Nitrate attenuation and the impact of pH on the predominant denitrifying microbial groups in the OR-IFRC subsurface.

Joel E. Kostka[1], Stefan J. Green[2], Om Prakash[1], Puja Jasrotia[3], Lavanya Rishishwar[1], Chris Schadt[4], David Watson[4], Scott Brooks[4]

[1]Georgia Inst. of Technology; [2]Univ. of Illinois-Chicago; [3]Florida State Univ.; [4]Oak Ridge National Lab

The goal of this research is to understand the fundamental mechanisms controlling the fate and transformation of uranium, nitrate, and technetium under natural attenuation conditions. The fate and transport of uranium and nitrate are intimately linked through a variety of biogeochemical reactions. We hypothesize that microbial denitrification is the only biogeochemical mechanism for permanently decreasing nitrate flux, where the denitrification rate is governed by pH and electron donor concentrations. Specific objectives are to: 1) quantify the relative roles of dilution, assimilatory uptake, and denitrification as mechanisms of nitrate natural attenuation along the Bear Creek watershed flow paths, 2) identify and quantify the denitrifying bacterial populations that catalyze nitrate removal in groundwaters on a watershed scale in the uranium-contaminated subsurface, and 3) directly link denitrification activity to the abundance and phylogenetic structure of nitrate-respiring organisms through a tight coupling between geochemical and microbiological approaches.

The site-wide distribution of overall bacteria as well as the dominant denitrifying bacteria in subsurface groundwaters of the main OR-IFRC contaminant plume has been conclusively tracked, and the results are published. Two site-relevant denitrifying bacterial isolates have been described as a novel species, *Rhodanobacter denitrificans*, the description is now published in IJSEM, and the strains have been deposited into public culture collections. We continue to inform our field efforts using genome-based approaches with site-relevant isolates. A genome sequencing project was initiated to further elucidate the in situ function of the *Rhodanobacter* group, which has been found to dominate the bacterial communities in the highly uranium-contaminated OR-IFRC subsurface. The genomes have been sequenced for 5 *Rhodanobacter* strains isolated from different regions of the OR-IFRC site, including the described species.

The metabolic potential of the aerobic and denitrifying microbial community response was assessed in the ORIFRC site subsurface in groundwater and sediment slurry experiments. These experiments demonstrate slow, but active denitrification even at acidic pH (<4), with higher rates and more complete denitrification (to N_2) at elevated pH. Low rates of aerobic respiration were also observed in the highly contaminated acidic, source zone. Background or pristine subsurface sediments showed higher rates of O_2 consumption but denitrification rates were below detection. In a pH threshold experiment, the maximum rate of denitrification in source zone subsurface sediments was observed at pH 5-6, matching the pH adaptation of *R. denitrificans*

To address their potential role in contaminant transformation, we expanded our site-wide microbial community characterization to include the fungal community composition across the site. Fungal diversity was less dramatically impacted by pH in comparison to bacteria, but some low pH samples had very low diversity. Other factors, such as sampling time, and depth also contribute to the observed fungal community dynamics. Fungal communities were often dominated by fungi from the order Coniochaetales. Site-relevant fungal isolates were obtained in parallel with molecular characterization of subsurface fungal communities. In particular, members of the Coniochaetales, which are abundant in sequence libraries were isolated and shown to mediate denitrification.