Enzymes Linked to CL-Out®
Aerobic Cometabolism of Halogenated Solvents

Cometabolism is the transformation of a non-growth substrate. A growth substrate is required to meet the nutrient and energy requirements of the cell. Cometabolism relies on the **relaxed specificity** of enzymes produced for the metabolism of other compounds to degrade analogous structures that may not produce carbon or energy for the benefit of the cell. The production of the enzymes may be induced by the substrate or cosubstrate. In CL-Out® bioremediation a metabolic pathway normally used for the degradation of aromatic compounds is used in the cometabolism of halogenated aliphatic hydrocarbons.

Enzymes are the key to cometabolism and understanding which organisms are potentially effective for bioremediation. *Pseudomonads*, such as those in CL-Out® produce a large number of metabolic enzymes that have a relaxed specificity thus facilitating the metabolism or cometabolism of many organic compounds.

Enzymes involved in the aerobic cometabolism of halogenated aliphatic compounds include:

- **naphthalene dioxygenase**
- **toluene dioxygenase**
- **methane monooxygenase**
- **phenol hydroxylase**
- **ammonia monooxygenase**

Organisms associated with aerobic cometabolism of halogenated aliphatic compounds include:

- ***Pseudomonas fluorescens***
- ***Pseudomonas stutzeri***
- ***Burkholderia cepacia***
- ***Pseudomonas putida***

Laboratory Results

A laboratory study was undertaken to verify the cometabolism of PCE by CL-Out®. As part of the investigation samples were taken for the detection and quantification of funtional genes present in the CL-Out® ammended microcosm. After the test period the treatability study showed 69% to 92% removal of the PCE. The functional gene analysis showed **naphthalene dioxygenase** concentrations in the Cl-Out® spiked microcosms ranging from 3.4E8 to 3.9 E10 cells per milliliter.

Field Results

Target functional enzymes were monitored following CL-Out® bioaugmentation to remove **chlorobenzene isomers** from ground water. Thirty days after the bioaugmentation the contaminant concentrations decreased by 62% to 82%. The biomass increased from 1.5 million to 12.8 million cells per milliliter. Tests for relevant funtional genes showed an increase in **naphthalene dioxygenase** from 7.1E4 to 4.3E5 cells per milliliter.

A similar field application of CL-Out® bioaugmentation to remove TCE from aerobic ground water showed an increased presence of **phenol hydroxylase** and **toluene dioxygenase** as the TCE removal rate ranged form 60% to complete removal.

Conclusions

Laboratory and field studies confirm the effectiveness of CL-Out aerobic bioremediation of halogenated solvents, identified functional genes associated with the cometabolism and suggest that monitoring for the genes may be an effective tool for remediation monitoring.

More complete description of aerobic cometabolism can be found in the following publications:

Deckard, L. A., Willis, J. C., and Rivers, D. B. 1994. Evidence for the aerobic degradation of tetrachloroethylene by a bacterial isolate. Biotechnology Letters 16: 1221-1224.

Ryoo, D., Shim, H., Canada, K., Barbieri, P., and Wood, T.K. 2000. Aerobic degradation of tetrachloroethylene by toluene-o-xylene monooxygenase of *Pseudomonas Stutzeri* OX1. Nature Biotechnology 18: 775-778.

Saul, M. T., Davis, G. A., and Mahaffey, W.R, 2009, Investigation of potential aerobic cometabolism of 13C-enriched PCE by a consortium of Pseudomonas. Proceedings, The Tenth Annual International In Situ and On-Site Bioremediation Symposium. Baltimore, Maryland.