Influence of Biogeochemistry and Engineering Controls on Microbial Growth, Membrane Features, and Interactions with Shale Matrices in Engineered Energy Systems

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Project Goals: Humans are currently engineering the deep subsurface through energy-capture technologies, energy-storage capabilities, and environmental mitigation strategies. Despite the potential for microorganisms to alter the effectiveness of these techniques, we lack a clear understanding of how microbial cells interact with fluids and solids under deep subsurface conditions. The overarching goals of this research are to *1) advance our understanding of the microscopic complexity of nonsterile engineered hydrocarbon extraction systems as they evolve into mature use and 2) build new partnerships between UNH researchers and DOE National Laboratories*. Microbial membranes act as the first line of defense in adaptation to environmental conditions and are responsible for critical cellular functions, including matrix interactions. The specific project objectives are to **1) characterize variables influencing growth parameters and membrane features of fractured shale taxa, 2) characterize the interactions between shale matrices and microorganisms and 3) elucidate engineered and environmental processes driving biogeochemical signatures at the field scale.**

Abstract: During the first few months of this project, post-doctoral researcher (Luek) and graduate student (Siew) have focused their efforts on characterizing variables influencing growth and membrane features of the Utica shale derived taxon *Halanaerobium* (Objective 1). Due to the sensitivity of these bacteria to changes in pH and metabolite accumulation, and in order to determine growth parameters under shale-relevant conditions, we are using the Sartorius Biostat Q system continuous culture (chemostat) system to control for pH, temperature, media flow rate/growth rate, and gas purge rate in six replicate anaerobic bioreactors. Once we optimize parameters to ensure chemostat stability, we will collect cell and media for intact polar lipids methods testing and preliminary analysis of proteomics, lipidomics, and metabolites at the Environmental Molecular Sciences Laboratory (EMSL).

In addition to cultivation efforts, we have made inroads toward optimizing methods for detection and quantification of microbial intact polar lipids (IPLs) using an LC-qTOF-MS accessed at the National Energy Technology Laboratory (NETL) in Pittsburgh, PA during a visit by Luek in early January 2019. She worked with NETL post-doctoral researcher (McAdams) to train and perform experiments on NETL's LC-qTOF-MS, testing an IPL-specific HILIC method to determine the sensitivity and retention times for four lipid standards (phosphoethanolamine, ubiquitin, phosphatidylglycerol, and cardiolipin). Retention times for two of these standards could be directly compared to published research using this HILIC method and were found to be comparable, indicating the tested HILIC method would be appropriate for a wide range of IPLs. Luek will visit NETL again in the coming months to determine detection limits for standards, assess the fragmentation patterns for IPLs, develop a parallel reverse-phase LC-qTOF-MS method for more nonpolar lipids, and test the methods on lipid extracts from cultured

Halanaerobium and/or the field. Fatty acid standards have been purchased to begin methods development for polyunsaturated fatty acids using the GC-MS at UNH in March

In parallel with laboratory efforts, we are working with West Virginia University and Northeast Natural Energy to prepare for field sampling efforts at MSEEL II, a DOE NETL funded hydraulic fracturing research site. Drilling and sidewall coring is scheduled at the site for March 2019 and well completion (fracturing) is slated for May. We will collect core, fluid, and other drill media throughout these upcoming field efforts for characterization of membranes and/or use in future experiments.

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