LABORATORY AND *IN SITU* ELECTRODE CULTIVATION OF SUBSURFACE MICROORGANISMS. Y. Jangir[1], L. M. Momper[2], B. K. Reese[5], G. Wanger[3,6], J. P. Amend[2,3] and M. Y. El-Naggar[1,2,4], [1]Department of Physics and Astronomy, University of Southern California (mnaggar@usc.edu), [2]Department of Biological Sciences, University of Southern California, [3]Department of Earth Science, University of Southern California, [4]Department of Chemistry, University of Southern California, [5]Department of Life Sciences, Texas A&M University, [6]Division of Geological and Planetary Sciences, California Institute of Technology.

Reduction-oxidation (redox) reactions and electron transfer are unifying themes in all biological energy conversion strategies, including respiration. Respiratory organisms extract free energy from their environment by coupling the oxidation of electron donors to the reduction of electron acceptors. Beyond soluble reductants and oxidants (e.g. oxygen), microbes are the ultimate energy scavengers of our planet, capable of utilizing a wide array of insoluble redox-active materials as electron donors or acceptors. For example, we now know that many microbes gain energy by performing extracellular electron transfer from or to natural minerals. This is clearly demonstrated in the case of iron oxidizers and reducers, among numerous other microbes [1]. These interfacial processes, which have special implications for life on iron-rich planets such as Mars, can also be mimicked at physical electrode surfaces, leading to new cultivation techniques that allow unprecedented control of the energetic conditions.

We here report recent progress in developing electrochemical platforms, based on physical electrodes that function as electron donors and acceptors for respiration, to cultivate slow-growing subsurface microbes. A comprehensive characterization of the subsurface biosphere is hindered because the majority of the resident archaea and bacteria appear 'unculturable' using traditional growth techniques.

We describe a bulk (multi-liter volume) bioelectrochemical cultivation platform based on carbon fiber cloth working electrodes. This platform mimics interfacial electron transport as a central mechanism for microbe-mineral energy transfer in the subsurface, while allowing the simultaneous exploration of multiple redox potentials in a single experiment.

Initial characterization was performed on samples from Nevares Deep Well-2 (NDW2) in Death Valley, California. This site is an artesian well drilled into dolostone rock, intersecting fault-associated, deeply sourced well water. Collected well water was used to enrich for subsurface microorganisms at four different redox potentials. Electrochemical cyclic voltammetry reveals redox activity at -200 mV vs Ag/AgCl, providing clues into the interfacial electron transfer mechanisms dominant in these reactors. 16S rRNA gene analysis and sequences from clone libraries closely matched *Dechloromonas* within *Rhodocyclaceae* family and *Delftia* within *Comamonadaceae* family. While alternate metabolisms from these organisms (e.g. denitrification) are well characterized, our electrode enrichments suggest that these microbes are also capable of extracellular electron transfer to insoluble electron acceptors. Isolation of pure strains for detailed mechanistic studies is currently in progress.

In addition to this laboratory demonstration, we have designed, constructed, and deployed a similar bioelectrochemical cultivation reactor *in situ*, 4850 ft below the surface at the Sanford Underground Research Facility (Lead, South Dakota). The system is now fully operational, and is enriching for microbes at 4 different redox conditions mimicking specific metabolisms predicted by recent modeling efforts [2]. Our studies demonstrate an alternate method for culturing microbes relevant to astrobiology exploration, while using physical electrodes to emulate the microhabitats, redox and geochemical gradients, and the spatially dependent interspecies interactions encountered in the subsurface.

References: [1] Nealson K. H. (1997) *Annu. Rev. Earth Planet. Sci., 25*, 403-434, [2] Osburn M. R. et al. (2014) *Frontiers in Microbiology,* 5:610.