

FTIR AND RAMAN SPECTROSCOPIC ANALYSIS OF NANOPHASE IRON (OXYHYDR)OXIDES BIOREDUCED BY HYPERTHERMOPHILIC ARCHAEA. Elizabeth C. Sklute¹, Srishti Kashyap², Peng Wang³, Thomas J. Tague Jr.³, M. Darby Dyar¹ and James F. Holden², ¹Mount Holyoke College, Dept. of Astronomy, 50 College St. South Hadley, MA. 01075, USA. ²University of Massachusetts, Dept. of Microbiology, 639 Pleasant St. Amherst, MA. 01003, USA. ³Bruker Optics Inc., 19 Fortune Dr. Billerica, MA. 01821, USA.

Introduction: Life in our Solar System beyond Earth would likely be microbial. However, the organic signatures of such life may degrade quickly on extra-terrestrial surface environments due to cosmic-ray exposure. Therefore, understanding the signatures of microbial life that could persist in the rock record or be preserved by mineralization processes is central to the detection of extraterrestrial life.

Nanophase iron (oxyhydr)oxides (FeNPOs) are prevalent and important phases on Earth, Mars, and potentially many other rocky Solar System bodies [1]. On Earth, these phases fuel microbial metabolisms across a multitude of environments [2]. Specifically, FeNPOs play a major role in the microbial metabolisms of warm and anoxic aqueous systems, like deep-sea vents and hot spring, which are compelling extra-terrestrial analogues. Still, very little is known about mineral transformations produced by the hyperthermophilic ($T_{opt} > 80^{\circ}\text{C}$) iron reducers that populate such environments. Understanding spectral characteristics of bioreduced mineral products formed by hyperthermophilic iron reducers and how they compare to similarly prepared abiotic samples is critical in the search for mineral biosignatures.

Here we present spectroscopic analyses for ferrihydrite, lepidocrocite, and akaganéite bioreduced by *Pyrobaculum islandicum*, a freshwater hyperthermophilic iron reducer isolated from a hot spring in Iceland, and *Pyrodictium delaneyi* Su06^T, a marine hyperthermophilic iron reducer isolated from a deep-sea hydrothermal vent. The bioreduction products are compared to abiotic controls and reference samples.

Methods: Ferrihydrite, nanophase lepidocrocite, and nanophase akaganéite were synthesized as in [1] and stored as a liquid suspension to maintain mineral-fluid surface properties. *P. delaneyi* and *P. islandicum* was grown as in [3, 4]. Four variations were performed for each experiment to add controls to the study: 1) a sample with no cells added (but with growth medium and oxide) left at room temperature (RT), 2) a sample with no cells incubated at 90 or 95°C, 3) a sample with cells left at RT, and 4) a sample with cells incubated at 90 or 95°C. Growth and Fe(II) were determined as in [4] and bioreduction products were harvested from late-exponential-early-stationary phase cultures.

Raman analysis was performed at Bruker Optics Inc. on a Bruker Senterra Raman microscope using a 20× objective (connected to a single elbow for fluid samples) and a 532 nm excitation laser powered to

either 0.2-0.25 or 2-2.5 mW depending on the sensitivity of the sample towards heat transformation. Fourier transform infrared (FTIR) attenuated total reflectance (ATR) data were acquired at Bruker Optics Inc. on a Bruker Vertex 70 FTIR using a diamond ATR accessory and an ultra-wide range beamsplitter and DTGS detector. Bioreduced samples, abiotic controls, and nanophase magnetite were anoxically dried and transported under nitrogen to Bruker optics and analyzed directly after opening. All other nanophase FeNPOs were filtered and dried aerobically immediately prior to analysis.

Results: *P. islandicum* and *P. delaneyi* cell concentrations and Fe(II) production are mineral-dependent with ferrihydrite > akaganéite ≥ lepidocrocite [5]. Both organisms partially reduce ferrihydrite to a black, magnetic product that is spectrally consistent with magnetite (**Fig. 1**). The FTIR spectrum of ferrihydrite bioreduced by *P. delaneyi* shows both magnetite peaks but that of *P. islandicum* is missing the low wavenumber peak, similar to what is observed in both heated control samples.

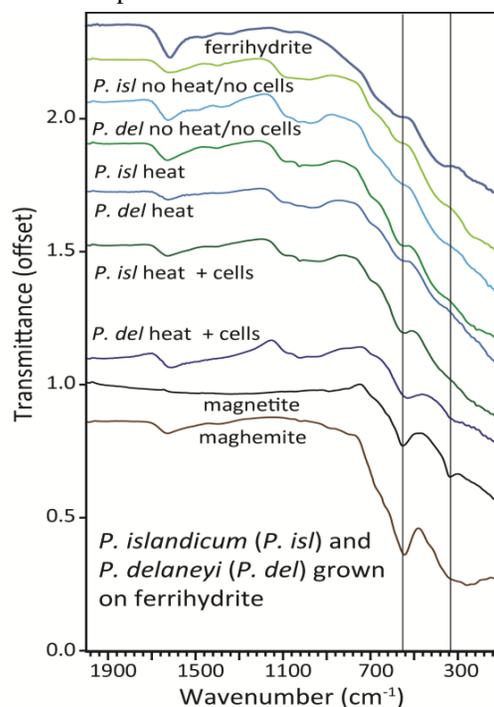


Fig. 1. FTIR data for anoxically dried ferrihydrite bioreduced by either *P. islandicum* or *P. delaneyi* along with control and reference samples. Vertical lines indicate position of magnetite absorptions.

Lepidocrocite bioreduced by *P. islandicum* is spectrally similar to both controls (Fig. 2). In contrast, lepidocrocite reduced by *P. delaneyi* shows evidence of a carbonate spectrally similar, but not identical, to siderite. Interestingly, when the sample is filtered directly prior to analysis rather than anoxically dried, the carbonate is much less evident.

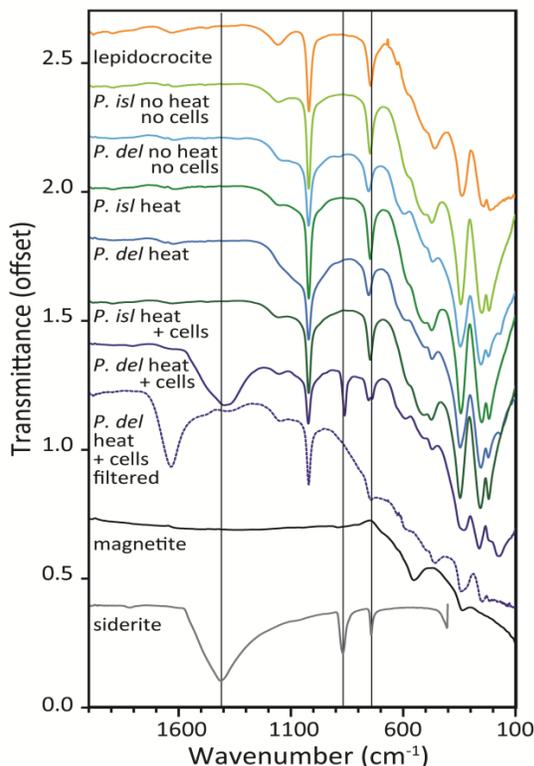


Fig. 2. FTIR data for anoxically dried lepidocrocite bioreduced by either *P. islandicum* or *P. delaneyi* along with control and reference samples. Vertical lines indicate position of siderite absorptions. *P. delaneyi* bioreduced lepidocrocite filtered directly prior to analysis also shown.

Akaganéite bioreduced by *P. islandicum* and *P. delaneyi* and the heated control for both organisms all show phosphate absorptions in $\sim 1030\text{-}930\text{ cm}^{-1}$ (Fig. 3). Positions of these features vary by organism and are much different between *P. delaneyi* heated control and bioreduced samples. While the phosphate absorptions are reminiscent of vivianite, they are not a perfect match and may indicate biological phosphate moieties binding on the mineral surface [6] or structural phosphorus in magnetite [7]. There is also evidence of a magnetite-like absorption in the *P. islandicum* heated control and both bioreacted samples, but the absorption is shifted to higher cm^{-1} . Here again, the filtered sample shows less evidence of the phosphate groups.

Raman data (not shown), acquired on multiple individual grains, show a slightly different story. Ferrihydrite bioreduced by *P. islandicum* clearly contains magnetite. For *P. delaneyi*, bioreduction is less definitive in anoxically dried samples but the filtered sample

does clearly show magnetite, albeit with a shifted peak position. *P. islandicum* bioreduction of akaganéite appears similar to abiotic controls, despite a visual color change. *P. delaneyi* bioreduction of akaganéite yields magnetite and a feature $\sim 300\text{ cm}^{-1}$ that may be a carbonate, though it lacks the expected $\sim 1090\text{ cm}^{-1}$ main absorption. *P. delaneyi* reduction of lepidocrocite also produces a $\sim 300\text{ cm}^{-1}$ feature, but lacks any vivianite absorptions.

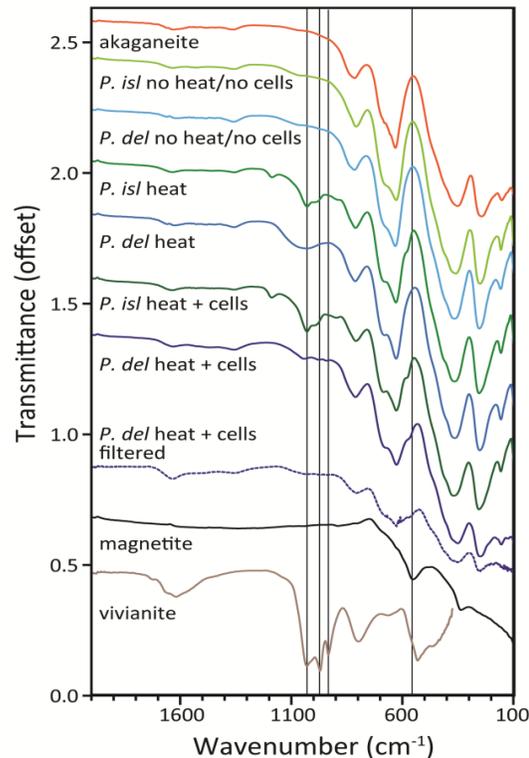


Fig. 3. FTIR data for anoxically dried akaganéite bioreduced by either *P. islandicum* or *P. delaneyi* along with control and reference samples. Vertical lines indicate position of siderite absorptions. *P. delaneyi* bioreduced akaganéite filtered directly prior to analysis also shown.

Implications: Bioreduced FeNPO products vary with organism, starting mineral, and preparation method when characterized by various types of spectroscopy. While several spectroscopic features could prove to be biosignatures, more work and equivalent abiotic systems are required to parameterize these transformations.

References: [1] Sklute E. C. et al. (2017) *Phys. Chem. Mineral.* doi:10.1007/s00269-017-0897-y [2] Schwertmann U. and Cornell R. M. (2000) *Wiley, New York*. [3] Kashyap, S. et al. (2016) 47th LPSC, #2192. [4] Lin T. J. et al. (2014) *Geobiol.*, 12, 200-211 [5] Kashyap, S. (2017) *AGU*, # P41B-2837. [6] Parikh S. J. and Chorover J. (2006) *Langmuir*, 22, 8492-8500. [7] Jurado M. J. et al. (2003) *J. Biol. Inorg. Chem.*, 8, 810-814.

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