

Microbial diversity, electrode cultivation, and metabolism energetics in the continental deep subsurface

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Quality samples from the marine deep subsurface biosphere are difficult to obtain, biomass there is often at very low concentrations, and slow metabolic activity thwarts many laboratory experiments. However, the host rock types are generally less diverse than their continental counterparts. The petrologic complexity of the continents is countered by the relative ease in access via mines, drill holes, and deeply-sourced springs. We are investigating the deep biosphere in the paleoproterozoic iron-rich metasediments at the Sanford Underground Research Facility in South Dakota (USA). We will present recent results in metabolic reaction energetics, microbial diversity analysis, and *in situ* electrode cultivation. Gibbs energy calculations of ~100 inorganic redox reactions show that per mole of electrons transferred, reactions with O₂, NO₃⁻, and Mn^{IV} are the most exergonic (-120 to -40 kJ). When normalized per kg H₂O, however, the most exergonic reactions are sulfur oxidation, essentially independent of the identity of the oxidant (including CO, CO₂, and Fe^{III}). Metagenomic analysis of fluid samples led to nearly closed genomes of several novel lineages within candidate phyla, including OP3 (Omnitrophica), OP11 (Microgenometes), and NKB19 (Hydrogenendentes). 16S sequences of DNA extracted from fresh rock core via sterile cryo-drilling share very few taxa with fluid samples from the same mine level (~1500 m). Enrichments from 5-month-long *in situ* electrode incubation experiments led to the isolation at several reducing potentials of novel bacteria that are related to known Fe and Mn oxidizers and reducers.