Competing evidence for enzymatic versus abiotic reduction of Cr(VI) in Hanford 100H flow-through columns

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Remediation of chromium contamination frequently involves reduction of the toxic and soluble hexavalent form, Cr(VI), to the relatively harmless and mostly immobile trivalent state, Cr(III). Microbially mediated Cr(VI) reduction at the Hanford 100H area was investigated by flow-through column experiments. The objective of this study is to identify the biogeochemical reactions that control *in situ* chromium reduction under different dominant electron-accepting conditions, i.e., nitrate-, Fe(III)-, and sulfate-reducing conditions. Replicate columns packed with natural sediments from the site were eluted with artificial groundwater containing lactate (5 mM) and Cr(VI) (5 μM) and kept under anaerobic conditions. Sulfate and nitrate solutions were added to selected columns to promote specific electron-accepting pathways. Native iron and manganese oxides were present in all the column sediments. Effluent concentrations of substrates and metabolites were analyzed regularly over a 1-year period using ICP-MS and IC. X-ray absorption spectroscopy was used to analyze the Cr phases formed during the experiment, while Hanford bacterial isolates were investigated under batch conditions to gain a detailed understanding of microbial metabolism and physiology.

Cr(VI) depletion was observed under all conditions, but the magnitude of the reduction varied significantly. Complete removal of the influent Cr was observed in the denitrifying columns and some of the sulfate-added columns in which fermentation was dominant. Despite the very different biogeochemical regimes in the denitrifying and fermentative columns, spectroscopic studies of both kinds of columns revealed very similar Cr(III)-containing minerals. Analysis of XANES spectra indicates that the Cr(III) particles most closely resemble standards produced by abiotically reacting Cr(VI) with biogenic Fe(II) and are distinct from abiotically synthesized mixed-phase Cr-Fe hydroxides. These results suggest that mixed $Cr_xFe_{1-x}(OH)_3$ material made under realistic subsurface conditions or in active bacterial cultures are structurally different from those materials synthesized through common abiotic synthetic methods.

Formation of these minerals is consistent with bacterial Fe(III) reduction and subsequent abiotic reduction of Cr(VI) by biogenic Fe(II). In contrast, other evidence supports enzymatic Cr(VI) reduction: (1) both denitrifying and fermentative Hanford bacterial isolates catalyze rapid Cr(VI) reduction in iron-free systems, (2) strong correlations between nitrate and chromate reduction suggest that Cr(VI) reduction is co-metabolic with denitrification, and (3) meta-transcriptomic data clearly indicate active denitrification, which is thermodynamically inconsistent with Fe(III) reduction. Current work is underway to understand the ability, or lack thereof, of two newly isolated Hanford organisms, one denitrifier and one fermenter, HCN1 and HCF1 respectively, to catalyze Fe(III) reduction under the column conditions in spite of the prevailing bulk thermodynamic conditions. These experiments will help to determine the extent to which a minor metabolic pathway, e.g., Fe(III) reduction, may or may not be disproportionately responsible for the fate of the redox-active contaminant Cr(VI).