

**SPECTROSCOPY OF MINERAL REACTION PRODUCTS FROM BIOREDUCTION BY HYPERTHERMOPHILES: POTENTIAL FOR REMOTE-SENSING BIOMARKERS.** M. D. Dyar<sup>1</sup>, E. C. Sklute<sup>2</sup>, J. K. Knutson<sup>1</sup>, T. D. Glotch<sup>2</sup>, C. Che<sup>2</sup>, S. L. Zelin<sup>3</sup>, T. J. Lin<sup>3</sup>, J. F. Holden<sup>3</sup>. <sup>1</sup>Dept. of Astronomy, Mount Holyoke College, South Hadley, MA 01075, mdyar@mtholyoke.edu; <sup>2</sup>Dept. of Geoscience, Stony Brook University, Stony Brook, NY 11794; <sup>3</sup>Dept. of Microbiology, University of Massachusetts, Amherst, MA 01003, jholden@microbio.umass.edu.

**Introduction:** Microbial Fe reduction is an important step in many biogeochemical processes. The ability of microbes to transform metals has major implications for bioremediation of toxic metals and metalloids, production of microbial fuel cells, and understanding microbe-mineral interactions in general. Geochemical evidence points to Fe<sup>3+</sup> reduction as one of the earliest forms of microbial metabolism; understanding this process helps to provide insights into the complex reactions occurring in geothermal environments today, both on Earth and potentially on other planets.

Most studies of microbial Fe<sup>3+</sup> reduction use the model organisms *Geobacter* and *Shewanella*, which are limited to a temperature range of about 15°-50°C and may not be broadly applicable to extraterrestrial environments. Studies of Fe<sup>3+</sup> reduction in hyperthermophilic archaea, microorganisms that grow optimally above 80°C, are needed to broaden biogeochemical understanding of Fe reduction mechanisms. They also provide important terrestrial analogs for bioreduction that may occur in hot spring-driven aqueous environments on other planets.

We are studying how energy transduction mechanisms used by hyperthermophilic microorganisms to reduce insoluble Fe<sup>3+</sup> oxides are assembled and maintained and the biochemical and physical processes that regulate energy-relevant chemical reactions at the microbe-mineral interface. We focus on the hyperthermophilic archaea *Pyrobaculum islandicum* and two related strains from deep-sea hydrothermal vents: *Hyperthermus butylicus* and *Hyperthermus* strain Ro04. We report here results of Mössbauer and infrared spectroscopies and x-ray diffraction (XRD) documenting metal transformations that occur during Fe<sup>3+</sup> oxide respiration by these organisms.

**Methods:** The organisms were grown on poorly crystalline iron gel, prepared by raising a 1 M FeCl<sub>3</sub> solution to pH 7 followed by several water rinses. Peptides were the carbon and energy sources for *P. islandicum*, Ro04 used H<sub>2</sub> and CO<sub>2</sub>, and *H. butylicus* used a combination of all three substrates. Cultures were grown anaerobically in liquid media at

92-95°C. Pre- and post-growth iron oxides were dried in an anoxic chamber.

**Characterization:** *Mössbauer spectra* were acquired at MHC using 16 different temperatures from 295 to 4K. Both the Mexdist and Mexfield programs from the University of Ghent, which solve for the full Hamiltonian, were used to model spectra. Results were compared with data from other iron oxides.

*Reflectance spectroscopy* was performed at SBU using a Nicolet 6700 FTIR spectrometer equipped with a FT-30 specular reflectance accessory with 30° incidence and emergence angles. For these measurements, samples were pressed into compact pellets with smooth surfaces using a Carver hydraulic press. All reflectance spectra were referenced to a first surface gold mirror.

*X-ray diffraction (XRD)* data were measured at SBU using a Thermo ARL Scintag PAD-X automated powder diffractometer with Cu-K radiation, collecting from 5° to 70° 2 θ at a 0.6 degree/minute. Samples for XRD analysis were prepared using the smear-mount method with ground sample powders.

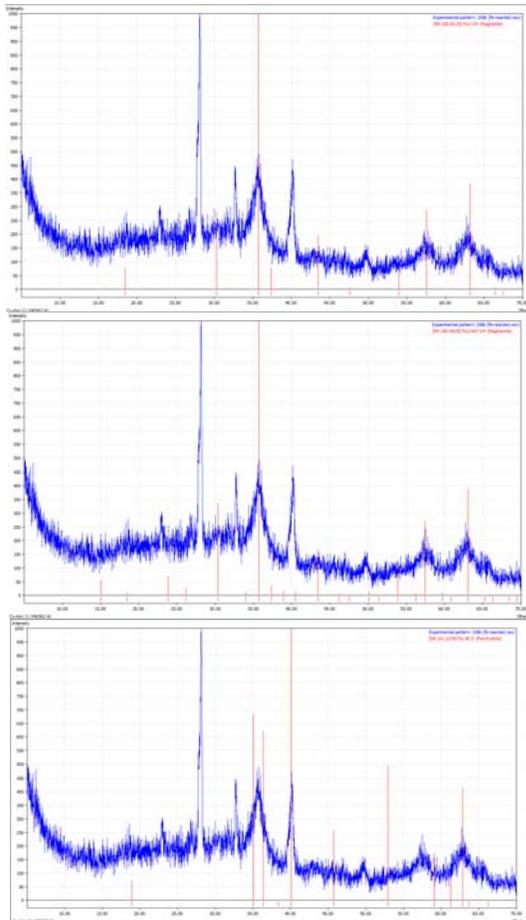
**Results and Discussion:** *8K Mössbauer parameters* of the starting gel used in the growth experiments, the reaction products of the three microbes, and Fe

**Table 1. 8K Mössbauer Parameters of Oxides and Reduction Products**

Param*	Hem	Aka	Fht	Gel	Pisl	Ro04	Hbut
IS	0.47	0.47	0.44	0.47	0.44	0.45	0.45
QS	-0.17	-0.18	-0.02	-0.13	-0.06	-0.08	-0.06
B <sub>Hf</sub>	51.2	45.5	46.1	46.5	47.4	46.0	47.3
Width	1.00	1.23	1.23	0.54	1.56	1.81	1.33
%Area	57	39	33	34	60	22	57
IS	0.49	0.49	0.47	0.48	0.47	0.45	0.46
QS	-0.19	-0.14	-0.01	-0.08	-0.06	-0.06	-0.04
B <sub>Hf</sub>	54.3	49.4	50.8	49.0	51.6	51.0	51.3
Width	0.82	1.30	1.35	0.62	1.30	2.03	1.15
%Area	43	61	67	46	40	78	43
IS				0.46			
QS				-0.09			
B <sub>Hf</sub>				42.8			
Width				0.72			
%Area				20			
χ <sup>2</sup>	13.10	5.03	4.00	5.14	8.33	1.42	6.18

\*IS (isomer shift), QS (quadrupole splitting) and peak widths in mm/s relative to the centroid of an Fe foil spectrum. Hyperfine fields are reported in Tesla.

oxides that are candidates for the reaction products are shown in Table 1. Magnetite (Mag) parameters are not listed because Mag has several hyperfine fields below the Verwey transition (ca. 119K). Above that, Mag has



**Fig. 1.** XRD patterns of *P. islandicum* reaction products showing red lines from magnetite (top), maghemite (middle) and hematite (bottom). The large peaks at  $2\theta = 28^\circ$  and  $40^\circ$  match (K,Na)Cl used in the reactions.

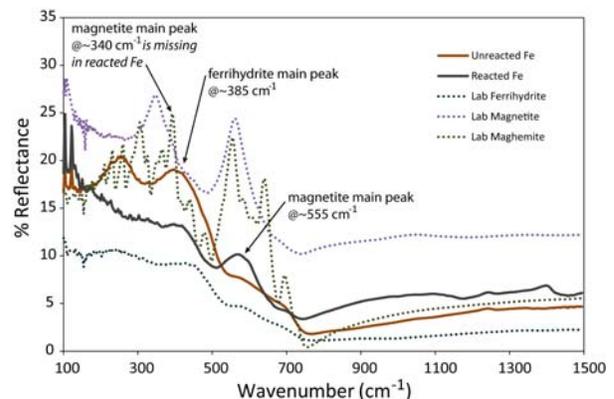
two sextets corresponding to the A and B sites, with isomer shifts of 0.26 and 0.67 mm/s at 295K.

The Mössbauer spectra of the starting gel used in all three growth experiments (gray in Table 1) is similar at all temperatures studied to both ferrihydrite (Fht) and akaganeite (Aka), and are indistinguishable from them because small differences may result from variable grain sizes. However, reflectance spectra and XRD confirm that this starting gel is Fht.

The Mössbauer spectra of the reaction products produced by all three organisms are clearly different from the starting gel. The Ro04 reaction product is most similar in both parameters and area to synthetic Fht. The hyperfine fields of the *P. islandicum* and *H. butylicus* spectra are slightly higher than those of synthetic Fht and the areas are much different. None of the three bio-reduced samples is in any way similar to

hematite (Hem), maghemite, or Mag, despite the fact that these samples are magnetic; these are *not* intimate mixtures of two distinct phases. It is possible that the observed changes in sextet areas and the increases in hyperfine field are representative of an intermediate phase in the process of undergoing a transformation to a different oxide structure/mineral.

The XRD pattern (Fig. 1) of the Fe-unreacted sample is consistent with Fht, though it is largely amorphous. The pattern of the *P. islandicum*-reacted sample appears to match a mixture of Mag, maghemite, Fht, and some contaminants (probably (K,Na)Cl from the growth medium); there is no good match in the database, suggesting the possibility that this sample is an intermediate between Fht and Mag.



**Fig. 2.** Reflectance spectra of starting gel and reaction product of *P. Islandicum* along with lab Fe oxides for comparison.

Reflectance spectra of the *P. islandicum* experiment before and after reaction are quite different (Fig. 2). The unreacted product looks very similar to Fht [1]. The reacted product has a peak at  $\sim 555 \text{ cm}^{-1}$  in the same position as one of two main Mag peaks, but it is missing the other Mag peak at  $340 \text{ cm}^{-1}$ . It also has another peak  $\sim 385 \text{ cm}^{-1}$  that is consistent with a Fht peak. Other strong features between about 1100 and  $1800 \text{ cm}^{-1}$  could not be assigned to any specific oxide; these might represent Fe-O or Fe-OH surface modes. This material cannot be just an intimate mixture of Mag and Fht, but seems to be an intermediate between the two phases, just as is seen in the Mössbauer data. Its characteristic pairing of the 555 and  $385 \text{ cm}^{-1}$  Mag/Fht peaks in the absence of the other Mag peak at  $340 \text{ cm}^{-1}$  might well be a useful biomarker in remote sensing studies if it proves to be unique to bio-reduced oxides. Further work is in progress to search for this spectral signature in the reduced oxides from *H. butylicus* and Ro04.

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**References:** [1] Glotch T. D. et al. (2009) *Icarus*, 204, 663-671.