In DBSEA Library (2)
1320 EPA

59064
PB89-219992

EPA/600/2-89/033
July 1989

# IN-SITU AQUIFER RESTORATION OF CHLORINATED ALIPHATICS BY METHANOTROPHIC BACTERIA

by

Paul V. Roberts, Lewis Semprini, Gary D. Hopkins,
Dunja Grbic-Galic, Perry L. McCarty, and Martin Reinhard

Research Staff:

Constantinos V. Chrysikopoulos, Mark E. Dolan, Franziska Haag,
Thomas C. Harmon, Susan M. Henry, Robert A. Johns, Nancy A. Lanzarone,
Douglas M. Mackay, Kevin P. Mayer, and Robert E. Roat

Department of Civil Engineering
Stanford University
Stanford, California 94305

CR-812220

7173

Project Officer

Wayne C. Downs
Processes and Systems Research Division
Robert S. Kerr Environmental Research Laboratory
Ada, Oklahoma 74820

U.S. ENVIRONMENTAL PROTECTION AGENCY
ROBERT S. KERR ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
ADA, OKLAHOMA 74820

Received by ER-RPF
SEP 03 1998
MA

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE

## TECHNICAL REPORT DATA
*(Please read Instructions on the reverse before completing)*

| | | |
|---|---|---|
| 1. REPORT NO. EPA/600/2-89/033 | 2. | 3. RECIPIENT'S ACCESSION NO. PB89 219992/AS |
| 4. TITLE AND SUBTITLE IN-SITU AQUIFER RESTORATION OF CHLORINATED ALIPHATICS BY METHANOTROPHIC BACTERIA | | 5. REPORT DATE July 1989 |
| | | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) Paul V. Roberts, Lewis Simprini, Gary D. Hopkins, Dunja Grbic-Galic, Perry L. McCarty, and Martin Reinhard | | 8. PERFORMING ORGANIZATION REPORT NO. |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Civil Engineering, Stanford University, Stanford, CA 94305 | | 10. PROGRAM ELEMENT NO. CBPC1A |
| | | 11. CONTRACT/GRANT NO. CR-812220 |
| 12. SPONSORING AGENCY NAME AND ADDRESS Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, P.O. Box 1198, Ada, OK 74820 | | 13. TYPE OF REPORT AND PERIOD COVERED Research Report (5/1/85-4/30/89) |
| | | 14. SPONSORING AGENCY CODE EPA/600/15 |

15. SUPPLEMENTARY NOTES

Project Officer: Wayne C. Downs FTS: 743-2272

16. ABSTRACT

This project evaluated the potential of enhanced in-situ biotransformation of chlorinated aliphatic solvents by a bacterial community grown on methane under aerobic conditions. The target chlorinated compounds were trichloroethene (TCE), cis - and trans -1,2-dichloroethene (DCE), and vinyl chloride (VC). Laboratory studies were conducted to improve understanding of the microbial growth and transformation rates and to characterize important transport properties. In field experiments, biostimulation was accomplished by introducing methane and oxygen into a shallow, confined, sand and gravel aquifer to encourage the growth of a native bacterial community. Methane utilization commenced within ten days in the first biostimulation attempt, and within one day in subsequent biostimulation episodes. Biotransformation of the target organic compounds ensued immediately after commencement of methane utilization, and reached steady-state values within three weeks. The approximate extents of transformation were as follows: VC, 95%; trans-DCE, 85%; cis-DCE, 40%; and TCE, 20%. Mathematical modeling of the transport and transformation process confirmed that the behavior observed in the field demonstration was consistent with the results of the laboratory research and theoretical expectations. This technology has been demonstrated to be effective in continuous operation under carefully controlled conditions in a real subsurface environment at small scale.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| | | |

| | | |
|---|---|---|
| 18. DISTRIBUTION STATEMENT RELEASE TO THE PUBLIC | 19. SECURITY CLASS (This Report) UNCLASSIFIED | 21. NO. OF PAGES 232 |
| | 20. SECURITY CLASS (This page) UNCLASSIFIED | 22. PRICE |

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

## EXECUTIVE SUMMARY

This project undertook the ambitious task of evaluating the potential of an innovative approach to in-situ aquifer restoration: enhanced biotransformation of chlorinated solvents. The chlorinated solvents investigated included trichloroethylene (TCE), cis- and trans-1,2-dichloroethylene (cis- and trans-DCE), and vinyl chloride (VC). Biotransformation was achieved by creating conditions that promote the growth of a group of bacteria known as methanotrophs, microorganisms that use methane as a source of food and energy under aerobic conditions. The growth of these bacteria can be stimulated by providing methane and oxygen in the proper amounts. These bacteria are able to transform the target chlorinated solvents as secondary substrates through a process termed cometabolism.

This report presents the results of a multidisciplinary investigation that evaluated the technical feasibility of biostimulating native populations of methane-oxidizing bacterial communities to degrade the target chlorinated compounds. The evaluation encompassed a small-scale demonstration of the method's effectiveness under natural conditions at a field site, as well as detailed investigations of the bacterial community's growth and transformation kinetics.

The laboratory studies improved understanding of the growth and transformation kinetics of the microbial population and also characterized the aquifer solids from the field site with respect to important transport properties. These laboratory investigations played an essential role in providing a foundation for designing and interpreting the field demonstration. Mixed cultures of methane-oxidizing bacteria and heterotrophs enriched from samples of the aquifer solids were capable of degrading TCE rapidly and completely under favorable conditions. Pure cultures of methane-oxidizers were isolated that also transformed TCE. It was demonstrated that nutrient media formulations could increase substantially the TCE transformation rates by mixed and pure suspended cultures. Studies with labelled compounds indicated that the transformation by mixed cultures proceeded to form $CO_2$ and cell mass as the main products, although there was a minor amount of intermediate product of trans-DCE transformation. Column experiments showed that high methane concentrations slowed the transformation of the target compounds through competition for enzyme sites. Sorption experiments showed that the equilibrium relation was approximately linear within the concentration range studied, and sufficiently strong to account for retardation factors on the order of two to ten for the various target organic compounds.

In the field experiments, biostimulation was accomplished by feeding the native population methane and oxygen. Methane utilization commenced rapidly, within ten days in the first biostimulation at the test site, and within one day in subsequent biostimulation episodes. Biotransformation of the target organic compounds ensued immediately after commencement of methane utilization, and reached steady state values within approximately two weeks. The extents of transformation at steady state were as follows: approximately 95% for VC, 85% for trans-DCE, 40% for cis-DCE, and 20% for TCE. These amounts of transformation were achieved in a relatively small biostimulated zone, with travel distances of 1 to 4 meters and residence times of 8 to 25 hours. Mathematical modeling of the transport and transformation processes confirmed that the behavior observed in the field demonstration was entirely consistent with the results of the laboratory research and theoretical expectations. The excellent agreement between the observed behavior and model predictions strengthens confidence in the validity of the results and conclusions.

This research has confirmed that biostimulation of a native population of methanotrophs is capable of substantially enhancing the transformation of halogenated aliphatic contaminants. The transformation rates are moderately high, with half lives on the order of several hours to several days under the conditions

that could be created and maintained in the subsurface environment. Transformation rates of this magnitude are substantially greater than the rates of most natural degradation processes in the subsurface, and if achieved over an extensive domain, would lead to complete degradation over the time spans corresponding to typical aquifer residence times.

Biostimulation of natural methanotrophic bacteria to achieve biotransformation of halogenated alkenes deserves full consideration as an alternative means of groundwater remediation in cases where this group of chemicals constitutes a major part of the contamination. This technology has been demonstrated to be effective in continuous operations in a real subsurface environment at small scale, and is ready for demonstration at a real contamination site where conditions are favorable.