

IN SITU ELECTROCHEMICAL ENRICHMENT OF SUBSURFACE BACTERIA AT THE SANFORD UNDERGROUND RESEARCH FACILITY. Y. Jangir¹, A. Karbelkar⁴, L. Zinke³, B. K. Reese⁵, G. Wanger⁶, C. Anderson⁷, J. P. Amend^{2,3} and M. Y. El-Naggar^{1,2,4}, ¹Department of Physics and Astronomy, University of Southern California (jangir@usc.edu), ²Department of Biological Sciences, University of Southern California, ³Department of Earth Science, University of Southern California, ⁴Department of Chemistry, University of Southern California, ⁵Department of Life Sciences, Texas A&M University, ⁶Division of Geological and Planetary Sciences, California Institute of Technology, ⁷Center for the Conservation of Biological Resources, Black Hills State University.

Continental subsurface environments can present significant energetic challenges to the resident microorganisms. While these environments are geologically diverse, potentially allowing energy harvesting by microorganisms that catalyze redox reactions, many of the abundant electron donors and acceptors are insoluble and therefore not directly bioavailable. Extracellular electron transfer (EET) is a metabolic strategy that microorganisms can deploy to meet the challenges of interacting with redox-active surfaces. Though mechanistically characterized in a few metal-reducing bacteria, the role, extent, and diversity of EET in subsurface ecosystems remain unclear. Since this process can be mimicked on electrode surfaces, it opens the door to electrochemical techniques to enrich for and quantify the activities of environmental microorganisms in the subsurface.

The Sanford Underground Research Facility (SURF, South Dakota), a state-run science facility, hosted in quartz-veined, sulfide-rich segments of an Early Proterozoic, carbonate-facies iron-formation [1, 2]. Hydrological modeling indicates older (>10,000 yrs) fluids reaching the deeper levels, especially on the northern ledges [3]. The mine provides access for *in situ* studies of a significant block of the Earth's Paleoproterozoic crust 2.7 x 3 x 5 km and 6.5 km of plunge length with >500 km of drift. Recently, by combining microbial phylogeny abundances (16S rRNA gene) with energy yields, the NASA Astrobiology Institute's Life Underground team identified putative chemolithotrophic metabolisms present in the mine, including oxidation of iron, sulfur, and methane, and reduction of iron [4]. With evidence of potentially chemolithotrophic microbes, SURF provides a unique deep subsurface portal to enrich EET-capable microorganisms using electrochemical methods.

We describe a potentiostatically controlled *in situ* electrochemical reactor installed at the 4850 ft level of SURF, with electrodes poised at electron-donating (reducing/cathodic) and electron accepting (oxidizing/anodic) redox potentials, to enrich for potential mineral oxidizing and reducing bacteria as predicted by Osburn *et al.* [4]. All active bacterial families present in the feeding borehole were represented on at least one of the poised electrodes of the reactor. This illustrates the

promise of electrochemical techniques for enriching and studying life in the subsurface. Both *in situ* and secondary laboratory electrochemical enrichments revealed the dominance of numerous unclassified bacteria at the cathode (poised at reducing potentials). The anodes (poised at oxidizing potentials) of the secondary enrichment were dominated by unclassified Firmicutes as well as *Desulfuromonadaceae* and *Geobacteraceae*. Among these, microbes belonging to the latter two families are well known to reduce electrodes. Finally, we successfully isolated several strains including *Bacillus*, *Anaerospira*, *Comamonas*, *Cupriavidus*, and *Azonexus* from these poised electrodes. Initial electrochemical studies on the electrode oxidizer *Comamonas* sp. (strain WE1-1D1) and electrode reducer *Bacillus* sp. (strain WE4-1A1-BC) hint towards novel EET mechanisms.

This study demonstrates the promise of exploring the hidden subsurface microbiota via bioelectrochemical techniques, and demonstrates *in situ* electrochemical enrichment as a convenient method to capture important bacterial lineages active in the subsurface.

References:

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