

## **Molecular Tools to Understand Intrinsic Aerobic Biodegradation of Chlorinated Contaminants in Groundwater Samples from the Wichita Northern Industrial Corridor**

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**Background/Objectives.** Groundwater Unit 1 (GWU1) within the Northern Industrial Corridor in Wichita, Kansas is contaminated with a mixture of chlorinated solvents and petroleum hydrocarbons. A monitored natural attenuation (MNA) study was performed to determine the efficacy of MNA as a remedial alternative for the contaminants at the site. As part of this effort, enzyme activity probes (EAPs) were used to probe for enzymes associated with cometabolic degradation of chlorinated solvents under aerobic conditions. Decades of research has shown that bacteria in the environment express oxygenase enzymes that have been shown to co-metabolically transform chlorinated solvents such as trichloroethene (TCE). A number of EAP that fluoresce when oxidized by these enzymes have been developed, not only showing the presence of bacteria with the enzyme in an environmental sample, but that the enzyme is active. When a groundwater sample is assayed using these EAPs, microbes with the active oxygenase enzymes fluoresce, which is measured using a fluorometer or epifluorescence microscopy and can be quantified. Positive results from the EAP analyses provide evidence for the potential of intrinsic aerobic remediation for contaminated sites.

**Approach/Activities.** Groundwater samples (14) from the Wichita Northern Industrial Corridor (NIC) were assayed using EAPs. Samples were filtered and EAPs for aromatic oxygenase enzymes (phenylacetylene, 3-hydroxyphenylacetylene and cinnamitrile) and one for soluble methane monooxygenase (coumarin) were applied. Bacteria labeled with the aromatic oxygenase enzymes were quantified using epifluorescence microscopy, while soluble methane monooxygenase (sMMO) was assayed using a fluorometer.

**Results/Lessons Learned.** Eight of the groundwater samples assayed showed the presence of enzymes that oxidized all three probes to a fluorescent product, while only three showed no significant fluorescence. The remaining three samples showed fluorescence with only one or two aromatic oxygenases. All samples showed some fluorescence when treated with coumarin, but only four showed moderate to high levels of fluorescence. Based on the EAP data, there is evidence of potential for significant intrinsic aerobic biodegradation at most locations tested, with eight of the sampling sites showing activities for all three aromatic EAPs under in situ conditions. All samples showed some sMMO activity, while three showed moderate activity and one groundwater sample showed high sMMO activity.

Enzyme activity probe analysis, along with other tools used to test for MNA mechanisms such as magnetic susceptibility and compound specific isotope analysis, appear to indicate conditions are favorable to support aerobic co-metabolism and/or biogeochemical transformation processes in several areas scattered throughout GWU1. Overall, the data appear to indicate that natural attenuation is occurring in most areas of GWU1 by one or a combination of mechanisms.