Implications of *Halanaerobium* Growth and Persistence in Hydraulically-Fractured Shale Ecosystems

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Bacterial *Halanaerobium* strains become the dominant microbial community member in produced fluids across geographically distinct hydraulically fractured (HF) shales. *Halanaerobium* is not native to the subsurface, but is inadvertantly introduced during the drilling and fracturing process. The accumulation of biomass in pipelines and shale formations is detrimental due to possible corrosion and bio-clogging that could negatively impact oil and gas recovery. Here, we used *Halanaerobium congolense* strain WG8 isolated from a HF well in the Utica Shale to identify metabolic and physioloigical responses to growth under high-pressure subsurface conditions. Laboratory incubations confirmed the capability of strain WG8 to grow under pressures representative of the subsurface (21-48 MPa). Shotgun proteomic measurements identified higher abundances of proteins assocaited with the production of extracellular polymeric substances (EPS), and utilization of 1,2 propanediol when strain WG8 was grown under pressure. Hydrogenase proteins were less abundant under the same growth conditions. Confocal laser scanning microscopy and scanning electron microscopy indicated that EPS production was associated with greater cell aggregation and attachment to shale surfaces under high pressure conditions. NMR and gas chromatography measurements of fermentation products revealed changes in strain WG8 central carbon metabolism under high pressure growth. Twice as much ethanol, acetate and propanol were generated per cell under high pressure conditions, while hydrogen production almost completely ceased. These metabolic shifts were associated with carbon flux through 1,2 propanediol in response to slower fluxes of carbon through stage 3 of glycolysis. Overall, these results revealed the potential for bio-clogging and corrosion (via organic acid fermentation products) associated with persistent *Halanaerobium* growth in deep, hydraulically-fractured shale ecosystems.