Biotransformation of Mixed Inorganic Ions: Biochemistry, and Contaminant and Species Interactions in Chromate-Reducing Consortia

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Mixtures of metallic and radioactive contaminants, including chromate, constitute a major environmental problem at DOE facilities. Direct microbial reduction of hexavalent chromium to trivalent chromium is one potential treatment technology for such sites. While previous research has shown the potential for this treatment, important questions still remain. Such questions include: (1) which members of an environmental consortium are responsible for chromate reduction; (2) for these active chromate reducers, what biochemical pathways are responsible for chromate reduction; (3) what are the effects of carbon source (electron donor) and of inorganic contaminant ions (e.g., SO42-, TcO4- and UO22+) on the system's chromate reduction capacity; and (4) can detailed knowledge of active chromate reducers and their biochemistry be used to facilitate preferential growth of subpopulations with the greatest specific contaminant reduction rates in aquifer systems?

During this year, bacterial enrichment cultures previously isolated from the Hanford site were examined in various ways. These cultures and an E. coli wild-type laboratory strain were assayed for their ability to reduce chromate under nitrate reducing and fermentative conditions.

Other studies have focused on the effect of co-contaminants and nutrients on the specific chromate reduction rate. The effect of various co-contaminant concentrations on the microbial growth rate and the chromate reduction rate is being assessed. In these studies, we are concentrating on the effect of sulfate, pertechnetate and uranyl on the growth rate and the specific chromate reduction rate. This community of chromium-tolerant microorganisms, enriched from the Hanford site, was also examined to assess its ability to anaerobically reduce Cr(VI) to Cr(III) using various electron donors. Growth occurred on a range of low-molecular-weight fatty acids and sugars that include acetate, pyruvate, lactate, succinate, citrate, glucose, sucrose and fructose. Chromate was reduced by cultures grown on glucose, sucrose, fructose, acetate and pyruvate — lactate, succinate and citrate are currently under examination. Other electron donors will be evaluated in the near future. Data suggests that this microbial community is made up of at least three cultivable strains. All isolated strains are comprised of oxidase negative, catalase positive, facultative Gram negative rods.

Laboratory-scale soil column studies are also being performed to test our understanding of chromium reduction in the presence of co-contaminants with various nutrients. The column (stainless steel) contains coarse sand inoculated with Hanford site, subsurface bacterial consortia. A clear lexan soil column has been developed that contains micro-oxidation/reduction (ORP) probes and sample collection ports at four locations. This column will allow data to be collected along the column length to enable a more complete understanding of biological activity.

To elucidate the actual enzyme(s) responsible for chromate reduction, two metal reducing cultures, Shewanella putrefaciens MR-1 and Pseudomonas aeruginosa PAO1, were selected for further experiments. Both of these bacteria have been fully sequenced, which allows for easier manipulation of the genome. We have also monitored and quantified the rate of chromate reduction during the transition from aerobic to anaerobic conditions. Initial results indicate an increase in the specific reduction rate as the culture grows under fumerate-reducing conditions. This result may indicate that the enzymes responsible for chromate reduction may be induced by such conditions.