Microbial Communities Associated with Anaerobic Metabolism and U(VI) Reduction in ORFRC Area 2 Sediment Amended with Acetate or Ethanol

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E. Roden (PI), B. Converse, T. Wu, and E. Shelobolina, *U. of Wisconsin*; R. Findlay, *U. of Alabama*; Q. Jin, *U. of Oregon*

This project examined bulk terminal electron accepting processes (TEAPs) and U(VI) reduction in ORFRC Area 2 sediments undergoing biostimulation through ethanol or acetate amendment. The central hypothesis is that patterns of U(VI) reduction will be linked to shifts in the predominant TEAP and to changes in abundance and activity of different types of respiratory microorganisms. Bulk TEAPs, U(VI) reduction, and microbial community composition were monitored in semicontinuous culture reactors (SCRs) (10 day residence time) amended with different amounts acetate or ethanol (either 0, 0.1 or 0.2 mM d^{-1}, in duplicate, for a total of 10 reactors) over a ca. 8-month period. Overall TEAP patterns were similar in the acetate and ethanol-amended reactors. Complete consumption of incoming nitrate was observed in all amended reactors. Complete reduction of Fe(III) phases (oxides and clays) took place in sediments receiving the higher levels of electron donor addition, in which sulfate reduction became active after 2-3 months. Roll tube enumerations confirmed major stimulation of Fe(III)- and sulfate-reducing populations. Patterns of U(VI) reduction indicated that the extent of reduction was similar in acetate vs. ethanol-fed reactors, and proportional to the amount of electron donor added. A major increase in the accumulation of residual HNO3-extractable U (presumably uraninite) took place during the transition between Fe(III) and sulfate reduction, indicating significant contribution of sulfate reducing bacteria to U(VI) reduction. The microbial community response to electron donor amendment in the SCRs was assessed via quantitative PCR (q-PCR) analysis of taxa-specific 16S rRNA genes and selected functional genes, 16S rRNA gene pyrosequencing, and ^{13}C stable isotope probing (SIP) of bacterial PLFAs. The q-PCR analyses revealed a ca. 10-fold increase in total 16S rRNA gene copies in the amended reactors. *Betaproteobacterial* 16S rRNA gene and *nosZ* functional genes were most abundant, increasing ca. 5-fold in the amended reactors and indicating a predominance of nitrate-reducing biomass in the reactors. Densities of *Deltaproteobacterial* 16S rRNA genes were lower, but increased more dramatically (ca. 100-fold) in response to electron donor addition. Although a full analysis of the 16S rRNA gene pyrosequencing results (7 time points for each of the 10 reactors) is not yet available, data from ca. 4 months into the experiment revealed communities dominated by known *Betaproteobacterial* nitrate-reducing taxa (e.g. *Dechloromonas*, *Herbaspirillum*, *Azoarcus*). Metal- and sulfate-reducing taxa (e.g. *Anaeromyxobacter*, *Desulfotomaculum*) were present in the libraries, but their relative abundance was generally much lower than putative nitrate-reducers. Bacterial PLFAs showed dramatic shifts with ethanol or acetate amendment. Profiles from unamended sediments were dominated by the fatty acids 16:0 and cy17:0, while amended sediments showed increased importance 16:1w7 and 18:1w7 and a reduction in cy17:0. Short-term (3-day) SIP (^{13}C-labled ethanol or acetate) assays conducted periodically during the experiment showed incorporation of ^{13}C values into some but not all PLFAs. The most heavily labeled fatty acids in assays conducted at day 150 were associated with metal/sulfate-reducing bacteria and other anaerobic taxa (br17:1a, 10Me16:0, i15:0). The collective geochemical and microbial community analysis results provide the basis for application of a population-based biogeochemical reaction model to the SCR experiments.