# Field Investigations of HRC®-Stimulated Bioreduction of Cr(VI) at Hanford 100H

*Terry C. Hazen[1] (PI), Dominique Joyner[1], Sharon Borglin[1], Boris Faybishenko[1], Jiamin Wan[1], Tetsu Tokunaga[1], Susan Hubbard[1], Ken Williams[1], Mark Conrad[1], Carlos Rios-Velazquez[1], Josue Malave-Orengo[1], Ramon Martinez-Santiago[1], Mary Firestone[2], Eoin Brodie[2], Philip E. Long[3], Anna Willett[4], and Stephen Koenigsberg[4]*

*[1]Lawrence Berkeley National Laboratory, Berkeley, CA; [2]University of California at Berkeley, Berkeley, CA; [3]Pacific Northwest National Laboratory, Richmond, WA; [4]Regenesis, San Clemente, CA*

The overall objective of this project is to carry out field investigations to demonstrate the feasibility of a cost-effective, in situ remediation technology, using lactate-stimulated bioreduction of dissolved Cr(VI) to form insoluble Cr(III) precipitates at the Hanford 100H area. Lactate will be provided in situ via injection of Hydrogen Release Compound (HRC®). Specific goals include (1) the design of a field test to develop design criteria for full-scale deployment of in situ Cr(VI) bioreduction via biostimulation for the use at DOE sites, (2) providing field testing and monitoring (including geophysical methods) of the effects of biostimulation on microbial community activity, redox gradients, transport limitations, and other reducing agents, and compare the field results with those of our previous laboratory work, (3) the assessment of kinetic rates and conditions that may cause reoxidation of Cr(III) to Cr(VI) after biostimulation is terminated, and (4) the assessment of the use of bioremediation in conjunction with other alternative remediation technologies, such as a pump-and-treat approach for the Hanford 100H site. Sediment samples from the field site have shown that the microbial densities are quite low ($<10^4$ cells/g), but are easily stimulated with HRC or sodium lactate to densities $>10^8$ cells/g in just a week. As part of that investigation, we collected sediment from the Hanford site and exposed it to 1,000 ppb Cr(VI) and to sodium lactate, HRC, HRC Primer, HRC-Extended Release (HRC-X™), Metals Remediation Compound (MRC™), and no carbon. HRC is a polylactate compound with different degrees of polymerization to control its viscosity and solubility in water. HRC Primer is a low viscosity polylactate, and provides a rapid release of lactate into groundwater. HRC-X is a high viscosity polylactate, and has an extended lactate release profile. MRC is an organosulfur and polylactate combination that reacts directly with the Cr, at least initially. In less than one week, MRC reduced Cr(VI) to undetectable concentrations, though the abiotic MRC control had only been reduced by 65%. By three weeks, all the biotic HRC and lactate combinations had undetectable Cr(VI), while the abiotic controls for each had 40–61% of the Cr(VI) remaining. The sediment with no additional carbon source also showed a 64% reduction in Cr(VI) after three weeks, whereas the abiotic control had only a 12% reduction in Cr(VI). The HRC compounds performed equally well, while the MRC gave a faster response, although much of this response was abiotic. Phospholipid fatty acid analyses (PLFA), terminal restriction fragment length polymorphisms (TRFLP), clone libraries, direct cell counts, and 16s rDNA microarray analysis demonstrated that the initial densities of microbes is very low (less than $10^4$ cells/g), but after biostimulation was typically greater than $10^8$ cells/g. The low densities made extraction for PLFA and DNA analysis difficult, so a cell concentration technique was developed to increase yields. These studies confirmed that the microbial diversity was low but that sulfate reducers, *Arthrobacter* spp. and *Geobacter* spp., dominated the samples. $^{13}$C-labeled lactate was also used to show use of the lactate by specific groups of bacteria. The results demonstrate that even in low biomass and diversity environments, biostimulation of Cr-reducers can occur and that their functional relationship can be evaluated by various molecular techniques. These studies, along with field studies in progress, will be used to develop a conceptual model of chromium bioreduction in groundwater at a field scale, and to provide recommendations for field deployment of engineered bioremediation.