SPECTROSCOPY OF NANOPHASE IRON (OXYHYDR)OXIDES BIOREDUCED BY *GEOBACTER METALLIREDUCENS*. Helena T. Valvur[1], Srishti Kashyap[2], Elizabeth C. Sklute[1], James F. Holden[2], Peng Wang[3], Thomas J. Tague, Jr.[3], and M. D. Dyar[1], [1]Dept. of Astronomy, Mount Holyoke College, South Hadley, MA 01075, valvu22h@mtholyoke.edu, [2]Dept. of Microbiology, Univ. of Massachusetts Amherst, 639 N. Pleasant St., Amherst, MA 01003. [3] Bruker Optics Inc. 19 Fortune Dr. Billerica, MA. 01821, USA.

Introduction: Dissimilatory iron reduction, or the extracellular reduction of Fe(III) to Fe(II), widely occurs in many anoxic environments, such as sediments and soils [1]. It may have also been an important process on the early Earth and could potentially serve as a means to support life elsewhere in our Solar System [2]. Mesophilic (T_{opt} 20-50°C) bacteria, particularly the *Geobacteraceae*, have been studied extensively to understand the physiology of microbes using extracellular terminal electron acceptors. Yet, our understanding of the mineral constraints for growth and reduction is nascent, as are the full suite of mineral transformation products. Here, we focus on evaluating the bioavailability of seven nanophase Fe(III) (oxyhydr)oxides (FeNPOs) for iron reduction by *Geobacter metallireducens* and spectroscopically characterizing the mineral transformation products that result from microbial metabolic activity. Our work provides insight into biogenically reduced minerals and their relevance as biosignatures in the search for life beyond Earth.

Methods: *G. metallireducens* was grown at 30°C in a freshwater medium using acetate as an electron donor as previously described [3]. Seven different FeNPOs – ferrihdydrite, lepidocrocite, akaganéite, hematite (two different syntheses), and goethite (two different syntheses) – were tested as electron acceptors. Syntheses were performed as detailed in [4]. Growth was confirmed by performing direct cell counts using epifluoresence microscopy and Fe(II) production determined using a ferrozine assay. An electron acceptor was considered bioavailable only after three successive transfers showed successful growth and Fe(II) production.

In order to characterize mineral transformations, we used a combination of visible and near infrared (VNIR) reflectance, Fourier transform infrared (FTIR) attenuated total reflectance (ATR), Raman, and Mössbauer spectroscopies. All samples for spectroscopy were prepared just prior to analyses using a vacuum filtration setup in an anaerobic chamber so as to prevent oxidation of biogenically reduced minerals. Raman analysis was performed at Bruker Optics Inc. on a Bruker Senterra Raman microscope using a 20× objective and 532 nm excitation laser (0.2 or 2 mW depending on the sensitivity of the samples to heat transformation). FTIR ATR spectroscopy was performed at Bruker Optics Inc. on a Bruker Vertex 70 FTIR using a diamond ATR accessory, ultra-wide range beamsplitter, and wide-range DTGS detector. VNIR spectroscopy was performed at Mount Holyoke College (MHC) on an ASD Fielspec 4 Max spectrometer (30° incidencen and 0° emissivity). Mössbauer spectroscopy was conducted at MHC on a WEB Research Co. (now See Co.) model W100 spectrometer (50–30 mCi ^{57}Co in Rh; referenced to α-Fe foil).

Results. *G. metallireducens* grew to the highest cell concentrations (~7×10^7 cells/ml) and produced the most Fe(II) (11 mM and 4 mM) on ferrihydrite and lepidocrocite, respectively (**Figure 1**). Maximum cell concentrations and Fe(II) produced were significantly lower (< 1 mM) for akaganeite, goethite, and hematite, despite showing no significant differences in growth rates. Ferrihydrite and lepidocrocite cultures were chosen for spectroscopy based on these results.

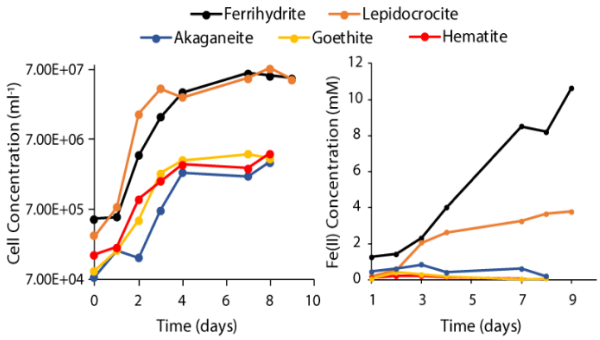

Figure 1. Growth and Fe(II) production profiles of *G. metallireducens* grown in batch cultures at 30°C on various nanophase Fe(III) (oxyhydr)oxides.

VNIR spectra for *G. metallireducens* bioreduced lepidocrocite and ferrihydrite (**Figure 2**) show the sample color changes that are visually apparent in reacted samples. Both the control and the bioreduced samples visibly darken; however, the bioreduced sample takes a green to black hue, indicating an admixing or coating of a dark phase. Spectrally, the bioreduced ferrihydrite sample is reminiscent of maghemite or magnetite, but the bioreduced lepidocrocite sample is unlike any available reference spectra. Based on the green hue, it may be converting to green rust. However, due to the highly unstable nature of that mineral, high-quality reference spectra are difficult to obtain.

Raman spectra for *G. metallireducens* bioreduced lepidocrocite and ferrihydrite (**Figure 3**) do not show any detectable differences from starting materials.

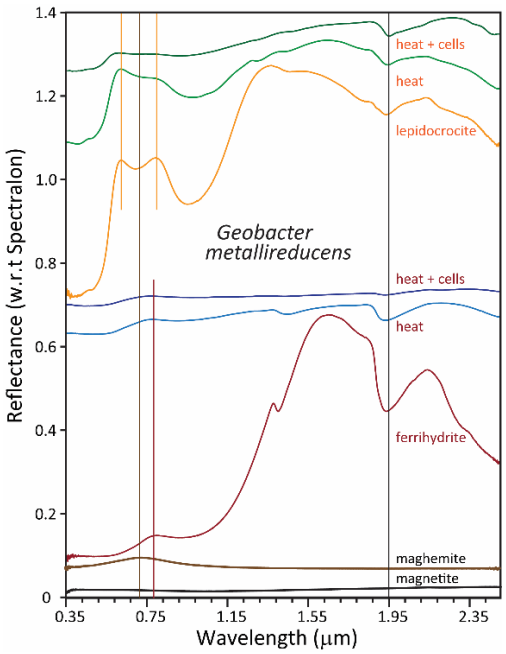

Figure 2. VNIR spectra for *G. metallireducens* reduced lepidocrocite (top) and ferrihydrite (bottom) along with reference samples. Color coded verticle lines indicate position of reference features. Black verticle line at 1.95 μm is meant to guide the eye.

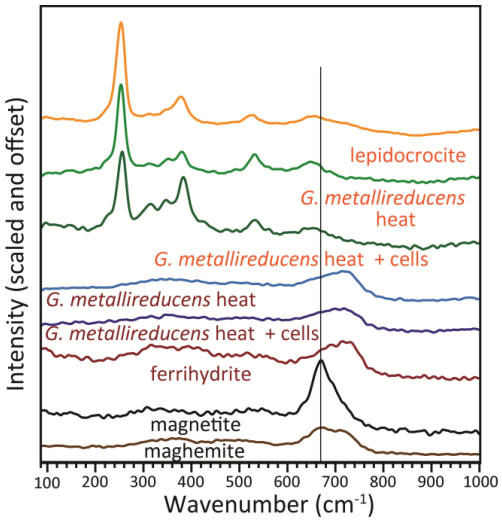

Figure 3. Raman spectra for *G. metallireducens* reduced lepidocrocite (top) and ferrihydrite (bottom) along with reference samples. Vertical line indicates position magnetite absorptions. No phosphate or green rust is detected.

FTIR spectra are shown in **Figure 4**. Lepidocrocite reduced by *G. metallireducens* displays phosphate absorptions between ~1030 and 890 cm^{-1}, reminiscent of the Fe(II) phosphate vivianite, but shifted in position. These phosphate absorptions may be due to a mineral phase but may also represent biological phosphate groups bonding to the mineral surface [5]. Ferrihydrite reduced by *G. metallireducens* also shows phosphate absoprtions, which are a much closer match to those of vivianite. This sample also displays an absorption at ~540 cm^{-1} consistent with magnetite.

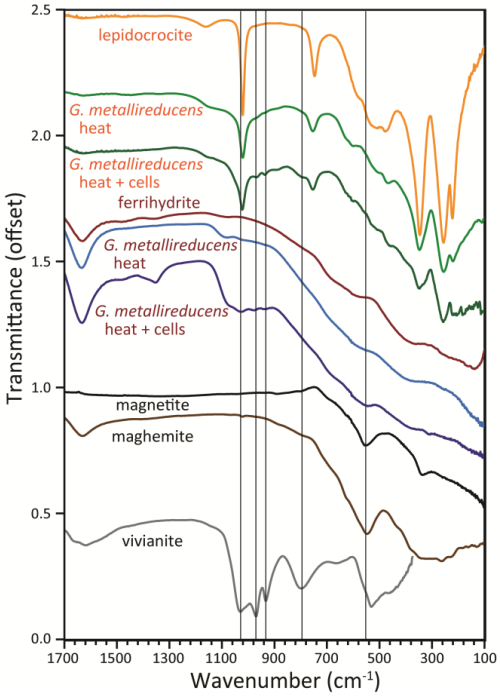

Figure 4. FTIR ATR spectra for *G. metallireducens* reduced lepidocrocite (top) and ferrihydrite (bottom) along with reference samples. Vertical lines indicate position of phosphate and magnetite absorptions.

Implications: While numerous reports exist in the literature on the mineralization products of *G. metallireducens*, all of these studies have examined minerals formed as a result of mere presence of unadapted and/or dying cells, rather than metabolically relevant growth. Here, we have shown the metabolic potential of *G. metallireducens* to grow in batch cultures on a range of FeNPOs, and spectrally characterized late-exponential growth phase mineralization products on the two most successful minerals for growth. Although further characterization of these products is necessary, our results clearly demonstrate the importance of distinguishing growth-related from organo-mineral-related mineralization processes when defining biogenic mineral signatures.

Acknowledgments: This work was supported by NASA Exobiology grant #NNX14AK25G. and NASA grant #80NSSC17K0243.

References: [1] Lovely D.R, et al. (2004) *Advances in Microbial Phys.*, 49, 219-286. [2] Vargas, M. et al. (1998) Nature, 395:65-67. [3] Lovely D.R., et al. (1993) *Arch. Microbio.*, 159, 336-344. [4] Sklute E. C. et al. (2017) *Phys. Chem. Mineral.* doi:10.1007/s00269-017-0897-y. [5] Parikh, S. J. et al. (2014) *Colloid. Surf. B*, 119, 38-46.