Research Brief from the
Western Region Hazardous Substance Research Center

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☆Summary of the Problem☆

Chlorinated solvents such as trichloroethylene (TCE) are used in a wide range of industrial processes and in the creation of consumer goods such as furniture coverings, plastic food wrap, and even decaffeinated coffee. Prior to their regulation, industrial wastes containing high levels of chloroethenes were disposed of haphazardly. These disposal practices have made chloroethenes common ground water contaminants.

Since the 1980's researchers have identified a number of microorganisms that degrade groundwater contaminants through a process called cometabolism. The microbes occur naturally in soil and groundwater and present an opportunity for *in situ* bioremediation. Oregon State University researchers Daniel Arp and Peter Bottomley are leading WRHSRC studies on cometabolism of chlorinated solvents such as trichloroethylene (TCE). Their goal is to maximize the contaminant degrading potential of several strains of bacteria.

Aerobic Cometabolism with Butane-Grown Microorganisms

Trichloroethylene (TCE) is a common and persistent contaminant in soil and groundwater. Human exposure to TCE has been linked to kidney and liver damage and TCE is a possible carcinogen. Because of the health risks associated with TCE exposure, considerable effort has been made to remove TCE contamination from soil and ground water.

Aerobic cometabolism is an emerging cleanup technology that utilizes microorganisms to degrade TCE and other chloroethenes. The term cometabolism indicates that transformation of the contaminants is a secondary reaction (Figure 1). The microbes consume a hydrocarbon, such as butane for their energy needs. In the process, they produce enzymes that fortuitously degrade other compounds such as chloroethenes. One cleanup approach is to add the microbes and/or their food source to an aquifer and exploit their ability to transform contaminants.

Oregon State University professors Daniel Arp and Peter Bottomley are leading studies of three types of bacteria that can cometabolize TCE and other chloroethenes. They are developing a better understanding of the biology of the bacteria and the chemistry of the cometabolic reactions – essential information for utilizing the bacteria in bioremediation.

The team's focus is on bacteria that use butane as an energy source and produce butane monoxygenase (BMO), an enzyme that degrades a wide range of substances including chloroethenes. In the case of TCE, the BMO enzyme oxidizes TCE to TCE epoxide (Figure 1). The oxidation of TCE is a reductant consuming process, it "uses up" the substance that provides electrons for the reaction. As a result, the sustained cometabolism of chloroethenes requires the presence of an appropriate substrate to donate reductant and support BMO enzyme activity.

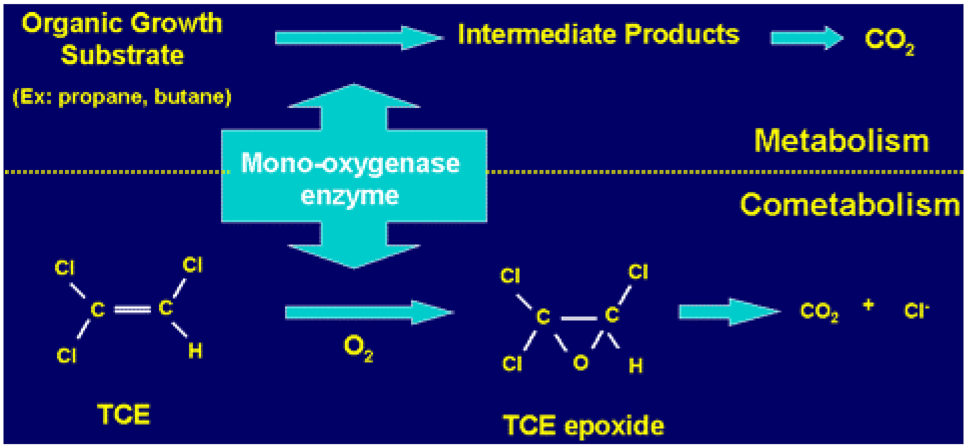

Figure 1. Diagram of the cometabolism of TCE.

One WRHSRC project compared the ability of different sources of reductant to drive the oxidation of dichloroethenes by the bacterium *P. butanovora*. Butane, the energy source for the bacteria, can serve as the source of reductant. However, this creates competition between the primary oxidation reaction that derives energy and the secondary oxidation reaction that degrades the chloroethenes. Adding an alternate substrate eliminates this competition and can increase the efficiency of the dechlorination reaction. In comparisons of several organic acids, graduate student Dave Doughty found that lactate supported the highest initial transformation rates and sustained transformation for the longest period of time (Doughty et al., in press).

About the WRHSRC

The Western Region Hazardous Substance Research Center (WRHSRC) is one of five university-based hazardous substance research centers in the United States. The Centers are funded by grants from the US EPA Office of Research and Development and Office of Solid Waste and Emergency Response. Our Research Briefs are designed to enhance our communication with environmental professionals and others interested in emerging technologies for hazardous substance cleanup. For more information about the WRHSRC visit: http://wrhsrc.orst.edu or call 541-737-2751.

Cometabolism of chloroethenes can stop or slow because the compounds and their breakdown products (such as TCE epoxide) inactivate the BMO enzyme or damage or kill the bacteria itself. In a second WRHSRC project, the team studied the TCE transformation ability of three strains of bacteria that each produces a different type of BMO enzyme. In a recent paper in *Applied Microbial and Cell Physiology*, the team compares the TCE transformation ability of each bacterium and evaluates its effect on BMO enzyme activity and cell physiology (Halsey et al., 2005). "One interesting finding," comments Graduate student Kim Halsey "is that *P. butanovora*, the bacterium that was initially most efficient at degrading TCE, was also the bacterium most negatively affected by TCE epoxide." In comparison, the bacterium that was the slowest TCE transformer, *M. vaccae*, was able to sustain TCE degradation the longest. This result suggests that strains that are slower co-metabolizers may be more effective for sustaining bioremediation.

The team is continuing to investigate the biochemistry of the BMO enzymes. For example, one current project focuses on making specific mutations within the BMO enzyme of *P. butanovora* and monitoring the mutation's effects on the bacteria's ability to transform TCE.

For More Information

Contact Dr. Peter Bottomley or Dr. Daniel Arp or review the following references:

Arp DJ (1999) Butane metabolism by butane-grown *Pseudomonas butanovora*. Microbiology 145:1173–1180.

Arp DJ, Yeager CM, Hyman MR (2001) Molecular and cellular fundamentals of aerobic cometabolism of trichloroethylene. Biodegradation 12:81–103.

Doughty DM, Sayavedra-Soto LA, Arp DJ, and Bottomley PJ (in press) Effects of dichloroethene isomers on the induction and activity of butane monooxygenase in the alkane-oxidizing bacterium, '*Pseudomonas butanovora*', Applied and Environmental Microbiology.

Halsey KH, Sayavedra-Soto LA, Bottomley PJ, Arp DJ (2005) Trichloroethylene degradation by butane-oxidizing bacteria causes a spectrum of toxic effects. Applied Microbiology and Biotechnology [Epub ahead of print 2005 Mar 8].

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