Biostimulation of Iron Reduction and Uranium Immobilization: Microbial and Mineralogical Controls

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Our overall objective to understand the microbial and geochemical mechanisms controlling the reduction and immobilization of U(VI) during biostimulation in shallow subsurface sediments cocontaminated with uranium and nitrate. The focus is on microbial populations (metal- and nitrate-reducing prokaryotes) that mediate electron flow as well as on iron minerals that are likely to make strong contributions to the transport/ transformation of U(VI) in the subsurface. Our integrated approach was applied to sediment cores, groundwaters, and microcosms of site materials from Area 2 of the Oak Ridge Field Research Center (ORFRC). In microcosms, pH and redox (electron acceptor/donor availability) were master variables controlling not only the rates but also the pathways of microbial metabolism. Nitrate, Fe(III), and sulfate reduction were the predominant terminal electron accepting processes. Iron minerals were characterized by Mössbauer spectroscopy over a wide range in temperature (4 to 298 K) in order to fully determine the form/speciation of Fe likely to impact the fate of U(VI). Mössbauer along with wet chemical analysis showed that phyllosilicates and goethite predominate, and phyllosilicate bound Fe is an important oxidant that is transformed by indigenous microbial populations. The mechanism of U(VI) reduction was electron donor dependent in microcosms, with substantial reduction occurring prior to Fe(III) reduction. Fe(III) and sulfate reduction cooccurred. While *Geobacter* sp. numerically dominated the cultivatable Fe(III)-reducing bacteria in microcosms, *Anaeromyxobacter* was detected more often in the metabolically active profiles of highly contaminated groundwaters collected near ORFRC source zone. Pure cultures were obtained from ORFRC subsurface sediments for at least four groups of anaerobic metal-reducing prokaryotes including *Geobacter*, *Desulfotomaculum*, *Anaeromyxobacter,* and *Clostridium*. *Geobacter* and *Desulfotomaculum* strains are physiologically distinct from close relatives. A database of > 1000 SSU rRNA gene sequences retrieved from ORFRC materials is now available and > 400 have been examined with *in silico* digestion for T-RFLP profiling. Our most recent work has focused on analysis of mRNA targets to determine the metabolically active metal- and sulfate-reducing prokaryotes as well as on using stable isotope probing (SIP) to directly link microbial function with phylogenetic structure. Community fingerprints of 16S rRNA genes from the SIP experiment revealed that the *Dechloromonas* group of the *Betaproteobacteria* incorporated ^{13}C-ethanol, suggesting that this group is active during both nitrate and metal reduction. The expression of key gene targets (dissimilatory bisulfite reductase, *dsrAB*; citrate synthase, *gltA*) was quantified in ORFRC microcosms. Levels of *dsrA* transcript were significantly higher and paralleled sulfate reduction rates in sulfate/ethanol amended sediments. The expression of *gltA* was significantly greater in glucose or ethanol amended sediments, when electron donors were depleted and acetate concentrations were high. Phylogenetic analysis of cDNA from mRNA extracts revealed clone sequences for *gltA* that are most closely related to *Geobacter bemidjiensis* and *Desulfuromonas acetexigens*.