

Title: Analysis of Microbiological and Geochemical Processes Controlling Biodegradation of Aromatic Hydrocarbons in Anaerobic Aquifers

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Background: Benzene, toluene, ethylbenzene and xylenes (BTEX) are among the groundwater contaminants of greatest concern because of their toxicity, their solubility in water, and their resistance to degradation in anaerobic environments. Prior to entry of hydrocarbon pollutants, shallow aquifers are often aerobic with relatively low levels of dissolved organic carbon. Indigenous aerobic bacteria readily metabolize hydrocarbon pollutants (e.g. fuels) entering these systems, and quickly deplete the available oxygen. Most enhanced bioremediation systems involve the addition of oxygen, however, the feasibility of aerobic biodegradation is often limited by its low water solubility; in addition, oxygen injection may induce iron oxide precipitation. Thus, bioremediation using alternate electron acceptors, such as NO_3^- , Fe^{+3} , and SO_4^{2-} , has been the subject of much recent research. A better understanding of underlying mechanisms of these anaerobic processes is needed for them to be effectively employed for aquifer bioremediation. This requires identification of the organisms mediating the reactions, the metabolic pathways by which they degrade BTEX, and the effects of available electron acceptors (and other environmental factors) on their activities.

The study aquifer at Fort McCoy, Wisconsin, underlies a former Petroleum, Oils, and Lubricants Station, and is contaminated with BTEX from two 12,000-gallon leaking underground storage tanks (UST's) that were installed in 1943 and removed in 1989. Residual non-aqueous phase liquid concentrated in a 0.3 to 0.6 m thick zone near the water table continuously supplies BTEX to groundwater. The current plume is approximately 100 m long and 30 m wide at its maximum, with BTEX concentrations generally less than 20 mg/l.

Objectives: The overall goal of this project was to determine the kinetics of BTEX biodegradation in the presence and absence of NO_3^- and to establish links to changes in the microbial community structure that accompanied BTEX biodegradation. The project was organized into two concurrent tracks of investigation. The first was designed to yield process-level information on BTEX biodegradation. The second track focused on characterizing the physiological and phylogenetic properties of the BTEX-degrading microbial community.

Methods: These studies were conducted with sediment material collected from the Ft. McCoy site. The approach used for the Track 1 experiments was to establish a series of microcosms to monitor the degradation of BTEX and the use of NO_3^- and iron in a controlled laboratory setting. Rates of TEX degradation and NO_3^- use were calculated and compared to previously conducted field experiments. For Track 2, at selected sampling points, the microcosm sediment was collected and used to inoculate enrichment cultures for BTEX degrading organisms. Aliquots of the sediment were also used for DNA extraction. The microbial community of the microcosms was analyzed by using molecular techniques including Amplified Ribosomal DNA Restriction Analysis (ARDRA) and Denaturing Gradient Gel Electrophoresis (DGGE). We also designed and tested *in situ* microcosms (ISMs). The ISMs were investigated as a means to conduct controlled experiments *in situ*. We used the ISMs to study the BTEX biodegradation rates and TEA use under field conditions at the site.

Results and Discussion: In the laboratory microcosms, only those amended with BTEX and NO_3^- exhibited BTEX degradation. Toluene, *o*-xylene ethylbenzene and *m,p*-xylenes degraded during the experiment. Benzene, however, was recalcitrant. Biodegradation rates were slightly higher in the field than in the lab. The sequence of TEX biodegradation in microcosms was similar to the field tracer experiments, but the lag times were different. The ISMs had a different sequence of TEX degradation, and had highly variable lag times. The differences in the lag times are most likely related to different competing electron donors in the field and in the lab, spatial heterogeneity in the field, and the re-establishment of acclimated microbial populations in the laboratory.

Molecular analysis indicated that the field samples and microcosm trials had distinctly different microbial communities. Nitrate amendment induced a significant alteration in the community structure. This structure appeared to be the same in microcosms treated with nitrate alone and in those receiving NO_3^- and BTEX. No organisms similar to known, cultured NO_3^- - or Fe^{+3} -reducing alkylbenzene degraders were identified in the microcosms. This suggests that the predominant TEX-degraders stimulated by NO_3^- amendment may comprise organisms not previously known to have alkylbenzene degrading abilities.

Conclusions/Recommendations: Results of this study suggest that NO_3^- can enhance overall BTEX biodegradation in the hydrocarbon-contaminated aquifer at Fort McCoy, WI, but it should be used in combination with other electron acceptors, either under intrinsic or enhanced conditions, to treat benzene. The molecular analysis indicated that distinct populations were enhanced by NO_3^- amendment, which probably use other electron donors as well as TEX. These banding patterns highlight populations in the microbial community that can be targeted for identification of the microorganisms involved with BTEX biodegradation and NO_3^- reduction in the field. Because the microbial communities of the microcosms and the field samples were different, future studies exploring impact of nitrate on specific microbial populations *in situ* would help describe impacts remediation efforts have on the microbial community.

Related Publications:

Schreiber, M.E. (1999) Experimental and modeling approaches to evaluating anaerobic biodegradation of petroleum-contaminated groundwater. Ph.D. dissertation. Department of Geology, University of Wisconsin-Madison, Madison, WI.

Taglia, P.J. (2001) Using *in situ* microcosms to evaluate the spatial heterogeneity of BTEX biodegradation under nitrate-reducing conditions. M.S. Thesis. Department of Geology, University of Wisconsin-Madison, Madison, WI.

Zwolinski, M.D., Harris, R.H., and Hickey, W.J. (2001) Microbial consortia involved in the anaerobic degradation of hydrocarbons. *Biodegradation* (in press).

Final Report: A final report containing more detailed information on this project is available for loan from Wisconsin's Water Library, University of Wisconsin - Madison, 1975 Willow Dr., Madison, WI 53706. (608)262-3069.