REACTIVE TRANSPORT MODELING AND DETECTION OF THERMOPHILES IN THE SUBSEAFLOOR. J. F. Holden[1], S. Kashyap[1], L. C. Stewart[1,2], C. K. Algar[3], E. C. Sklute[4], and M. D. Dyar[4], [1]Department of Microbiology, University of Massachusetts, Amherst, MA 01003 USA (Email: jholden@microbio.umass.edu), [2]GNS Science, Wellington 5010, New Zealand, [3]Department of Oceanography, Dalhousie University, Halifax, B3H 4R2, Canada, [4]Department of Astronomy, Mount Holyoke College, South Hadley, MA 01075 USA.

Introduction: Hot (> 65°C), rocky subsurface environments are ideal for biogeochemical modeling and biosignature detection development. They have predictable mineralogy and microbial metabolisms, low microbial diversity, and spatially restricted habitats relative to many other Earth environments. Thermophilic and hyperthermophilic microbes present are limited to relatively few genera of aphotic anaerobes that reduce various sulfur and metal species, nitrate, H^+, and CO_2. However, key questions remain, such as determining the biomass and activity of these microbes, the impact of microbe-microbe and microbe-mineral-fluid interactions, and the spatial distribution of microbes in their habitats.

Here, we use reactive transport modeling to predict the abundance and biogeochemical impact of thermophilic and hyperthermophilic methanogens within the basalt subseafloor associated with a diffuse hydrothermal vent. We also examine the growth of a hyperthermophilic iron reducer on various forms of synthetic nanophase Fe(III) (oxyhydr)oxides to determine environmental limits on the growth of this organism and search for potential mineral biomarkers for their detection in nature using various spectroscopic techniques.

Reactive Transport Modeling: Microcosm incubations at 55°C and 80°C of diffuse hydrothermal fluids collected from Axial Seamount in the NE Pacific Ocean demonstrated that growth of the thermophilic and hyperthermophilic methanogens present was limited primarily by H_2 availability rather than by N, vitamin, or metal limitations [1]. The methanogens present in the fluids were almost exclusively *Methanocaldococcus* and *Methanothermococcus* spp. These results prompted us to determine the Arrhenius and Monod CH_4 production kinetics, cell-specific CH_4 production rates, and limiting H_2 concentrations of *M. jannaschii* and *M. thermolithotrophicum* using a chemostat. The results were used to develop a reactive transport model for the growth and CH_4 production of methanogens along a one-dimensional flow path of end-member 350°C hydrothermal fluid progressively diluted with 2°C seawater. The model was constrained by field measurements of methanogen, H_2, and CH_4 concentrations in 20-40°C hydrothermal fluids from Axial Seamount. Thermophilic-to-hyperthermophilic methanogen ratios determined by metagenomics and direct cell counts were used to predict the shape function of fluid residence times along the flow path. As few as 2×10^{10} methanogens (~ 1 mg) were needed along the flow path to produce 15-20 µM of CH_4 in the exiting fluid, which matched field CH_4 measurements. The findings suggest that methanogenesis at hydrothermal vents is catalyzed by a modest number of cells that have a large biogeochemical impact along their flow path.

Bioreduction and Detection of Nanophase Iron Oxides: Our reactive transport modeling of methanogenesis points to the need to detect potentially low amounts of biomass or metabolic end products in environmental samples. Our previous detection of microbial pigments in the interiors of hydrothermal metal sulfide deposits using spectroscopic techniques suggested that this analytical approach has promise [2]. Because of the need to distinguish microbes from the mineral matrix in which they are found, we chose to examine the hyperthermophilic iron reducer *Pyrodictium delaneyi* for its growth on various synthetic nanophase Fe(III) (oxyhydr)oxides and its ability to transform the minerals in a predictable and discernable manner.

Seven nanophase Fe(III) (oxyhydr)oxides (ferrihydrite, lepidocrocite, akaganéite, hematite, goethite, maghemite, magnetite) were synthesized and analyzed using TEM, XRD, and Mössbauer, Raman, VNIR, and MIR spectroscopies to determine their spectral and morphological characteristics [3]. *P. delaneyi* was then incubated separately at 90°C on each of these iron oxides (except magnetite). It grew the fastest and to the highest cell concentration on ferrihydrite, with up to 18-20 mM Fe(II) produced. It also grew modestly on lepidocrocite and akaganéite (up to 2 mM Fe(II) produced) and poorly on goethite, hematite, and maghemite (< 1 mM Fe(II)). The mineral end product produced from the bioreduction of ferrihydrite was black and magnetic, whereas none of the controls showed magnetism. Spectrally, the bioreduced product was similar to nanophase magnetite using Raman, Mössbauer, VNIR, and MIR. This product was only produced when both heat and cells were present in the incubation. Mixed samples analyzed by only one or two techniques produce equivocal results. Only integrated spectral studies can positively identify iron oxides.

References: [1] Topçuoğlu B. D. et al. (2016) *Front. Microbiol., 7,* 1240. [2] Lin T. J. et al. (2016) *Geochem. Geophys. Geosyst., 17,* 300-323. [3] Sklute E. C. et al. (submitted) *Am. Mineral.*.