



## **Spatial Correlation and Stochastic Simulation of Water, Uranium, and Biocolloid Fluxes in Natural Zones of U(VI) Bioreduction at the Rifle Integrated Field-Scale Subsurface Research Challenge (IFRC) Site**

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Knowledge of the subsurface natural attenuation capacity is critical to understanding where active and natural attenuation can be relied upon for the remediation of uranium plumes. An important component of understanding the natural attenuation capacity of a site is to quantify the indigenous extent and rate uranium bioreduction as inferred from ambient measures of uranium and biocolloid fluxes. The effect of naturally occurring zones of bioreduction on the fate and transport of uranium in the subsurface is likely key to accurate prediction of natural attenuation of U in alluvial aquifers with high organic matter. During the summer of 2008 geophysical surveys were done in order to locate zones of natural bioreduction at the Rifle IFRC site. Five 4-inch wells were installed in three of the most promising areas identified by the geophysical surveys. Sediment from well LQ-107 was chosen for initial characterization due to apparent sulfide precipitates. Geochemical results showed the total Fe content of sediment from the 20ft depth was 10.5  $\mu\text{mol/g}$ , Fe(II) was 8.28  $\mu\text{mol/g}$  and total U was 3.36  $\mu\text{grams/g}$ . The amount of total reduced inorganic sulfur (TRIS) extracted from LQ-107 at the 20ft depth was 6.85  $\mu\text{mol/g}$  and was similar to that extracted from biostimulated sediments after prolonged (100+ day) acetate amendment. Microbial quantitative polymerase chain reaction (qPCR) analysis targeting total Eubacteria provided an estimate of 106 cells/g at the 20ft depth and 104 cells/g of  $\Delta$ -proteobacteria. Previous results from another bioreduced zone at Rifle contained a significant amount of solid phase carbon, even relative to the amounts utilized during active biosimulation of iron and sulfate reducing bacteria. The high carbon fractions in the natural bioreduced zones may have the potential to maintain long-term reducing conditions and thus uranium stability.

Given the existence of these bioreduced zones, a thorough understanding of how these zones impact the overall Uranium flux at the Rifle site will be critical to decision making processes and management of these plumes. A first step, passive flux meters (PFMs) were deployed in each of the five wells to quantify ambient biocolloid, water, and Uranium fluxes. The PFMs provide direct in situ measures of both the water and uranium flux; whereas biocolloid fluxes are obtained from the product of measured water flux and measured biomass concentrations in sampled water.

Water and Uranium flux increased with well depth and were highest in the bioreduced zone. Flux-averaged uranium concentrations were consistent with observed aqueous concentrations: an indication PFM measures were valid. The higher fluxes in the bioreduced zones were not expected. To characterize the transport of biocolloids, 16S rRNA gene sequencing was used to obtain the initial characterization of bacterial ecology again as a function of well depth. This was followed by qPCR to measure total microbial biomass and specific microbial groups associated with iron, sulfate, and Uranium reduction. Several types of bacteria were identified in water samples including those known for nitrate reduction *Dechloromonas* spp, Fe reduction *Geobacter* spp. and *Rhodoferrax* spp, and sulfate reduction *Desulfotomaculum* spp. and *Desulfovibrio* spp.

Given spatial data on uranium flux, water flux, and biocolloid fluxes specific to several types of bacteria, this paper presents for the first time spatial correlations of these fluxes and results of stochastic simulations conducted to investigate possible roles these bacteria play in the natural bioreduction of Uranium at Rifle.