New Isolates of *Geobacter, Desulforegula, Desulfovibrio* and *Pelosinus* and Their Roles in a Low Diversity Consortia During Sustained *In Situ* Reduction of U(VI)

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Subsurface amendments of slow-release substrates (*e.g.*, emulsified vegetable oil; EVO) are potentially a pragmatic alternative to using short-lived, labile substrates for sustained bioimmobilization within contaminated groundwater systems. We previously tracked the dynamic changes in geochemistry and microbial communities for 270 days following a one-time EVO injection that resulted in decreased groundwater U concentrations that remained below initial levels for approximately 4 months. Pyrosequencing and quantitative PCR of 16S rRNA from monitoring well samples revealed a rapid decline in groundwater bacterial community richness and evenness after EVO injection, concurrent with increased 16S rRNA copy levels, indicating the selection of a very narrow group consisting of 10-15 dominant OTUs. Members of the Firmicutes family *Veillonellaceae* dominated after injection and most likely catalyzed the initial oil decomposition and utilized the glycerol associated with the oils and possibly energy sources associated with the small amounts of yeast extract in the EVO product. Sulfate-reducing bacteria from the genus *Desulforegula*, known for LCFA oxidation to acetate, also increased greatly shortly after EVO amendment and are thought to catalyze this process. Acetate and H_2 production during LCFA degradation appeared to also stimulate NO_3^-, Fe(III), U(VI), and SO_4^{2-} reduction by members of the *Comamonadaceae*, *Geobacteriaceae*, and *Desulfobacterales*. Methanogenic archaea flourished late in the experiment and at points constituted over 25 % of the total microbial community.

Subsequent to the experiment we were able to isolate several of these organisms into pure culture including representatives of phylogenetically distinct isolates and/or species of *Geobacter* and *Desulforegula*, *Pelosinus* and *Desulfovibrio*. Physiological investigations into the substrate utilization patterns and end products in pure cultures of these isolates have largely verified the hypothesized model for the functioning of these limited communities that had been developed during the community investigations. *Pelosinus* IFRC1214 has the ability to ferment glycerol from triglycerides and produce acetate, while *Desulforegula* IFRC300 has the ability to use LCFAs producing acetate or acetate + propionate depending on the carbon chain length of the LCFA's while reducing sulfate. The endproducts above (largely acetate) have in turn been shown to support growth by *Geobacter* IFRC128 and *Desulfovibrio* IFRC170 isolates in laboratory studies of the isolates. Currently, in collaboration with the JGI, representative strains of the above isolates are undergoing full genome sequencing and future studies are planned to reconstitute the community and verify its function in synthetic consortia derived from the pure cultures above.