

## Metagenomic Monitoring of Microbial Community Evolution in a Tetrachloroethene Contaminated EPA Superfund Site.

Rebecca Reiss, Ph.D., New Mexico Tech and Peter Guerra, M.S., AMEC, Inc.

Contamination of groundwater with chlorinated solvents is a major threat to potable water supplies. Fortunately, microbial communities evolve to utilize these compounds for energy. Although our knowledge of the microbes and the biochemical pathways that result in chlorinated solvent biodegradation is limited, *in-situ* augmentation protocols that create favorable environmental conditions for degrading consortia are available. The North Railroad Avenue Plume (NRAP) Superfund Site in Española, New Mexico is currently undergoing such augmentation protocols. The Site consists of an approximately 58-acre, 260-foot-deep plume of tetrachloroethene (also known as perchloroethylene or PCE) that is contaminating the drinking water aquifer for Española, the Santa Clara Pueblo, and nearby populations. PCE is a possible carcinogen and is used as a dry-cleaning fluid and degreaser. AMEC is an international environmental engineering company with an office in Albuquerque that is contracted by the New Mexico Environmental Department (NMED) to carry out the EPA-mandated remediation. The remediation using augmentation (NPL #NMD986670156) is under the direction of Peter Guerra (M.S., Environmental Engineering, NMT, 2000). The test plan including the field monitoring of water quality parameters such as dissolved oxygen, temperature, pH, and redox potential as amendments were added to the aquifer. Laboratory analyses include the following geochemical parameters: chloride, sulfate, sulfide, nitrate, arsenic, manganese, total and dissolved iron, ortho and total phosphorus, carbon dioxide (as free calcium carbonate), methane, total organic carbon, alkalinity (total, as calcium carbonate), bicarbonate (as calcium carbonate), and carbonate (as calcium carbonate), PCE, trichloroethylene (TCE), cis and trans 1,2-dichloroethene (DCE), vinyl chloride, ethane, and ethane. The changes in these parameters were monitored to determine the success of each treatment. A baseline for each parameter was established prior to augmentation.



**NRAP EPA superfund site in Española, New Mexico.** Groundwater sampling is facilitated by a series of wells and a manifold system built as part of the pilot project.

The NRAP site provides a unique opportunity to use metagenomic analysis to monitor the evolution of the microbial communities in response to the selection pressure of the remediation efforts. AMEC submitted ground-water samples to Microbial Insights, Inc. (Rockford, Tennessee) for analyses to determine the presence or potential for halocarbon-degrading microorganisms. Denatured Gradient Gel Electrophoresis (DGGE) of 16S rRNA genes, phospholipid fatty acids (PFLA) analysis, and qualitative PCR (qPCR) can screen for dechlorinating bacteria and provides a low-resolution snapshot of the microbial community. Three dechlorinating genera were detected; *Dehalococcoides*, *Desulfuromonas*, and *Dehalobacter*. Complete genome sequences of

**Metagenomic Monitoring of Microbial Community Evolution in a  
Tetrachloroethene Contaminated EPA Superfund Site.**

Rebecca Reiss, Ph.D., New Mexico Tech and Peter Guerra, M.S., AMEC, Inc.

the first two genera are available and can serve as scaffolds for high-resolution metagenomic analyses.

The objective of this project is to determine the biological changes in the contaminated groundwater using metagenomic techniques as regions in the aquifer are treated by augmentation. Our long-term goal is to understand the microbial biodegradation of chlorinated solvents. Short-term goals include the identification of biomarkers that can be used as rapid indicators for risk assessment. Protocols for the isolation of microbes from ground-water and the extraction of protein and DNA have been under development in the Reiss lab at NMT since 1998 in collaboration with Peter Guerra. Research projects funded by the New Mexico Water Resource Institute (WRRI) and the New Mexico Waste Education Research Consortium (WERC) facilitated the development of molecular techniques on microbial communities from gasoline-contaminated aquifers in Ribera and Socorro. Two wells at the NRAP site have been sampled three times. The first sampling occurred on June 13, 2007, prior to treatment, the second, on November 19, 2007, five months after augmentation, and the most recent occurred on June 15, 2009. Preliminary genomic DNA (gDNA) extractions from these sampling events indicate that adequate yields of DNA were obtained for massively parallel sequencing-by-synthesis (MPSS).

The DNA will be submitted for sequencing on the Solexa 1G MPSS instrument at the New Mexico Genomic Sequencing Center (NMGSC) at the National Center for Genome Resources (NCGR) in Santa Fe. Three samples will be sequenced, one pre and two post-treatment for one well. The sequence data will be aligned to selected scaffolds and annotated using NCGR's Alpheus software. Further analyses will be preformed using New Mexico's recently established Center for High Performance Computing to develop novel bioinformatics tools necessary to compare the metagenomes that evolve in response to each treatment and over time. The geochemical and water quality changes monitored as part of the bioremediation field test plan will be included in the analysis as representative of the exometabolome, or metabolic footprint, of the community. Students taking the Fall 2009 Advanced Genetics course will investigate the relationship between the genetic structure and the geochemical parameters.

Using these new bioinformatics tools, we will test hypotheses regarding the nature of the microbial response to bioaugmentation. For example, if bioaugmentation is considered positive selection pressure, the phylogenetic and metabolomic response can be calculated. Not only will this project add metagenomics to the bioremediation toolbox, it will provide a method to determine additional microbes and genes responsible for intrinsic biodegradation of chlorinated solvents without any reliance on culturing. An increased understanding of degradation pathways can be used to design novel bioremediation strategies for the reclamation of polluted water sources. This preliminary data will be incorporated into peer-review publications and proposals for continued funding.