

**DETECTION OF MINERAL PRODUCTS FOLLOWING FE(III) OXIDE REDUCTION BY A THERMOPHILIC AUTOTROPHIC BACTERIUM USING REFLECTANCE SPECTROSCOPIES.** D. A. Lepo<sup>1</sup>, E. C. Sklute<sup>2</sup>, K. A. Neat<sup>3</sup>, J. F. Holden<sup>1</sup>, M. D. Dyar<sup>2,3</sup>, <sup>1</sup>Dept. of Microbiology, Univ. of Massachusetts, 639 North Pleasant Street, Amherst, MA 01003, dleopo@umass.edu, <sup>2</sup>Planetary Science Institute, 1700 East Fort Lowell, Tucson, AZ 85719, <sup>3</sup>Department of Astronomy, Mount Holyoke College, 50 College St., South Hadley, MA. 01075.

**Introduction:** One of the goals of exobiology is to search for habitable environments and past or present microbial life using instruments that characterize the texture, chemistry, and mineralogy of extraterrestrial rocks and organisms [1]. This includes the identification and characterization of mineral assemblages associated with microbial mineral transformations using a suite of instruments relevant to planetary exploration.

To support this goal, we seek to understand the mineral biosignatures of past and present microbial life. To this end, we examined reduction of the nanophase Fe(III) (oxyhydr)oxide minerals (hereafter iron oxides) ferrihydrite, akagenéite, and lepidocrocite by an obligately autotrophic and anaerobic thermophile, '*Candidatus* Desulfothermobacter ferrireducens' strain Ax17. It was isolated from Axial Seamount, a deep-sea hydrothermal vent located on the Juan de Fuca Ridge. These vents, where seawater and hot rock interact, are known to produce mineral deposits and gases, such as H<sub>2</sub> and CO<sub>2</sub>, that fuel microbial metabolism in the absence of oxygen and sunlight. Ax17 uses H<sub>2</sub> as an electron donor and iron oxides (ferrihydrite, lepidocrocite, akagenéite, goethite, and hematite) and sulfur compounds (SO<sub>4</sub><sup>2-</sup>, SO<sub>3</sub><sup>2-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, and S<sup>0</sup>) separately as terminal electron acceptors.

We used visible-near infrared (VNIR) and Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopies to characterize the products of ferrihydrite, akagenéite, and lepidocrocite reduction by Ax17. Using a multi-instrument approach, we previously distinguished nanophase iron oxides (1-100 nm) from bulk iron oxides (> micrometer sized) as well as from each other [2]. Here, we gain insight into potential mineral biosignatures in terrestrial and extraterrestrial rock samples and biosignature interpretation.

**Methods:** Ax17 was grown at 55°C in modified DSM 981 growth medium [3] using 2 atm of 80% H<sub>2</sub>-20% CO<sub>2</sub> in the headspace as the energy and carbon sources. Ferrihydrite, akagenéite, and lepidocrocite were synthesized and separately used as terminal electron acceptors at concentrations of 100 mmol/l [2, 4].

To identify the mineral products that resulted from the growth of Ax17, three experimental variations were performed (with two controls): 1) a sample with iron oxide and Ax17 cells incubated at 55°C, 2) a sample with iron oxide and growth medium incubated at 55°C without Ax17, and 3) a sample with iron oxide and growth

medium left at room temperature without cells. Each condition had three replicates.

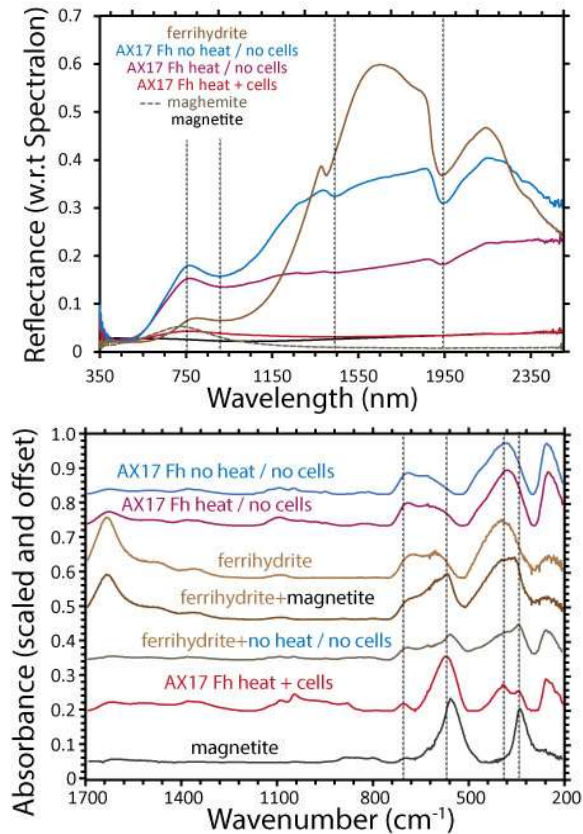
All samples were incubated for five days, which was the amount of time needed for Ax17 to complete its growth on the iron oxides. Samples were filtered onto 0.2 µm pore size Millipore filters in an anoxic chamber to prevent oxidation by air, then left to dry in the anoxic chamber for ~12 h before spectroscopic analyses.

VNIR spectra were acquired on an Analytical Spectral Devices (ASD) Fieldspec 4 Max spectrometer with incidence and emission angles set to 30° and 0°, respectively (3 nm resolution at 700 nm). Each spectrum was the average of 300 scans taken over three rotations to ensure homogenous scattering.

FTIR data were acquired on a Bruker Alpha FTIR with a diamond ATR attachment (4 cm<sup>-1</sup>, Mertz, Norton-Beer medium, 128 scans) inside the anoxic chamber. Additional FTIR spectra were acquired in air on a Bruker Vertex 80 FTIR with diamond ATR attachment and a wide-angle beamsplitter and detector (30-6000 cm<sup>-1</sup>, 4 cm<sup>-1</sup>, Mertz, Norton-Beer medium, 128 scans). The two sets of FTIR data were compared to verify that no oxidation had occurred during the oxic measurements. MIR ATR data were processed using Bruker's OPUS software. Spectra were cropped to remove noise and then baseline-corrected using a concave rubberband algorithm (64 points, iterations varied by mineral) [5]. Raw minerals spectra from [3] were processed identically and used for identification.

**Results:** The bioreduced mineral product of Ax17 grown on ferrihydrite was a dark brown (dry) or black (wet) magnetic material. None of the controls showed any magnetism. In the VNIR, the bioreduced sample showed minimal reflectance, similar to magnetite (Figure 1, top). The FTIR spectrum displayed features consistent with both magnetite and ferrihydrite but cannot be replicated by linearly mixing those two end members (Figure 1, bottom).

The bioreduced mineral product of Ax17 grown on akagenéite was a dark brown (dry) or brown (wet) material that displayed no magnetism. In the VNIR, it is spectrally dark, like bioreduced ferrihydrite, with a slight maximum at ~550 nm (Figure 2). The FTIR spectrum of the bioreduced sample, however, differed only slightly from the controls. A new feature at 337 cm<sup>-1</sup> is consistent with one magnetite peak, but the main peak at 557 cm<sup>-1</sup> is missing, and the sample is not magnetic.



**Figure 1.** VNIR (top) and FTIR spectra (bottom) for Ax17 grown on ferrihydrite. The FTIR spectrum for bioreduced ferrihydrite cannot be replicated through linear spectral mixing of magnetite with ferrihydrite or the unheated control.

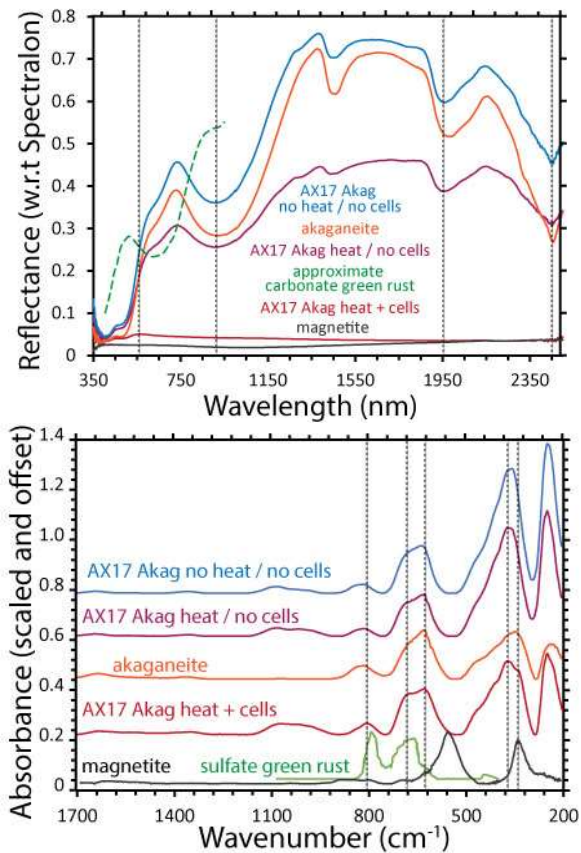
Literature reports for possible mineral analogs, such as various green rusts, are scarce at these wavelengths, but curves approximated from [6] for the MIR and [7] for the VNIR are shown for comparison.

The bioreduced mineral product of Ax17 grown on lepidocrocite was a dark brown (dry) or dark green (wet) nonmagnetic material. It was nearly spectrally flat in the VNIR, displaying a small maximum at ~550 nm (Figure 3). In the MIR (data not shown), there was no discernable difference between experimental conditions.

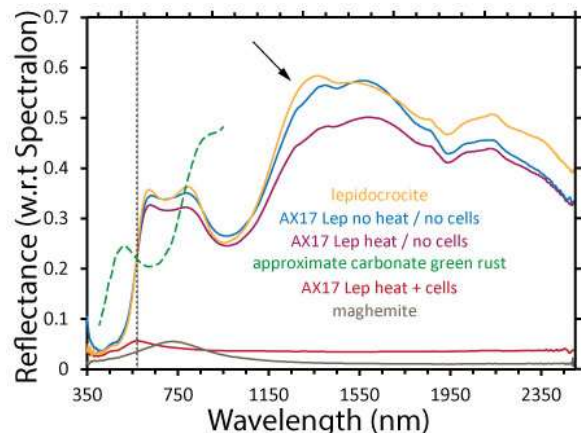
**Conclusions:** The bioreduced mineral products of Ax17 vary by mineral. Ferrihydrite was reduced to magnetite and showed a potential biosignature similar to that seen in our earlier work [8,9]. In contrast, akaganéite and lepidocrocite were reduced to non-magnetic minerals that were not identified here. These spectral features, to date, cannot be reproduced abiotically.

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**References:** [1] Farley K. et al. (2020) *Space Sci Rev*, 216, 1-41. [2] Sklute E.C. et al. (2018) *Phys Chem Miner*, 45, 1-26. [3] Kashefi, K. et al. (2002) *Int J Syst*



**Figure 2.** VNIR (top) and FTIR spectra (bottom) for Ax17 grown on akaganéite.



**Figure 3.** VNIR spectra for Ax17 grown on lepidocrocite.

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