

Electrode Cultivation of Subsurface Microorganisms

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The finding that genetically and metabolically diverse microbial communities thrive in the deep subsurface raises fundamental questions. While culture-independent molecular methods are increasingly shedding light on these questions, a comprehensive characterization of the subsurface biosphere is hindered because the majority of the resident archaea and bacteria appear to be ‘unculturable’. We here report our 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.

We describe two electrode cultivation platforms: (1) a bulk (multi-liter volume) bioelectrochemical reactor based on carbon cloth working electrodes, and (2) a microfluidic ‘on chip’ platform based on microfabricated electrodes compatible with *in situ* microscopy. Both platforms mimic 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.

Preliminary characterization was performed on samples from Nevares deep well-2 (NDW2) in Death Valley, CA. This site is an artesian well drilled in travertine spring mound without drilling fluid, intersecting fault-associated, deeply-sourced water. Collected well water was used to enrich for subsurface microorganisms at four different redox potentials (50 mV, 150 mV, 250 mV, and 350 mV vs. Ag/AgCl) using multichannel potentiostats. Preliminary electrochemical, microscopy, and 16S rRNA analyses point to the growth of a few metal reducers, including *Rhodoferrax ferrireducens* and other unknown organisms currently being characterized. Additional molecular biological and electrochemical measurements are ongoing to assess the microbial diversity and interfacial electron transport mechanisms dominant in these reactors.

Our study demonstrates an alternate method for culturing subsurface microbes, while using physical electrodes to emulate the microhabitats, redox and geochemical gradients, and the spatially dependent interspecies interactions encountered in the subsurface.