Environmental and Engineered Factors Influence Membrane Features of Shale Taxa

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Project Goals:

The overall aim of this project is to advance our understanding of the microscopic complexity of non-sterile engineered hydrocarbon systems as they evolve from completion into mature use. Here, we characterize the effects of salinity and hydraulic retention time (HRT) on membrane features of persistent shale taxa.

Deep subsurface shale reservoirs are increasingly being engineered in the United States and globally using unconventional techniques including horizontal drilling and hydraulic fracturing, to meet rising demands for natural gas which is a cleaner alternative energy source to other fossil fuels. Halotolerant anaerobic microbial communities, dominated by *Halanaerobium*, colonize fractured shale and adapt to extreme subsurface conditions such as anoxia, elevated pressure, brine-level salinities and high temperatures. Despite the role these microorganisms play in altering biogeochemical reactions, effective energy capture, and well longevity, *in situ* growth kinetics and activities, including interactions with fluids and shale matrices are poorly understood. The microbial membrane protects the cell from external stressors and mediates critical physiologies, including transport, metabolism, aggregation and cell-surface interactions. Membrane functions are associated with the activities of peripheral and integral proteins, which in turn depend on biophysical properties such as bilayer symmetry, viscosity, curvature, elasticity and thickness. These properties are collectively dictated, to a large extent, by the membrane lipidome comprised of polar head groups and hydrophobic fatty acid tails.

For *Halanaerobium* and other persistent microbial taxa of fractured shale, salinity and hydraulic retention time (HRT) are important perturbants of cell membrane structure. Hence, we used a suite of analytical techniques including gas chromatography-mass spectrometry (GC-MS), dark-field hyperspectral imaging, dynamic light scattering and atomic force microscopy to investigate the effects of salinity (7%, 13% and 20% NaCl) and HRT (19.2 h, 24 h and 48 h) on membrane fatty acid composition and mechanics of *Halanaerobium congolense* WG10 and mixed enrichment cultures from hydraulically fractured wells in West Virginia. For these experiments, cultures were grown in chemostat vessels operated in continuous flow mode under strict anoxia and constant stirring. Our findings show that salinity and HRT induce changes in membrane fatty acid chemistry and biophysical properties of *H. congolense* WG10 in distinct and complementary ways. Notably, under suboptimal salt concentrations (7% and 20% NaCl), *H. congolense* WG10 elevates the proportion of polyunsaturated fatty acids (PUFAs) in its membrane, and this results in an apparent increase in fluidity (homeoviscous adaptation principle) and an experimentally-determined decrease in bilayer elasticity (Figure 1). Taken together, these results provide insights into our understanding of how environmental and engineered factors might disrupt the physical and biogeochemical equilibria of fractured shale by inducing physiologically relevant changes in the membrane of persistent microbial taxa.

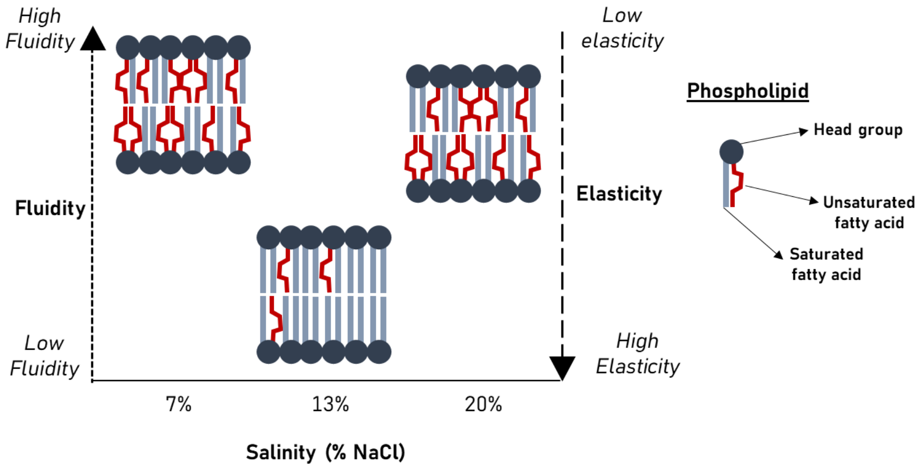

Figure 1. Salinity induces changes in membrane fluidity and elasticity of *Halanaerobium congolense* WG10.

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