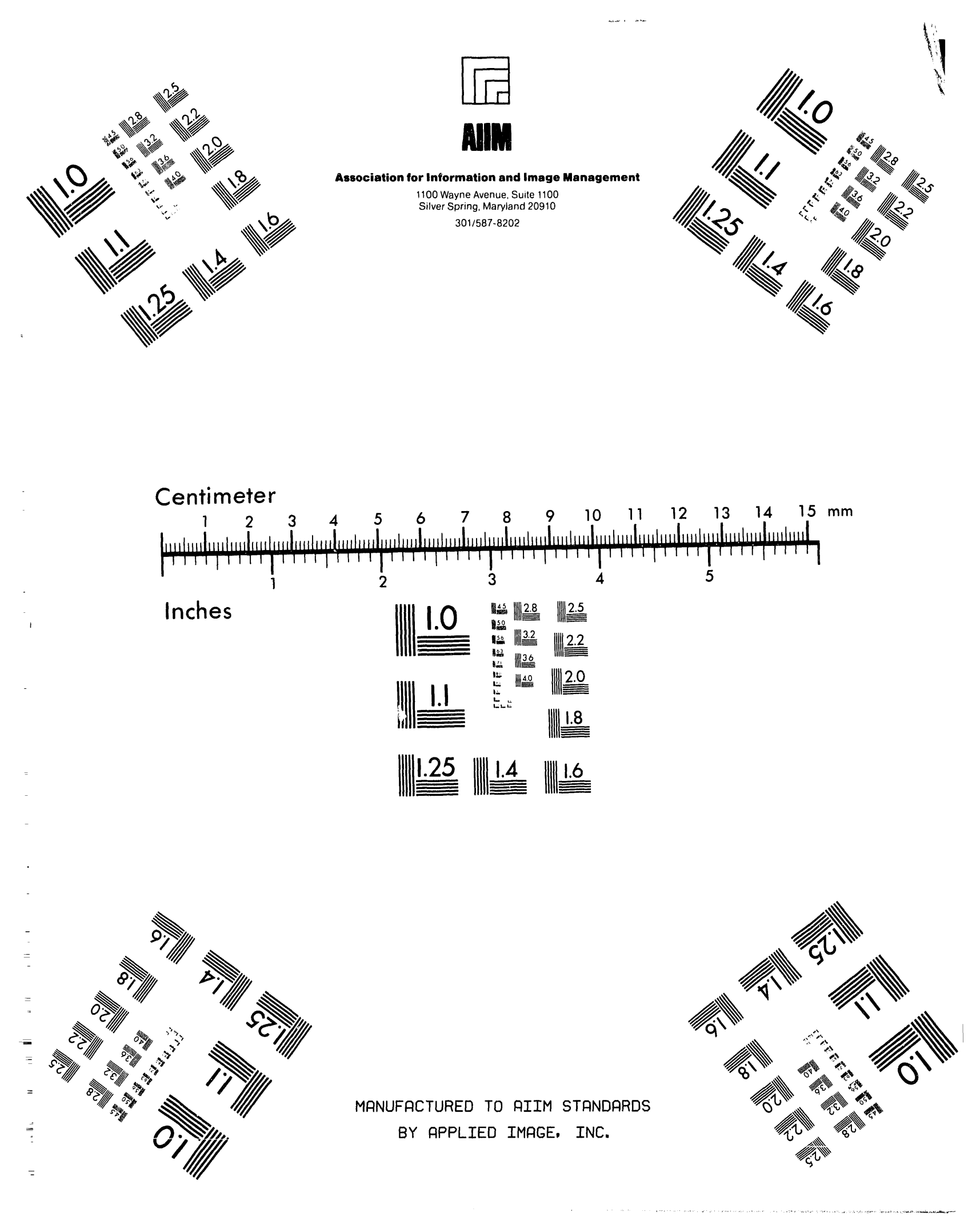
AIIM
Association for Information and Image Management
1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202
Centimeter
Inches
MANUFACTURED TO AIIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

Conf-930794--2

2

DOE/MC/29467-93/C0192

In-Situ Bioremediation of Chlorinated Solvents - A Review

Authors:
W.A. Sack
K.D. Jones
J.E. Cuddeback
A.K. Shiemke

Contractor:
West Virginia University
P.O. Box 6101
Morgantown, WV 26506-6101

Contract Number:
DE-FC21-92MC29467

Conference Title:
25th Mid-Atlantic Industrial & Hazardous Waste Conference

Conference Location:
College Park, Maryland

Conference Dates:
July 7-9, 1993

Conference Sponsor:
University of Maryland

DISCLAIMER

IN-SITU BIOREMEDIATION OF CHLORINATED SOLVENTS-A REVIEW

WILLIAM A. SACK, KEVIN D. JONES, AND JOAN E. CUDDEBACK
Department of Civil and Environmental Engineering
West Virginia University
P.O.B. 6101
Morgantown, WV 26506-6101

ANDREW K. SHIEMKE
Department of Biochemistry
West Virginia University
Morgantown WV 26506

BACKGROUND

It is estimated [1] that hazardous waste remediation and site restoration costs in the United States may approach $1.7 trillion over the next 30 years. There are a variety of technologies available for treatment of contaminated sites and groundwaters. In the past, the conventional method of remediation has been pump-and-treat (P&T) technology where groundwater is pumped from the contaminated aquifers, treated and then discharged. While P&T technology may effectively contain the dissolved-phase contaminant plume and reduce the amount of contamination in an aquifer, cleanup is often far from complete.

Alternative remediation technologies are under development to enhance or replace P&T methods. While no single technology will be applicable to all contaminants or site conditions, in-situ bioremediation is expected to play a major role in many cases. In-situ bioremediation has a number of advantages for destruction of organics. Many other processes such as sorption or volatilization do not destroy contaminants, but rather just concentrate them or transfer them to another medium. Abiotic treatment (chemical transformation) is not normally cost-effective in

groundwaters and may even result in production of more toxic chemical species. Soil flushing methods, as noted earlier have a number of limitations and may actually increase the risk of health hazards by bringing the contaminants to the surface with potential human exposure.

This review focuses on the in-situ bioremediation of chlorinated organic solvents. This group of compounds is one of the most widespread contaminant classes and one of the most troublesome to remediate. They are found nationwide in municipal and industrial wastewaters, landfills and landfill leachates, industrial sludges, waste disposal sites, and groundwaters. Chlorinated alkanes and alkenes, such as trichloroethane (TCA) and trichloroethylene (TCE), are used as dry cleaning fluids, refrigerants, degreasing agents, solvents, and in the the production of decaffinated coffee [2]. The review will include a discussion of laboratory-scale research, some field application considerations, and a review of a full-scale remediation study.

Heavy usage in a variety of applications has led to widespread contamination of shallow aquifers since past practice has often been to dump these chemicals into unlined trenches. While it is true that a very significant fraction of the solvents will evaporate in a few days due to the high vapor pressure, some will inevitably migrate through the soil profile and reach the groundwater table. Once captured in the soil-groundwater system, rates of volatilization are greatly diminished and these compounds may persist for long periods of time. The widespread occurrence of halogenated aliphatic compounds in aquifers reflects their resistance to microbial attack under aerobic conditions.

Members of this group of compounds such as TCE and tetrachloroethylene (PCE) are typically reasonably soluble in water and may move as a dissolved phase in flowing water. However, most are significantly denser than water and will tend to migrate vertically downward through an aquifer as a dense non aqueous phase liquid (DNAPL). They have relatively low octanol water coefficients and yet will sorb to some degree on organic soils. Finally, they are quite volatile.

BIODEGRADATION OF CHLORINATED SOLVENTS

As noted earlier, the chlorinated solvents are both toxic and persistent in the environment. As a result, a significant amount of effort has recently gone into understanding the biodegradation of these compounds. At present, the microbial degradation of trichloroethylene (TCE) has been more thoroughly characterized than that of other chlorinated solvents. For the purposes of this review we assume that the biodegradation of other chlorinated solvents takes place by a similar mechanism. Where appropriate, the biodegradation of other chlorinated solvents will be compared and contrasted with the biodegradation of TCE.

Mineralization refers to the conversion of toxic compounds to innocuous products, such as CO_2, H_2O, and Cl^-. TCE and other chlorinated

solvents are extremely resistant to chemical mineralization. However, several pathways are available for biomineralization, some of which have been demonstrated in laboratory experiments. Aerobic mineralization is catalyzed by the oxygenases found in many types of bacteria. Methanotrophic bacteria are organisms that have an obligate requirement for methane as the sole carbon source [3]. These bacteria contain a non-specific monooxygenase (referred to as methane monooxygenase, or MMO) that functions in vivo to fix methane and oxidize it to methanol [4]. The further oxidation of methanol provides cell-carbon and energy for the bacteria. Methane monooxygenase also initiates the biodegradation of TCE by methanotrophs [5,6]. An ammonia monooxygenase enzyme (AMO) occurs in ammonia oxidizing bacteria and has many similarities to MMO [7]. Accordingly, ammonia oxidizing bacteria can also degrade TCE. The propane monooxygenase found in propane oxidizing bacteria appears to be a more efficient catalyst of TCE oxidation than either MMO or AMO [8]. The toluene and phenol dioxygenase enzymes found in toluene and phenol oxidizing bacteria confer TCE biodegradation activity as well [9,10,11,12]. Lastly methanogenic bacteria can catalyze the reductive dehalogenation of TCE and many other chlorinated solvents, producing compounds that can be readily mineralized by a variety of organisms, including those mentioned above [13]. All of these organisms have in common the general ability to degrade TCE. However, the specifics of their behavior toward TCE differ, as discussed below.

Aerobic Degradation. The degradation of TCE by methanotrophic bacteria is typical of the aerobic biomineralization of chlorinated solvents. MMO is known to catalyze the epoxidation of small alkenes [4], and is responsible for the initial oxidation of TCE, to TCE epoxide [5]. It has been shown that TCE epoxide is unstable in aqueous solutions, decomposing spontaneously in a pH dependent manner [14]. In acidic solution the products of this decomposition are dichloroacetate and glyoxalate, whereas the products are carbon monoxide and formate under basic conditions. Carbon monoxide and formate can be readily converted to CO_2 by methanotrophs [4], and in studies using pure methanotroph cultures CO_2 is the major product of TCE degradation at pH > 7. In contrast, when the solution is acidic the initial products of TCE degradation by methanotrophs are water soluble, and persistent [5]. Evidently, the methanotrophs are unable to further degrade either the dichloroacetate or the glyoxalate, or convert these initial compounds to others that are persistent. However, it is expected that the initial products would be degraded by other organisms in the wild.

The aerobic degradation of TCE by methanotrophs, and the other organisms mentioned above, is cometabolic, meaning that the organisms do not derive energy or cell carbon from TCE oxidation [15]. For this reason no acclimation period is necessary; as long as growth substrates are provided the TCE will be coincidentally degraded. However, the toluene and phenol dioxygenase enzymes that initiate TCE degradation by

toluene and phenol utilizing bacteria are not constitutively expressed. Before these bacteria can degrade TCE the dioxygenase must be induced by pre-incubation with either toluene or phenol [9,11]. Other small chlorinated aliphatic compounds are also degraded by methanotrophs, ammonia oxidizing bacteria, propane oxidizing bacteria, and toluene/phenol utilizing bacteria. However, it has been shown that compounds with fewer chlorine substituents are more readily degraded by these aerobic organisms than more heavily chlorinated congeners [16]. For example, perchloroethylene (PCE) is more resistant to aerobic degradation than TCE. However, PCE is rapidly degraded under anaerobic conditions [13], but complete mineralization requires aerobic organisms as well. In fact, it appears that the most efficient pathway for biomineralization of highly chlorinated solvents would be initiated by anaerobic degradation for removal of the halogens, followed by aerobic oxidation of the resulting mono- and di-chlorinated hydrocarbons.

Anaerobic Degradation. The anaerobic degradation of TCE is very different from the aerobic pathway described above. TCE degradation by methanogenic bacteria is among the best understood of these types of reactions and proceeds by reductive dehalogenation [16]. The TCE is initially converted to 1,2-dichloroethylene and then to vinyl chloride. The vinyl chloride is persistent under anaerobic conditions and is very slowly converted to ethylene by anaerobic bacteria. However, vinyl chloride is rapidly degraded by many aerobic organisms, including the methanotrophic bacteria mentioned above [15]. Thus, it appears that a consortium of methanogenic and methanotrophic bacteria would be quite effective for mineralization of TCE and other chlorinated solvents. The optimal system would be engineered such that degradation occurred in a sequential manner, with the anaerobes performing the dehalogenation and the aerobes oxidizing the dehalogenated products. The number of chlorines dramatically effects the rate of anaerobic degradation: the more chlorines the faster the rate of degradation [13]. A higher number of halogen substituents results in a more oxidized compound making it more susceptible to biological reduction. This behavior is a result of the chlorine atoms increasing the redox potential of the pollutant, and thereby increasing the driving force of the reductive dehalogenation reaction. Although methanogenic bacteria will not grow in the presence of oxygen, the reductive dehalogenation reaction is somewhat oxygen tolerant. The rate of TCE degradation is reduced under micro-aerophilic conditions, but not completely blocked [16]. Under these conditions a suitable source of reducing equivalents must be provided (eg. methanol, hydrogen, acetate, and formate).

Metabolic Factors Affecting Degradation. Reducing equivalents must also be supplied for aerobic TCE degradation. The oxygenase enzymes that initiate TCE degradation require reducing equivalents to activate O_2 for substrate oxidation. MMO from methanotrophic bacteria is quite non-

specific, relative to most other monooxygenases [4]. It will oxidize most small (<8 carbons) straight-chain hydrocarbons, and many aromatic and heterocyclic compounds. Furthermore, the rate of substrate oxidation by MMO is inversely proportional to the size of the substrate. Reducing equivalents can be supplied to methanotrophs in the form of methane, or any of the intermediates in their methane oxidation pathway (methanol, formaldehyde, or formate) [17]. For TCE degradation, methanol is observed to work best [5,6]. The MMO preferentially binds methane, causing inhibition of TCE degradation at moderate methane concentrations of greater than 15% in air [1]. In contrast, methanol is a weak inhibitor, and only affects TCE degradation rates at very high concentrations [17]. The bacteria are unable to use the products of TCE degradation as a source of energy; in most cases an exogenous reductant is present, and TCE degradation stops when the reductant is depleted. However, some strains of methanotrophs are able to synthesize polymers of β-hydroxybutyrate when methane is present in excess. These organisms can then use this storage polymer as an energy source, and consequently will continue TCE degradation in the absence of exogenous reductants [17].

Two forms of MMO are known to be expressed in certain strains of methanotrophic bacteria [18]. One is found in the cytosol and is referred to as soluble MMO (sMMO), whereas a completely unrelated form is found in the plasma membrane and is referred to as the particulate MMO (pMMO). Most species of methanotrophs that have been characterized appear to express only pMMO, which has been less well characterized. Of the two forms of MMO, the soluble enzyme appears to be more effective for TCE degradation [6]. Expression of the soluble enzyme is repressed by copper, and the presence of ~0.25 μM copper sulfate has been shown to significantly inhibit TCE degradation by those methanotrophs that are able to express both forms of MMO. TCE degradation by propane oxidizing bacteria probably proceeds by a mechanism similar to that of the methanotrophs. The propane monooxygenase probably initiates the degradation, and appears to operate at a faster rate than MMO [12]. As with the methanotrophs, the propane oxidizers can also degrade vinyl chloride, the major product of anaerobic reductive dehalogenation.

FIELD APPLICATION CONSIDERATIONS

Just as it is necessary to provide suitable environmental conditions for biological activity in a surface reactor, it is often necessary to enhance treatment in the subsurface by creation of a more suitable growth environment.

Typical methods used to stimulate bioremediation include:

1) control of factors such as moisture, pH, and nutrients, to optimize microbial activity;

2) addition of organic amendments (such as methane) to stimulate

cooxidation or cometabolism;
3) control of subsurface oxygen to accomplish aerobic or anaerobic biodegradation;
4) addition of electron acceptors such as nitrate, gaseous oxygen or hydrogen peroxide to increase the concentration of terminal electron acceptors in the soil and enhance aerobic degradation;
5) augmentation with exogenous acclimated or specialized microorganisms or cell-free enzymes.

Factors That May Limit In-situ Bioremediation. Many factors may impact the feasibility of biodegradation of chlorinated aliphatic compounds. Even though a specific organic constituent has been shown to biodegrade under laboratory conditions, it may be mineralized slowly or not at all under field conditions in a specific soil/site system.

The supply of oxygen is almost always the rate limiting factor for in-situ bioremediation when aerobic conditions are required [19]. The presence of even nominal amounts of organic contaminants will deplete the subsurface of oxygen, thereby creating anaerobic conditions which do not favor the degradation of lower halogenated compounds as rapidly as sustained aerobic conditions. Thus, in-situ bioremediation typically requires that the subsurface be artificially oxygenated.

The concentration of contaminants and pH are also examples of parameters that influence the feasibility of using biological treatment processes. However, treatment systems can be designed and engineered to accomodate waste with high contaminant concentrations and extreme pH values. Neutralization may be used to adjust the pH to within a range conducive to biological treatment. Likewise, if concentrations of contaminants are high enough to inhibit microbiological activity, a dilution step can be introduced to reduce the concentrations to within ranges conducive to biological treatment.

The presence of metals in the subsurface may be limiting to biological treatment. However, in some cases, metals may be leached or complexed to reduce microbial toxicity and improve the potential for contaminant treatment. Addition of amendments such as methane may also result in localized growth inhibition if it reaches too high a concentration.

Another general limitation for in-situ bioremediation involves low soil permeability , which can hinder supplementation of air, moisture, organic amendments, microorganisms and nutrients. Again, it is possible in some soils to control or modify the existing conditions in order to overcome these limitations. Dense non-aqueous phase liquids, such as the chlorinated solvents, have a specific gravity greater than water and may accumulate in isolated pools in low spots of an aquifer. This may make bioremediation more difficult. However, other methods of remediation may also be impacted.

BIOREMEDIATION AT THE MOFFETT NAVAL AIR STATION

A field-scale evaluation of in-situ bioremediation of halogenated organic contaminants, to test the feasibility of methane monooxygenase induction to degrade alkyl halides, was performed at the Moffett Naval Air Station in Mountain View, California. Several halogenated organic contaminants, including trichloroethene (TCE), cis- and trans-dichloroethene (cis- and trans-DCE), and vinyl chloride (VC), were evaluated with regard to transformation under biostimulation conditions. The study was assessed by means of controlled addition of chemicals, frequent sampling, quantitative analysis, and mass balance comparisons [20]. Site conditions at Moffett Naval Air Station were near-ideal. Representative of a typical situation in the San Francisco Bay area, the shallow aquifer was contaminated with chlorinated aliphatics which had been used as solvents [21]. The hydrologic system was a shallow (4-6 meter), highly permeable sand-gravel aquifer that was confined by overlying and underlying silts and clays [2].

Retardation factors for the organic solutes were determined in the field and found to be in the range of two to ten [21]. Sorption was strongest for TCE and weakest for vinyl chloride. Biostimulation and biotransformation experiments were performed at the Moffett site by creating induced-gradient conditions by injecting and extracting the groundwater. Biostimulation was enhanced by addition of methane (primary substrate) and oxygen (electron acceptor). Initially, concentrations of both methane and dissolved oxygen increased at well S2 in response to the pulsed additions. After a lag period of about 200 hours, the concentration of both DO and methane began to decrease. This indicated the growth of methanotrophic bacteria and consequent methane oxidation.

After the initial biostimulation was performed, subsequent field studies were performed, during which methane and oxygen uptake occurred very rapidly with essentially no lag. The results indicated that some of the methanotrophs stimulated previously were capable of utilizing methane and oxygen immediately even when a period of no addition occurred. Results from the study [21] found that the methanotrophs, which are indigenous to the subsurface environment, may be successfully biostimulated to degrade a variety of chlorinated organics. Partial transformation of VC, 90 to 95%; trans-DCE, 80 to 90%; cis-DCE, 45 to 55%; and TCE, 10 to 20%, occurred over a relatively short flow path of 1 to 2 m at residence times of 1 to 2 days. The rate of biotransformation was dependent on structure with the less chlorinated compounds being transformed more rapidly.

In addition to the above results, another study was performed [22] that evaluated enhancement of in-situ aerobic biodegradation of cis- and trans-1-trichloroethylene and cis- and trans-1,2-dichloroethylene by phenol-utilizing bacteria at the Moffett site. Research has demonstrated that aerobic microorganisms grown in phenol or toluene can initiate the cometabolic oxidation of chlorinated aliphatic compounds to stable nontoxic

end products [12]. These microorganisms, which possess toluene oxygenase, (TO) have good potential for bioremediating aquifers contaminated with halogenated organics and their anaerobic and abiotic transformation products. The objective of this study performed at the Moffett site was to evaluate the TO system for in-situ biodegradation of TCE, c-DCE, and t-DCE by phenol and oxygen addition [22].

This set of tests was performed alongside of the previous zone of study to allow for a comparison of results. The test zone was contaminated with TCE, c-DCE, and t-DCE at concentrations of 30 µg/l, 40 µg/l, and 40 µg/l, respectively. Biostimulation was achieved by injecting phenol after steady-state contaminant concentrations were achieved. Phenol was pulse injected for 1 hour in 8 hour pulse cycles at concentrations of 50 mg/l. The phenol injection concentration was doubled after 520 hours, and then was reduced to the original concentration after 840 hours. Significant degradation of c-DCE and TCE was observed during the low level of phenol addition. The c-DCE concentration decreased by approximately 60 to 70% and TCE by 20 to 30%. Doubling the phenol concentration resulted in even greater transformations of both TCE and c-DCE. During this period TCE was transformed by 85 to 90 percent and c-DCE by over 90 percent.

Based on the tests performed at the Moffett site, it is evident that a phenol-utilizing and methane-utilizing population that effectively degraded TCE and c-DCE could be stimulated in-situ.

SUMMARY

Our current understanding of the metabolic pathways utilized by methanotrophic and methanogenic bacteria for degradation of volatile chlorinated solvents suggests that the efficiency of bioremediation could be greatly improved if a symbiotic sequential relationship between the two were encouraged. The methanotrophs have an obligate requirement for methane, which is complemented by the methane production of methanogenic organisms. Furthermore, the methanogens ability to dehalogenate more oxidized (more halogenated) pollutants, generating vinyl chloride, is complemented by the ability of the methanotrophs to mineralize vinyl chloride and other small mono-chlorinated hydrocarbons. The rate limiting step of mineralization under anaerobic conditions is the dehalogenation of vinyl chloride. Thus, it would be advantageous to induce methanotrophic growth at the point where all (or most) of the more chlorinated compounds have been dehalogenated to vinyl chloride. Initial stimulation of methanogenic growth could be subsequently inhibited by the addition of oxygen, at a determined optimum time. The methane remaining from methanogenic metabolism, together with the exogenous oxygen should suffice to stimulate growth of methanotrophic and heterotrophic bacteria, thus completing the mineralization process.

A number of field-determined factors may limit the application of in-

situ bioremediation of chlorinated solvents. These include the ability to deliver nutrients, electron acceptors and gases, especially where soil permeability is limited. Toxicity problems may also occur either due to substances in the formation such as heavy metals or due to high concentrations of amendments added. However, a variety of solutions are being evaluated to mitigate unfavorable site conditions. These include modification of site conditions and improved methods of delivery of needed amendments.

A review of an actual field study showed that it is possible to successfully stimulate the growth of desired organisms for degradation of chlorinated aliphatic compounds achieving signifcant levels of removal.

REFERENCES

1. Russell et al., (1992) "TCE Removal from Contaminated Soil and Groundwater", EPA/540/S-92/002.
2. Chapelle, F.H. (1993) Ground-Water Microbiology & Geochemistry, John Wiley and Sons, NY.
3. Hanson, R. S. (1980) "Ecology and Diversity of Methylotrophic Organisms ", Adv. Appl. Microbiol., Vol. 26, pp 3-40.
4. Anthony, C. (1986) "Bacterial Oxidation of Methane and Methanol" Adv. Microb. Physiol., Vol. 27, pp 113-209.
5. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer (1988) "Trichloroethylene Biodegradation by a Methane-Oxidizing Bacterium" Appl. Environ. Microbil., Vol. 54, pp 951-956.
6. Tsien, H.-C., G. A. Brusseau, R. S. Hanson, and L. P. Wackett (1989) "Biodegradation of Trichloroethylene by Methylosinus trichosporium OB3b", Appl. Env. Microbiol.,Vol. 55 ,pp 3155-3161.
7. Vannelli, T. et al. (1990) "Degradation of Halogenated Aliphatic Compounds by the Ammonia Oxidizing Bacterium Nitrosomonas europea", Appl. Env. Microbiol., pp 1169-1171.
8. Wackett, L. P. et al. (1989) "Survey of Microbial Oxygenases: Trichloroethylene Degradation by Propane-Oxidizing Bacteria", Appl. Env. Microbiol., pp 2960-2964.
9. Folsom, B.R., P.J. Chapman, and P.H. Pritchard (1990), "Phenol and Trichlorethylene Degradation by *Pseudomonas cepacia* G4: Kinetics and Interactions between Substrates", Appl. Env. Microbiol., May 1990, 1279-1285.
10. Zylstra, G. L. et al. (1989) "Trichloroethylene Degradation by Escherichia coli Containing the cloned Pseudomonas putida F1 Toluene Dioxygenase Genes", Appl. Env. Microbiol., pp 3162-3166.
11. Nelson, J. K. et al. (1987) "Trichloroethylene Metabolism by Microorganisms that degrade Aromatic Compounds", Appl. Env. Microbiol.,pp 604-606.
12. Wackett, L. P. and D. T. Gibson (1988) "Degradation of Trichloroethylene by Toluene Dioxygenase in Whole-Cell Studies with

DATE
FILMED
9/8/93

END