Biological Uranium (VI) Reduction/immobilization Near Source Zone at the ERSP Field Research Center, Oak Ridge, TN

Wei-Min Wu[1], Jack Carley[2], Matthew A. Ginder-Volgel[1], Jian Luo[1], Chaichi Hwang[3], Shelly D. Kelly[4], Mary Beth Leigh[5], Erick Cardenas[5], Liyou Wu[6], Chuangmin Ruan[2],Wenshui Luo[2], Margaret E. Gentile[1], Terry Gentry[2], Jennifer Nyman[1], Michael Fienen[1], Sue Carroll[2], Kenneth Lowe[2], Tonia Mehlhorn[2], Terrence L. Marsh[5], David Watson[2], Matthew W. Fields[3], Baohua Gu[2], Jinzhong Zhou[6], Kenneth M. Kemner[4], James Tiedje[5] , Phillips M. Jardine[2], Scott Fendorf[1], Peter K. Kitanidis[1], **Craig S. Criddle (PI)**[1]

[1]Stanford University, Stanford, CA94305, [2]Oak Ridge National Laboratory, Oak Ridge, TN 37831, [3]Argonne National Laboratory, Argonne, IL60439, [4]Miami University, Oxford, OH 45056. [5]Michigan State University, East Lansing, MI 48823, [6] University of Oklahoma, Norman, OK73019

The objectives of the studies were to investigate the feasibility of in situ bioremediation of uranium contaminated subsurface near source zone in the DOE ERSP Field Research Center at the Y-12 National Security Complex in Oak Ridge, TN. *In situ* biological reduction and immobilization of uranium(VI) was evaluated in a long term experiment (>3 years) conducted at a highly contaminated aquifer. The soil at the site contained up to 1,000 mg U/kg, and groundwater contained ~40 mg U /L. The groundwater also contained high levels of nitrate (8-10 g/l), calcium (0.9 g/l), and aluminum (0.5 g/L) at a pH of 3.4. A step-wise strategy was employed to assess the potential for U(VI) reduction: (1) flushing with acidified clean water to remove clogging agents and inhibitors (Al, Ca, nitrate); (2) flushing with clean water at pH 6 to create a pH condition suitable for robust microbial growth; and finally (3) ethanol addition to remove residual nitrate by denitrification and to stimulate U(VI) reduction. Reduction of U(VI) to U(IV) occurred after nitrate had decreased to a low level (< 1.0 mM). After one year of intermittent injection of ethanol, nitrate levels fell below 0.05 mM, and aqueous U(VI) levels fell below the USA EPA drinking water maximum limit (<30 µg/l). U(VI) reduction was concomitant with sulfate reduction. Ethanol was consumed with acetate as an intermediate. Reduction of U(VI) to U(IV) in soil was confirmed by X-ray absorption spectroscopy, and the structures of uranium in the sediments before and after biostimulation was investigated. The reduced and immobilized uranium was stable under anaerobic conditions but sensitive to oxygen. Most-probable-number analyses indicated proliferation of denitrifying populations, sulfate-reducing bacteria and iron-reducing bacteria. Groundwater bacterial communities were analyzed using 16S rRNA gene clone libraries, and predominant genera related to U(VI) reduction were *Geobacter* spp. and *Desulfovibrio spp*. Microarray analysis indicated that uranium reductions significantly correlate with the cytochome content and the abundance of *Desulfovibrio* and *Geobacter* species.

PI: Craig S. Criddle, Department of Civil & Environmental Engineering, Stanford University, Stanford, CA 94305-4020. Phone: 650-723-9032. Fax:650-725-3164. Email: criddle@stanford.edu