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ABSTRACT TITLE: Proteins in the subsurface - understanding microbial function and metabolism using proteogenomics

ABSTRACT: Identifying the active mechanisms that drive microbial biogeochemical cycles in subsurface environments is key for obtaining a better predictive understanding of both short-term and long-term system behavior. Shotgun proteomics, the analysis of all proteins within a sample, can be coupled with genomic information to reveal potential active pathways and organisms that catalyze reactions and processes of interest.

At the Rifle site in Western Colorado, this approach has been utilized to track *Geobacter* physiology and metabolism over a period of active carbon amendment to the subsurface. This biostimulation promotes the growth and activity of *Geobacter* strains that subsequently catalyze the reduction of both Fe(III) phases and U(VI). These species therefore play a key role in the subsurface cycling of metal cations. To investigate how *Geobacter* strains respond to increased carbon flux in the aquifer, twelve planktonic biomass samples were recovered using filtration over a 30-day period from a downgradient monitoring well. Across these samples, 16S rRNA analyses were used to track the rapid enrichment of *Geobacter* species; concomitantly, shotgun 2D-LC-MS/MS analyses identified approximately 1,200 proteins from dominant *Geobacter* species, and revealed active central metabolism pathways through acetyl-CoA and pyruvate that allowed the efficient use of acetate for growth and energy generation. Over the experimental period, we infer potential shifts in carbon flux from biomass synthesis to respiration, and note the activity of both hydrogenase and nitrogenase enzymes.

During a subsequent experiment that stimulated sulfate reduction within the Rifle aquifer, another series of temporally resolved biomass samples were recovered. Subsequent shotgun proteogenomic analyses have identified a significant phage signature in some samples, suggesting that viral predation may play a role in microbial community dynamics under certain conditions.

Most recently, background planktonic biomass samples have been recovered by filtering large volumes of groundwater. Coupled to metagenomic assemblies, analyses of proteins from these samples will aid the understanding of active biogeochemical cycles under non-stimulated conditions at the site, and link to modeling efforts that aim to predict microbially-catalyzed processes occurring at the floodplain scale.