest lines for magnetite ( $d = 2.53, 1.48, 2.97, \text{ and } 1.62 \text{ \AA}$ ) are either missing or very weak. Magnetite crystals that are not in contact with cells show typical SAED pattern. As bioreduction proceeded, siderite formed in the space that was previously occupied by bacterial cells, and cells appeared to have lysed (Fig. 1). High-resolution TEM lattice fringe image showed randomly oriented magnetite nanoparticles with a fringe spacing of approximately 2.9 Å and discrete fringes for siderite with spacing of 2.5 Å (Fig. 2). Newly formed siderite SAED pattern shows typical siderite reflections ( $d = 2.80, 3.60, 1.96 \text{ \AA}$ ) as well as a strong line at  $d = 2.57 \text{ \AA}$  corresponding to the strongest line of magnetite. These characteristics suggest that microbially mediated magnetite to siderite transformation within our experimental time frame (14 days) was not complete, and

siderite and magnetite co-existed at the nano- to atomic scale. Quantitative analytical electron microscopy (AEM) analyses show non-stoichiometric Fe/O ratios for magnetite and siderite, illustrating a transitional nature of these two minerals. Siderite characteristically contains a minor amount of Ca. The transitional nature of magnetite and siderite is further confirmed by EELS. Magnetite at the cell-magnetite interface shows 50-60% of Fe(III), as opposed to 66%. Conversely, siderite shows ~90% of Fe(II) in its structure, as opposed to 100% Fe(II).

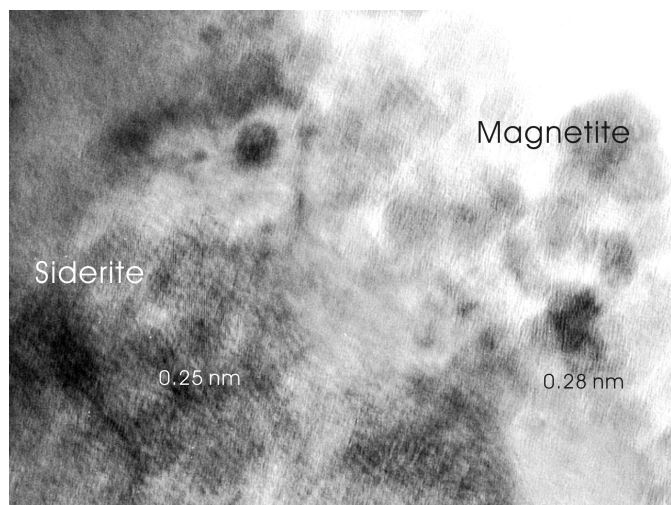
In conclusion, microbial reduction of Fe(III) in magnetite resulted in the magnetite to siderite transformation. Considering the drastic difference in the structure of these two minerals, we infer that such a biomineralization may have involved dissolution of magnetite and precipitation of siderite. Multiple lines of evidence suggest that intermediate products, such as magnetite with some characteristics of siderite, or siderite with some characteristics of magnetite resulted. Such a complex biomineralization process may be common in nature where environmental conditions are much more variable.

#### References:

- [1] Dong, H., Jaisi, D.P., Kim, J.W., and Zhang, G. (2009) Microbe-clay mineral interactions. *American Mineralogist*, 94, 1505-1519.
- [2] Melton, E. D., Swanner, E. D., Behrens, S., Schmidt, C. & Kappler, A (2014). The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle. *Nat. Rev. Microbiol.* 12, 797-808.



**Figure 1.** TEM image showing siderite inside a thick coating of magnetite particles. M: magnetite; S: siderite.



**Figure 2.** High-resolution TEM lattice fringe image of magnetite and siderite at the interface showing the interlocking texture between these two phases.