Molecular Analysis of Rates of Metal Reduction and Metabolic State of *Geobacter* Species during In Situ Uranium Bioremediation

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Previous studies have demonstrated that *Geobacter* species may account for over 90% of the microorganisms in the groundwater during active in situ uranium bioremediation. In order to optimize this uranium bioremediation strategy and broaden its application to other sites, it is important to have information on the rates of metabolism and metabolic state of the *Geobacter* species responsible for the bioremediation.

A search for the most suitable gene to monitor the rates of metabolism in *Geobacter* species found that in chemostat cultures there was direct correspondence between levels of transcripts for *gltA*, which encodes for a novel eukaryotic-like citrate synthase, and rates of Fe(III) reduction. In contrast, transcript levels for other genes were not directly related to Fe(III) reduction rates, and/or their levels varied considerably, depending upon whether metabolism was limited for electron donors or electron acceptors.

In order to better understand the physiology of *Geobacteraceae* during in situ uranium bioremediation, levels of mRNA for key *Geobacteraceae* genes were measured every other day during a study at the NABIR study site in Rifle, CO, in which acetate was injected into the subsurface to promote in situ bioremediation of uranium. As expected from previous studies at this site, the addition of acetate-stimulated removal of U(VI) from the groundwater and this was associated with a bloom of *Geobacteraceae*. In fact, *Geobacteraceae* accounted for more than 99% of the microbial population at the height of U(VI) reduction, and analysis of highly conserved *Geobacteraceae* genes demonstrated that the number of *Geobacteraceae* increased by more than 3 orders of magnitude. There was a remarkable correspondence between acetate levels in the groundwater and levels of transcripts for *gltA*. As acetate rose, *gltA* transcript levels increased. Both acetate and *gltA* transcript levels dropped during a rain event that diluted the acetate with rainwater recharge, and then *gltA* levels increased concurrent with a renewed increase in acetate over time. This contrasted with the constant expression, relative to total RNA, of *Geobacteraceae* housekeeping genes such as *recA*, *rpoD*, and *proC*. Numbers of transcripts of *ompB*, which encodes for a novel outer-membrane protein involved in Fe(III) reduction, tracked with numbers of *Geobacteraceae*, but transcripts of the homolog, *mofA*, thought to encode for a metal oxidation protein, were not detected. These results are consistent with the finding that metal reduction, not oxidation, is the predominant terminal electron-accepting process. Expression of *Geobacteraceae nifD* followed a pattern similar to that of *gltA*. This provided further evidence that the metabolism of *Geobacteraceae* was controlled by the availability of acetate and also demonstrated that the growth of *Geobacteraceae* during bioremediation was limited by the availability of fixed nitrogen. These results demonstrate that quantifying levels of mRNA in subsurface environments can provide insight into the in situ metabolism of microorganisms to aid in modeling and optimizing bioremediation.