# Vadoze Zone Microbiology Research at the Hanford Site Sponsored by the EMSP and Microbial Genome Program

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While numerous techniques exist for remediation of contaminant plumes in groundwater or near the soil surface, remediation methods in the deep vadose zone are less established due to complex transport dynamics and sparse microbial populations. There is a lack of knowledge on how physical and hydrologic features of the vadose zone control microbial growth and colonization in response to nutrient delivery during bioremediation. The overall objective of the projects was to increase knowledge of the feasibility of engineered bioremediation in the deep vadose zone, particularly at arid western sites where microbial populations and activities are low. Accomplishments include the following.

For the first time, numeric values have been defined for microbial colonization under nonflowing unsaturated conditions. How sand particle size and water content controls these values was also determined. Motile bacteria were able to rapidly migrate in response to nutrient addition. The work showed that aqueous nutrient delivery to vadose zones with patchy and low density microbial populations appears to be an effective technology for driving rapid microbial colonization in the field. Microbial characterization of vadose zone sediments beneath the 216-Z-9 Trench at the DOE Hanford Site, where large amounts of carbon tetrachloride and radionuclides were disposed. The analysis showed that the highest carbon tetrachloride concentrations were co-located with the highest microbial populations and highest sediment moisture content. These sediments were further used in laboratory experiments to investigate the potential of the microbial community to utilize gaseous hydrocarbons and potentially transform CT under unsaturated conditions.

Five hydrocarbons (methane, ethane, propylene, propane, and butane) and sources of nitrogen and phosphorus that could be delivered to the vadose zone during engineered bioremediation via vapor-phase transport were tested. Eighty percent of the sediments degraded at least one of the hydrocarbons; 20–45% of the sediment samples were able to biodegrade a specific hydrocarbon; more than 3 hydrocarbons were degraded in 70% of the sediments. Gaseous nitrogen and phosphorus did not stimulate removal of gaseous C sources compared to no addition of N and P. The ability to successfully deliver the gases to the in situ subsurface microsites where microbes exist should be tested in intact Hanford deep vadose zone cores. Sediment samples that displayed the ability to use gaseous hydrocarbons were tested for carbon tetrachloride-degrading ability. CT degradation occurred in only 5% of the bottles and only 50-60% was degraded after a 10 month period. It is likely CT degradation would occur more readily in the subsurface vadose zone where porosity and oxygen diffusion rates are lower and anoxic sites would be more readily formed as a result of aerobic degradation of gaseous hydrocarbons. The microbial community beneath a leaking high-level radioactive waste tank was characterized by 16S rDNA sequencing, and metagenome sequencing was performed on pooled enrichments from the sediments. Protein hits were largely consistent with amplified and sequenced 16S rDNA phylogenies from both pooled enrichments and sediments. The 16S data from pooled enrichments showed 10 genera from the Micrococcineae, Propionibacterineae, and Steptomycineae suborders within the Actinobacteria (high GC gram-positive) phylum with nucleotide identities of 93–97%; and one genera (*Pseudomonas*) within the gamma class of the Proteobacteria phylum. A total of 3,840 metagenome clones were sequenced. After removing higher eukaryote clones (contamination), 8,762 nonredundant prokaryotic protein hits were found, representing 489 different EC classes and 113 different KEGG maps. Of these, 6,053 hits were associated with TIGR role categories. Protein hits were 62% Proteobacteria, 36% Actinobacteria, and 1% Bacilli and Clostridia classes of the Firmicutes (low GC gram-positive) phylum. With the exception of the genera *Pseudomonas*, the community at this contaminated site was very similar to previous studies of the noncontaminated vadose zone at the Hanford Site (i.e., strong dominance by high GC gram-positive microbes).