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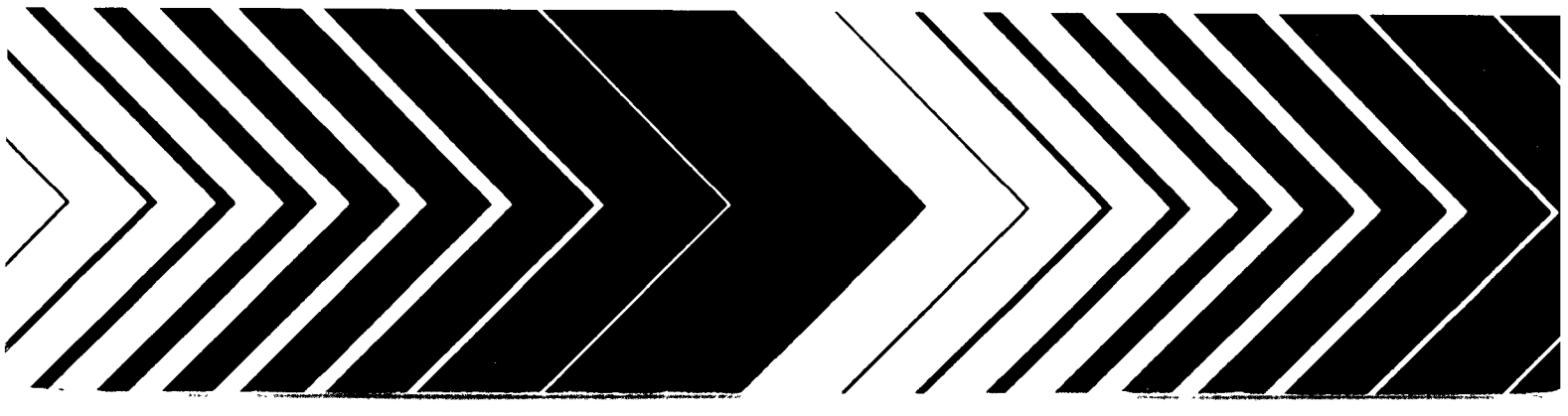
Methodologies for Evaluating *In-Situ* Bioremediation of Chlorinated Solvents



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METHODOLOGIES FOR EVALUATING IN-SITU BIOREMEDIATION OF CHLORINATED SOLVENTS

by

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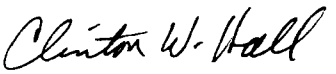
FOREWORD

EPA is charged by Congress to protect the Nation's land, air and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment; b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; c) develop techniques for predicting the effect of pollutants on ground water, soil, and indigenous organisms; and d) define and demonstrate the applicability and limitations of using natural processes indigenous to the soil and subsurface environment, for the protection of this resource.

This report summarizes the results of several research projects where methodologies were developed for evaluating *in-situ* bioremediation of chlorinated solvents, which are widely encountered as groundwater pollutants.



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ABSTRACT

This report summarizes the behavior of and requisite conditions for a class of natural biological processes that can transform chlorinated aliphatic compounds. These compounds are among the most prevalent hazardous chemical contaminants found in municipal and industrial wastewaters, landfills and landfill leachates, industrial disposal sites, and groundwater. Biological degradation is one approach that has the potential for destroying hazardous chemicals so that they can be rendered harmless for all time. Methodologies are presented that are useful for evaluating the potential for biorestoration of groundwater contaminated with chlorinated aliphatic compounds.

The report is composed of six sections. Section 1 provides an introduction and an overview of the problems with chlorinated aliphatic compounds in groundwater. Section 2 presents a review of the processes affecting the movement and fate of chlorinated aliphatics in the subsurface, including advection, dispersion, sorption and relative mobility, diffusional transport, and immiscible transport. Section 3 provides a thorough review of the microbial transformation of organic pollutants. Basic microbial metabolic processes are reviewed, focusing on anaerobic and aerobic transformations of chlorinated aliphatic compounds. Laboratory studies of aerobic cometabolic transformation and degradation of TCE by methanotrophs and methanotrophic communities are summarized. In Section 4 transport and microbial process models are presented and incorporated into a model for the aerobic cometabolic transformation of chlorinated aliphatics by methanotrophic communities. Section 5 presents pilot-scale results of enhanced *in-situ* biotransformation of halogenated alkenes, including TCE, cis- and trans-DCE, and vinyl chloride by methanotrophic bacteria along with model simulations of the results. Section 6 presents an example study to evaluate the potential and limitations for groundwater bioremediation at a Superfund site by methanotrophs. Methodologies and results are presented for evaluating the presence of a native methanotrophic community and its ability to degrade the contaminants of concern; determining the sorption of contaminants to the aquifer material; and preliminary designing of an *in-situ* treatment approach using the model previously described.

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ACKNOWLEDGMENTS

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SECTION 1

INTRODUCTION

PURPOSE AND OVERVIEW

Chlorinated aliphatic compounds are among the most prevalent hazardous chemical contaminants found in municipal and industrial wastewaters, landfills and landfill leachates, industrial sludges, waste disposal sites, and groundwater. Several of these chemicals have been widely used as solvents for household, commercial, and industrial cleaning and degreasing operations. Included here are carbon tetrachloride (CT), tetrachloroethylene (PCE), trichloroethylene (TCE), 1,1,1-trichloroethane (TCA), and methylene chloride (MC). In the past, used solvents were often discharged indiscriminately into the environment. A characteristic of these man-made solvents is their relative resistance to breakdown by natural microorganisms, and hence they have tended to persist and accumulate in the environment. While efforts are now being made to clean with other less harmful and less environmentally persistent chemicals, or to clean and recycle used chlorinated solvents, there is a legacy of chlorinated-solvent-contaminated environments that need to be remediated.

Most current cleanup methods for chlorinated aliphatic compounds employ physical processes which tend to transfer the compounds to another medium or to concentrate the compounds for burial elsewhere. For example, groundwater contaminated by chlorinated solvents is often pumped to the ground surface and air stripped, which sends the solvents into the atmosphere. Now that this method is being restricted in some areas, the air stripped solvents sometimes are sorbed and concentrated onto activated carbon, which then may be hauled to a hazardous chemical disposal site. In such schemes the chemicals are simply moved from one part of the environment to another, where they may cause other problems. Biological decomposition is one approach that has the potential for destroying hazardous chemicals so that they are rendered harmless for all time. This natural process is widely used for more easily biodegraded compounds, especially where chemicals exist in relatively dilute form (mg/kg levels) in waste water or sludges, or in the environment. Although chlorinated aliphatic compounds have been known for quite some time to be resistant to normal biological transformations, many natural organisms have now been found that can bring about their decomposition. This raises the possibility that these chemicals can also be destroyed by engineered biological processes.

This report provides an overview of the characteristics of the natural biological processes that can transform chlorinated aliphatic compounds. It also describes research and field studies that have been directed towards taking advantage of these natural processes for *in-situ* restoration of groundwaters. Methodologies are presented that might be applied for evaluating the potential for biorestoration of contaminated groundwater. An example study to evaluate the potential and limitations for groundwater bioremediation at a Superfund site is also included.

CHLORINATED ALIPHATIC COMPOUNDS

Table 1 contains a listing of the reported frequency of occurrence of several volatile organic chemicals (VOCs), which includes the halogenated aliphatic compounds of interest here. The four most prevalent VOCs in groundwater used as a source of drinking water supply are the trihalomethanes, which are formed from chlorination for disinfection. As such, these compounds are not major contaminants of the groundwater itself. The trihalomethanes are also among the dominant VOCs in treated municipal wastewater, again a result of chlorination of drinking water supplies. The next most prevalent VOCs are the three major chlorinated solvents PCE, TCE, and TCA. Not far down on the list is the chlorinated solvent CT, which has seen decreased usage in recent years but was a major solvent in the past.

TABLE 1. FREQUENCY OF OCCURRENCE OF VOLATILE ORGANIC CHEMICALS

	Order of Frequency		
VOC	Groundwater[a]	Treated Municipal Wastewater[b]	CERCLA Sites[c]
Chloroform	1	3	7
Bromodichloromethane	2	6	
Dibromochloromethane	3	7	
Bromoform	4	9	
Tetrachloroethylene	5	2	4
Trichloroethylene	6	5	1
1,1,1-Trichloroethane	7	1	6
1,1-Dichloroethane	8		9
1,2-Dichloroethylene	9		12
Carbon tetrachloride	10		15
1,1-Dichloroethylene	11		10
m-Xylene	12	8	5
o,p-Xylene	13	11	15
Toluene	14		3
p-Dichlorobenzene	15	4	11
1,2-Dichloropropane	16		13
Dichloroiodomethane	17		
Benzene	18		2
Ethylbenzene	19	10	7
Bromobenzene	20		
Vinyl chloride	21		8

[a] Based upon survey of treated groundwater used for drinking-water Supply (Westrick et al., 1984).

[b] Survey of four treatment plants in Arizona, California, and Colorado (McCarty, 1990).

[c] U.S. EPA, 1990.

In contaminated groundwater, another group of chlorinated aliphatic compounds appear that are not generally prevalent in municipal wastewaters. These are 1,1-dichloroethane (1,1-DCA), 1,2-dichloroethylene (1,2-DCE), 1,1-dichloroethylene (1,1-DCE), and somewhat further down the list, vinyl chloride (VC). These compounds often are produced slowly in the environment under appropriate conditions as daughter products from chemical and biological transformations of chlorinated solvents, which are the original contaminants.

Another group of prevalent VOCs consist of the aromatic hydrocarbons: benzene, xylene, and ethylbenzene. These compounds represent the soluble components of gasoline and are indicators of contamination by petroleum products. These compounds, however, are not the subject of this report.

Table 2 provides a listing of the major one- and two-carbon halogenated VOCs that are considered in this report, the chemical formulas for each, acronyms that are used, and U.S. drinking water maximum contaminant limits (MCL) that have been established. Federal MCLs are not available for compounds without MCLs specified in Table 2, but some states have established regulations for at least some of them. Reaching the low contaminant levels represented by the federal drinking water standards presents a significant challenge for any bioremediation process, especially when contaminants occur two to four orders of magnitude higher in concentration at many contaminated sites of concern.

TABLE 2. COMMON HALOGENATED ALIPHATIC CONTAMINANTS

Chemical	Formula	Acronym	U.S. Drinking-Water MCL (µg/l)
Trihalomethanes			
Chloroform	$CHCl_3$	CF	$\Sigma = 100$
Bromodichloromethane	$CHBrCl_2$		
Dibromochloromethane	$CHBr_2Cl$		
Bromoform	$CHBr_3$		
Other Chlorinated Methanes			
Carbon tetrachloride	CCl_4	CT	5
Methylene chloride	CH_2Cl_2	MC	
Chlorinated Ethenes			
Tetrachloroethylene	$^{Cl}_{Cl}C = C^{Cl}_{Cl}$	PCE	5
Trichloroethylene	$^{Cl}_{Cl}C = C^{H}_{Cl}$	TCE	5
cis-1,2-Dichloroethylene	$^{H}_{Cl}C = C^{H}_{Cl}$	c-1,2-DCE	70
trans-1,2-Dichloroethylene	$^{H}_{Cl}C = C^{Cl}_{H}$	t-1,2-DCE	100
1,1-Dichloroethylene	$^{Cl}_{Cl}C = C^{H}_{H}$	1,1-DCE	7
Vinyl chloride	$^{Cl}_{H}C = C^{H}_{H}$	VC	2
Chlorinated Ethanes			
1,1,1-Trichloroethane	CCl_3CH_3	TCA	200
1,1-Dichloroethane	$CHCl_2CH_3$	1,1-DCA	
1,2-Dichloroethane	CH_2ClCH_2Cl	1,2-DCA	5
Chloroethane	CH_2ClCH_3	CA	

SECTION 2

PROCESSES AFFECTING MOVEMENT AND FATE

OVERVIEW

Once released onto the land surface or underground, synthetic organic chemicals, such as the halogenated aliphatics, are subject to a variety of influences that lead to an extraordinarily complex pattern of behavior. The processes influencing the transport, distribution and fate of these chemicals include the following:

1) Advection, the miscible transport in aqueous solution under the influence of the hydraulic potential gradient;

2) Dispersion, the mixing and spreading of concentration fronts, that arises largely from differential rates of movement along the myriad individual flow paths through the porous medium;

3) Sorption, the partitioning of a compound between the moving solution and the stationary solid phase;

4) Immiscible transport, the migration of slightly soluble chemicals as a separate liquid phase, often driven downward by density difference in the case of halogenated aliphatics; and

5) Diffusional transport, the slow migration of solute molecules into the matrix rock or dead-end pores under the influence of a concentration driving force.

The influence of these factors on contaminant behavior has been summarized in several recent reviews (McCarty et al., 1981; NAS, 1984; Anderson, 1984; Mackay et al., 1985; Goltz and Roberts, 1986). The present section focuses on the principles underlying the transport, distribution, and fate of halogenated aliphatic compounds; transformation processes are described in Section 3. Field studies of organic contaminant behavior are emphasized, as these often reveal the surprising significance of phenomena that occur in real subsurface environments over long time periods in a way that could not be demonstrated from theoretical and laboratory studies alone (Roberts et al., 1982b, 1986; Anderson, 1987).

ADVECTION

The transport of nonreactive, dissolved contaminants is driven by the same hydrostatic forces responsible for groundwater movement. The governing principles have been expounded in standard references on groundwater movement, such as Freeze and Cherry (1979), where methods of investigation are also summarized. In most instances of groundwater flow through fine- to medium-grained porous media, Darcy's Law applies:

$$v = -K\frac{dh}{dL} = \bar{v}n \quad (1)$$

where v = specific discharge or Darcy velocity, an average velocity based on the total cross-sectional area [L/t], $\bar{v}$ = average linear velocity [L/t], n = porosity [–], K = hydraulic conductivity [L/t]; and dh/dL = hydraulic gradient [–].

The porosity, n, represents the intergranular pore volume expressed as a fraction of the total void volume. The value of n depends on the grain shape and size distribution, with typical values on the order of n = 0.25 to 0.5 for silt, sand, and gravel, and somewhat higher values, n = 0.4 to 0.7 for clay.

The hydraulic conductivity, K, depends mainly on the grain size of the porous medium, although fluid density and viscosity play a minor role, according to

$$K = \frac{Cd^2\rho g}{\mu} = \frac{k\rho g}{\mu} \quad (2)$$

where C = empirical drag coefficient [–], d = grain diameter [L], ρ = fluid density [M/L^3], μ = viscosity, and k = intrinsic permeability = Cd^2. Accordingly, both the intrinsic permeability and the hydraulic conductivity are strong functions of the grain diameter, increasing approximately as d^2. Approximate values of the hydraulic conductivity and intrinsic permeability can be found in Table 2.2 of Freeze and Cherry (1979). For example, the hydraulic conductivity for a clean sand usually lies between 10^{-4} and 1 cm/s, whereas values for silty sand range about an order of magnitude lower. The magnitude of the hydraulic conductivity is significantly reduced by the presence of fine material, which tends to fill the interstices between the coarser grains, thus reducing the effective pore size.

Under natural conditions, the hydraulic gradient is influenced by the presence of recharge sources and discharge sinks as well as their separation distances and relative elevations. In areas of relatively flat topography, values of the hydraulic gradient often are in the range from 10^{-3} to 10^{-5}, with higher values encountered in areas of high recharge and steep slope. The hydraulic conductivity also exerts an indirect effect on the hydraulic gradient, introducing a complexity that sometimes greatly complicates data interpretation and modeling. However, the hydraulic gradient is influenced significantly by injection or extraction wells, which may increase the hydraulic conductivity by several orders of magnitude.

Considering the combined effects of hydraulic conductivity, hydraulic gradient, and porosity, it can be expected that linear velocities of groundwater transport in uniform sand and gravel aquifers in areas of gentle topography are on the order of 1 to 1000 m/yr (Mackay et al.,1985). Values in this range, frequently on the order of 10 to 100 m/yr, are often observed at hazardous waste sites in such settings. Thus, groundwater movement is relatively slow in most instances, a fact of salient importance in remediation efforts.

DISPERSION

Dispersion refers to the sum effect of processes that tend to spread an advancing solute concentration wave or pulse. The underlying processes are thought to be the following: molecular diffusion, hydromechanical mixing, and differences in hydraulic conductivity along different flow paths. The dispersive mixing can be longitudinal, i.e., in the direction of flow, or transverse, i.e.,

orthogonal to the flow. Thus, dispersion tends to dilute contaminant concentrations by spreading the contaminant, but also hastens the arrival of concentration fronts.

The extent of dispersion is highly dependent on the heterogeneity of the porous medium, because a wide range of hydraulic conductivities results in large spatial variation of velocities along different flow paths. The dispersion coefficient, D [L^2/t], the basic rate coefficient governing the extent of dispersive mixing, can be estimated as the product of the fluid velocity, $\bar{v}$ [L/t], and a dispersive mixing length scale, α [L]

$$D = \bar{v} \cdot \alpha \tag{3}$$

The dispersivity, α, captures the effects of the porous medium's heterogeneity, and must be estimated by interpretation of tracer experiments. Estimates of α based on field measurements typically exceed those from laboratory data by several orders of magnitude, owing to the greater heterogeneity of the field settings (Freeze and Cherry, 1979). Moreover, comparison of estimates based on field measurements at different scales shows that the dispersivity estimates usually increase with increasing scale of observation. Anderson (1984) suggests that the estimated dispersivity increases approximately in proportion to the scale of observation, as shown in Figure 1.

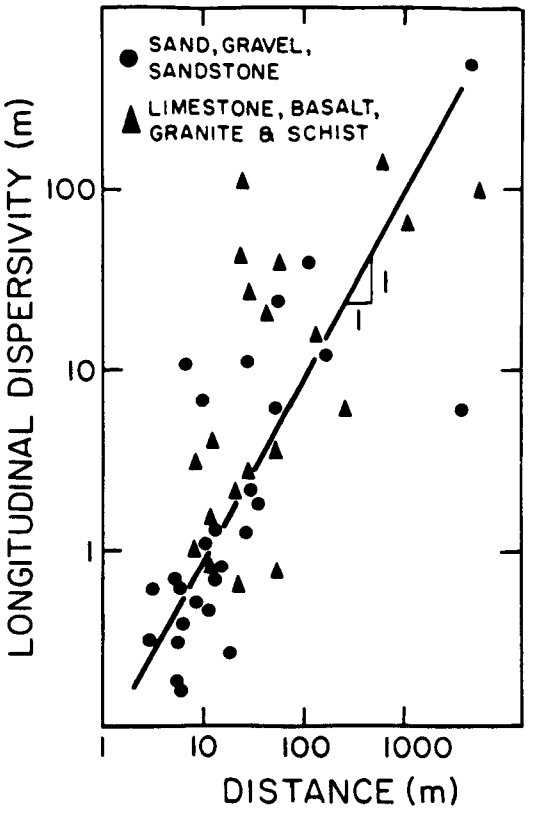


Figure 1. Variation of dispersivity with distance (from Anderson, 1984).

The values of α for a given observation distance, Z, are distributed over an order of magnitude above and below the fitted straight line in Figure 1, presumably because some sites exhibit considerably more heterogeneity than others.

The extent of dispersive mixing or spreading can be quantified in terms of a dimensionless group known as the Peclet Number, $Pe = X/\alpha$, where X is the integral length scale of observation in the longitudinal direction. The larger the Peclet Number, the smaller the dispersive length scale compared to the total travel distance, and hence the smaller the role of dispersion relative to advection. If $Pe > 100$, dispersion exerts a minor influence on contaminant transport and distribution, whereas if $Pe < 10$, dispersion has a major effect. If the linear relationship between log α and log X in Figure 1 is valid, then the values of Pe for the collection of field sites fall within an order of magnitude of $Pe = 10$, suggesting that dispersion is of moderate importance at typical field sites. However, it can be fairly stated that dispersion does not directly influence the fate of contaminants in solution, since it acts only to spread out the contaminant mass over a greater volume.

SORPTION AND RELATIVE MOBILITY

The halogenated aliphatic compounds do not sorb to soils and aquifer materials as readily as do many pesticides, but nevertheless, sorption in aquifer systems is sufficient to retard the rate at which they move in groundwater in relation to the movement of groundwater itself. This relative movement can be expressed mathematically by the retardation equation (Freeze and Cherry, 1979):

$$\frac{\bar{v}}{\bar{v}_c} = 1 + \frac{\rho_b K_d}{n} \tag{4}$$

where $\bar{v}$ = average linear velocity of groundwater, $\bar{v}_c$ = average linear velocity of the contaminant, ρ_b = mass density of solids in aquifer, n = porosity, and K_d = distribution coefficient.

The term $(1 + \rho_b K_d/n)$ is commonly known as the retardation factor. For aquifer materials, ρ_b is approximately 1.7 g/cm^3, and n generally varies between 0.2 and 0.4 (Freeze and Cherry, 1979). With these units, K_d should be expressed in units of cm^3/g. K_d is defined as follows:

$$K_d = \frac{\text{Contaminant mass on solid phase per unit mass solid phase}}{\text{Concentration of contaminant in solution}} \tag{5}$$

Roberts et al. (1980, 1982b) measured the retardation of various halogenated aliphatic compounds that were present in reclaimed municipal wastewater that was injected into a confined aquifer in Palo Alto, California. The data demonstrated how differences in sorption affinity (i.e. K_d) cause differences in mobility that are reflected as a chromatographic separation of the concentration fronts arriving at an observation well (Figure 2). The retardation factors, estimated as the ratio of the center of mass for the respective concentration response relative to that of chloride (Roberts et al., 1980) were as follows: $CHCl_3$, 3; $CHBr_3$, 6; C_6H_5Cl, 33; and CH_3CCl_3 (not shown in Figure 2), 12.

In another field experiment at the Borden Air Force Base, Ontario, Canada, a 12-m^3 plug of water contaminated with 5 organic chemicals and a chloride tracer was injected into an unconfined relatively uniform aquifer with small-scale horizontal bedding (Mackay et al., 1986; Roberts et al., 1986). Evidence of chromatographic separation of halogenated aliphatics was observed in both temporal and spatial sampling (Figures 3 and 4, respectively). The complementarity of temporal and spatial data is an important concept in understanding solute transport (Goltz and Roberts,

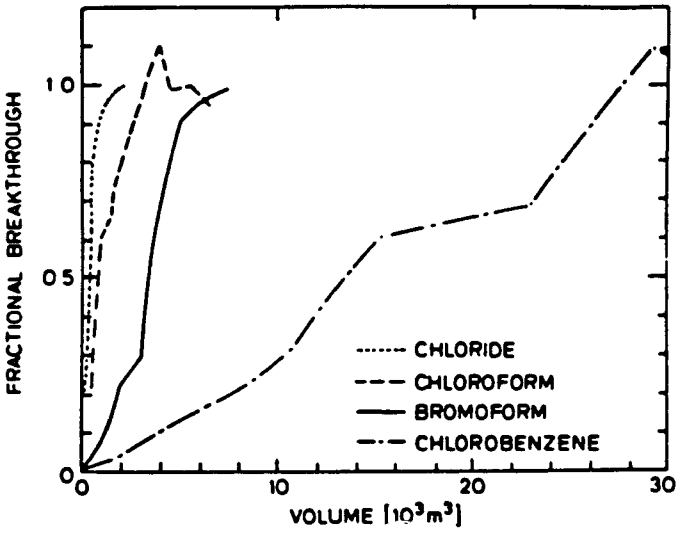


Figure 2. Sequential breakthrough of solutes at an observation well during the Palo Alto groundwater recharge study (Roberts et al., 1982b).

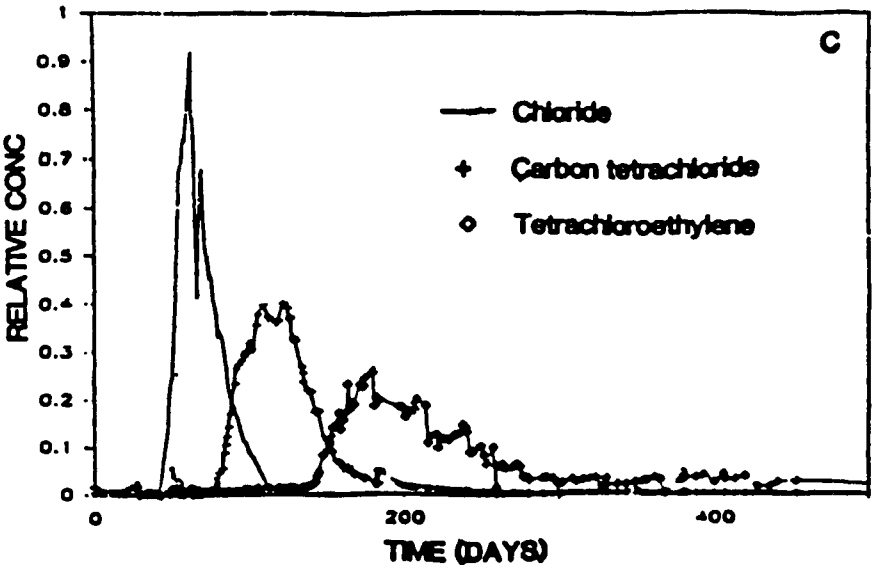


Figure 3. Breakthrough responses for chloride, carbon tetrachloride, and tetrachloroethylene at time-series sampling points a, b, c during the Borden transport experiment (Roberts et al., 1986).

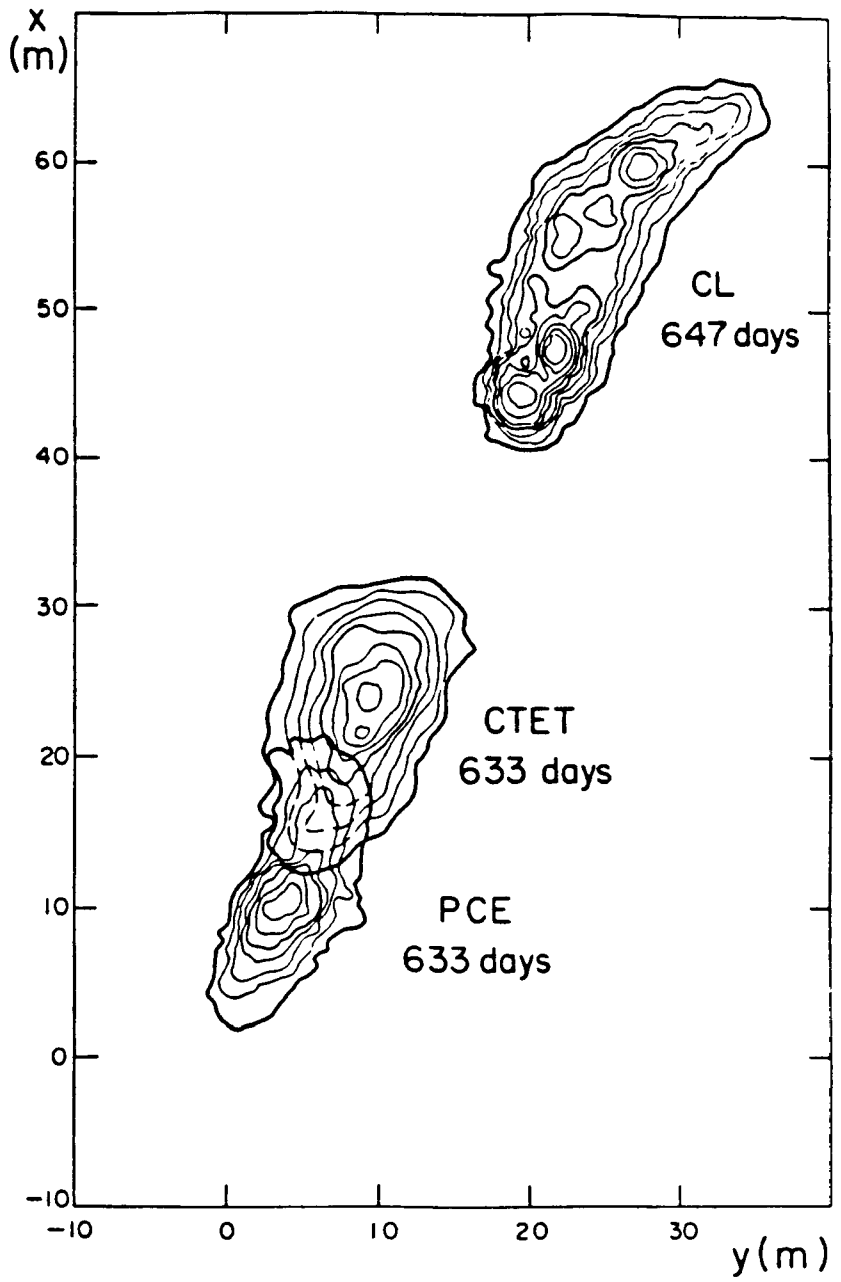


Figure 4. Chromatographic separation of spatial plumes at Borden: chloride (Cl, 647 days); carbon tetrachloride (CTET, 633 days); and tetrachlorethylene (PCE, 633 days). Contour interval depicted for Cl is 5 mg/l beginning at an outer contour of 10 mg/l. Contour intervals depicted for CTET and PCE are 0.1 µg/l beginning at an outer contour of 0.1 µg/l (Roberts et al., 1986).

1987). The aquifer material at Borden had a low organic content (0.02%), and consisted of quartz (58%), amphiboles (7%), feldspars (19%), carbonates (14%), and miscellaneous materials (2%). Measured values of K_d for the halogenated aliphatic compounds injected are listed in Table 3 together with calculated and measured retardation coefficients (Curtis et al., 1986a). Although the retardation factors inferred from short-term field observations (10–30 days) were consistent with

the distribution coefficients measured in the laboratory (Curtis et al., 1986a), it was found that the retardation factor appeared to increase with time and distance from the point of injection. This appears to be related to a slow rate of diffusion of the contaminants into the aquifer solids, the characteristic time scale for which can be measured in terms of weeks to months rather than hours as commonly assumed (Ball and Roberts, 1991b). To measure sorption equilibrium with such extended contact periods without introducing artifacts, a special protocol employing flame-sealed ampules has been developed (Ball and Roberts, 1991a); alternatively, aquifer materials can be pulverized to expedite the equilibrium measurements.

TABLE 3. DISTRIBUTION COEFFICIENTS AND RETARDATION FACTORS

			Retardation			
					Field Data[c]	
	$\log_{10}K_{ow}$	$K_d \times 10^6$ (m^3/g)	Predicted[a]	Batch Experiments[b]	Temporal	Spatial
CTET	2.7	0.15	1.3	1.9	1.6-1.8	1.8-2.5
BROM	2.3	0.17	1.2	2.0	1.5-1.8	1.9-2.8
PCE	2.6	0.45	1.3	3.6	2.7-3.9	2.7-5.9
DCB	3.4	0.76	2.3	6.9	1.8-3.7	3.9-9.0
HCE	3.6	0.81	2.3	5.4	4.0	5.1-7.9

[a] Calculated from the regression by Schwarzenbach and Westall (1981) with $f_{oc} = 0.02\%$.
[b] Curtis et al. (1986a).
[c] Roberts et al. (1986).

While others have shown a relatively good correlation between K_d and the aquifer organic content and the contaminant's octanol/water partition coefficient, K_{ow} (Karickhoff et al., 1979; Schwarzenbach and Westall, 1981), this correlation is relatively poor for aquifers with low organic content ($f_{oc} < 0.1\%$, McCarty et al., 1981), and for similar chemicals as represented by the chlorinated aliphatic compounds summarized. Generally aquifers are fairly poor in organic matter content so that the retardation noted appears to be more a function of sorption to inorganic rather than organic materials (Curtis et al., 1986b). Figure 5 (Curtis et al., 1986a) indicates that the correlation between the distribution coefficient normalized to the organic content ($K_{oc} = K_d/f_{oc}$) and the octanol/water partition coefficient is relatively poor within this group of compounds: bromoform (BROM), tetrachloroethene (PCE), carbon tetrachloride (CTET), 2-dichlorobenzene (DCB), and hexachloroethane (HCE), in order of increasing K_{ow}.

Retardation is an important process in groundwaters for at least two reasons. Firstly, since chemicals have different retardation coefficients, their relative rates of movement through aquifers will differ widely (Roberts et al., 1982a). Thus, if an aquifer were to become contaminated with several compounds at one location, they would tend to move at different speeds and would arrive at a downgradient well at different times. This would make the contaminant composition of the extracted water different than that at the point of contamination, often making it difficult to verify the original source of contamination. The other important aspect of retardation is that, as noted, retardation provides a basis for estimating the relative amount of the contaminant present in the aqueous phase as compared with that sorbed to the aquifer solids. For example, for a retardation factor of 5, one-fifth of the contaminant would be present in the aqueous phase and four-fifths would be sorbed onto the aquifer solids. Restoration of a contaminated aquifer would require that

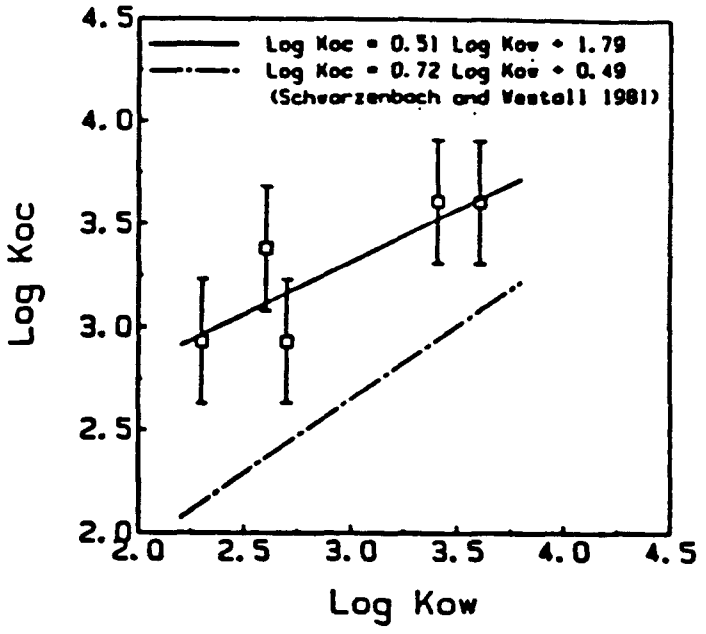


Figure 5. Correlation between the distribution coefficient and the octanol water partition coefficient (from Curtis et al., 1986a).

the contaminants be removed from the solid phase as well as from the aqueous phase. Also, sorption is an important factor in damping concentration fluctuations that arise as packets of water with differing composition move through an aquifer; sorption tends to strengthen the tendency of such fluctuations to be smoothed during groundwater transport (Roberts and Valocchi, 1981; Valocchi and Roberts, 1983). Finally, sorption of contaminant may also affect contaminant transformation either by hindering or accelerating the transformation process. For these various reasons, sorption is an important process in aquifers; and more basic information is needed about factors affecting it.

DIFFUSIONAL RATE LIMITATIONS

Mass transport between the moving groundwater and the stationary solids is sufficiently rapid under ideal circumstances to assure equilibrium distribution of contaminants between the pore water and solids. However, both theory and recent field evidence suggest strongly that the fate of organic contaminants in natural hydrogeologic settings may depend critically on rates of transport between the pore water and stationary solid phases. For example, a field-scale study suggested that organic contaminant sorption equilibrated very slowly, over a period of several years (Roberts et al., 1986; Goltz and Roberts, 1986). Diffusional rate limitations are especially likely to arise under circumstances where the sorption capacity of the solids is large and the porous medium is heterogeneous, containing extensive zones of low permeability (Brusseau et al., 1989; Valocchi, 1985). Serious rate limitations can occur at the grain scale even in relatively homogeneous media, if the sorbing solids are microporous (Ball and Roberts, 1991a,b) or aggregated (Rao et al., 1982).

The implications for aquifer remediation are obvious, and mostly adverse (Criddle et al., 1991; Reijnaarts et al., 1990). Deviations from local equilibrium can lead to situations in which 1) a

large proportion of sorbed contaminant remains in the solid phase long after aqueous concentrations have subsided to low levels as a result of purging with clean fluid, and 2) the local solution concentrations are governed by the slow release of previously sorbed contaminants. These complications are likely to hinder both pump-and-treat and *in situ* remediation efforts, by substantially reducing the rate of contaminant removal by either purging or reaction, thus extending the time duration of remediation efforts. Such conditions are especially likely to exert a significant impact on remediation performance if 1) the contaminants sorb strongly, and/or 2) the porous medium is significantly heterogeneous.

It would be premature, given present knowledge, to propose quantitative criteria for deciding whether diffusional rate limitations will affect remediation significantly. Judging from field observations of contaminant behavior at the Borden and Moffett Field sites, we surmise that retardation factors greater than approximately three can lead to significant rate limitations if a substantial portion of the sorption capacity is associated with zones having permeability significantly (e.g., more than an order of magnitude) below average (Roberts et al., 1986; Semprini and McCarty, 1992). These conditions are believed to apply to many, if not most, remediation situations. Hence there is an urgent need to improve methods for characterizing remediation sites with respect to the factors controlling the rates of sorbed contaminant release from solid phases, and to employ those methods systematically in site investigations.

IMMISCIBLE TRANSPORT

Transport of immiscible liquid phases, known colloquially as non-aqueous phase liquids or NAPLs, has come to be recognized as a major route of contaminant spreading at hazardous waste sites and as a significant factor affecting contaminant distribution (Huling and Weaver, 1991). The principles of immiscible contaminant movement and the role of contaminant properties have been reviewed recently (Mercer and Cohen, 1990; Huling and Weaver, 1991). The halogenated aliphatic compounds emphasized in this document are susceptible to pronounced vertical transport as immiscible phases: almost all are specifically dense, slightly miscible liquids under conditions typical of the subsurface, with solubilities on the order of 0.1 to 10 g/l and specific gravities on the order of 1.1 to 2 [Table 4 (from Huling and Weaver, 1991)]. Such compounds that are more dense than water are commonly referred to as DNAPLs. As shown by Schwille (1988) in physical model experiments, compounds of this kind tend to migrate downward through the vadose and groundwater zone, driven by gravity forces which dominate the hydrostatic forces that impel groundwater movement and solute transport (Figure 6).

In contrast to groundwater transport, DNAPL movement is primarily vertical, except where the descending immiscible organic liquid encounters a barrier posed by a stratum of low permeability. Hence, the DNAPL transport is uncoupled from the usual mode of advective solute transport in several ways: with respect to direction, velocity, and pathways. Research on the transport and distribution of DNAPLs in porous media is still in its infancy. Most investigations have centered on relatively homogeneous glass bead and sand packs; and it is altogether uncertain how immiscible liquids behave in markedly heterogeneous media, especially where layers or fractures abound. Further research is needed to develop practical means of characterizing hazardous waste sites from the viewpoint of processes governing immiscible liquid behavior before the ramifications for bioremediation can be assessed realistically.

TABLE 4. MOST PREVALENT CHEMICAL COMPOUNDS AT U.S. SUPERFUND SITES WITH A SPECIFIC GRAVITY GREATER THAN UNITY
(from Huling and Weaver, 1991)

Compound	Density	Water Solubility (mg/l)
Halogenated Volatiles		
Chlorobenzene	1.1060	4.9 E+02
1,2-Dichloropropane	1.1580	2.7 E+03
1,1-Dichloroethane	1.1750	5.5 E+03
1,2-Dichloroethylene	1.2140	4.0 E+02
1,2-Dichloroethane	1.2530	8.7 E+03
Trans-1,2-Dichloroethylene	1.2570	6.3 E+03
Cis-1,2-Dichloroethylene	1.2480	3.5 E+03
1,1,1-Trichloroethane	1.3250	9.5 E+02
Methylene Chloride	1.3250	1.3 E+04
1,1,2-Trichloroethane	1.4436	4.5 E+03
Trichloroethylene	1.4620	1.0 E+03
Chloroform	1.4850	8.2 E+03
Carbon Tetrachloride	1.5947	8.0 E+02
1,1,2,2-Tetrachloroethane	1.6	2.9 E+03
Tetrachloroethylene	1.6250	1.5 E+02
Ethylene Dibromide	2.1720	3.4 E+03

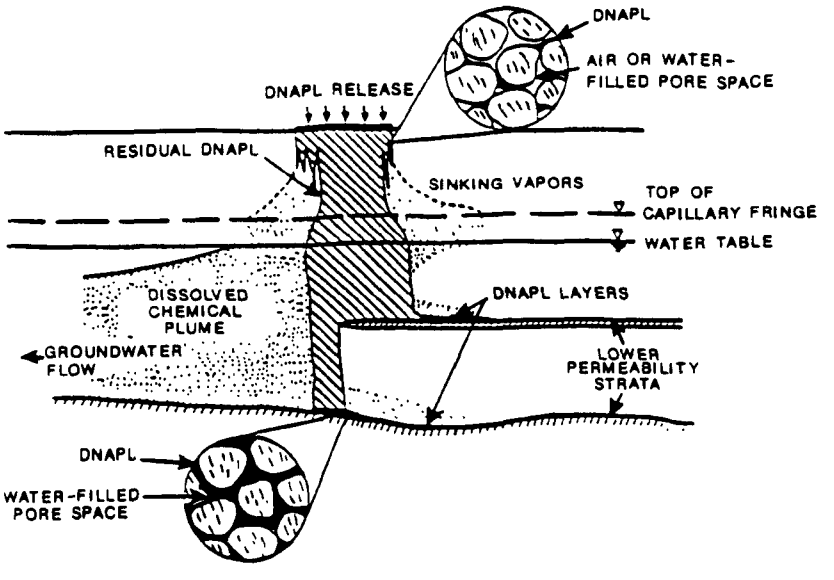


Figure 6. DNAPL infiltration schematic (from Mercer and Cohen, 1990).

SECTION 3

BIOTRANSFORMATION

MICROBIAL TRANSFORMATION OF ORGANIC POLLUTANTS

Microbiological processes have a great significance in determining the fate and transport of environmental pollutants. Chemoorganotrophic bacteria and fungi are especially prominent in this respect (Alexander, 1980; Bumpus et al., 1985). These microorganisms produce natural catalysts (enzymes) which break down organic substrates to obtain carbon, energy, reductants, and sometimes also nutrients necessary for growth. Frequently, these degradative (catabolic) enzymes can also transform (i.e., partially change), or degrade (i.e., completely destroy) organic pollutants. The degree of chemical change of the pollutant molecule may vary and will depend on the chemical and physical properties of this compound, as well as on the physiological types of microorganisms involved, and physical and chemical environmental factors (Boethling and Alexander, 1979; Alexander, 1981, 1985).

The outcome of the pollutant-microorganism interaction is affected by the environment to such an extent that no transformation will occur if the environment is not conducive to microbial growth, or if it renders the pollutant unavailable to microorganisms. The most important environmental variables are the following: presence/absence of microbial growth substrates, electron acceptors, nutrients, inhibitors, other microorganisms, and toxic compounds; water activity; ionic strength; chemical composition of the medium; pH; oxidation-reduction potential; temperature; and adsorptive surfaces (Alexander, 1965; Morrill et al., 1982).

Some pollutants, however, especially xenobiotics (man-made chemicals with novel structures, Leisinger, 1983), persist for long periods of time in various types of environments, or are intrinsically nonbiodegradable because of their physical and chemical properties. Such compounds are termed recalcitrant pollutants (Alexander, 1973). The notion of recalcitrance and the classification of substances in that regard are subject to change: new microorganisms are constantly being discovered that exhibit degradative or transforming capabilities towards pollutants previously believed to be recalcitrant (Brown et al., 1987), or under environmental conditions previously considered unconducive to such transformations (Wilson and Wilson, 1985; Grbić-Galić, 1990; Lovley and Lonergan, 1990).

Microbial Metabolism

Provided favorable environmental conditions, microorganisms will grow and multiply, and their population will increase. Microbial metabolism is the basis for growth. Degradative (catabolic) reactions yield energy, reductant supply, and simple building blocks necessary for synthesis of new cells; biosynthetic (anabolic) reactions put all of these together to synthesize cellular constituents (Gottschalk, 1986). In order for the metabolism to function, several essential requirements need to be satisfied: electron donor, electron acceptor, carbon source, energy source, water, and nutrients.

Chemoorganotrophs (Table 5) are the most important class of microorganisms with regard to the transformation and degradation of organic pollutants. These microorganisms frequently satisfy several of their requirements (e.g., carbon, energy, and electron donor demands) from the same organic compound. This organic compound is then termed primary substrate. Since the carbon source for these organisms is organic, they are frequently called heterotrophs (because they build their organic constituents from other organic compounds). On the contrary, chemolithotrophs, bacteria with equally powerful enzymes but different preferences, obtain their energy and electrons from inorganic chemicals such as ammonia, nitrite, hydrogen sulfide, sulfur, molecular hydrogen, etc. Their carbon demand is satisfied from an inorganic source, carbon dioxide, and therefore they are called autotrophs (capable of synthesizing their organic carbon from an inorganic source). Other physiological groups exist, but they are not so important in degradation of organic pollutants (except maybe photoorganotrophs) and are not within the scope of this report.

TABLE 5. CLASSIFICATION OF MICROORGANISMS BY MAJOR CATABOLIC REQUIREMENTS

Microorganisms	Energy Source	Carbon Source	Electron Donor (Reductant)
Chemoorganotrophs	Organics	Organics	Organics
Chemolithotrophs (Chemoautotrophs)	Inorganics (e.g., NH_4^+, NO_2^- H_2, Fe^{2+}, H_2S, S, etc.)	Carbon dioxide (CO_2)	Inorganics (e.g., NH_4^+, NO_2^- H_2, Fe^{2+}, H_2S, S, etc.)
Photoorganotrophs	Light	Organics, CO_2	Organics
Photolithotrophs (Photoautotrophs)	Light	Carbon dioxide (CO_2)	Water (H_2O), or other reduced inorganics (e.g., H_2, H_2S, S)

Regardless of the physiological group, microorganisms also need an electron acceptor which, when coupled to the electron donor, establishes an electron transfer (oxidation-reduction reaction) that plays an important role in generation of useful energy (Table 6). Aerobic microorganisms use molecular oxygen as an electron acceptor; anaerobic microorganisms respire other compounds, such as nitrate or nitrous oxide (denitrifiers and nitrate reducers), ferric iron (iron reducers), sulfate, thiosulfate or sulfur (sulfate reducers), protons (obligate proton-reducing acetogens), or carbon dioxide (methanogens). Recently, microorganisms have been found which can utilize selenate (Oremland et al., 1989) or chlorinated organic compounds such as chlorinated aromatic acids (Dolfing and Tiedje, 1987) as electron acceptors and can obtain useful metabolic energy through such reactions.

Finally, there are bacteria which utilize their primary substrate not only as a carbon and energy source and an electron donor but also as an electron acceptor. In this process, which happens in the absence of exogenous electron acceptors, an incomplete degradation of the primary substrate occurs; some of the products are more reduced than the primary substrate, whereas the others are more oxidized. The bacteria which carry out this type of catabolism are called fermenters.

TABLE 6. CLASSIFICATION OF MICROBIAL PHYSIOLOGICAL GROUPS BY CATABOLIC ELECTRON ACCEPTOR

Catabolic Process	Electron Acceptor
Aerobic respiration	Oxygen (O_2)
Anaerobic respirations:	
Denitrification, nitrate reduction	Nitrate (NO_3^-), nitrous oxide (N_2O)
Sulfate reduction	Sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$)
Sulfur reduction	Sulfur (S)
Ferric iron reduction	Ferric iron (Fe^{3+})
Obligate proton reduction	Protons (H^+)
Methanogenesis	Carbon dioxide (CO_2)
Organic respiration	Organics (e.g., fumarate)
Fermentation	Organics as both donors and acceptors of electrons

Types of Pollutant Transformation (Table 7)

Bacteria can frequently use numerous organic pollutants as their primary substrates (energy sources, carbon sources, and/or electron donors). As the result, the microorganisms grow on the pollutants, their populations increase, and consequently the rate of pollutant degradation also increases if the biomass concentration is rate-limiting (Alexander, 1980). This is the best possible outcome in the battle of environmental detoxification and reclamation, because it usually results in complete degradation (mineralization) of the pollutant molecule: an organic compound is converted to innocuous inorganic products such as carbon dioxide, water, ammonia, chloride, etc. For example, simple aromatic and aliphatic constituents of petroleum, and even some polynuclear aromatic hydrocarbons (PAH), represent excellent primary substrates for bacteria (see reviews by Britton, 1984, and Gibson and Subramanian, 1984). Indigenous microorganisms have been successfully used for *in-situ* bioreclamation of gasoline-contaminated aquifers following injection of oxygen or hydrogen peroxide (Raymond et al., 1975) or nitrate (Battermann and Werner, 1984) as electron acceptors for microorganisms.

Frequently, however, organic pollutants which could be used as primary substrates by bacteria are present in such low (trace) concentrations that they cannot support microbial growth. The microorganisms can deal with this situation if there is another, sufficiently abundant, primary substrate present in their environment; this substrate is utilized for growth, and the pollutant can be simultaneously degraded through a process termed secondary substrate utilization (Rittmann et al., 1980a; McCarty et al., 1981). This type of pollutant degradation also usually results in mineralization.

Unfortunately, mineralization does not always happen; numerous pollutants are only partially transformed by microorganisms. Fungi, typically, catalyze various detoxification reactions (partial transformations) rather than catalyzing catabolic breakdown of xenobiotics (Gibson and Subramanian, 1984). Even bacteria often catalyze only a partial chemical change which can result in a loss of toxic properties (detoxification), or no loss of toxicity, or even generation or amplification of toxicity (activation). A well-known example of activation is reductive dechlorination of tetrachloroethylene (PCE) and trichloroethylene (TCE) to vinyl chloride (VC) under methanogenic conditions (Vogel and McCarty, 1985).

TABLE 7. TYPES OF ORGANIC POLLUTANT TRANSFORMATION ACCORDING TO THE ROLE OF THE POLLUTANT IN MICROBIAL METABOLISM

Metabolic Role	Type of Metabolism	Outcome
Primary substrate (energy source, carbon source, electron donor)	Primary substrate utilization	Usually mineralization (complete breakdown)
Secondary substrate (could be a carbon or energy source, but present in too low concentrations)	Secondary substrate utilization; occurring only in the presence of a primary substrate	Usually mineralization
Electron acceptor	Electron acceptor utilization (respiration)	Partial reductive transformation (detoxification, or no change in toxicity, or activation)
None	Cometabolism	Partial transformation (detoxification, or no change in toxicity, or activation)

In bacteria, incomplete chemical modifications of organic pollutants frequently result from a special type of metabolism, called cometabolism. This phenomenon, which was first described (and named) by Jensen (1963) and then fully analyzed by Horvath (1972), represents a partial chemical change of the pollutant molecule which happens fortuitously and yields no benefit to the transforming microorganism. The transformation of a cometabolic substrate happens while microorganisms grow on their primary substrate. This transformation is due to the relaxed specificity of catabolic enzymes with compromised active sites which will accommodate numerous structures, sometimes only remotely related chemically. Aerobic transformation of chlorinated solvents, which is one of the main topics of this report, is often cometabolic, such as TCE transformation by methanotrophs (Little et al., 1988). It is important to emphasize, however, that cometabolism in a natural setting may sometimes end as a complete degradation of the initially cometabolic substrate. Natural habitats are inhabited by microbial communities which frequently practice interspecies transfer of various metabolites; a product of cometabolic change, created fortuitously by the transformation-initiating microorganism, may represent a useful resource for another microorganism(s) which will catabolize it to inorganic products. Methanotrophs and heterotrophs together completely degrade TCE, 1,2-dichloroethylene (1,2-DCE), VC, and other chlorinated compounds through such cooperative routes (Semprini et al., 1990, 1991; Henry and Grbić-Galić, 1990, 1991a,b; Alvarez-Cohen and McCarty, 1991a,b; Lanzarone and McCarty, 1990).

Under anaerobic conditions, halogenated compounds can undergo reductive dehalogenation (Bouwer and McCarty, 1982; Suflita et al., 1982), a partial transformation which sometimes detoxifies but in other cases activates the pollutant. This process is also included in cometabolic reactions, with the active bacteria not getting any benefit from it. However, recent investigations indicate that, in some cases, microorganisms may be utilizing the halogenated compounds as electron acceptors, which implies generation of a useful form of energy when the halogenated compound is coupled to a suitable electron donor. It has been suggested that PCBs may represent such electron acceptors and that the microorganisms which reductively dechlorinate PCBs in anoxic environments may be at an advantage relative to other members of the microflora (Brown et

al., 1987). For some other chlorinated compounds, such as 3-chlorobenzoic acid, it has been proven that the dehalogenating microbial communities indeed obtain energy from this dechlorination (Dolfing and Tiedje, 1987).

Acclimation Lag

Regardless of the type of microbial transformation of a pollutant, there is frequently an initial period ("acclimation lag") during which no obvious changes of the pollutant occur. This period may be due to various reasons (Linkfield et al., 1989). Sometimes the causes are in the indigenous microbial communities. The starting biomass may be so low that no appreciable degradation can happen until a critical biomass concentration is reached; or the total microflora may be abundant but the specific active populations need to be enriched, or plasmids-coding for the active enzymes must spread through the population. On other occasions, the pollutant must induce a requisite enzyme, or a new enzyme needs to be synthesized, which will involve a genetic event such as mutation and gene rearrangement. Sometimes, the reasons for the initial lag period may lie in the pollutants themselves. The pollutants may be present in such low concentrations that they will not induce the relevant enzymes or their chemical structure may be so unusual that they cannot interact with the enzymic active sites. Finally, the environment will determine the duration of an acclimation period. The lag can occur because preferential microbial substrates must be depleted first before the degradation of a pollutant can start (in this case the pollutant is used as a primary substrate). Sometimes, the temporarily inhibitory environmental conditions need to be changed; or, simply, not all the necessary microbial nutrients are present, and microbial activity cannot be supported.

We can sometimes influence or change the environmental impacts on the acclimation lag; for example, nutrients can be added to a contaminated habitat to stimulate microbial growth. If pollutants are present in very low concentrations, suitable primary substrates can be injected into the contaminated site to facilitate secondary substrate utilization. Further, if the desired physiological group of microorganisms is not present, addition of acclimated or genetically modified microorganisms to the site may be considered. Although this type of environmental biotechnology is not yet practiced at large, it will be one of the most important technologies in the future. However, if a pollutant is recalcitrant because of its xenobiotic chemical structure, as is the case with some pesticides, little can be done once the contamination has occurred. The only viable option in the case of recalcitrant pesticides is prevention (rather than cure): the use of biodegradable alternatives with a similar range of activity.

ANAEROBIC TRANSFORMATIONS

The potential for anaerobic biological transformations of brominated and chlorinated (halogenated) aliphatic compounds was demonstrated in 1981 (Bouwer et al., 1981). Subsequently, halogenated aliphatic compounds in general have been found to transform under a variety of environmental conditions in the absence of oxygen. In addition, research has indicated that some transformations occur chemically (abiotic), as well as through the direct action of microorganisms (biotic). The major abiotic and biotic transformation processes occurring in natural systems are summarized in Table 8 (Vogel et al., 1987). The abiotic processes most frequently occurring under either aerobic or anaerobic conditions are hydrolysis and dehydrohalogenation, while the anaerobic biotic processes are generally reductions through hydrogenolysis or dihalo-elimination. The biotic reductive processes appear to be occurring mostly through cometabolism. Here the compounds are not used for energy or growth by the microorganisms, but are transformed fortuitously by enzymes being used for other normal metabolic processes. Cometabolism generally occurs when other chemicals are also present that can be used for energy and growth. This helps explain why transformations occur at some sites but not at others.

TABLE 8. TRANSFORMATIONS OF HALOGENTED ALIPHATIC COMPOUNDS
(after Vogel et al., 1987)

Reactions	Examples
I. Substitution	
a. solvolysis, hydrolysis $RX + H_2O \rightarrow ROH + HX$	$CH_3CH_2CH_2Br + H_2O \rightarrow CH_3CH_2CH_2OH + HBr$
b. other nucleophilic reactions $RX + N^- \rightarrow RN + X^-$	$CH_3CH_2Br + HS^- \rightarrow CH_3CH_2SH + Br^-$
II. Oxidation	
a. α-hydroxylation $-\overset{|}{\underset{H}{C}}-X + H_2O \rightarrow -\overset{|}{\underset{OH}{C}}-X + 2H^+ + 2e^-$	$CH_3CHCl_2 + H_2O \rightarrow CH_3CCl_2OH + 2H^+ + 2e^-$
b. epoxidation $>C=C< + H_2O \rightarrow >C(O)-C< + 2H^+ + 2e^-$	$CHClCCl_2 + H_2O \rightarrow CHClOCCl_2 + 2H^+ + 2e^-$
III. Reduction	
a. hydrogenolysis $RX + H^+ + 2e^- \rightarrow RH + X^-$	$CCl_4 + H^+ + 2e^- \rightarrow CHCl_3 + Cl^-$
b. dihalo-elimination $-\overset{|}{\underset{X}{C}}-\overset{|}{\underset{X}{C}}- + 2e^- \rightarrow >C=C< + 2X^-$	$CCl_3CCl_3 + 2e^- \rightarrow CCl_2CCl_2 + 2Cl^-$
c. coupling $2\,RX + 2e^- \rightarrow R\text{-}R + 2X^-$	$2\,CCl_4 + 2e^- \rightarrow CCl_3CCl_3 + 2Cl^-$
IV. Dehydrohalogenation	
$-\overset{|}{\underset{X}{C}}-\overset{|}{\underset{H}{C}}- \rightarrow >C=C< + HX$	$CCl_3CH_3 \rightarrow CCl_2CH_2 + HCl$

Figure 7 illustrates the anaerobic biotic and abiotic pathways that chlorinated aliphatic compounds may undergo at contaminated sites. For example the chlorinated solvent TCA may be transformed abiotically to form 1,1-DCE and acetic acid. The rates are relatively slow, with a half life for TCA on the order of one year (Vogel et al., 1987; Cline and Delfino, 1989; Jeffers et al., 1989). Also, under anaerobic conditions, TCA may be biologically transformed into 1,1-DCA, which can be further reduced to CA. CA is relatively stable biologically; but abiotically it can be transformed into acetate and chloride, thus rendering it relatively nontoxic. Thus, when TCA is discharged to soil, a variety of abiotic and biotic transformation products may be found there in later years. As another example, TCE can be reduced anaerobically to either cis- or trans-1,2-DCE, both of which can further be transformed into vinyl chloride (VC). Recent research has indicated that VC can even undergo reduction into ethylene (Freedman and Gossett, 1989), which is essentially harmless.

The pathways outlined in Figure 7 suggest that it is possible to render harmless numerous chlorinated aliphatic compound under anaerobic conditions. Unfortunately, there are several problems that hinder this potential approach to bioremediation. First, the biotic transformations generally involve cometabolism such that other organic compounds must be present to serve as primary substrates for organism growth. Second, the rates of anaerobic transformation are much greater for the highly chlorinated compounds than for the less-chlorinated compounds, so that the less chlorinated ones persist longer in the environment. Third, some of the anaerobic transformation products are more hazardous than the parent compounds. Examples here are TCE transformation to VC, and TCA transformation to 1,1-DCE. Fourth, reaction rates tend to be greater under highly reducing conditions associated with methane formation than under the less reducing conditions associated with denitrification (Bouwer and Wright 1988). The latter is the main anaerobic process occurring when excess nitrates are present. Reductive transformation rates are somewhat intermediate between the two under conditions favoring sulfate reduction (sulfate, but no nitrate present). Fifth, when proper environmental conditions are present, microorganisms that can bring about the transformations through cometabolism must also be present.

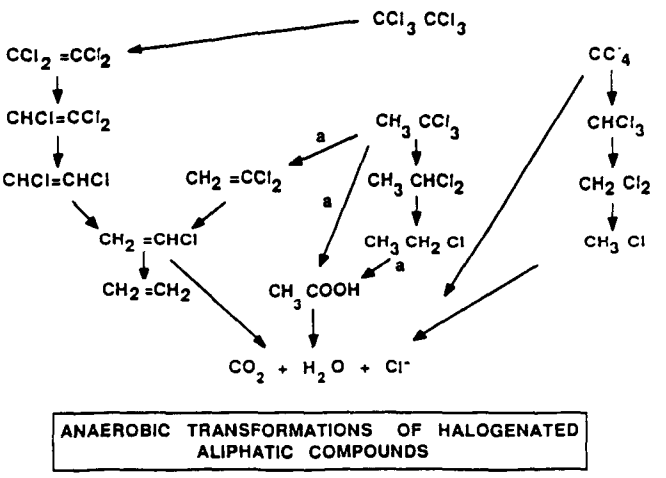


Figure 7. Chemical and biological transformation pathways of selected chlorinated aliphatic compounds under anaerobic conditions. Arrows with "a" indicate chemical transformations; other arrows represent biological transformations (after Vogel, et al., 1987).

Thus, one cannot count upon sufficiently high rates and complete transformation to harmless products to occur in groundwater unless all the right conditions are present. On the other hand, anaerobic transformation processes do frequently occur, converting chlorinated aliphatic compounds into less chlorinated species that are more readily transformed by aerobic microorganisms. It is for this reason, as well as to help understand the environmental fate of compounds, that knowledge of anaerobic pathways is important.

AEROBIC MICROBIAL TRANSFORMATION OF C_1 AND C_2 CHLORINATED ALIPHATIC HYDROCARBONS

Under aerobic conditions, halogenated aliphatic compounds with one or two carbons per molecule can be transformed by three types of microbial enzymes: dehalogenases, hydrolytic dehalogenates, and oxygenases. Dehalogenases, which require reduced glutathione as a cofactor, dehalogenate the substrates by means of nucleophilic substitution (Stucki et al., 1981; Kohler-Staub and Leisinger, 1985). The first product of this degradation pathway is an S-chloroalkyl-glutathione, which is probably nonenzymatically converted to glutathione and an aldehyde (Stucki et al., 1981). Hydrolytic dehalogenases hydrolyze their substrates, yielding alcohols (Goldman et al., 1968; Keuning et al., 1985). Oxygenases use molecular oxygen as a reactant for the attack on the halogenated compounds (Dalton, 1980; Hou, 1984; Nelson et al., 1987); the products could be alcohols, aldehydes, or epoxides, depending on the structure of the compound. Numerous halogenated short-chain aliphatic hydrocarbons have been demonstrated to undergo aerobic transformation. However, compounds which have all the available valences ontheir carbon atoms substituted by halogens, such as PCE or carbon tetrachloride, have never been shown to transform through any other but reductive pathways (Vogel et al., 1987; Henson et al., 1988; Oldenhuis et al., 1989). Generally, as the degree of halogenation increases, the likelihood of aerobic transformation decreases (Figure 8); the opposite is true for anaerobic (reductive) transformations (Vogel et al., 1987).

Among C_1 compounds, dichloromethane (DM) and chloroform (CF) have been found susceptible to aerobic microbial transformation. DM can be completely mineralized under aerobic conditions by sewage sludge microorganisms (Rittmann and McCarty, 1980a; Klecka, 1982) and by mixed methanotrophic cultures enriched from soil (Henson et al., 1989). Pure cultures of the genera *Pseudomonas* and *Hyphomicrobium* have been isolated which can grow on DM as the sole carbon and energy source (Brunner and Leisinger, 1978; Brunner et al., 1980; Stucki et al., 1981; La Pat-Polasko et al., 1984; Kohler-Staub et al., 1986). CF degradation has been reported for soil communities consisting of methanotrophs and heterotrophs (Strand and Shippert, 1986; Alvarez-Cohen and McCarty, 1991b). Pure cultures of methanotrophs, such as *Methylosinus trichosporium* OB3b, partially transform (oxidize) DM and CF (Oldenhuis et al., 1989, 1991).

Alkylhalides (haloalkanes), such as 1,2-dichloroethane (1,2-DCA), are frequently hydrolytically dehalogenated (Stucki et al., 1983). *Xanthobacter autotrophicus* utilizes 1,2-DCA as sole carbon source (Janssen et al., 1985). A hydrolytic dehalogenase isolated from this microorganism dehalogenates 1,2-dibromoethane (EDB) as well, but the bacterium cannot grow on this compound (Keuning et al., 1985). An *Acinetobacter* sp., isolated from sewage sludge, was shown to dehalogenate and grow on ethylbromide (EB); EDB was dehalogenated, but not utilized for growth (Janssen et al., 1987b). Complex communities consisting of methanotrophs and heterotrophs which inhabit groundwater aquifers mineralize 1,2-DCA (Lanzarone and McCarty, 1990). A *Pseudomonas fluorescens* strain isolated from water and soil contaminated by chlorinated aliphatic hydrocarbons was shown to utilize 1,2-DCA, 1,1,2-trichloroethane (1,1,2-TCA), and TCE, but not PCE or 1,1,1-TCA (Vandenbergh and Kunka, 1988). On the other hand, *Methylosinus trichosporium* OB3b could transform not only 1,1-DCA and 1,2-DCA, but also 1,1,1-TCA

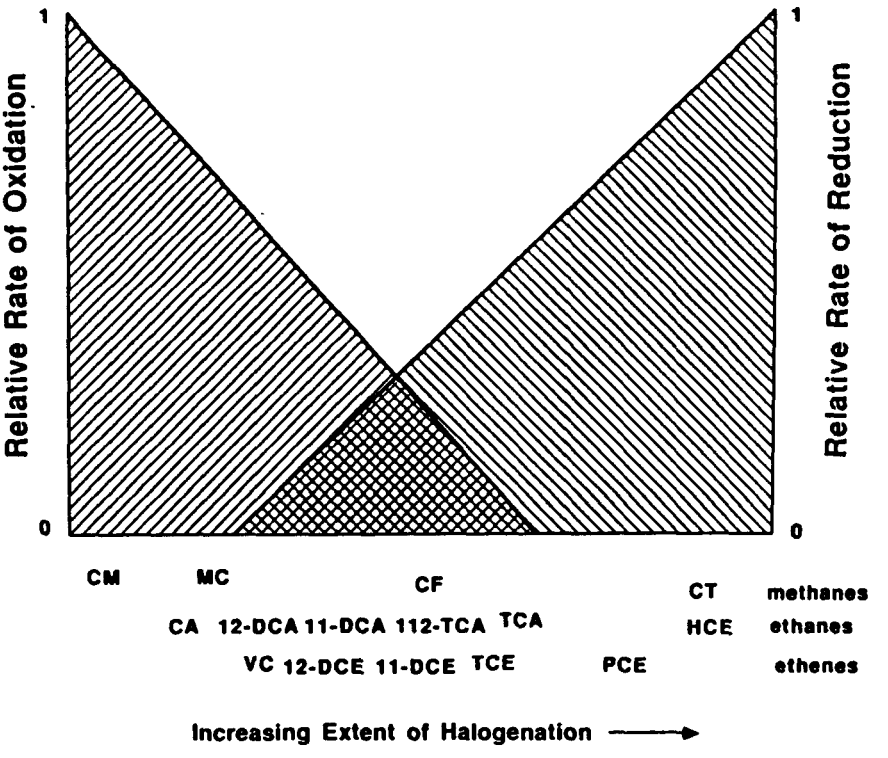


Figure 8. Relative rates of oxidation and reduction of a range of C_1 and C_2 chlorinated compounds. CM = chloromethane; MC = dichloromethane; CF = chloroform; CT = carbon tetrachloride; CA = chloroethane; 12-DCA = 1,2-dichloroethane; 11-DCA = 1,1-dichloroethane; 112-TCA = 1,1,2-trichloroethane; TCA = 1,1,1-trichloroethane; HCE = hexachloroethane; VC = vinyl chloride; 12-DCE = 1,2-dichloroethylene; 11-DCE = 1,1-dichloroethylene; TCE = trichloroethylene; PCE = tetrachloroethylene (from Vogel et al., 1987).

(Oldenhuis et al., 1989); similar results were obtained with mixed methanotrophic cultures and soil microcosms containing methanotrophic/ heterotrophic communities (Henson et al., 1988; Strand et al., 1990).

Haloalkenes, such as TCE, cis- and trans-1,2-DCE, 1,1-DCE, and VC, are also transformed by several different physiological groups of aerobes. Methanotrophic communities consisting of methanotrophs which initiate the oxidative transformation, and heterotrophs which utilize the products of oxidation and hydrolysis, are very active in this respect, and can achieve complete degradation of chlorinated alkenes (Fogel et al., 1986; Henson et al., 1989; Roberts et al., 1989; Alvarez-Cohen and McCarty, 1991a,b; Henry and Grbić-Galić, 1990, 1991a,b; Lanzarone and McCarty, 1990). The same communities fail to transform PCE, however, because this compound is too oxidized (Fogel et al., 1986; Henson et al., 1988). Pure cultures of methanotrophs, such as *Methylosinus trichosporium* OB3b or *Methylomonas* sp. MM2, have been shown to partially transform TCE, *trans*-1,2-DCE, and *cis*-1,2-DCE (Oldenhuis et al., 1989, 1991; Henry and Grbić-Galić, 1990, 1991a,b). Other microorganisms capable of transforming halogenated alkenes belong to the genera *Pseudomonas*, *Alcaligenes*, *Mycobacterium*, and *Nitrosomonas* (Nelson et al., 1986,

1987, 1988; Arciero et al., 1989; Wackett et al., 1989; Folsom et al., 1990; Harker and Kim, 1990; Vannelli et al., 1990). All of these microorganisms, except the genus *Nitrosomonas*, are heterotrophs which grow on various organic substrates (e.g., aromatic hydrocarbons, phenols, propane, etc.); *Nitrosomonas* is a chemolitotroph which derives energy from oxidation of ammonia. All of them cometabolize chlorinated compounds such as TCE or 1,2-DCE while growing on their respective growth substrates; the haloalkenes are only fortuitously transformed, not utilized for growth. However, vinyl chloride seems to be an exception. It has been demonstrated that a *Mycobacterium* strain isolated from soil contaminated by VC, could grow on VC as sole carbon and energy source (Hartmans et al., 1985).

AEROBIC TRANSFORMATION AND DEGRADATION OF TCE

In 1985, Wilson and Wilson showed that TCE may be susceptible to aerobic degradation (by soil microbial communities fed natural gas). Since then, much scientific research addressing this phenomenon has been performed, including numerous laboratory experiments but also directed field experiments (Roberts et al., 1990; Semprini et al., 1990, 1991). So far, the groups of bacteria capable of transforming TCE comprise methanotrophs (Fogel et al., 1986; Little et al., 1988; Mayer et al., 1988; Tsien et al., 1989; Oldenhuis et al., 1989, 1991; Henry and Grbić-Galić, 1990, 1991a,b; Alvarez-Cohen and McCarty, 1991a,b; Lanzarone and McCarty, 1990), propane oxidizers (Wackett et al., 1989), ethylene oxidizers (Henry, 1991), toluene, phenol, or cresol oxidizers (Nelson et al., 1986, 1987, 1988; Wackett and Gibson, 1988; Folsom et al., 1990; Harker and Kim, 1990), and ammonia oxidizers (Arciero et al., 1989; Vannelli et al., 1990). All of these microorganisms have catabolic oxygenases that catalyze breakdown of their respective growth substrates, but have nonspecific active sites which can accommodate TCE (and a variety of other non-growth substrates) as well.

Thus TCE can be transformed (upon the induction of the oxygenase enzyme by its substrate) in the presence of the microorganismal growth substrate (cometabolism), or in its absence (resting cells transformation). However, TCE is not utilized by the bacteria as a carbon, energy, or electron source; this transformation is only fortuitous. Based on the findings with methanotrophs (Little et al., 1988; Henry and Grbić-Galić, 1991a), it can be concluded that TCE is most likely oxygenated to TCE-epoxide. The epoxide is unstable and is quickly nonenzymatically rearranged in aqueous solution to yield various products including carbon monoxide, formic acid, glyoxylic acid, and a range of chlorinated acids (Miller and Guengerich, 1982). Recent findings with purified MMO from *Methylosinus trichosporium* OB3b indicate that TCE-epoxide is indeed a product of TCE oxygenation (Fox et al., 1990). In nature, where cooperation between the TCE oxidizers and other bacteria (most prominently heterotrophs) occurs, TCE can be completely mineralized to carbon dioxide, water, and chloride (Fogel et al., 1986; Henson et al., 1989; Roberts et al., 1989; Henry and Grbić-Galić, 1991a).

Toluene, phenol, and cresol oxidizers, such as *Pseudomonas putida* or *P. cepacia*, express the TCE transformation activity upon induction by their aromatic substrates (Nelson et al., 1988; Folsom et al., 1990). These bacteria have a great potential for remediation of groundwater aquifers which are contaminated by mixtures of gasoline or jet fuel (or other petroleum derivatives), and chlorinated solvents, such as TCE, DCE, or VC. If the aromatic contaminants are not present, however, bacterial growth substrates need to be injected into the site in order to stimulate the transformation of chlorinated solvents. In this situation, methanotrophs become more attractive agents of bioremediation: methane, their preferred substrate, is a nontoxic and inexpensive chemical. Once methane and oxygen are injected into the site, methanotrophs (if present) will start cometabolizing chlorinated solvents, as well as a great number of other contaminants (see below), and the accompanying heterotrophs will mineralize their transformation products.

Transformation and Degradation of TCE by Methanotrophs and Methanotrophic Communities

Methanotrophs grow on C_1 compounds as sole carbon and energy sources (Anthony, 1982). Their catabolic oxygenases are methane monooxygenases (MMO) which incorporate one atom of oxygen from the oxygen molecule into methane to yield methanol. This alcohol is further oxidized via a series of dehydrogenation steps, through formaldehyde and formic acid, to CO_2 which is the final product of catabolism. MMO enzymes utilize molecular oxygen as a reactant, and require a reduced electron carrier (e.g., NADH + H^+) to reduce the remaining oxygen atom to water. NADH + H^+ is regenerated via dehydrogenation of catabolic intermediates. MMO enzymes have relaxed substrate specificity, and will oxygenate many compounds which are not growth substrates for methanotrophs. Such compounds include various alkanes, alkenes, ethers, alicycles, aromatics, nitrogen heterocycles, and halogenated alkanes, alkenes, and aromatics (Colby et al., 1977; Higgins et al., 1979; Stirling and Dalton, 1979; Stirling et al., 1979; Hou, 1984).

Two types of MMO have been suggested: a particulate (membrane-bound), and a soluble enzyme (Dalton et al., 1984). The soluble MMO (purified from *Methylosinus trichosporium* OB3b and *Methylococcus capsulatus* [bath]), which is produced under the conditions of copper limitation and increased oxygen tension (Stanley et al., 1983), has been considered to have broader substrate specificity (Colby et al., 1977; Burrows et al., 1984). It has been stated that only the soluble MMO can transform TCE (Oldenhuis et al., 1989; Tsien et al., 1989). However, recent findings indicate that the particulate MMO in some methanotrophs may be as effective in the transformation of chlorinated solvents as the soluble MMO (Henry and Grbić-Galić, 1991a). Since the soluble MMO is not constitutively expressed whereas the particulate MMO is, the latter methanotrophs (*Methylomonas* sp.) have a significant potential for *in-situ* bioremediation.

On the basis of certain morphological and physiological differences, methanotrophs can be classified as type I, type II, and Type X (Anthony, 1982). Types I and II have been shown to oxidize TCE, presumably to an epoxide (Little et al., 1988; Oldenhuis et al., 1989; Tsien et al., 1989; Henry and Grbić-Galić, 1990, 1991a). Some of the suggested products of TCE-epoxide hydrolysis, such as carbon monoxide, formic acid, glyoxylic acid, and chlorinated acids, have indeed been detected in the methanotrophic culture fluid (Little et al., 1988; Henry and Grbić-Galić, 1990, 1991a,b). These products are quickly consumed by heterotrophic bacteria, so the overall process brings about complete destruction of TCE (Henson et al., 1989; Alvarez-Cohen and McCarty, 1991a; Henry and Grbić-Galić, 1991a). In groundwater *in situ*, such degradations have been demonstrated at a moderate efficiency for TCE and cis-1,2-DCE, and at a great efficiency for trans-1,2-DCE and VC (Semprini et al., 1990, 1991).

Significant Findings Concerning the Fortuitous Transformation of TCE by Methanotrophs

Like any other microbial process affecting an environmental pollutant, methanotrophic transformation of TCE has certain peculiarities and also requirements which need to be satisfied in order for the process to function. Furthermore, the transformation is susceptible to environmental influences. In the course of the laboratory investigations of TCE transformation at Stanford University, under the auspices of the Moffett Field project (Roberts et al., 1989), a number of interesting phenomena were observed. Several of these phenomena were confirmed through parallel work of researchers at other institutions. The laboratory batch cultivation techniques used and the most important of the findings are described below.

Batch Cultivation Techniques--For microcosm work, glass columns (40×2 cm) were packed with sandy aquifer material. The columns were batch-fed methane and oxygen (or hydrogen peroxide) dissolved in groundwater. Cold or ^{14}C-labeled TCE was added to the feed after the microbial communities had become established in the columns. The sequential exchanges of the pore water

were performed at intervals of 3 days, one week, or two weeks. The effluent samples were analyzed for methane concentration (gas chromatography, GC), dissolved oxygen (dissolved oxygen probe), hydrogen peroxide concentration (titration with ceric sulfate), number of bacteria (acridine-orange direct counting--Ghiorse and Balkwill, 1983), TCE concentration, and other ^{14}C-labeled species (GC, scintillation counting). A total of six columns were operated; two of them were live controls that received no methane (Mayer et al., 1988).

The suspended mixed cultures were enriched from aquifer material, groundwater, or laboratory column effluent. The enrichments were achieved by inoculating continuously stirred reactors containing defined mineral medium. The reactors were incubated under a continuous flow of approximately 25% methane in air, at room temperature (21–23°C). The transfers to new reactors were repeated 5–10 times, until stable methanotrophic consortia were obtained (as determined by micro- and macroscopic observations). Pure methanotrophic cultures were isolated from these mixed cultures on agarose plates with mineral medium. The plates were incubated in desiccators filled with 25% methane in air, at room temperature. Purity was determined through repeated colony isolation and observation of constant macro- and micromorphology (Henry, 1991). The isolates were tested for the lack of growth on multicarbon substrate in liquid and solid media, and for the type of biosynthetic enzymes (Large and Quayle, 1963; Dahl et al., 1972). Both mixed and pure cultures were examined by light microscopy (including staining procedures for the determination of membrane structure and presence of internal storage granules), and scanning and transmission electron microscopy (Henry and Grbić-Galić, 1990).

For TCE transformation experiments, the cultures were first raised to mid-logarithmic growth phase in defined mineral medium in continuously stirred reactors under a continuous stream of 30-35% methane in air, at room temperature. The pure culture received a mixture of vitamins for growth in liquid medium (Henry, 1991). The inocula from the reactors were then transferred to 250-ml screw-cap bottles containing 150-ml headspace. The bottles were incubated upside-down on a rotary shaker in a 21°C environmental chamber, for resting-cell transformation studies. Formate, when added to some of the cultures as an electron donor, was provided as 2 mM sodium formate. The cultures were appropriately diluted (to prevent mass-transfer limitations) with either phosphate buffer or the same mineral medium in which they had been grown. Cell biomass was determined as dry weight. TCE concentration and TCE transformation products were evaluated in the bottle headspace and in the aqueous fraction by GC, gas partitioning, ion chromatography, and reduction gas detector (for carbon monoxide). Scintillation counting was used in the experiments with ^{14}C-labeled TCE (Henry and Grbić-Galić, 1990). Autoclaved controls were used in all the experiments.

Competitive Inhibition--MMO is the enzyme which is implied in oxidation of both methane and TCE (or other organic pollutants transformed by methanotrophs). Therefore, methane and TCE will compete for the same active site on the MMO. Depending on the affinities of the enzyme for methane and TCE (K_S), which vary in different methanotrophs (Oldenhuis et al., 1989; Tsien et al., 1989; Alvarez-Cohen and McCarty, 1991a; Henry and Grbić-Galić, 1991a), and on the concentrations of methane and TCE, methane will interfere more or less efficiently with TCE transformation (and *vice versa*) if both compounds are simultaneously present.

The phenomenon of competitive inhibition by methane in cometabolism of TCE has been shown for all methanotrophic systems examined so far. This inhibition can be relaxed if the methane concentration is kept low (Henry and Grbić-Galić, 1990), or if the resting-cells transformation of TCE (in the absence of methane) is employed (Oldenhuis et al., 1989; Alvarez-Cohen and McCarty, 1991a; Henry and Grbić-Galić, 1991a). For example, resting cells of *Methylomonas* sp. strain MM2 (Henry and Grbić-Galić, 1991a) transform TCE at low concentrations (30-60 $\mu g\ l^{-1}$), in the presence of 2 mM formate as an electron donor, at a rate as high as $2.3 \pm 0.05\ l\ mg^{-1}\ d^{-1}$

(pseudo-first-order rate constant, $k' = k/K_S$) at 21°C. The competitive inhibition of TCE transformation by methane has to be taken into account when the process is considered for application (Semprini et al., 1991).

The Importance of Endogenous or Exogenous Electron Donors--In the presence of methane, $NADH+H^+$ is regenerated through dehydrogenations of methanol, formaldehyde, and formate. In the absence of methane, it is not possible to renew the reduced electron carriers necessary for the oxygenation of TCE. However, some methanotrophs will continue transforming TCE in the absence of methane (resting cells transformation) for extended periods of time (longer than 24 hr; Henry and Grbić-Galić, 1991a; see Figure 9.B), whereas others will lose the transformation capability within several hours (Alvarez-Cohen and McCarty, 1991a). The former group has been shown to form intracellular lipid storage inclusions while growing on methane (Henry and Grbić-Galić, 1990, 1991a). This storage can be used as a source of electrons and protons for the oxygenation reactions in resting cells.

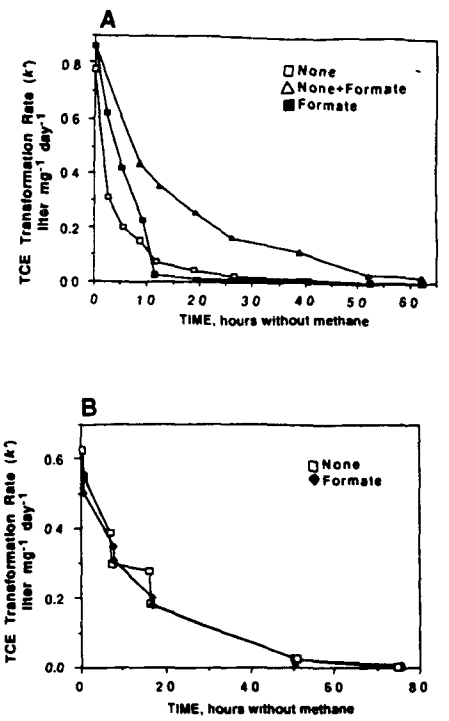


Figure 9. The influence of formate addition on TCE transformation rates in the absence of methane, in the pure culture *Methylomonas* sp. MM2 (A) and mixed culture MM1 (B), both derived from the Moffett Field groundwater aquifer. MM1 contains methanotrophs with lipid storage inclusions, and can transform TCE for a prolonged period of time in the absence of methane, but it does not react to formate addition (B). *Methylomonas* sp. MM2, on the other hand, does not have internal storage granules, but its resting-cell transformation of TCE can be prolonged by formate addition (A). "None" = subcultures not amended with formate. "None+formate" = subcultures incubated without formate, but receiving 2 mM formate at the time of TCE addition. "Formate" = subcultures incubated with formate, and receiving 2 mM formate at the time of TCE addition (from Henry and Grbić-Galić, 1991a).

The methanotrophs which do not have the endogenous electron donor storage can be aided in their resting-stage fortuitous transformations if an external electron donor is added, such as formate (Oldenhuis et al., 1989; Semprini et al., 1991; Alvarez-Cohen and McCarty, 1991a; Henry and Grbić-Galić, 1991a) or methanol (Mayer and Grbić-Galić, 1989; Semprini et al., 1991). Figure 9 shows the behavior of a pure methanotrophic culture, *Methylomonas* sp. MM2 , and a mixed methanotrophic culture, MM1, both derived from the Moffett Field groundwater aquifer (Henry and Grbić-Galić 1990), in the absence of methane but with the formate addition. *Methylomonas* sp. MM2 (Figure 9.A) does not contain internal storage granules, whereas the mixed culture MM1 (Figure 9.B) does. As the result, formate increases the TCE transformation rates in *Methylomonas* sp. MM2 under starvation conditions, while MM1 remains insensitive to the formate addition.

The methanotrophs containing storage granules which represent an internal electron donor source may be of a great significance for *in-situ* treatment of contaminated aquifers. Such organisms would be capable of prolonged contaminant transformation in the absence of methane, thus circumventing competitive inhibition, and would not require the addition of alternate reductants. Although formate or methanol can be used as alternate electron sources by some methanotrophs, they are also utilized by heterotrophs; the addition of these compounds to the subsurface could thus shift the delicate balance of the active microbial community.

Product Inhibition--Carbon monoxide (CO) is one of the products of TCE transformation by methanotrophs: *Methylomonas* sp. MM2 was shown to transform approximately 9 mol% of the TCE to CO, which was subsequently oxidized (Henry and Grbić-Galić, 1991b; see Figure 10). It is known that CO can be oxidized by methanotrophs, and that the oxidation is catalyzed by MMO (Ferenci, 1974; Hubley et al., 1974; Ferenci et al., 1975; Henry and Grbić-Galić, 1991b). Consequently, the CO will compete for the MMO active site; and it will scavenge reduced electron/proton donors (Henry and Grbić-Galić, 1991b, have shown that the CO oxidation rate doubles upon addition of formate as an electron source). As the result, a competitive inhibition of TCE transformation by CO occurs (Figure 11). Our experiments with *Methylomonas* sp. MM2 indicated that CO is a much more powerful competitive inhibitor of TCE transformation than is methane (Henry and Grbić-Galić, 1991b): the measured inhibition constant (K_i) for CO inhibition of TCE oxidation was 4.2 μM, and that for methane inhibition of TCE oxidation, 116 μM. The CO itself was not toxic to methanotrophs, but it interfered with methane oxidation by competing for the same resources. The influence of CO may be significant in treatment of chlorinated solvents by pure cultures of methanotrophs, and will depend on the affinities of the microorganisms for the chlorinated solvent and CO, respectively, on the amount of CO produced, and the availability of the reductant. However, this effect has not been demonstrated in mixed microbial communities (Henry and Grbić-Galić, 1991b).

Chemistry of the Medium--The composition of the nutrient medium which is used to grow methanotrophs can have a profound influence on subsequent TCE transformation. For example, the presence or absence of copper will significantly influence the formation of soluble MMO in methanotrophs which possess this type of enzyme, and consequently affect TCE transformation (Oldenhuis et al., 1989). In the presence of copper, the soluble MMO is not formed, and the subsequent TCE transformation becomes negligible.

Furthermore, the presence or absence of a metal chelator, such as EDTA, in the growth medium can result in a change in affinity of the methanotrophic enzymes (K_S) for TCE. It can also result in more than an order of magnitude difference in the TCE oxidation rate (k/K_S) under resting cell conditions (Henry and Grbić-Galić, 1990; see Table 9). EDTA can chelate a variety of metal ions, such as calcium, magnesium, copper, iron, and various trace metals (Bridson and Brecker, 1970; Stumm and Morgan, 1981). Some of these metals become more available through chelation, whereas others are rendered unavailable (O'Sullivan, 1969). The most important factors

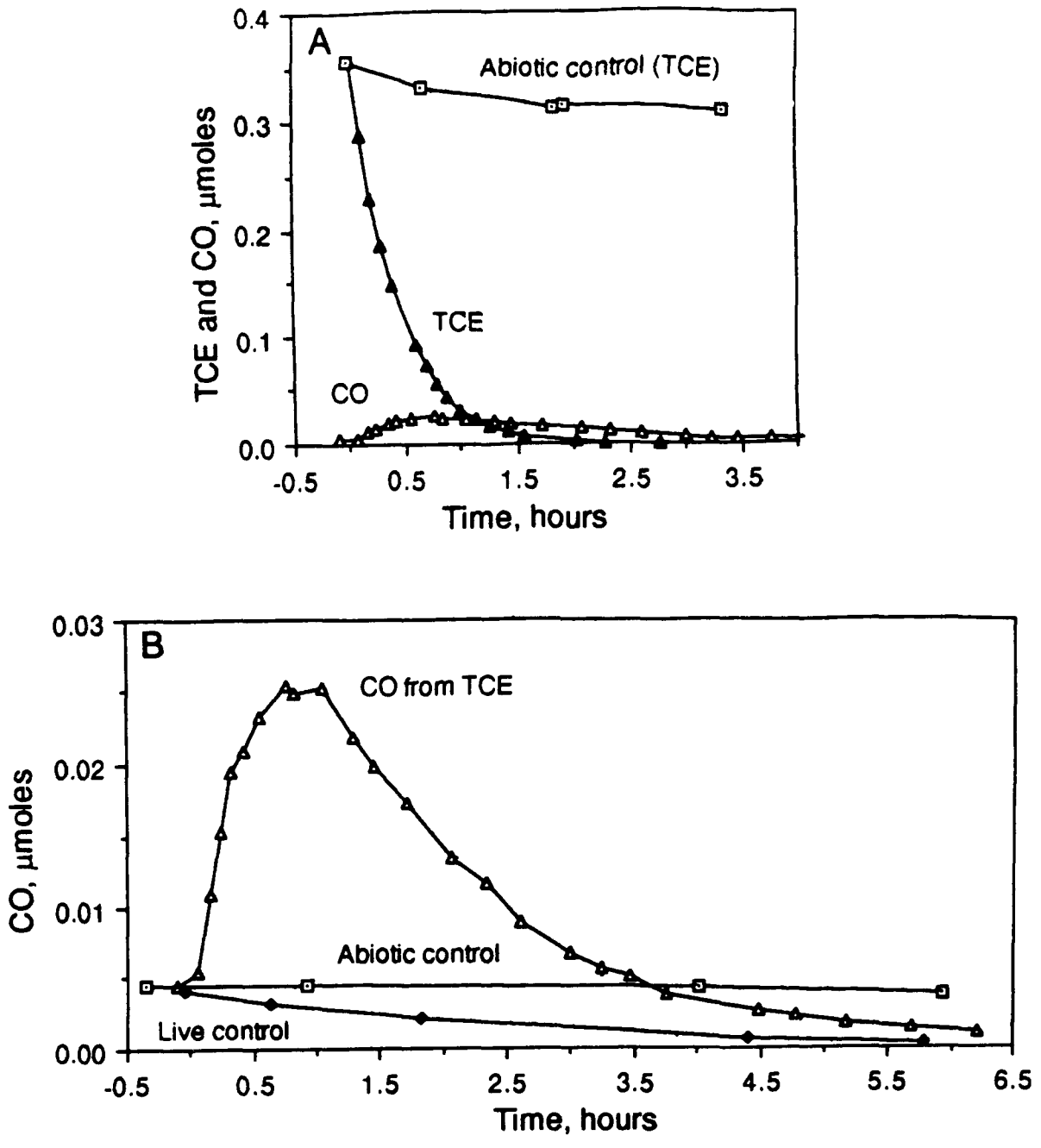


Figure 10. Production (from TCE) and subsequent oxidation of CO by *Methylomonas* sp. MM2 [(B) is an expanded view of the CO data from (A)]. Cell density: 0.11 g cells (dry weight) l^{-1}. Abiotic control: sterile mineral medium and TCE. Live control: a subculture of *Methylomonas* sp. MM2, without TCE (from Henry and Grbić-Galić, 1991b).

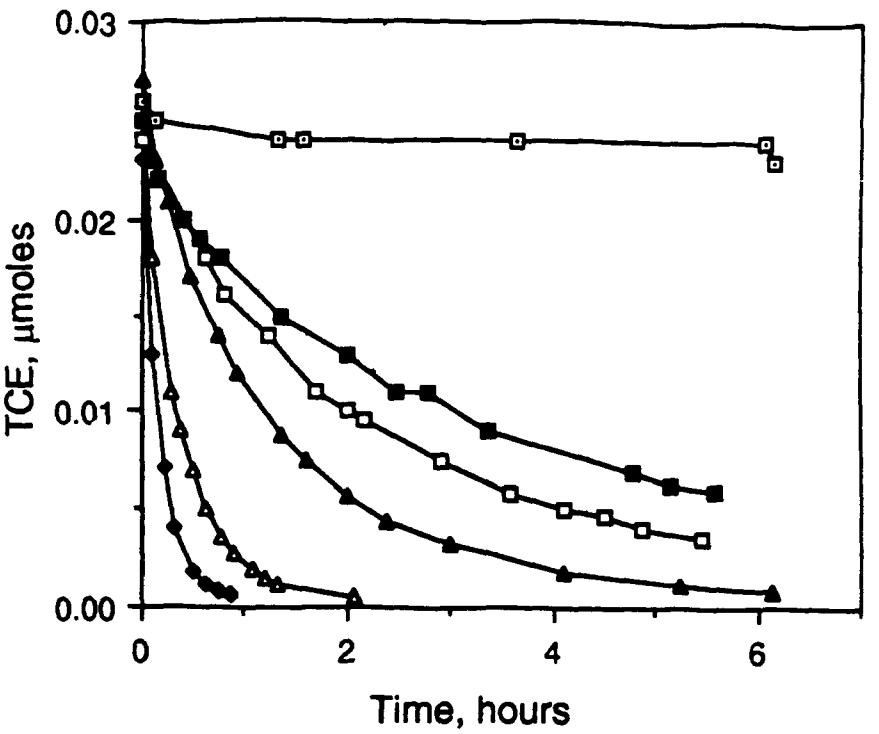


Figure 11. Competitive inhibition of TCE oxidation in *Methylomonas* sp. MM2 by CO. Cell densities: 0.18 to 0.21 g cells (dry weight) l^{-1}. The cultures were amended with 2 mM formate. Symbols: (◆) no CO; (Δ) 6.7 µM CO; (▲) 25.6 µM CO; (■) 53.5 µM CO; (□) 57.3 µM CO; (⊡) control (sterile mineral medium). From Henry and Grbić-Galić, 1991b.

in this complex process are the stability constants of the metal-EDTA complexes, and the concentrations of metals in the medium. EDTA potentially could affect the methanotrophic performance in various ways, and the specific mechanism has not been elucidated yet, but there are indications that copper, calcium, or magnesium availability may be crucial (Henry and Grbić-Galić, 1990). These results stress the significance of water chemistry in the TCE transformation process *in situ*: the composition of solutes in groundwater will substantially affect the transformation of the contaminant.

Particulate Versus Soluble MMO--Although it is commonly believed that only the soluble form of MMO (produced by *Methylosinus trichosporium* OB3b and *Methylococcus capsulatus* [bath]) is capable of catalyzing TCE transformation (Oldenhuis et al., 1989; Tsien et al., 1989), our recent work suggests that the particulate form of MMO in some methanotrophs may be at least as efficient (Henry and Grbić-Galić, 1990, 1991a). *Methylomonas* sp. MM2, isolated from Moffett Field groundwater aquifer, has never been shown to produce the soluble MMO (Henry and Grbić-Galić, 1991a), and it is relatively insensitive to variations in copper concentration. The pseudo-first-order rate constant (k/K_S) for TCE transformation in *Methylomonas* sp. MM2 (1.6 ml mg^{-1} min^{-1}) is comparable to that of *Methylosinus trichosporium* OB3b (2.14 ml mg^{-1} min^{-1}).

TABLE 9. KINETIC PARAMETERS FOR TCE TRANSFORMATION BY THE PURE CULTURE *METHYLOMONAS* SP. MM2 GROWN IN THE PRESENCE OR ABSENCE OF A METAL CHELATOR, EDTA
(from Henry and Grbić-Galić, 1990)

Mineral Medium	k (day^{-1}) [a]	K_S ($mg\ l^{-1}$) [b]	k/K_S ($l\ mg^{-1}\ day^{-1}$) [c]
Whittenbury[d]	0.29	0.51	0.57
Whittenbury, no EDTA	0.046	1.35	0.033

[a] k = maximum specific substrate utilization rate.
[b] K_S = half saturation coefficient.
[c] k/K_S = second order (pseudo-first-order) rate coefficient.
[d] Whittenbury = mineral medium formulation after Whittenbury et al. (1970).

The particulate form of the enzyme could be very significant in environmental applications, because the expression of this enzyme does not require the conditions of rigorous copper limitation. The environmental strains with the constitutively expressed particulate MMO which has a broad substrate specificity, such as *Methylomonas* sp. MM2 (Henry and Grbić-Galić, 1990), are ideally suited for *in-situ* treatment of contaminated groundwater aquifers.

TCE Oxidation Toxicity--TCE in high concentrations (e.g., 50 $mg\ l^{-1}$) has been shown to inhibit methane utilization and TCE transformation (Oldenhuis et al., 1989). The results of our research show that some of the products of TCE oxidation by methanotrophs are in fact toxic to these bacteria (Alvarez-Cohen and McCarty, 1991a; Henry and Grbić-Galić, 1991a), even when the TCE concentrations are considerably lower (below 10 $mg\ l^{-1}$; Henry and Grbić-Galić, 1991a). Similar findings have been reported for *Pseudomonas putida* F1, which transforms TCE upon stimulation with toluene (Wackett and Householder, 1989). Previous work with mammalian liver enzymes had shown that TCE epoxide and its products interacted with proteins and other macromolecules in mammalian cells, causing damage (Bolt and Filser, 1977; Bergman, 1983).

Our results indicate that the ^{14}C from ^{14}C-labeled TCE becomes incorporated into the cells of *Methylomonas* sp. MM2, although it is not likely that the products of hydrolysis of TCE-epoxide could be utilized by the pure methanotrophic culture (Henry and Grbić-Galić, 1991a). Furthermore, the methanotrophic cells which had transformed TCE have great difficulties in subsequent utilization of their growth substrate, methane (Figure 12), and the overall active cell numbers are reduced by more than an order of magnitude. The oxygenase enzymes in *Methylomonas* sp. MM2 and *Pseudomonas putida* F1 seem to activate TCE in a similar fashion as mammalian enzymes, and the effect seems to be comparable: the inactivation of the transforming cells.

This phenomenon should be a very important consideration for application of methanotrophic processes: it must be kept in mind that TCE oxygenation inactivates methanotrophs and that this effect will be more pronounced at higher TCE concentrations and lower cell densities.

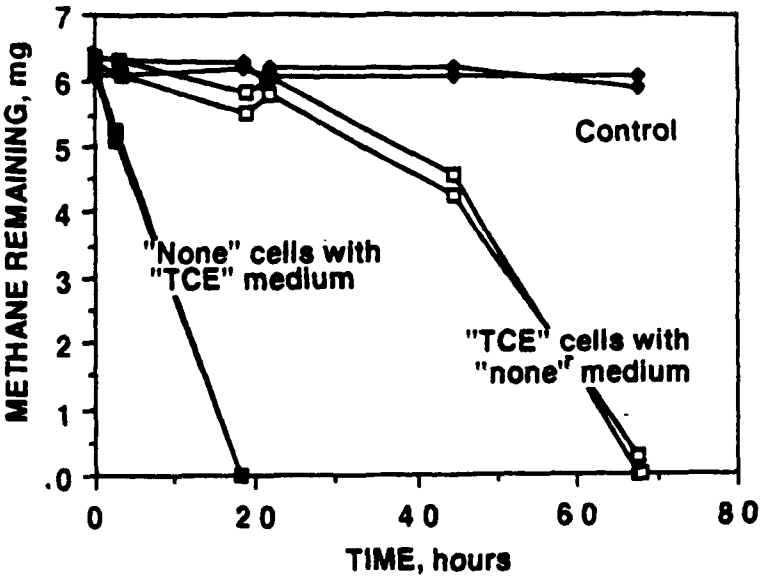


Figure 12. The effect of TCE oxidation on subsequent methane utilization by *Methylomonas* sp. MM2. Culture medium from the subcultures incubated with 6 mg of TCE per liter ("TCE" subcultures) was switched with that from the subcultures incubated without TCE ("None" subcultures). Only the "TCE" subcultures amended with "None" supernatant were affected, indicating that the TCE transformation itself was toxic to the methanotroph. The aqueous intermediates of TCE transformation, on the other hand, were not inhibitory for methane utilization (after Henry and Grbić-Galić, 1991a).

The Influence of Hydrogen Peroxide--Hydrogen peroxide is frequently used as a substitute for oxygen in groundwater remediation (Lee et al., 1988). However, our experiments with column microcosms consisting of saturated aquifer material from the Moffett Field site indicate that H_2O_2 may be inhibitory to methanotrophs (Mayer et al., 1988). Heterotrophs are less affected; however, since in this particular case the methanotrophs are the initiating microorganisms in the TCE degradation chain, the TCE-degrading activity is lost. The field experiments in the aquifer itself did not show an improvement in TCE transformation efficiency upon addition of H_2O_2 either (Semprini et al., 1990). This should be another important consideration for the application of methanotrophs in *in-situ* treatment of chlorinated solvents.

Important Considerations--Summary

Table 10 summarizes the most important factors to be considered in methanotrophic *in-situ* treatment of chlorinated solvent contamination.

TABLE 10. SUMMARY OF THE IMPORTANT FACTORS FOR *IN-SITU* TREATMENT OF CHLORINATED SOLVENTS BY METHANOTROPHIC COMMUNITIES

Methane concentration (competitive inhibition of chlorinated solvent transformation)

Transformation in the absence of methane (resting-cells) -- alternate pulses of CH_4 and oxygen

Oxygen concentration

Influence of **hydrogen peroxide** as an oxygen substitute

Chlorinated solvent concentration (inhibition of microorganisms)

External (formate) or internal (lipid storage granules) electron donors in resting-cells transformation

Particulate vs. soluble methane monooxygenase (particulate seems to be less susceptible to external influences, such as medium chemistry)

Robustness of microorganisms vs. high transformation rates (in a subsurface environment, robustness may be far more important)

Groundwater chemistry (influence on microbial growth and transformation capabilities)

Chlorinated solvent oxidation toxicity (reduction in number of viable microorganisms)

Product inhibition of chlorinated solvent transformation (percent conversion of the parent compound to a particular product, and the affinity of methanotrophs for the product oxidation)

SECTION 4

PROCESS MODELS

INTRODUCTION

Models used in simulating biotransformation processes in porous media are useful tools in designing *in-situ* remediation systems. The models may be used in evaluating different remediation alternatives, determining effective means of implementing remediations, and providing estimates on clean-up times, chemical demands, cost for pumping, etc. This section will present kinetic models for microbial processes including cometabolic transformations. The incorporation of these microbial process models into the advective-dispersive transport equation will also be introduced. Models are still being developed for cometabolic transformation processes, so it is premature to present details of different models that have been developed. A model that was used in evaluating the results of a field demonstration of *in-situ* biodegradation of chlorinated aliphatics (Section 5) will be discussed. In Section 6 the model will be used as a tool for evaluating the bioremediation potential of the methanotrophic process at a Superfund site in Michigan.

The enhanced *in-situ* biotransformation approach requires creating a biologically reactive zone in the subsurface. Conceptual models for *in-situ* treatment systems are given by Lee et al. (1988) and McCarty (1985). The conceptual model shown in Figure 13 presents several forms of biological treatment: 1) surface treatment, 2) a well bore reactor, and 3) *in-situ* treatment of the contaminated aquifer. The biological treatment of the halogenated aliphatics may be accomplished by one or a combination of these forms of treatment. There is as yet insufficient experience with biological treatment of chlorinated aliphatics to determine the appropriate conditions for *in-situ* treatment, and to judge categorically when *in-situ* or above ground treatment should be applied. Models that include the key microbial and transport processes can be useful in determining the important factors to consider in selecting a treatment process and whether there may be an advantage to using one treatment process or a combination of processes.

Bioremediation depends on a combination of complex processes including advective and dispersive transport, sorption, microbial growth, utilization of electron donors and acceptors, and for cometabolic transformations, the transformation of the contaminant itself. Modeling of these coupled processes is the only means for evaluating their interactions and for determining how bioremediation will be affected by the combination of the different processes.

The main processes that need to be considered are advection, dispersion, sorption and biodegradation (Figure 14). Transport processes include advection (idealized as plug flow in Figure 14) and dispersion which acts to spread the contaminants compared to idealized plug flow. Sorption partitions the contaminants between the aqueous and solid phases. Biodegradation acts as a sink term that removes contaminants, and is usually considered to occur in the aqueous phase. Figure 14 shows conceptually the concentration histories of contaminants at an observation location that result from their continuous injection into the subsurface. Concentration histories are shown for contaminants that do and do not sorb, and are and are not biodegraded. Sorption onto the aquifer

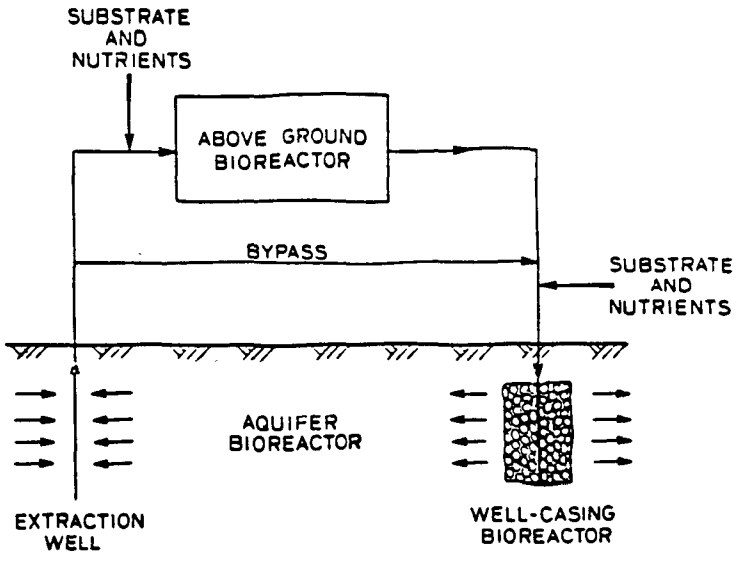
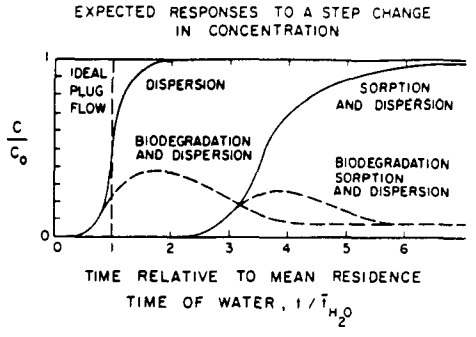


Figure 13. Conceptual diagram of above-ground well casing and aquifer (*in-situ*) bioreactor (from McCarty, 1985).



Ideal Plug Flow $\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x}$

Dispersion (Fick's Law) $\frac{\partial C}{\partial t} = D_h\frac{\partial^2 C}{\partial x^2}$

Sorption (Retardation) $R = \left(1 + \rho_b\frac{k_d}{\theta}\right)$

Biodegradation (Sink) λ

$$R\frac{\partial C}{\partial t} = \left(D_h\frac{\partial^2 C}{\partial x} - v\frac{\partial C}{\partial x}\right) - \lambda$$

biodegradation sink term

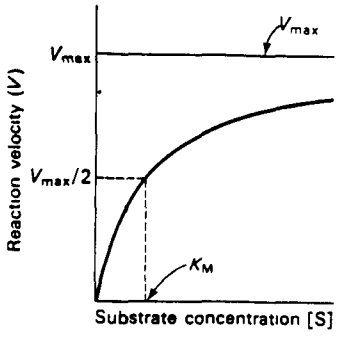
Figure 14. Conceptual model of transport and biodegradation processes that must be considered (from McCarty et al., 1981).

solids results in slower transport through the aquifer and longer transport times, both in the presence and absence of biotransformation. Biodegraded contaminants initially break through like the nondegraded contaminants. As time proceeds, the contaminant concentrations decrease as microbes grow that can degrade the contaminants. Methane as a growth substrate would be expected to behave like the nonsorbing, biodegraded contaminant. The chlorinated aliphatics would be expected to behave as contaminants that are sorbed and biodegraded.

As indicated in Figure 14, many processes must be incorporated in simulating the degradation of contaminants in the subsurface. To illustrate the processes, one-dimensional representations of the mathematical terms are shown. Here sorption is represented by a retardation model as described in Section 2. The biological sink term (λ) represents the rate of transformation of the contaminants. For cometabolic transformations, this will be shown to be a complicated term, dependent on many factors.

MICROBIAL PROCESSES

Monod kinetics and variations of Monod kinetics are most commonly used in the formulation of rate expressions for microbial processes, including microbial growth, utilization of the electron donor and acceptors, and for the cometabolic transformations of contaminants. Monod kinetics are derived from Michaelis-Menten enzyme kinetics (Figure 15). Here the reaction velocity (V) is plotted versus substrate concentration (S). At high substrate concentrations ($S >> K_m$), the rate of reaction (V) approaches a maximum rate (V_{max}) that is independent of substrate concentration; while at low substrate concentrations ($S << K_m$), the rate of reaction, $V = V_{max}S/K_m$, is first order with respect to substrate concentration. This first-order reaction rate dependence has implications on transformation rates at low concentrations, as the reaction rates become progressively slower with decreasing substrate concentration.



$$V = \frac{V_{max}\,S}{K_m + S}$$ Michaelis-Menten (1913)

V = rate = (mol/(liter-sec)

V_{max} = maximum utilization rate (mol/liter-sec)

K_m = Michaelis constant (mol/liter)

$S << k_s \quad V = \frac{V_{max}\,S}{K_m}$

$S >> k_s \quad V = V_{max}$

Figure 15. Michaelis-Menten enzyme kinetics (from Stryer, 1981).

The Monod equation is commonly used for the rate of primary substrate utilization:

$$\frac{dC_D}{dt} = -kX\left(\frac{C_D}{K_{SD} + C_D}\right) \tag{6}$$

where X = concentration of microbes (mg/l), k = maximum substrate utilization rate (day^{-1}), K_{SD} = substrate saturation constant (mg/l), and C_D = electron donor concentration (mg/l).

The rate of substrate utilization (electron donor) is proportional to the microbial concentration, assuming that the enzyme concentration is also proportional to the microbial concentration (X). The substrate concentration term is the same as the enzyme kinetic model. For utilization by methanotrophs, C_D is the methane concentration. When the utilization of the primary substrate is dependent on the presence of oxygen, as is the case for methanotrophic bacteria, a dual form of Monod kinetics is often used:

$$\frac{dC_D}{dt} = -kX\left(\frac{C_D}{K_{SD} + C_D}\right)\left(\frac{C_A}{K_{SA} + C_A}\right) \tag{7}$$

where C_A = oxygen concentration (mg/l) and K_{SA} = contaminant saturation constant for oxygen (mg/l). Thus, the rate of substrate utilization can be limited by both the methane or oxygen concentration.

Kinetic models for the cometabolic transformation of chlorinated aliphatics are currently under investigation. One model that appears to be appropriate for the transformation of chlorinated aliphatics by methanotrophs is a form of the Monod model that includes terms for competitive inhibition. Competition between the growth substrate and the non-growth substrates for the active site of MMO enzyme can affect the rate of transformation of the non growth substrate as discussed in Section 3. Hou et al. (1979) and Patel et al. (1982) found methane to inhibit the rate of transformation of other hydrocarbons. Recent studies have shown that methane can inhibit the transformation rates of chlorinated aliphatics (Strand et al. 1990, Lanzarone and McCarty, 1990, Oldenhuis et al., 1991, Semprini et al., 1991). Equation 8 is a dual Monod kinetic model that has been adapted for competitive inhibition (Semprini et al., 1991).

$$\frac{dC_2}{dt} = -Xk_2\left[\frac{C_2}{K_{S2} + C_2 + \frac{C_i}{K_i}}\right]\left[\frac{C_A}{K_{SA} + C_A}\right] \tag{8}$$

where X = concentration of contaminant degrading microbes (mg/l), k_2 = maximum contaminant transformation rate (day^{-1}), K_i = inhibition constant (–), K_{S2} = contaminant saturation constant (mg/l), C_2 = concentration of non-growth contaminant (mg/l), C_i = inhibitor concentration (mg/l), and C_A = oxygen concentration (mg/l).

For methanotrophs, methane inhibits the rate of contaminant biodegradation; thus C_i in Eq. 8 is the methane concentration C_D. According to this model, the rate of contaminant transformation decreases with increasing methane concentration. The effect is more pronounced the lower the value of K_i. A second Monod factor is included for the electron acceptor, since the presence of dissolved oxygen is required for contaminant transformation.

Equation 8 illustrates that many factors can influence the rate of cometabolic transformation by methanotrophs. The goal of enhanced *in-situ* bioremediation is to increase the biomass concentration, X, to enhance the rate of the cometabolic transformation. For methanotrophs this requires the addition of methane as a primary substrate for growth. As indicated in Eq. 8, the addition of high methane concentrations to increase the methanotrophic biomass in the aquifer could adversely affect transformation rates due to competitive inhibition by methane.

The rate of the cometabolic transformation by methanotrophs, both spatially and temporally, depends on the microbial mass, methane and oxygen concentrations (Eq. 8); consequently, in mathematical model computations all dependent variables must be calculated simultaneously. The microbial processes that must be considered include methanotrophic growth, the utilization of methane as a primary substrate (electron donor) and of oxygen as an electron acceptor, and the cometabolic transformation of the halogenated aliphatic.

The rate of microbial growth and decay of microbial populations can be represented by:

$$\frac{dX}{dt} = XkY\left(\frac{C_D}{K_{SD} + C_D}\right)\left(\frac{C_A}{K_{SA} + C_A}\right) - bX\left(\frac{C_A}{K_{SA} + C_A}\right) \tag{9}$$

where Y = yield coefficient (mg bacteria/mg substrate) and b = decay coefficient (day^{-1}). The dual Monod kinetic formulation shows that the growth of methanotrophic bacteria can be limited by the absence of methane or oxygen. In this equation, the rate of decay also depends on the oxygen concentration.

Equation 7 gives the dual Monod expression for electron donor utilization. The dual Monod expression is also used for the utilization of the electron acceptor:

$$\frac{dC_A}{dt} = -kFX\left(\frac{C_D}{K_{SD} + C_D}\right)\left(\frac{C_A}{K_{SA} + C_A}\right) - d_c f_d\, bX\left(\frac{C_A}{K_{SA} + C_A}\right) \tag{10}$$

where F = stoichiometric factor (mg acceptor/mg donor), d_c = cell decay oxygen demand (mg acceptor/mg cells), and f_d = degradable cell fraction. For methanotrophs, the stoichiometric factor represents the amount of oxygen consumed for the amount of methane utilized. The rate of oxygen consumption due to biomass decay is also included in Eq. 10.

Biodegradation models based on the variations of the Monod equation presented above have been developed for simulating the utilization of organics and oxygen in porous media. A review of how these models are incorporated into transport equations is given by Baveye and Valocchi (1989). Tables 11 and 12 give citations and brief descriptions of some of the models that have been developed. The simplified models given in Table 11 are for shallow biofilms, and the more complicated biofilm models are listed in Table 12. The model developed for simulation of the results of the Moffett Field study, which will be discussed later, used the simplified model of shallow biofilm kinetics.

COUPLING WITH TRANSPORT PROCESSES

The transport of the microbes, electron donors, electron acceptors, and halogenated contaminants is an important component in modeling subsurface biotransformation processes. In the microbial kinetic models, source and sink terms are added to the advective-dispersive transport equation. Details of this approach are given in the papers cited in Tables 11 and 12.

TABLE 11. SELECTED SHALLOW BIOFILM MODELS FOR MICROBIAL TRANSFORMATION IN POROUS MEDIA

Borden and Bedient (1986) -- Developed model for the instantaneous reaction of a primary substrate with oxygen.

Borden et al. (1986) -- Applied model of Borden and Bedient (1986) to aquifer contaminated with fuels.

Corapcioglu and Haridus (1985) -- Developed a model that considers biofouling as a result of biogrowth.

Kindred and Celia (1989) -- Developed model that includes aerobic and anaerobic reactions plus cometabolic transformations.

Molz et al. (1986) -- Developed non-steady-state model for growth of micro-colonies from the utilization of electron donor and acceptor.

Semprini and McCarty (1989) -- Results of a field study simulated using a model that considers co-metabolic transformations and rate-limited sorption-desorption.

Srinavasan and Mercer (1988) -- Developed model that considers different sorption processes along with primary substrate utilization.

TABLE 12. SELECTED BIOFILM MODELS FOR MICROBIAL TRANSFORMATIONS

Bouwer and McCarty (1984) -- Applied model of Rittmann and McCarty (1980b) to tracer organic biotransformation in the subsurface.

Bouwer and McCarty (1985) -- Used model of Rittmann and McCarty (1980b) for transformation in laboratory columns of trace halogenated organics as secondary substrates.

Bouwer and Wright (1988) -- Modeled the transformation of chlorinated aliphatics in laboratory column operated under different electron acceptor conditions.

Bryers (1988) -- Modeled biofilm accumulation of mixed cultures.

Kissel et al. (1987) -- Modeling mixed culture biofilms with different electron acceptors used in the biofilm.

Rittmann and McCarty (1980b) -- Analytical model developedfor steady-state biofilms and secondary-substrate utilization.

Rittmann et al. (1980) -- Applied model of Rittmann and McCarty (1980b) to results of a field experiment.

Rittmann et al. (1988) -- Modeled transformation of chlorinated aliphatics in a laboratory column operated under denitrifying conditions.

Speitel et al. (1987) -- Biofilm modeling of GAC columns including sorption and radial diffusion into the activated carbon.

Different assumptions are made in models of how to represent the microbial mass in these models as discussed by Baveye and Valocchi (1989). Most models assume that the microbial mass is attached to the porous media and is immobile. Most models also assume that biodegradation occurs only in the aqueous phase. The sorbed contaminants must desorb in order to be transformed, as indicated in Figure 16 (McCarty, 1988).

Partitioning of the contaminants between the aqueous and solid phases must therefore be considered. Sorption can cause a large fraction of the contaminant mass to be partitioned onto the aquifer solids. Sorption also lowers the aqueous concentration, thus reducing the rate of reaction (Eq. 9). Biodegradation in the aqueous phase reduces the aqueous concentration, creating a driving force for contaminant desorption from the aquifer solids. The desorption is a physical process that is being driven by a biological process.

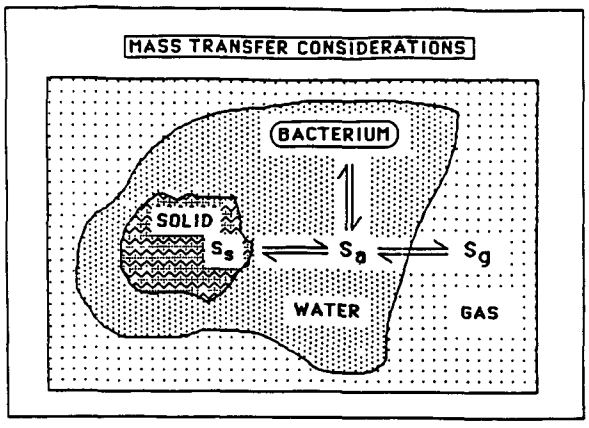


Figure 16. Mass transfer considerations (from McCarty, 1988).

The sorption and desorption of a contaminant can be modeled as an equilibrium or rate-limited process (Section 2). Laboratory studies, presented in Section 2, indicate that sorption-desorption of aquifer solids can be rate-limited. Thus, models should consider sorption-desorption as a rate-limited process, when appropriate.

A model must also be selected for the partitioning of the contaminants onto the aquifer solids. As discussed in Section 2, the simplest model, and probably the most adequate for halogenated aliphatics, is a linear and reversible sorption model (linear isotherm), with the equilibrium sorbed-phase concentration given by:

$$\bar{C} = K_d C \tag{11}$$

where K_d is the partition coefficient (l/kg) and $\bar{C}$ is the sorbed-phase concentration (mg/kg).

This simple sorption model is easily incorporated into a model that includes rate-limited transfer between the solid and aqueous phases. One of the simplest forms of a rate-limited sorption model that is easily incorporated into the advection-dispersion equation is the first-order linear nonequilibrium model, also known as the chemical kinetic model:

$$\frac{d\bar{C}}{dt} = \alpha\left(K_d\, C - \bar{C}\right) \tag{12}$$

where α is the rate coefficient for mass transfer between phases. The driving force for mass transfer is the concentration difference between the aqueous and sorbed phase, where the aqueous-phase concentration is expressed in terms of the equilibrium sorbed-phase concentration using the linear sorption model. Desorption occurs when the expressed equilibrium-sorbed concentration is less than the actual concentration on the solids, and sorption occurs when the opposite conditions hold true. This simple kinetic model represents a reasonable approximation of more complex sorption models that include diffusive transfer between mobile and immobile zones (van Genuchten, 1985). This kinetic model was used in simulating the Moffett Field chlorinated organic responses without biotransformation (Harmon et al., 1990) and with biotransformation (Roberts et al., 1989; Semprini and McCarty, 1992).

Equation 12 is incorporated into the advection-dispersion equation as a source sink term:

$$\frac{\partial C}{\partial t} = D_h \frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \frac{\rho_b}{\theta}\alpha\,(K_d C - \bar{C}) \tag{13}$$

Upon desorption ($K_d\, C < \bar{C}$), it acts as a source term to the aqueous phase, while for sorption ($K_d C > \bar{C}$), it acts as a sink term. Equation 12 must also be solved along with Eq. 13.

The microbial rate expression for electron donor utilization, electron acceptor utilization, and cometabolic contaminant transformations are added as source and sink terms to the advection-dispersion. For example, the equation that describes 1-D advective-dispersive transport, the cometabolic contaminant transformation, and rate-limited sorption results from the addition of Eq. 8 to Eq. 13 to give:

$$\frac{\partial C_2}{\partial t} = D_h \frac{\partial^2 C_2}{\partial x^2} - v\frac{\partial C_2}{\partial x} - \frac{\rho_b}{\theta}\alpha\,(K_d C_2 - \bar{C}_2) - Xk_2\left[\frac{C_2}{K_{S2} + C_2 + \frac{C_D}{K_i}}\right]\left[\frac{C_A}{K_{SA} + C_A}\right] \tag{14}$$

Equations 12 and 14 must be solved simultaneously, along with similar partial differential equations for the electron donor (C_D), electron acceptor (C_A), and the biomass concentration. These equations and a method for their solutions are given by Semprini and McCarty (1991). Other solution methods and different model formulations are given in the citations listed in Tables 11 and 12.

This section briefly reviews microbial process models required for modeling cometabolic transformations. More detailed kinetic models, especially for the cometabolic transformations, are currently being investigated in the laboratory. Thus, improvements in the models presented here are likely. Despite these limitations, it is important that models like those presented here be tested to determine how well they reproduce field observations and to establish the model limitations. Results of such a modeling exercise will be presented in Section 5, where the results of the Moffett Field pilot demonstration experiment are presented. In Section 6, results of a preliminary evaluation of *in-situ* bioremediation at a Superfund site, using the cometabolic transformation will be presented.

SECTION 5

RESULTS OF A PILOT-SCALE STUDY OF ENHANCED BIOTRANSFORMATION OF HALOGENATED ALKENES BY METHANOTROPHIC BACTERIA

MOFFETT FIELD STUDY

A pilot-scale field study was performed to assess under field conditions the capacity of native microorganisms, i.e., bacteria indigenous to the groundwater zone, to degrade halogenated organic contaminants when proper conditions were provided to enhance bacterial growth. Specifically, the growth of methanotrophic bacteria was stimulated in a field situation by providing ample supplies of dissolved methane and oxygen. Under biostimulation conditions, the transformation of representative halogenated organic contaminants, including trichloroethene (TCE), cis- and trans-1,2-dichloroethene (cis- and trans-DCE), and vinyl chloride (VC), was assessed by means of controlled addition, frequent sampling, quantitative analysis, and mass balance comparisons.

This section summarizes the results of the field study. Detailed descriptions of the results are presented by Roberts et al. (1990) and Semprini et al. (1990, 1991).

Field Demonstration Methodology

An effective methodology was developed to evaluate objectively and quantitatively the effectiveness of the biorestoration approach for stimulating the growth of the desired bacterial population and transforming the target organic compounds under natural conditions at a field site. The methodology entails creating a flow field dominated by pumping from an extraction well, while introducing solutes in known amounts at a nearby injection well and measuring concentrations regularly at the injection, extraction, and intermediate observation points (Figure 17). Interpretation of biotransformation behavior could then be made by qualitative examination of the concentration histories of the various solutes at the several monitoring points, comparing results under biostimulation conditions with results obtained under similar conditions in the absence of biostimulation measures. These interpretations were substantiated by quantitative mass balances.

A specially designed, automated data acquisition and control system (Figure 18), constructed for this purpose, proved capable of providing continuous records of high-accuracy data over sustained periods that enabled us to compute mass balances with relative errors of only a few percent. Details of the system design and operation are presented by Hopkins et al. (1988).

Site Characterization

The site chosen for the field demonstration, at Moffett Naval Air Station (Figure 19), offered a near-ideal combination of characteristics. The site was representative of a typical situation of groundwater contamination in the San Francisco Bay area and elsewhere, in which a shallow sand-and-gravel aquifer is contaminated by chlorinated aliphatic compounds widely used as solvents.

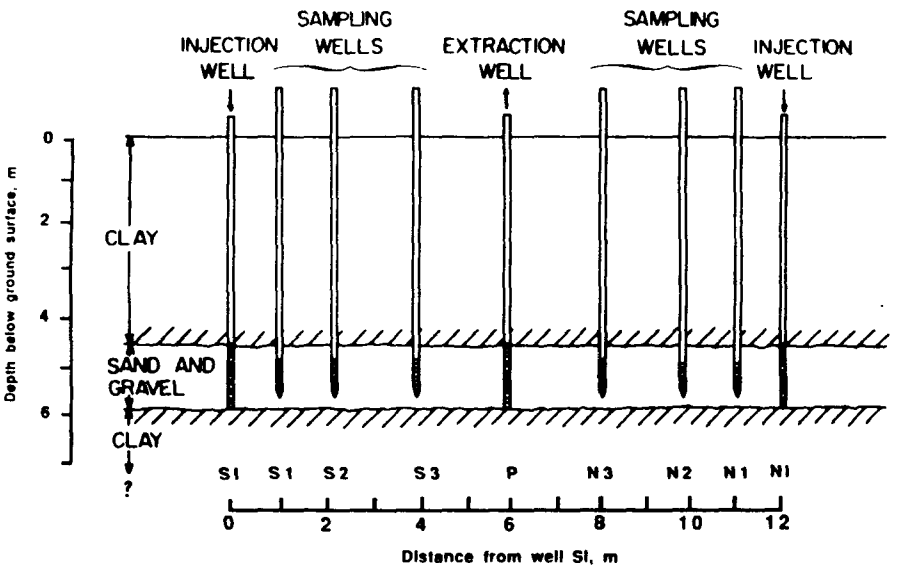


Figure 17. A vertical section of the test zone (from Roberts et al., 1990).

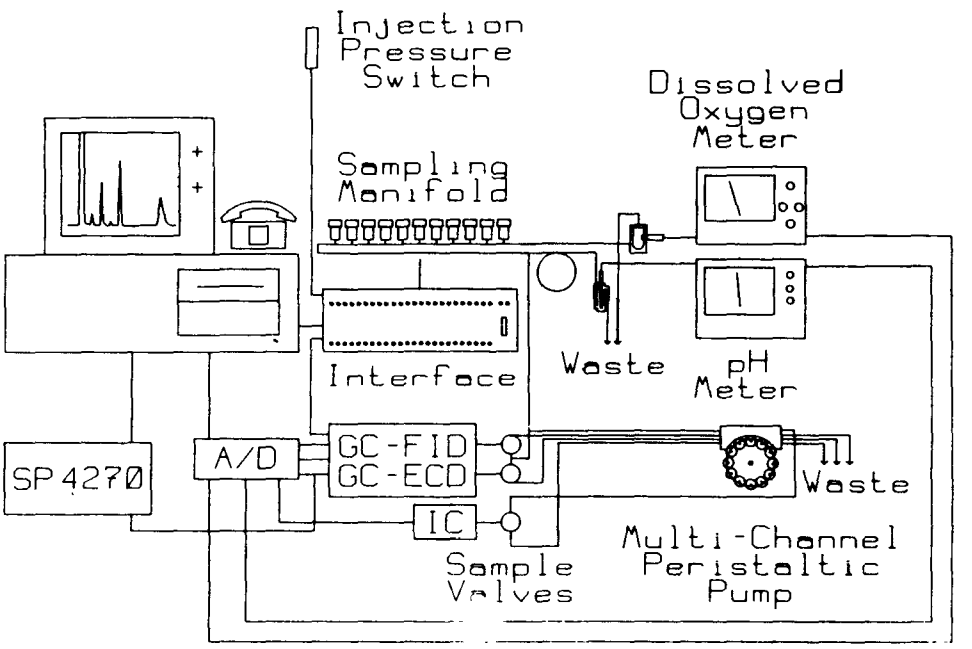


Figure 18. Schematic of the automated Data Acquisition and Control system (from Roberts et al., 1990).

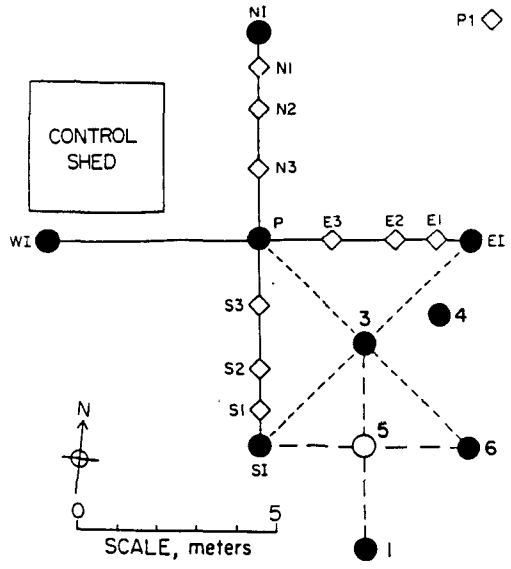


Figure 19. Map of the well field installed at the field site (from Roberts et al., 1990).

Drilling logs revealed that the aquifer at the test site consisted of a layer of silt, sand, and gravel, approximately 1.2 m thick, at shallow depth (approximately 5 m below the ground surface), well confined above and below by a silty clay layer of low permeability. The solids exhibited a wide size range, with approximately 70 wt% > 2 mm and 10 wt% < 0.1 mm. The organic carbon content of the aquifer solids was 0.11% and the specific surface area was 5 m^2/g. Details of methods used in characterizing the solids are given by Ball et al. (1990).

The formation groundwater was also of appropriate composition for the field experiments. The water was moderately saline (TDS of 1500 mg/l) and was substantially contaminated by chlorinated organic compounds, mainly 1,1,1-trichloroethane, but was devoid of the chlorinated alkenes--TCE, 1,2-DCE isomers, and VC--chosen as target compounds for this study. There were no appreciable amounts of toxic metals (Roberts et al., 1989). Nitrate was present in adequate amounts in the native groundwater (25 mg/l) as a source of nitrogen. Phosphorus concentrations were low (< 0.1 mg/l) but near solubility limits of common phosphorus minerals, which probably were the source of phosphorus.

Sustained pump tests showed that the transmissivity was sufficiently high (approximately 100 m^2/day) to permit extracting water at the design rate (approximately 10 l/min) without excessive drawdown at the extraction well. Detailed analysis of the pump tests showed the aquifer behaved as a leaky aquifer. Model comparisons found a water-table aquitard model best fit the pump test observations (Johns et al., 1992).

Preliminary tracer tests under natural gradient conditions showed that the local groundwater velocity was approximately 2 m/day. Preliminary mathematical modeling of the flow field with RESSQ, (Javandel et al., 1984), imposing a forced gradient on the natural flow field to simulate injection/extraction operations, showed that injection and extraction rates of approximately 1 l/min

and 10 l/min, respectively, would be sufficient to satisfy the two main requisites for the field experiment from the hydraulic point of view: 1) complete permeation by injected fluid of the aquifer in the observation zone between the injection and extraction points (i.e., minimum dilution by native groundwater in that zone); and 2) complete recovery of the injected fluid at the extraction well (to assure accurate mass balances).

Extensive bromide tracer tests were undertaken to quantify transport velocities and residence times in the test zone. Bromide tracer breakthroughs from one of the tests (TR8) are shown in Figure 20. Results summarized in Table 13 confirmed that the aquifer was virtually completely permeated by the injected fluid in the observation zone, as evidenced by complete steady-state breakthrough of bromide tracer at the observation wells, under the chosen experimental conditions. Further, the overall mass balances, comparing the amounts of tracer injected and extracted, demonstrated that the tracer recovery in the extracted water was essentially complete: after raising the injection and extraction rates in the second and third seasons of field work, the amount of bromide extracted agreed within six percent with the amount injected (Table 13). This assured the validity of the experimental approach, which relied on quantifying the extent of biotransformation of the organic solutes by comparing instantaneous concentrations at the injection and monitoring points and, over the long term, by mass balances of the quantities injected and extracted.

The hydraulic residence times (Table 13) between the injection well and the two nearest observation wells (S1 and S2), quantified by tracer tests under the forced gradient conditions, were found to be in the range of 8 to 23 hr, depending on the pumping rate. The residence time between the injection and extraction wells was 25 to 40 hr. These residence times were later found to be suitable for quantifying the transformation rates of interest in this work. The retardation factors for the organic solutes, evaluated from relative mobility data obtained in the field, were in the range of two to ten (Table 14). Details of the results of the tracer tests and modeling the tracer breakthroughs are given by Roberts et al. (1990) and Chrysikopoulos et al. (1990).

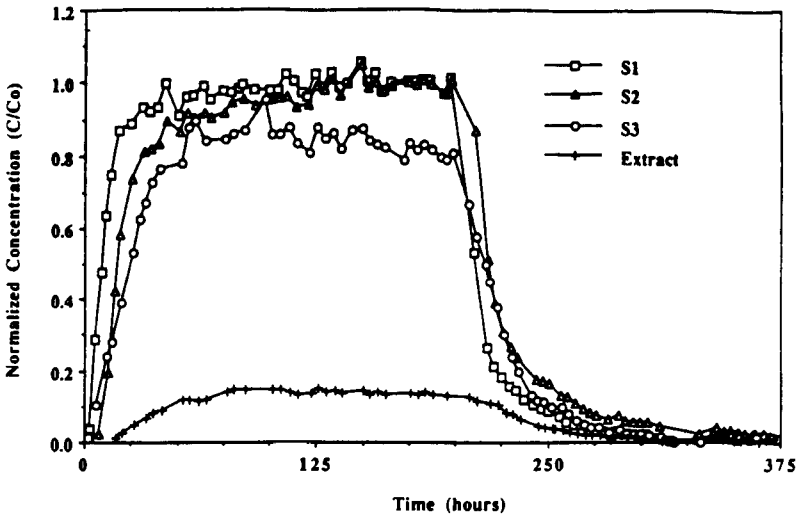


Figure 20. Bromide tracer breakthrough and elution in the Tracer8 experiment. (from Roberts et al., 1990).

TABLE 13. COMPARISON OF BROMIDE TRACER TESTS UNDER INDUCED GRADIENT CONDITIONS

	Test[a]	TR4	TR5	TR8	TR11	TR12
	Field Season	1	1	2	3	4
Injection Rate (l/min)		1.1	0.66	1.36	1.5	1.5
Extraction Rate (l/min)		8.0	8.0	10.0	10.0	10.0
Percent Steady-State Breakthrough	Well S1	95	94	100	102	100
	Well S2	95	72	98	100	99
	Well S3	80	57	84	96	95
	Extraction	9	5	13	14	15
Time to 50% Breakthrough (hr)	Well S1	8	9	7.5	9	8
	Well S2	20	17	16	23	21
	Well S3	20	7	20	27	26.5
	Extraction	26	20	30	40	42
Percentage Recovered at the Extraction Well		66	59	105	94	ND

[a]TR4 = Tracer4 experiment, etc.

TABLE 14. RESIDENCE TIMES AND RETARDATION FACTORS FOR THE CHLORINATED ORGANIC COMPOUNDS BASED ON THE TIME REQUIRED TO ACHIEVE 50% FRACTIONAL BREAKTHROUGH

Experiment	Compound	Well S1 t50% (hr)	Well S2 t50% (hr)	R (S1)	R (S2)
Tracer4	1,1,1-TCA	10	30	1.3	2.0
Tracer5	TCE	40	160	5	9
Tracer8	TCE	60	150	7	8
	trans-DCE	50	150	6	8
	cis-DCE	30	70	3	4
Tracer11	TCE	50	175	6	8
	trans-DCE	120	280	13	12
	cis-DCE	45	90	5	4
Tracer12	Vinyl chloride	13	42	1.6	2.0

Sorption

The sorption of the organic solutes by aquifer core samples from the Moffett site was studied in batch laboratory experiments. Details of these studies and the methods used are given by Roberts et al. (1989) and Harmon et al. (1990). The studies confirmed that sorption equilibrium was approximately linear, justifying the use of a distribution coefficient for interpreting and reporting the sorption equilibrium data. The retardation factors quantified from the field data (Table 14) were consistent with the results of laboratory studies of sorption. Sorption was strongest for TCE and weakest for VC, among the compounds studied. The retardation factors calculated from the laboratory sorption data agreed within a factor of two with those estimated from the transport experiments conducted in the field. The extent of sorption was approximately equal for all grain size fractions, but equilibrium was reached much more slowly in large grains than in small ones. This finding points out that deviations from sorption equilibrium owing to rate limitations may be an important factor influencing transport behavior. The slow rates of adsorption and desorption need to be taken into account by incorporating the rate limitation into transport models used for simulation and design (Section 4).

Results of Biostimulation and Biotransformation Experiments

The experimental methodology used in the biostimulation and biotransformation experiments is discussed in detail by Roberts et al. (1990) and Semprini et al. (1990, 1991). Biostimulation and biotransformation experiments were performed under induced-gradient conditions created by the injection and extraction of groundwater. The well field used for this purpose is shown in Figure 17. The experiments were performed as a series of stimulus-response tests. The stimulus was the continuous injection of measured concentrations of the chemicals of interest into the test zone, and the response was the concentration history of the chemicals in the groundwater sampled from the monitoring wells and the extraction well.

To enhance the effectiveness of biostimulation, methane (primary substrate) and oxygen (electron acceptor) were dissolved separately in groundwater and introduced as alternating timed pulses. This was done in order 1) to avoid clogging of the injection well and borehole interface, and 2) to achieve as uniform a distribution of the microbial growth as possible throughout a substantial portion of the aquifer. The system used for this purpose is shown in Figure 21. Groundwater was saturated with methane or oxygen using two counter-current gas sorption columns, one for oxygen and the other for methane. The columns achieved effluent concentrations ranging from 16 to 20 mg/l methane and 33 to 38 mg/l oxygen, approximately 80 percent of the saturation values at 20°C, and atmospheric pressure. The injection solenoids and a pulse timer permitted the alternated injection of groundwater containing either methane or oxygen, with varying pulse lengths.

The *in-situ* biostimulation of a native population of methane-oxidizing bacteria was achieved in three successive field seasons through the introduction of methane and oxygen dissolved in groundwater, without any other supplementary nutrients (N and P). Figure 22 shows the concentration history of methane and oxygen at the S2 observation well during the initial biostimulation experiment. At early time (0 to 100 hr) methane and oxygen behaved like the conservative bromide tracer, indicating no retardation and minimal consumption. During the period of 200 to 430 hr, methane and oxygen concentrations rapidly decreased, indicating the population of methane utilizers had grown to the point of utilizing substantial amounts of methane and oxygen. The ratio of oxygen consumption to methane consumption was 2.5 g/g, which is significantly lower than the ratio of 4 that would be required for complete methane oxidation. The lower ratio was also expected, however, since biological growth assuredly incorporated some of the methane's carbon into cell mass.

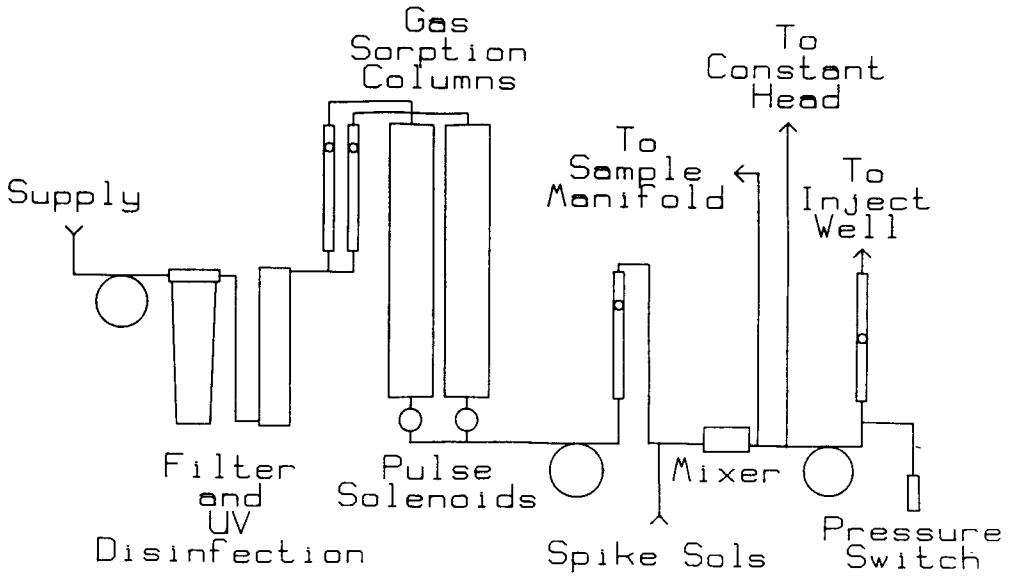


Figure 21. Schematic of the chemical injection system (from Roberts et al., 1990).

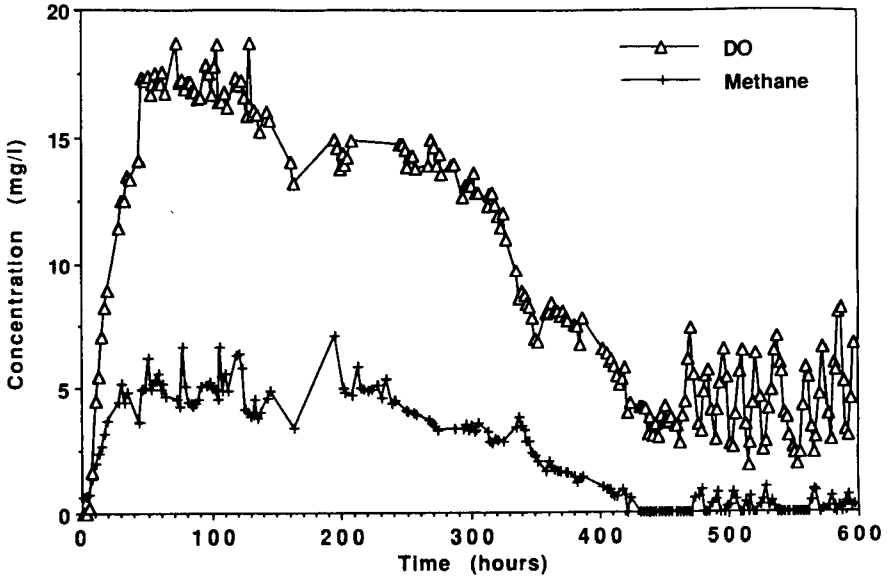


Figure 22. Methane and DO response at the S2 observation well due to the biostimulation of the test zone (from Semprini et al., 1990).

In order to control the clogging of the injection well and borehole interface, the alternate pulse injection of methane and oxygen containing groundwater was initiated at 430 hr, with a pulse cycle time of 4 and 8 hr, respectively. The arrival of methane and DO pulses at the S2 well was observed at a later time. Based on continued methane breakthrough at the observation wells, the pulsing is believed to have promoted a spatially distributed microbial population in the test zone. Biofouling of the near well-bore region was thus limited by the pulsing methodology, as anticipated in the experimental design; operation run times on the order of six months were feasible, before clogging forced shutdown and redevelopment of the injection well.

In subsequent field seasons, the uptake of methane and oxygen occurred very rapidly with essentially no lag. The results indicated that some of the methanotrophs stimulated in previous seasons were present and capable of utilizing methane and oxygen immediately, despite a six month hiatus since the end of the previous biostimulation. The results indicated that the methanotrophic population could survive for long periods without being fed methane, especially under conditions where no oxygen was present in the aquifer.

In order to evaluate biotransformation, the chlorinated compounds were added to the injected water (at concentrations in the range of 50 to 100 µg/l), in the absence of methane, until the soil was saturated, as evidenced by complete breakthrough at the monitoring wells. The feed was then supplemented with dissolved oxygen and methane. Transformation of the organic target compounds ensued immediately following the beginning of methane utilization, increasing with time as the bacterial population grew, and ultimately reaching a steady-state value that differed among the compounds. Figure 23 shows the response of the target compounds at the S2 observation well in the third season of field testing. The steady-state transformations observed during the third year's field work (Table 15), quantified by normalization to the bromide fractional breakthrough, were as follows: TCE, 10 to 19%; cis-DCE, 31 to 47%; trans-DCE, 85 to 90%; and VC, 85 to 95%. Of

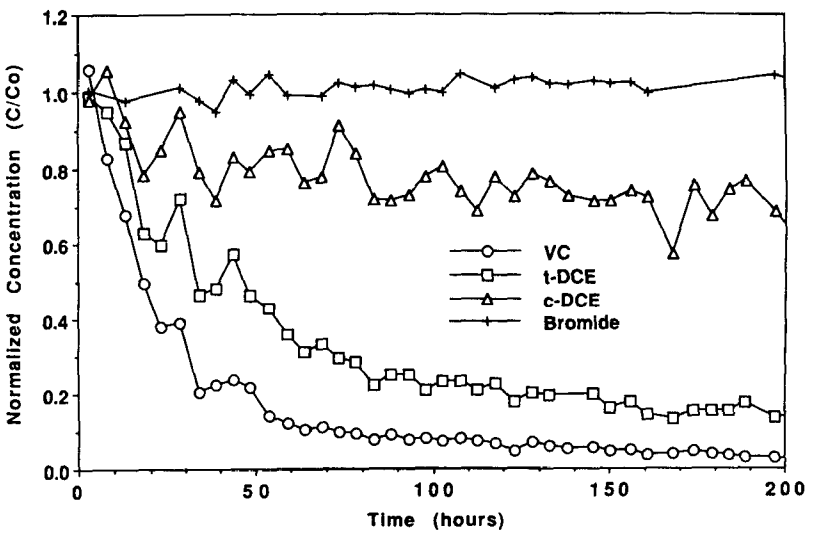


Figure 23. Decreases in normalized concentration of vinyl chloride, trans-DCE, and cis-DCE at the S2 well in response to biostimulation in the third season (from Semprini et al., 1990).

the values cited, the lower end of the range represents the nearest observation point (1 m distant, 8 hr fluid residence time), whereas the upper end of the range represents more distant observation points with longer residence times (2 to 4 m; 16 to 27 hr). The contaminants' residence times in the test zone are longer than those of the fluids due to sorption (Table 14). A saturated compound present as a background contaminant, 1,1,1-trichloroethane (TCA), was not degraded to any appreciable extent.

TABLE 15. EXTENT OF BIOTRANSFORMATION--THIRD FIELD SEASON

	Percent Transformed[a]			
Well	VC	t-DCE	c-DCE	TCE
S1	85	85	31	10
S2	96	90	41	17
S3	95	90	43	19
Ext	87	80	47	10

[a]Estimated by adjusting for bromide.fractional breakthrough.

The field results also indicated that the presence of methane inhibited the rate of transformation of the chlorinated aliphatics. Periodic changes in methane concentration, which was pulsed, result in the pulses in concentration VC and t-DCE, which were not pulsed. This effect was most pronounced at the closest observation well S1, where the largest variations in the methane concentration were observed (Figure 24). High concentrations of VC and t-DCE are associated with high methane concentrations, indicating slower rates of transformation in the presence of methane.

Transient tests were also performed to determine whether effective transformation rates would be achieved through the addition of alternative substrates, which unlike methane were not expected to inhibit transformation rates (Section 3). Figure 25 shows the concentration responses that resulted when formate and methanol were substituted for methane. Formate and methanol temporally increased the rates of transformation, while reducing oscillations caused by competitive inhibition. The ability of formate to keep the system stimulated, thus enhancing transformation rates, decreased within a few days. Methane was then added to restimulate the population's transformation of the chlorinated aliphatics. When the addition of electron acceptor was terminated, the concentrations of the chlorinated aliphatic compounds rapidly increased, indicating that the methanotrophic population required an input of reducing power (energy) for transformation to be maintained. The transient tests demonstrated that the reducing power may be supplied by methane or other utilizable energy substrates such as formate and methanol.

GC analysis of water samples during active biotransformation of trans-DCE provided evidence of an intermediate transformation product identified in laboratory studies to be the epoxide of trans-DCE (Reinhard et al., 1989), which was present in amounts equivalent to a few percent of the parent compound. The formation of epoxides is a well-known step in the oxidation of aliphatic compounds (Patel et al. 1982). The epoxides of TCE or VC were not observed, since these epoxides are much less stable in water and hydrolyze very rapidly, with half-lives on the order of seconds. The epoxide of trans-DCE hydrolyzes much more slowly in water with a half-life on the order of 50 to 70 hr (Janssen et al., 1987a and Reinhard et al., 1989) and hence was observed as a transformation intermediate. No other intermediate products were identified.

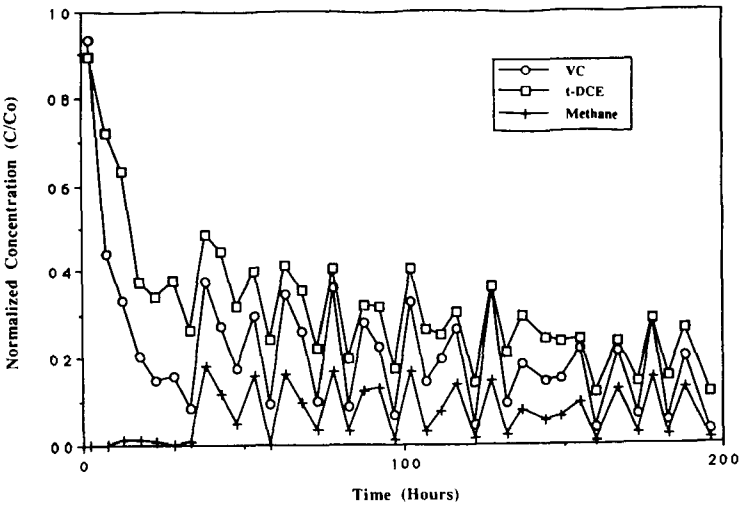


Figure 24. Decreases in normalized concentration of vinyl chloride, trans-DCE, and cis-DCE at the S1 well in response to biostimulation in the third season. Note the pulsing in concentrations of VC and trans-DCE that result from the pulsing in methane concentrations (from Semprini et al., 1991).

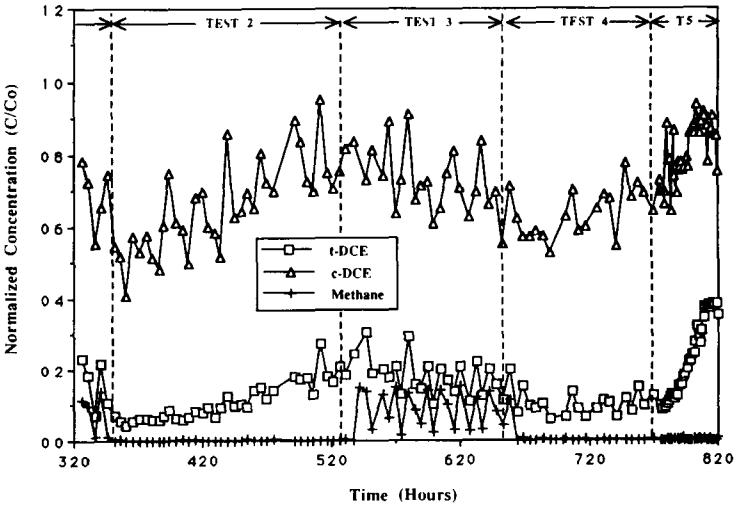


Figure 25. Response of trans-DCE and cis-DCE at the S1 well to the injection of (1) methane, (2) formate, (3) methane and formate, (4) methanol, and (5) no electron donor (from Semprini et al., 1991).

In one set of experiments, hydrogen peroxide was applied as a means of increasing the electron acceptor dose. The addition of hydrogen peroxide permitted operating at a higher rate of methane feed and increased biological growth but did not enhance the rate of transformation of the target organic compounds.

Summary

The field experiments at the Moffett site have shown that microbial transformation processes observed in the laboratory can be promoted and effectively tested *in situ*, under conditions typical of many contamination incidents. Stimulation of a specific population of indigenous bacteria that degrade selected compounds of interest can be accomplished when the proper conditions are promoted in the subsurface. In this pilot study, the population of methanotrophic bacteria was enhanced through the addition of methane as a primary substrate for growth.

The biostimulation and biodegradation experiments demonstrated that:

1) A specific class of microorganisms, the methanotrophs, which are indigenous to the subsurface environment, can be successfully biostimulated to promote the degradation of certain chlorinated aliphatic compounds.

2) 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 in a field test with fluid residence times of 1 to 2 days.

3) The rate of biotransformation was dependent on the structure of the chlorinated organic compounds, with less chlorinated compounds being transformed more rapidly.

4) An intermediate transformation product, trans-DCE oxide, was produced as a result of trans-DCE oxidation, which is consistent with the proposed transformation pathway.

5) Methane competitively inhibited the rates of transformation of the chlorinated aliphatics.

6) The substitution of formate and methanol for methane temporally enhanced the rates of transformation of the chlorinated aliphatics.

7) Active utilization of an energy source (methane, formate, or methanol) in the biostimulated zone was required for chlorinated aliphatic biotransformation to occur.

MODEL INTERPRETATION

The non-steady-state model for simulating the results of the field experiment proved an extremely useful tool in interpreting the results and comparing them with the laboratory data. A brief description of this model was given in Section 4. More detailed descriptions are given by Semprini and McCarty (1991, 1992). The model incorporated advection, dispersion, sorption with rate limitation, and the microbial processes of substrate utilization, growth, halogenated aliphatic transformation, and competitive inhibition. The transport was simplified by assuming one-dimensional, uniform flow, as a computational compromise to permit a more rigorous representation of the biological processes. Input parameters were estimated based on the results of the laboratory research of the biological processes, or within ranges of values from the literature, with some adjustments made to achieve fits to the field results.

Figure 26 illustrates a comparison between model simulations and the experimental data from the field-site's second monitoring well (2.2 m from the injection well) during the first year following the introduction of both methane and oxygen into the aquifer. The good match obtained indicates that the basic processes included in the model are good representations of those occurring in the field. The decreasing concentrations that resulted after about 220 hr (9 days) indicate the growth of methanotrophic bacteria in the aquifer. An important fitting parameter for the lag phase was the initial concentration of methanotrophic bacteria, as discussed by Semprini and McCarty (1991). After about 450 hr, pulsing of methane and oxygen at cycles of 4 and 8 hr respectively, was introduced in order to better distribute the biomass throughout the aquifer.

The simulated increase in methanotrophic biomass computed by the above simulation for a point 2.2 m from the injection well is presented in Figure 27. The biomass rapidly increases during the period when the methane and oxygen concentrations decrease. A decline in biomass concentration at approximately 450 hr, due to the lack of methane, is predicted. The decline in biomass concentration slows when the pulsing of methane and oxygen is initiated. The model simulations indicated that the alternate pulsing of methane and DO helped to distribute the biomass in the aquifer and prevented the bioclogging of injection well and well-bore interface. Additional details of simulations of biostimulation with pulsing of methane and oxygen are given by Semprini and McCarty (1991).

When the model was used to simulate the distribution of bacterial mass within the aquifer, the computed results appeared to correspond with the field response, although there was no direct way the model predictions could be verified. Model simulations of the methanotrophic population response to restimulation of the test zone in the second and third seasons of testing supported the hypothesis that the population, once developed, does not decrease in size rapidly, especially under anoxic conditions.

Figure 28 compares model simulations of the degradation of three organics with data from the third season of testing: a good match to the field observations was achieved. In order to improve the fit between the model and the field results, the use of both rate-limited sorption/desorption and competitive inhibition kinetics was required. Details of these models are discussed in Section 4. The rates used in the model simulation for methanotrophic growth, methane utilization, and halogenated hydrocarbon degradation were in good agreement with those derived under laboratory conditions that most closely mimicked the field tests (Section 3). One of the main fitting parameters used in the model calibration was the initial mass of methanotrophs present. Other biological parameters and rate parameters include stoichiometry between methane and oxygen, Monod rate parameters, decay coefficient, and values for competitive inhibition. Model parameters are given by Semprini and McCarty (1991, 1992).

Figure 29 shows the field results and the model simulations of the response of the chlorinated aliphatics at the nearer observation well (S1) to biostimulation in the third season of field testing. Model simulations of the effects of competitive inhibition by methane and rate-limited sorption-desorption also agreed well with the observed dynamic behavior in response to the pulsed injection of methane and oxygen. Oscillations in t-DCE, and VC concentration resulted from methane competition, with rate-limited sorption causing greater amplitudes in the oscillations.

The model simulations showed that the responses for the different chlorinated aliphatic compounds resulted from the compounds having different maximum transformation rates and different sorption rates and K_d, as summarized in Table 16. The transformation rate parameter values suggest that vinyl chloride and t-DCE were transformed about as rapidly as methane, whereas c-DCE and TCE were transformed one and two orders of magnitude less rapidly, respectively. Rates for TCE

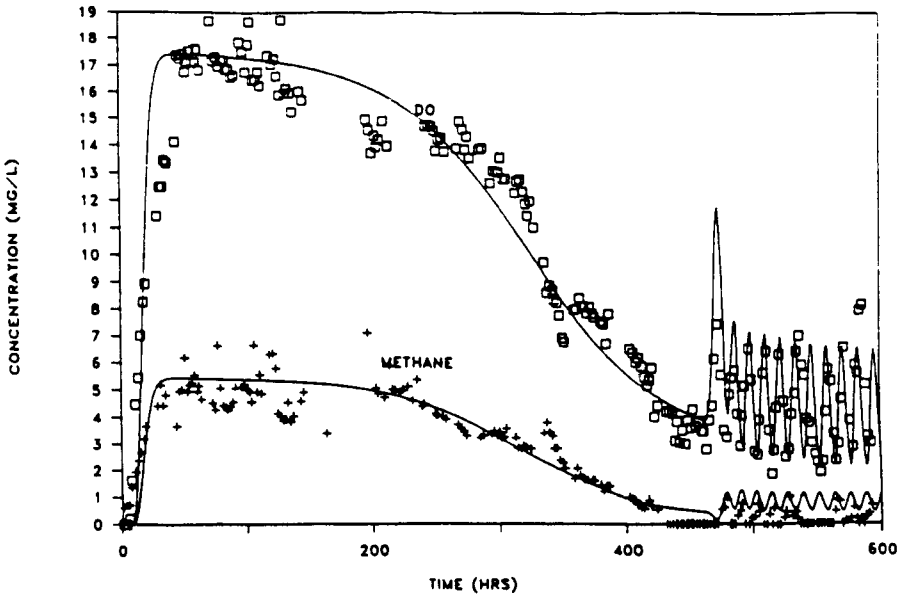


Figure 26. Model simulation and observed methane and DO response at the S2 observation well during the first season of field testing (adapted from Semprini and McCarty, 1991).

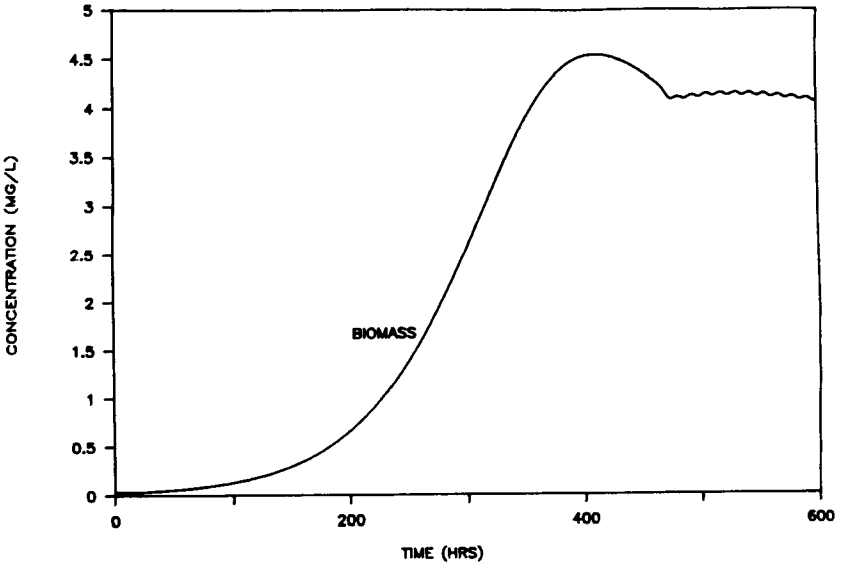


Figure 27. Predicted biomass concentration at a node 2.2 m from the injection well due to stimulation with short and long pulses (from Semprini and McCarty, 1991).

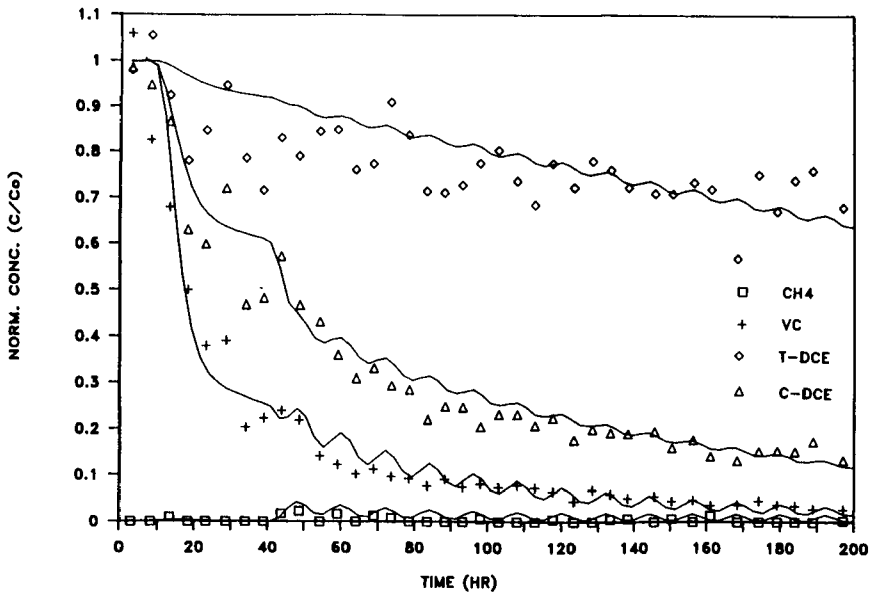


Figure 28. Simulations of the response of methane, VC, t-DCE, and c-DCE, at the S2 well to biostimulation of the test zone in the third season of field testing. Simulations used competitive inhibition kinetics and rate-limited sorption (from Semprini and McCarty, 1992).

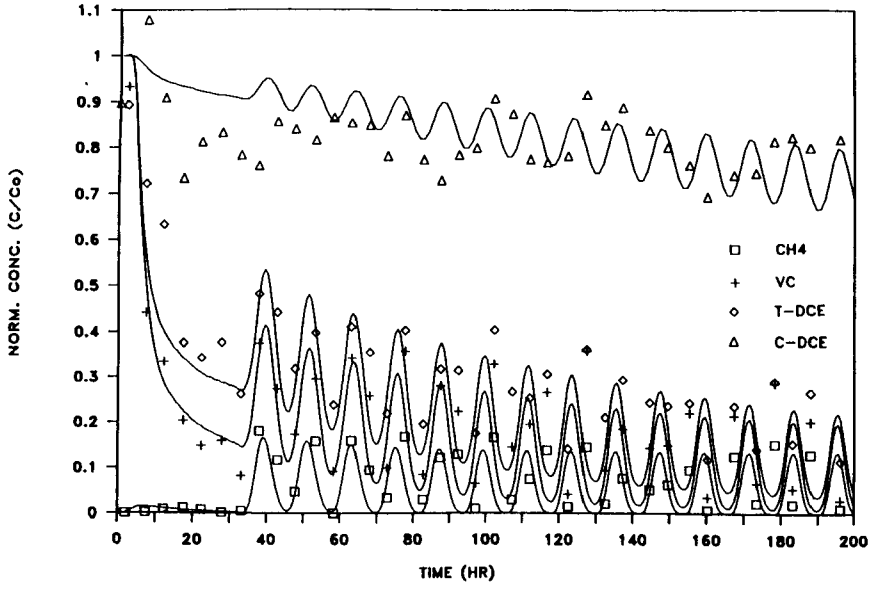


Figure 29. Model simulations and the response of methane, VC, t-DCE, and c-DCE at the S1 well using competitive inhibition kinetics and rate-limited sorption (from Semprini and McCarty, 1992).

TABLE 16. MODEL PARAMETERS FOR SIMULATION OF CHLORINATED ORGANICS IN BIOSTIM3 SHOWN IN FIGURE 29

Compound	K_d (l/mg)	α (d^{-1})	k (d^{-1})	K_S (mg/l)	k/K_S (l/mg-d)
Methane	0.0	0.0	2.0	1.0	2.0
VC	0.4	0.5	1.5	1.0	1.5
trans-DCE	1.6	0.3	1.5	1.0	1.5
cis-DCE	1.6	0.3	0.1	1.0	0.1
TCE	2.0	0.2	0.02	1.0	0.02

K_d = sorption distribution coefficient (l/mg).
α = rate coefficient for sorption (d^{-1}).
k = maximum transformation rate (d^{-1}).
K_S = half-saturation coefficient (mg/l).

transformation (k/K_S) are in the range of those determined in the laboratory under non-optimal growth conditions (Table 9).

The model simulations of the Moffett Field studies demonstrate the usefulness of models that incorporate fundamental microbial and transport processes. The processes of microbial growth, utilization of methane and oxygen, competitive inhibition of the cometabolic transformation, and rate-limited sorption, must be considered along with advection and dispersion. In these simulations, the model was greatly simplified by assuming 1-D transport, as well as a shallow biofilm; even with these simplifications, the model simulated most aspects of the field test quite well. However, 2-D and 3-D models may be necessary at other locations, where conditions are more complex, assuming this can be justified by the availability of sufficient data on aquifer characteristics. The matter of adequately characterizing highly heterogeneous sites for purposes of bioremediation assessment is so challenging as to be virtually intractable, given the limits of present knowledge. In any case, mathematical model representations of such highly complex situations is beyond the scope of this paper.

This model that was developed, validated, and calibrated in the Moffett Naval Air Station field study will be used in preliminary evaluations of bioremediation scenarios for a Superfund site in Michigan. These model simulations are presented in Section 6.

SECTION 6

FEASIBILITY STUDIES FOR A SITE

INTRODUCTION AND OBJECTIVES

The Moffett field evaluation demonstrated that indigenous microorganisms could be stimulated through injection of methane and oxygen to degrade TCE, DCE, and VC. As part of that evaluation, methodologies for determining the potential for methanotrophic *in-situ* biorestoration of sites contaminated with chlorinated solvents were developed. In order to illustrate the use of these methodologies , to further evaluate the potential of this process for bioremediation of contaminated groundwater, and to better consider the engineering factors involved in system design, a contaminated site was sought that would provide a full-scale test case for the process. The St. Joseph Superfund site appeared to be a good candidate for this evaluation.

At the St. Joseph site, the aquifer is relatively homogeneous and heavily contaminated with the same three contaminants for which degradation by a methanotrophic community has been demonstrated at the Moffett site. Trichloroethylene (TCE), 1,2-cis-dichloroethylene (c-DCE), 1,2-trans-dichloroethylene (t-DCE), and vinyl chloride (VC) are present at groundwater concentrations from 0.8 to 8.0 mg/l (Figure 30). It appears from the data available that the latter compounds were formed through anaerobic transformation of TCE as described under Section 3 on Biotransformation. These compounds were also found at the Moffett test site to be more readily degraded than TCE.

The specific objectives of this evaluation and the methodologies developed were to:

- Determine whether a native population of methanotrophic bacteria existed in the St. Joseph aquifer, and if so, to evaluate this population's ability to degrade the contaminants of concern.
- Determine the degree of sorption of the contaminants to the aquifer material.
- Evaluate likely designs for *in-situ* treatment through use of the non-steady-state model developed for this process.

To simulate results for *in-situ* bioremediation, the presence of an indigenous population and its size must be known. This is the basis for the first objective. Knowledge of the sorptive properties of contaminants at a given site is also important for any bioremediation scheme as this affects how the contaminants move relative to the water. The rates of biodegradation are also functions of the amount of compound sorbed, as well as that in solution.

The third task was to evaluate possible designs for the remediation system. The model developed and verified for simulation at the Moffett test site (Section 5) was believed suitable for initial evaluations at the St. Joseph site. In addition, a regional flow model was developed to evaluate the important parameters affecting flow of water into and through the St. Joseph aquifer system.

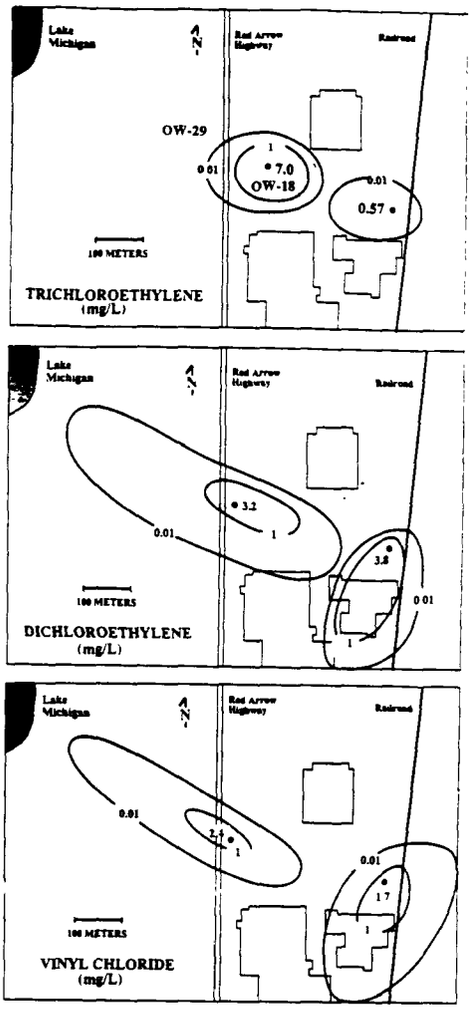


Figure 30. Distribution of TCE, DCE, and VC at the St. Joseph site (after Keck, 1988).

PROCEDURES

Collection of Aquifer Samples

The U.S. Environmental Protection Agency's (EPA) Robert S. Kerr Environmental Research Laboratory provided their drilling crew and drilling rig for collection of aquifer cores according to a protocol designed to avoid sample contamination (Wilson and Leach, 1989). Samples were taken near sampling wells OW-18 and OW-29 (Figure 31), where the maximum concentrations of DCE and VC were found in the western portion of the plume, migrating toward Lake Michigan. Here, the groundwater lies about 10 m below the surface, and the center of the contaminant plume is about 20 m below the surface.

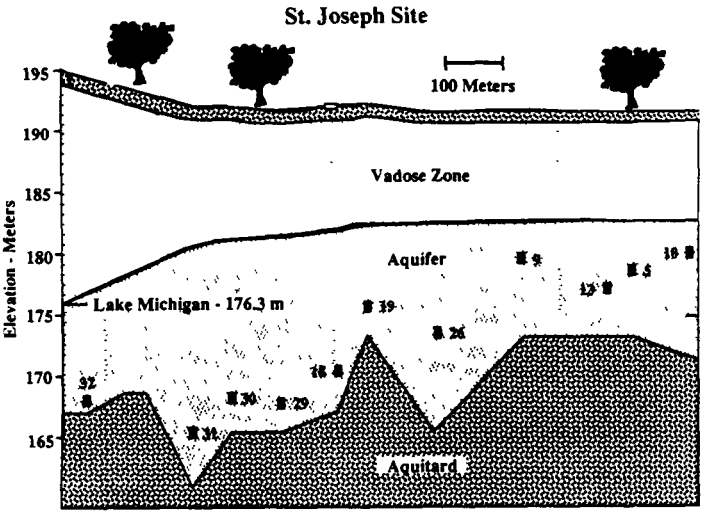


Figure 31. Vertical profile of subsurface at St. Joseph (from McCarty et al., 1991).

Aseptically obtained samples of aquifer material were collected using a 15-cm diameter hollow-stem auger equipped with a cover plate to exclude the entrance of aquifer material into the hollow stem during drilling. When the auger reached the desired level, a 10-cm pitcher barrel, equipped with a plunger to prevent loss of aquifer material, was driven first to open the cover plate and then into previously undisturbed aquifer material below the auger (Wilson and Leach, 1989). The pitcher barrel was then removed from the auger, plugged with a rubber stopper to prevent loss of material, and attached by the upper end in a horizontal position to a drive device. The stoppered end of the barrel was placed in a glove box, nitrogen from a cylinder was allowed to fill the glove box to exclude oxygen. The stopper was removed, the drive device was activated to force the plunger in the barrel to move forward a short distance, exposing about 10 cm of the core which was tapped free, leaving a surface of fresh aquifer material. A sterile circular cover plate with a hole in the center was then attached to the barrel. The auger plate had a sharp circular edge around the hole that permitted only the central material in the core to pass, while excluding the exterior core material that had come into contact with the barrel wall. The central portion of the core, which was free from extraneous contamination due to sampling, was forced out by activating the drive device and was collected in sterile 1-liter Mason® jars for transport to the Stanford laboratory, where they were stored in the dark at 4°C until used. Because of difficulties with the fine sandy aquifer material, aseptically collected core samples were obtained only from the upper 4 m at OW-29 and the upper 2 m of the aquifer at OW-18 in initial sampling. Later, the cover plate on the auger was modified to prevent the intrusion of fine sand during drilling; and samples from near the center of the plume were obtained. Material was also obtained between 10 and 12 m below the water surface near OW-29 for physical characterization.

Column Microcosm Studies

The objectives of column microcosm studies were 1) to determine whether indigenous methanotrophic bacteria are present at the St. Joseph site, 2) to quantify the time required to increase the population of such bacteria to an adequate level for bioremediation, and 3) to assess the capability of such a stimulated population to degrade TCE, t-DCE, and VC.

The 2-cm diameter glass column microcosms (Figure 32) had a volume of 133 ml, contained about 200 g of aquifer material, had a void volume of 50 ml, and were constructed and operated as described previously (Roberts et al., 1989, Lanzarone and McCarty, 1990). The microcosms were autoclaved and aquifer material was aseptically added, as was filter-sterilized groundwater from an uncontaminated location at the St. Joseph site. The columns were operated by exchange of column fluid after periods ranging from a few to 30 days. Here, replacement of column fluid was effected by upward flow of about 140 ml of amended and filter-sterilized groundwater over about a 30-min period (Figure 33).

Five columns (B, C, D, E, F) contained aquifer material from near well OW-18 and two (G, K) from near well OW-29. Column flow characteristics were determined by exchange of water containing a bromide tracer, the concentration of which was monitored by ion chromatography (Figure 34). Then, some columns were exchanged with water containing only methane and oxygen to determine whether methane-consuming methanotrophs were present. Once stimulation of methanotrophs was in evidence, VC was added to the exchange water and the possible degradation of VC was determined. Later, TCE, t-DCE, as well as VC were added to evaluate degradation potential.

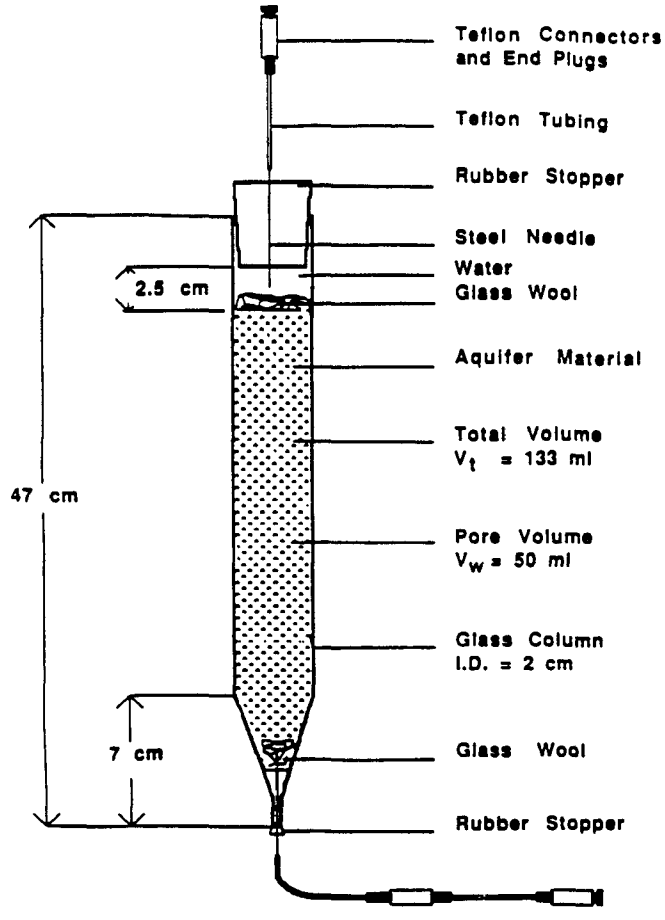


Figure 32. Schematic diagram of column microcosms (from Lanzarone and McCarty, 1990).

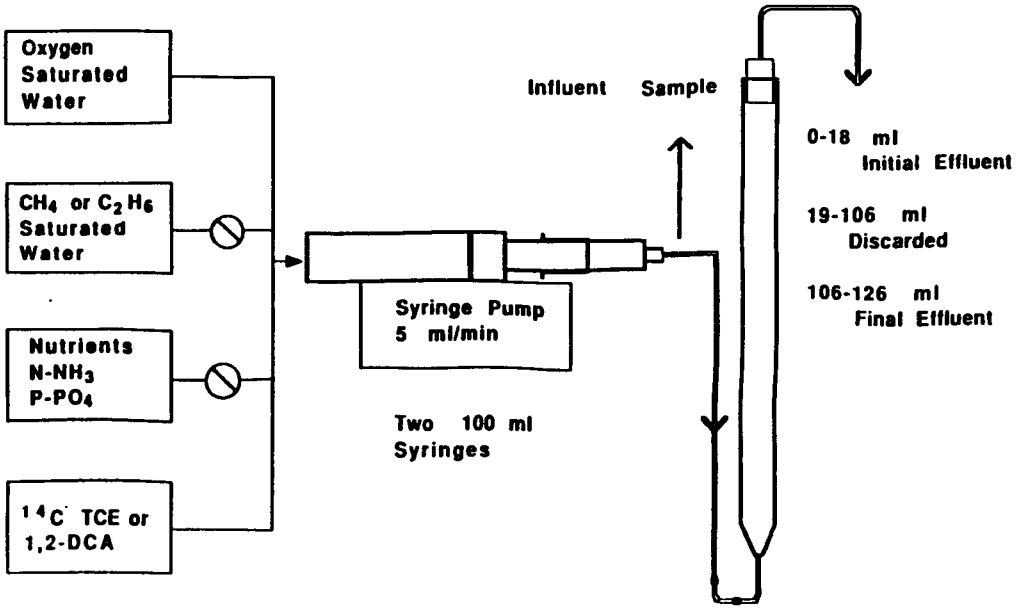


Figure 33. Schematic figure of procedure used in exchanging column fluids (from Lanzarone and McCarty, 1990).

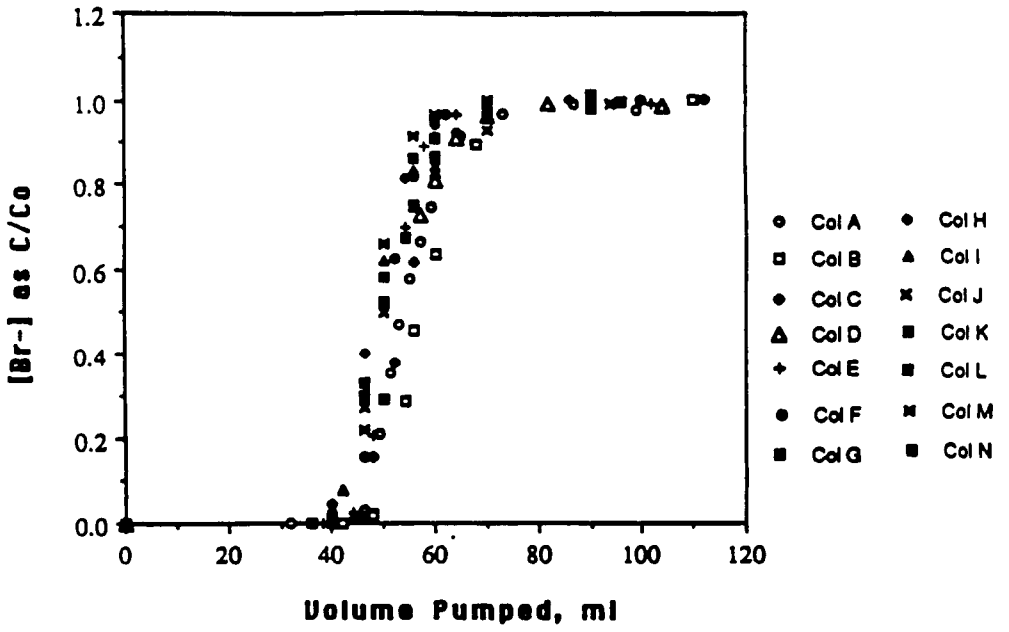


Figure 34. Breakthrough curves of bromide tracer for the different columns.

COLUMN MICROCOSM RESULTS

Bromide Tracer

No breakthrough of the nonreactive and nonsorptive bromide tracer was observed in preliminary column microcosm studies until the passage of about 30 ml of fluid. All effluents reached the influent concentration after about 90 ml passed through (Figure 34). The estimated average column pore volume is about 50 ml. The sharp bromide breakthroughs for all columns are consistent with the uniform and fine grain size of the aquifer material. These results indicated that the first 30 ml of column effluent was representative of concentrations present within the column and the absence of the influence of short-circuiting by the feed solution. Thus, the first 30 ml from each column during each exchange were taken for analysis to represent column fluid characteristics before the exchange. The results also indicated that a total exchange of 90 ml of fluid during each period was required to insure that the influent materials were distributed throughout the columns.

Methane and Oxygen Consumption

Methane (about 3.5 mg/l) and oxygen (about 25 mg/l) were added initially to four columns (C, E, G, K), which were then exchanged similarly at various times to determine whether methane consumption had begun. Methane consumption in Column C was complete within 31 days, and in Column E within 41 days (Figure 35). The columns contained aquifer material taken near well OW-18, but at somewhat different depths. No data reveal methane utilization before day 28, but methane was consumed in Column C by day 31. Figure 35 illustrates the averages of concentrations in two samples taken at each time period, one for the first 15 ml and the other for the second 15 ml.

The time intervals for the onset of methane consumption in Columns G and K, with aquifer material from near well OW-29, were similar, although initially methane consumption was not uniform throughout the columns. Methane concentration in the initial effluent was little changed from the influent values; but in the sample from the second 15 ml, it was nearly completely removed in both columns. This suggests that the methanotrophic population may not have been evenly distributed throughout the columns. Since the activity was higher in both columns at the

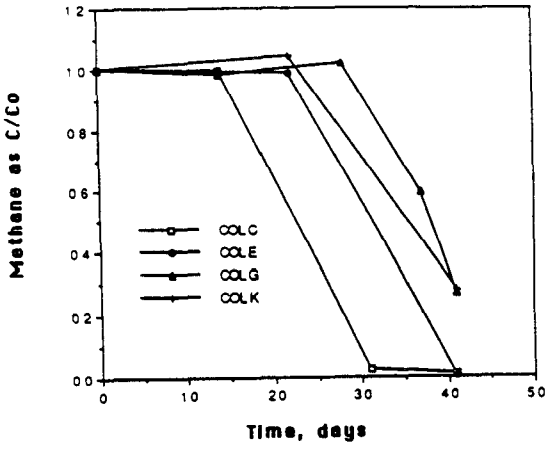


Figure 35. Methane removal in column microcosms (from McCarty et al., 1991).

influent end, it is also possible that the columns might have become contaminated with methanotrophs during an exchange. Assuming this did not occur, the population levels in all the columns appeared similar. The methanotrophic populations in St. Joseph aquifer materials were much lower than in Moffett material, which exhibited noticeable methane consumption within two weeks. Since the doubling time for methanotrophs is about one day, this difference indicates that the St. Joseph methanotrophic population is four to five orders of magnitude smaller than the Moffett population. The previously mentioned observation that the distribution of methanotrophic bacteria appears patchy would be consistent with this finding that the indigenous population of methanotrophs is extremely sparse.

Vinyl Chloride Degradation

After methane consumption occurred, approximately 0.175 mg/l vinyl chloride (VC) together with methane and oxygen were added to Columns C and E through fluid exchange. The control Column B was then exchanged with fluid containing dissolved oxygen and VC, but no methane. Periodically, the pore fluid was exchanged with similarly amended fluid, and the initial effluents were analyzed for dissolved oxygen, methane, and VC. The VC results are presented in Figure 36. Time zero represents the time of initial VC addition. At the first exchange one week after VC addition, 20 percent of the VC in the control column was removed, probably from sorption to the aquifer material. In the methane-stimulated columns, however, 75 percent VC removal was obtained. The second exchange, carried out one week later, showed no VC removal in the control column, indicating that VC had reached sorption equilibrium. Columns C and E both showed increased VC removal to about 80 percent, possibly as a result of the increasing size of the methanotrophic population. Complete methane consumption was obtained between each exchange in all cases.

The influent VC concentration for the third exchange (t = 28 days) was increased to 0.78 mg/l to determine whether higher VC concentrations similar to those found at the St. Joseph site would be inhibitory. The results again showed about 80 percent VC removal in Columns C and E over a period of two weeks and only small sorptive removal in the control column. The columns were exchanged once a week for two additional weeks while maintaining a higher influent concentration. The exchange results (t = 35 and 42 days) showed a consistent 75 to 80 percent VC removal from Columns C and E with little or no removal in the control column. These data indicate that the higher VC concentrations were not toxic, and that the degree of removal with higher concentrations was similar to that with lower concentrations.

Following the above exchanges (i.e., t > 42 days), the influent VC concentration was reduced to 0.28 mg/l and left in the columns for two weeks. After this period, the control column showed VC concentrations 20 percent greater than the influent concentration resulting from desorption of VC from the aquifer solids equilibrated previously at the higher VC levels. Both Columns C and E showed 95 percent VC removal during this period. When analyzed 30 days later, VC was removed to below the detection limit in Columns C and E. However in control Column B, it was two times greater than the influent concentration because of the desorption of VC from the aquifer solids, which had been equilibrated previously at higher VC levels. This confirms that methane consumption was required for VC degradation to occur.

Trans-1,2-Dichloroethylene and Trichloroethylene

On day 54, 0.16 mg/l t-DCE and 0.07 mg/l TCE were added together with 0.1 mg/l VC to the feed for Columns B, C, and E. The results are presented in Figure 36, with time zero for the lower two graphs representing day 54 on the upper graph when t-DCE and TCE were added. A comparison

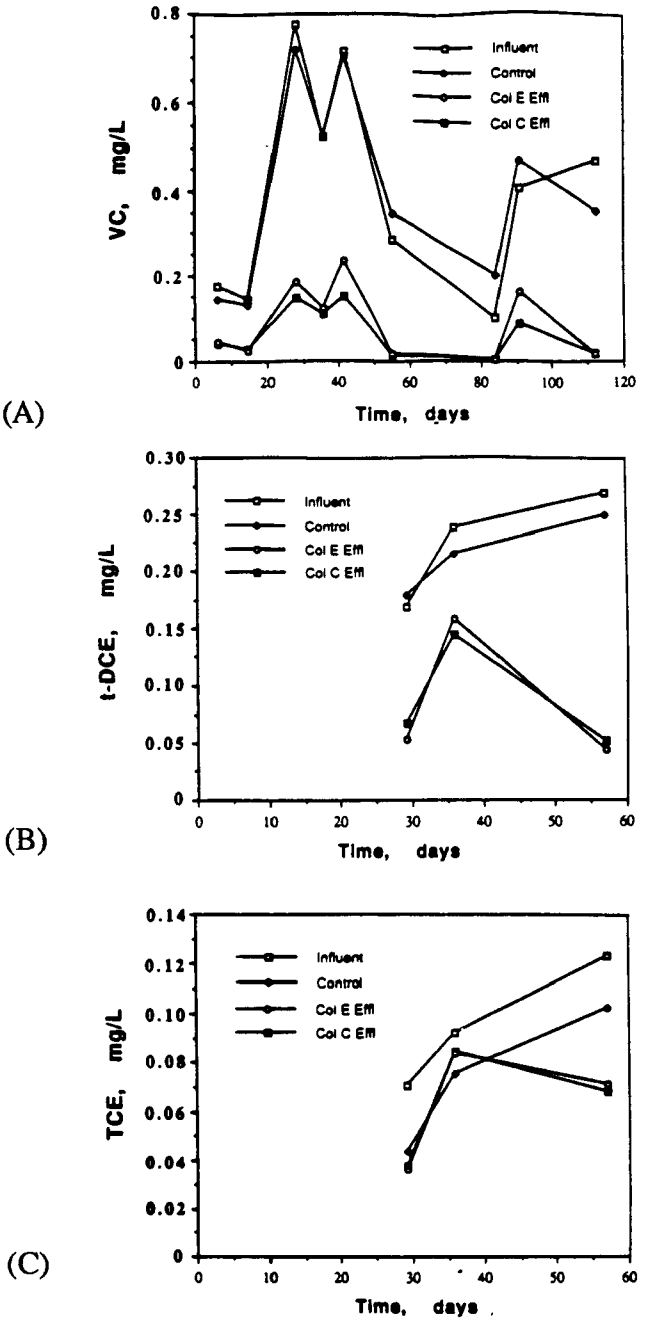


Figure 36. VC, t-DCE, and TCE removal in column microcosms. The origin on the time scale in (B) and (C) corresponds to t = 54 days in (A) (from McCarty et al., 1991).

between the t-DCE concentration in the effluent from the control column and Columns C and E suggests a t-DCE removal of 60 percent by the methanotrophs. TCE levels were the same in effluents from Columns C, E, and the control.

Influent concentrations of t-DCE and TCE were then increased to 0.25 and 0.1 mg/l, respectively, and left in the columns for one week. Exchange results showed removals of 60 to 75 percent for VC, 35 to 40 percent for t-DCE, and 0 percent for TCE in the active columns. The columns were fed again and left for 21 additional days, after which the active columns showed removals of 95 percent for VC, 80 percent for t-DCE, and 30 percent for TCE. The extent of VC transformation before and after the addition of t-DCE and TCE to the columns appears to be approximately the same.

Long-Term Vinyl Chloride Results

One study was conducted to simulate field conditions in which methane, oxygen, and VC would be mixed together initially and allowed to incubate in the aquifer. Here, VC was added to two previously unexchanged columns and left to incubate at room temperature. Dissolved oxygen (25 mg/l) and methane (4.3 mg/l) were added with VC (0.95 mg/l) to Column D, but only oxygen and VC were added to control Column F. After ten weeks of incubation, the columns were exchanged and the initial effluents analyzed. The control column showed a 17 percent decrease in VC, probably from sorption, whereas the methanated column showed 90 percent removal of VC. All the methane and about 15 mg/l of the dissolved oxygen were consumed in the methanated column. The theoretical oxygen demand from the methane and VC is in the range of 13 to 18 mg/l (depending on the oxygen-to-methane ratio assumed), which brackets the actual oxygen usage. In the control column, about 6 mg/l dissolved oxygen was used over the ten-week period. This test illustrates the potential effectiveness of a single aquifer exchange for removing a major portion of the VC contamination in place. VC removals in the range of 95 percent were obtained when three weeks rather than one week was allowed between exchanges. The fact that additional removal can be obtained after the depletion of methane is consistent with other column studies conducted at Stanford (Roberts et al., 1989).

REMEDIATION SCENARIOS

Model simulations will be presented to show how biotransformation models can be useful tools in preliminary designs of *in-situ* and on-site bioremediation processes. As a case study, model simulations were performed to investigate biorestoration schemes for the St. Joseph site using the numerical code that had been developed, validated, and calibrated in the Moffett Naval Air Station field study (Sections 4 and 5). The model includes the transport processes of advection, dispersion, and sorption combined with microbial processes for the growth of methanotrophic bacteria, utilization of methane as the growth substrate and oxygen as the electron acceptor, and microbial kinetics for the cometabolic transformation of chlorinated aliphatic compounds.

The cometabolic transformation of chlorinated aliphatic compounds by methanotrophs is a fairly complex process with many factors contributing to the effectiveness of this form of treatment. Such cometabolic transformation is governed by competitive inhibition kinetics, where high methane concentrations can reduce the transformation rates of the cometabolized substrates. The biotransformations are also limited in the absence of oxygen, thus the model reaction kinetics also include an expression for oxygen concentration. The ability of methanotrophs to degrade chlorinated aliphatic compounds is closely associated with their utilization of methane. During periods when methane is not being consumed, the ability to transform chlorinated compounds is rapidly lost. The model also incorporates this activity-loss process through a first-order deactivation rate expression.

The simulations used input parameters obtained from the calibration with the Moffett Field test results (Roberts et al., 1989, Semprini and McCarty, 1991, Semprini and McCarty, 1992). Additional parameters obtained from the St. Joseph study were also entered into the simulation

model. These parameters include the extent of sorption onto the aquifer solids and the estimate of initial concentration of methanotrophs in the treatment zone. The latter was estimated through the model by selecting a population that would best provide a simulation of the column microcosm results. Simulations were then performed using rate coefficients derived from the Moffett Field study to determine whether the Moffett-derived parameters were consistent with results from the laboratory column studies with St. Joseph aquifer material. This served to help verify the applicability of the Moffett results to the St. Joseph site.

The model described above has been shown to be capable of reproducing all aspects of the Moffett Field experiments (Section 5). The model, which was calibrated using the results from the Moffett study, thus provided a strong framework for conducting simulations of *in-situ* treatment for the St. Joseph site. Incorporation of the specific measured sorption parameters (K_d, α) for the chlorinated aliphatic compounds of interest on St. Joseph aquifer solids, the initial methanotrophic population appropriate for the St. Joseph site, and transformation rate coefficients consistent with the laboratory studies, provides the necessary information for the model suitable for the St. Joseph site evaluations.

This preliminary modeling study did not attempt to devise optimal designs for the *in-situ* remediation system. Remediation criteria and adequate information on site hydrogeology that are needed for developing an optimal design were not available. Rather, the process design developed here is one of perhaps several feasible configurations that might be used for comparison with alternative clean-up strategies, such as simple pump-and-treat remediation.

The remediation system for which model simulations of bioremediation were performed (Figure 37) is conceived as a pump-and-treat system. However, all of the treatment is carried out biologically in the aquifer itself. Here, groundwater is extracted through a series of wells spanning the contaminant plume in a direction perpendicular to that of groundwater flow. At the surface, methane and oxygen are added to the extracted groundwater, either together or in alternating pulses, depending upon the extent to which a stimulated methanotrophic population has been developed. The alternating pulses are used to distribute the microbial growth throughout the test zone. The groundwater containing the methane, oxygen, and extracted contaminants is reinjected into the treatment zone through a series of downgradient monitoring wells, distributed parallel to the extraction wells. In the subsurface biotreatment zone, both the in-place contaminants and the reinjected contaminants are biologically degraded.

The above *in-situ* biotreatment system was directly compared with a pump-and-treat system as illustrated in Figure 38. In the latter case, an unspecified form of surface treatment is assumed to remove contaminants quantitatively before the water is reinjected. Otherwise, the two systems are operated identically, and thus can be compared directly through model simulations.

For the two alternate simulations, a complete cross-section segment through the contaminant plume was chosen for treatment. The cross section assumed was 200 m wide, which is expected to span the width of the plume. The length and depth of the assumed remediation segment are 120 m and 20 m, respectively. In this segment the contaminants VC, c-DCE, and t-DCE were assumed each to be initially present at a uniform aqueous concentration of 1 mg/l, which is within the general range of observed concentrations.

The simulations were performed using rate parameters from the Moffett study (18°C) and the laboratory study with St. Joseph material (22°C), that were adjusted for the lower temperature of 10°C for the St. Joseph aquifer. Based on the laboratory sorption study and the comparison with

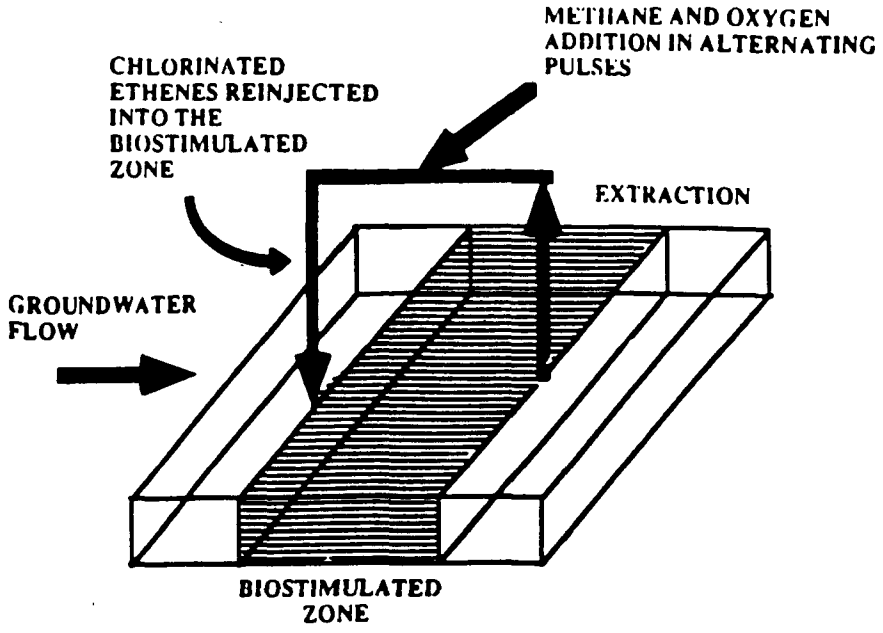


Figure 37. *In-situ* bioremediation case simulated (from McCarty et al., 1991).

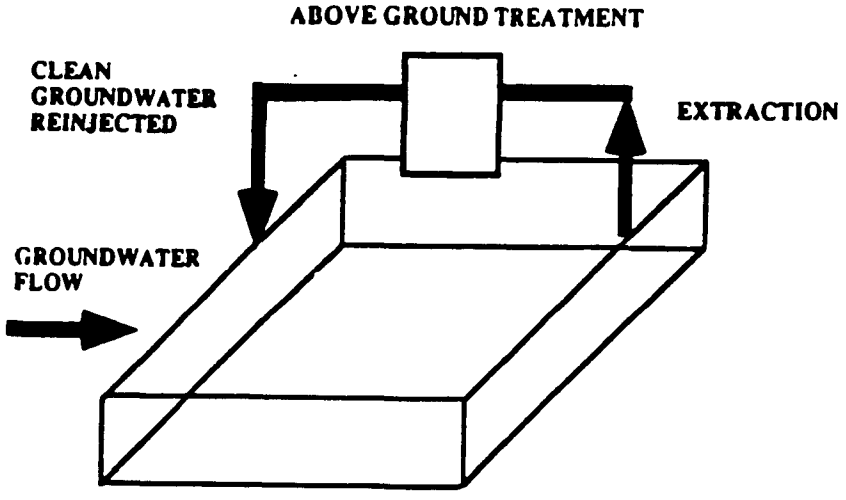


Figure 38. Pump-and-treat system for comparison with bioremediation (from McCarty et al., 1991).

Moffett Field results, a retardation factor for VC of 1.7 was assumed. Based upon results summarized in Table 15, c- and t-DCE were assumed to be more strongly sorbed, with retardation factors for both of 4.

The injection and extraction flow rates used in the simulation model provide a fluid residence time in the treatment zone of 50 days. This residence time was estimated based on the laboratory column estimates of initial methanotrophic population and a time of 30 to 50 days for significant growth to occur. At the colder St. Joseph groundwater temperature, this time would probably be twice as great. Thus, the chosen residence time of 50 days should be sufficient to permit the mixed methane and oxygen in the injection water to completely permeate the treatment zone before observable methane and oxygen uptake occurs. After the breakthrough of methane and oxygen occurs at the extraction well(s), the induced flow (pumping) is stopped to wait for the onset of methanotrophic growth and the initial transformation of the chlorinated compounds to occur. After that, injection would again commence, but with alternating pulses of water containing methane and oxygen.

Figure 39 shows the results of the model simulation for VC treatment. Shown are the concentrations of VC and methane as a function of time at a node adjacent to the extraction well. The treatment is operated initially as a batch process as described above. Groundwater is extracted and methane and oxygen are added to the reinjected fluid until breakthrough of methane and oxygen is observed at the extraction well after about 40 to 50 days. To minimize both the amount of water extracted and injected and the quantities of methane and oxygen added, all pumping is then stopped. The growth of methanotrophic bacteria is enhanced through consumption of the injected methane and oxygen, and at the same time, the cometabolic degradation of the VC begins. After 100 days (50 days of no pumping), all of the methane should be consumed; and the model indicates that 80% of the VC will be biodegraded. The simulations show that despite the presence of methanotrophic bacteria, once methane is completely utilized, the transformation of VC rapidly ceases. The laboratory column-study results, however, suggest that VC transformation may continue at a lesser rate, so that as much as 90% or more may actually be decomposed. Thus the model simulations may somewhat underestimate the actual transformations. Nevertheless, in line with the model simulations, once methane is depleted in the aquifer, more methane and oxygen are then added to the treatment zone to accomplish additional degradation. Here, methane breakthrough occurs once again after 120 to 140 hr of injection, and the subsequent biodegradation brings VC to the drinking-water standard.

The results of this simulated, semi-continuous batch treatment of the test zone agree well with the results of the batch column studies presented earlier. In the column studies the initial batch addition of 3.5 mg/l of methane resulted in approximately a 75% reduction in VC concentration. A slightly greater reduction was calculated in the field treatment simulation. However, in the column studies with longer exchange periods, VC decomposition of 90% occurred. Thus, the column results bracket the simulations. The combined results indicate that the rate coefficients derived from the Moffett Field study, when used in the model simulations, agree well with the results of the laboratory experiments with St. Joseph materials. This indicates that the model simulations were performed with a reasonable set of input parameters for the St. Joseph site.

The above bioremediation simulation system is compared with a simulation of a conventional pump-and-treat approach in Figure 40. Here, the systems were operated exactly the same with respect to hydraulic conditions, that is, with the same amount of pumping and over exactly the same time periods. The biostimulation treatment is shown to be slightly better than the pump-and-treat method, based on the pumping time required to reduce VC concentration in the treatment zone. Since VC is weakly sorbed (R = 1.7), a pump-and-treat system should also work fairly well. The effect of possible aquifer heterogeneities are not included in the simulations, however.

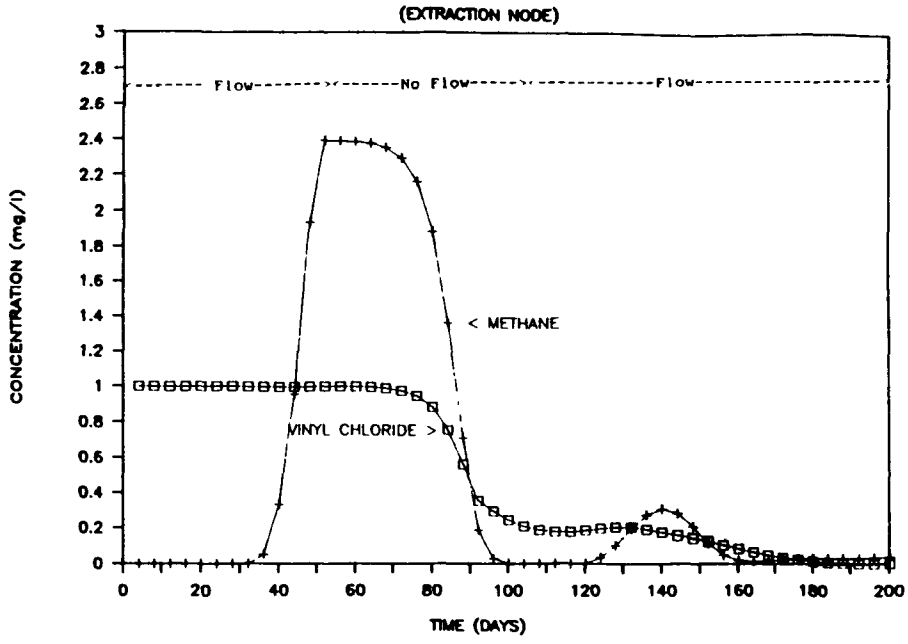


Figure 39. Simulation response to biostimulation with methane for VC remediation.

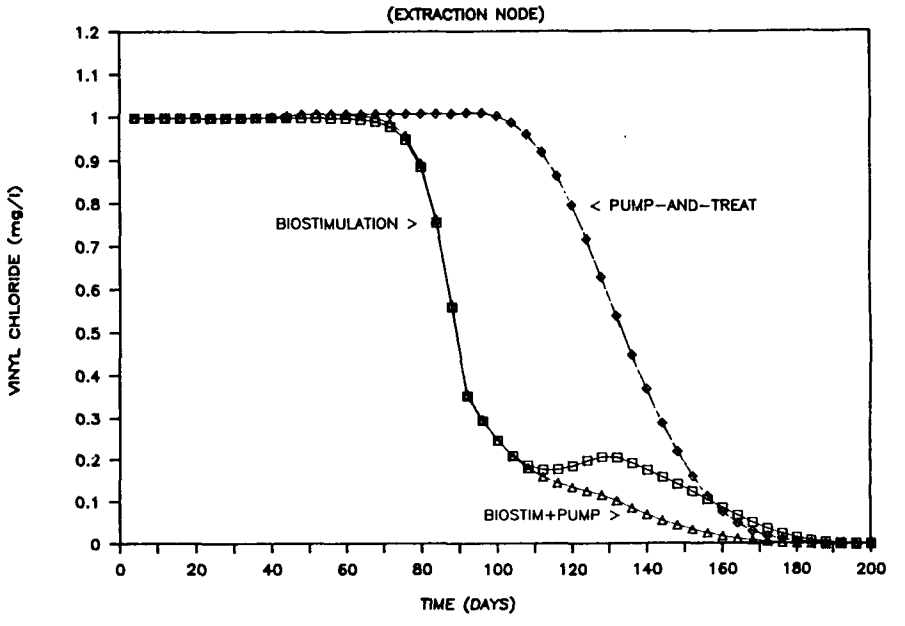


Figure 40. Comparison of *in-situ* bioremediation of VC with pump-and-treat. Biostim+pump is a combination of surface treatment and *in-situ* treatment (from McCarty et al., 1991).

Heterogeneities could result in significant tailing in the pump-and-treat response that might make the bioremediation method even more effective by comparison. Heterogeneities may also affect the efficiency with which O_2, CH_4, etc., can be distributed within the aquifer and reduce the effectiveness of bioremediation as well.

The model simulations illustrated in Figure 40 also indicate the advisability of recycling the contaminants through the treatment zone rather than removing them through treatment at the surface. The dashed line with triangles (Biostim+pump) shows a simulation in which the contaminants in the extracted water are removed using surface treatment before reinjection. Methane and oxygen are added to the surface-treated reinjected water. Some, but not a significant, enhancement of removal is achieved by adding surface treatment. Moreover, the *in-situ* bioremediation process also degrades the reinjected contaminants to nontoxic end products, which is an advantage over some forms of surface treatment.

Simulations for t-DCE are shown in Figure 41. In the Moffett Field study, t-DCE had the same degradation rate coefficient as VC, but was more strongly sorbed onto the aquifer solids. This resulted in a slower rate of treatment for t-DCE. The Moffett transformation rate coefficient (corrected for temperature) was used in the St. Joseph simulations, along with a sorption retardation factor of 4.0. The simulation model indicates that a 50% reduction in t-DCE concentration would occur during the initial methane injection phase. As in the Moffett study, the lower reduction with t-DCE than with VC results because of stronger sorption by the former compound. The simulation results, however, suggest that *in-situ* bioremediation for t-DCE is more attractive than pump-and-treat alone. Thus, some degree of sorption to keep compounds in the aquifer favors *in-situ* bioremediation.

The corresponding simulation for c-DCE is illustrated in Figure 42. In the Moffett Field study c-DCE was degraded at a rate an order of magnitude lower than t-DCE. With this lower rate, a longer *in-situ* treatment period is required to pump and treat. However, the c-DCE that is biodegraded within the aquifer is permanently destroyed, and thus *in-situ* treatment has important benefits. Also, the proposed drinking-water MCL for c-DCE is much higher than for VC. Thus, treatment that is adequate for bringing VC and t-DCE into compliance may be adequate for c-DCE as well. This possibility will not be known until clean-up requirements are established. Nonetheless, the simulation does demonstrate that in the treatment of mixtures of compounds by *in-situ* bioremediation, reaction rates and degrees of sorption, as well as remediation goals, must all be considered before the best strategy can be formulated.

A summary of the engineering aspects of the *in-situ* bioremediation scheme, as described above and illustrated in Figure 37, and based upon the assumptions already described is contained in Table 17. For this case, a total of 480,000 m^3 of aquifer would be treated in 400 days, using a total extraction rate of 400 gpm. A total of 1375 kg of the original 1617 kg of contaminants present in the treated volume would be biodegraded to nontoxic end products. This treatment would require 5,200 kg of methane and 19,200 kg of oxygen.

These simulations show how model simulations provide a support for evaluating *in-situ* processes at a given site. Simulations permit comparisons between remediation via bioremediation, bioremediation in combination with pump-and-treat, and pump-and-treat. For the example shown, pump-and-treat might be satisfactory for remediation as well. Here, a suitable treatment process at the surface must be used. The hydrogeology was also greatly simplified, and effects of aquifer heterogeneities on pump-and-treat and bioremediation were neglected. In order to make the best selection, however, more information is required on the hydraulic and transport characteristics of the site, methanotrophic population distribution, and remediation goals, as well as other site-related clean-up criteria.

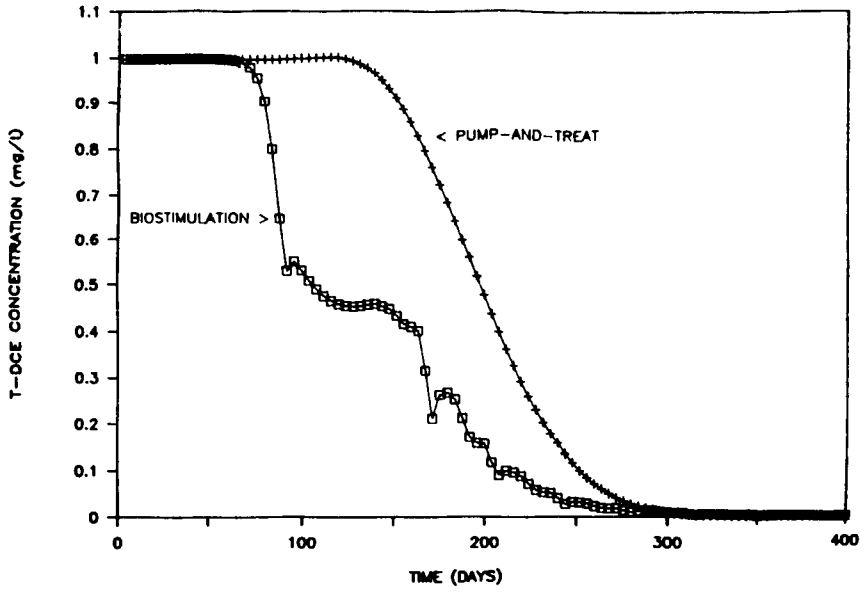


Figure 41. Comparison of *in-situ* bioremediation of t-DCE with pump-and-treat.

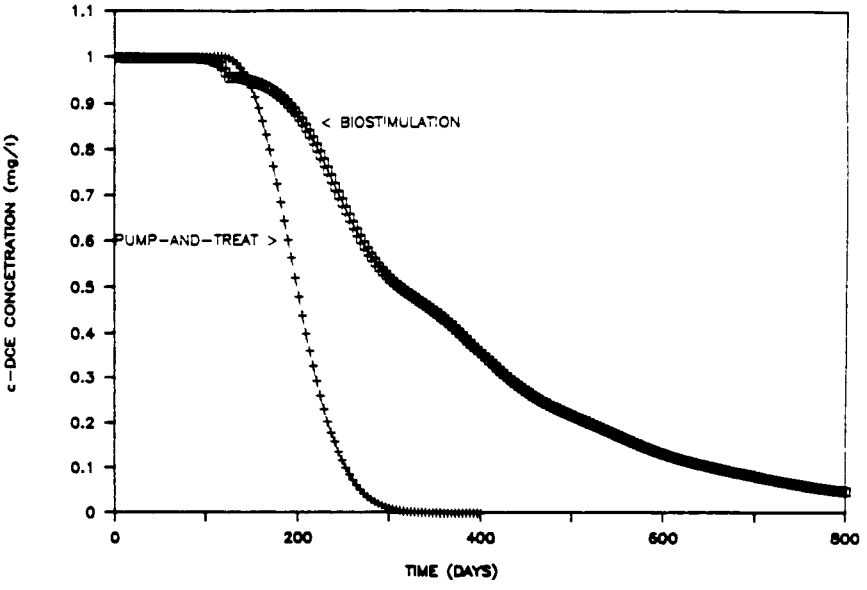


Figure 42. Comparison of *in-situ* bioremediation of c-DCE with pump-and-treat (from McCarty et al., 1991).

TABLE 17. ENGINEERING SUMMARY OF *IN-SITU* BIOLOGICAL TREATMENT
(from McCarty et al., 1991)

Dimensions of the Treated Zone

120 m × 200 m × 20 m = 480,000 m^3
length width depth volume

Mass Biodegraded in 400 Days (1 mg/l initial aqueous)

	Initial Aqueous (kg)	Initial Solids (kg)	Initial Total (kg)	Treated (kg)	Percent Treated
VC	158	117	275	274	> 99
t-DCE	158	513	671	665	> 99
c-DCE	158	513	671	436	65

Methane and Oxygen Requirements

Methane	5,200 kg
Oxygen	19,200 kg

Extraction and Injection Rates

1.8 m^3/min (400 gpm)

It should be emphasized again that no attempt was made to develop an optimized treatment system with the simulations since insufficient information is presently available to do so. The simulations serve primarily to indicate the factors involved in an *in-situ* treatment scheme and the time scales that must be considered. Since *in-situ* bioremediation in this way has not been attempted before, there are many possibilities for improvement on the design assumed for the simulations. The simulations do help to bracket the probable range of outcomes, and thus should help enormously in the design and interpretation of demonstrations, such as the one planned for the St. Joseph site.

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16. ABSTRACT

This report summarizes the behavior of and requisite conditions for a class of natural biological processes that can transform chlorinated aliphatic compounds. These compounds are among the most prevalent hazardous chemical contaminants found in municipal and industrial wastewaters, landfills and landfill leachates, industrial disposal sites, and groundwater. Biological degradation is one approach that has the potential for destroying hazardous chemicals so that they can be rendered harmless for all time. Methodologies are presented that are useful for evaluating the potential for biorestoration of groundwater contaminated with chlorinated aliphatic compounds. The report is composed of six sections. Section 1 provides an introduction and an overview of the problems with chlorinated aliphatic compounds in groundwater. Section 2 presents a review of the processes affecting the movement and fate of chlorinated aliphatics in the subsurface, including advection, dispersion, sorption and relative mobility, diffusional transport, and immiscible transport. Section 3 provides a thorough review of the microbial transformation of organic pollutants. Basic microbial metabolic processes are reviewed, focusing on an aerobic and aerobic transformations of chlorinated aliphatic compounds. Laboratory studies of aerobic cometabolic transformation and degradation of TCE by methanotrophs and methanotrophic communities are summarized. In Section 4 transport and microbial process models are presented and incorporated into a model for the aerobic cometabolic transformation of chlorinated aliphatics by methanotrophic communities. Section 5 presents pilot-scale results of enhanced in-situ biotransformation of halogenated alkenes, including TCE, cis- and trans-DCE, and vinyl chloride by methantrophic bacteria along with model simulations of the results. Section 6 presents an example study to evaluate the potential and limitations for groundwater bioremediation at a Superfund site by methanotrophs. Methodologies and results are presented for evaluating the presence of a native methantrophic community and its ability to degrade the contaminants of concern; determining the sorption of contaminants to the aquifer material; and preliminary designing of an in-situ treatment approach using the model previously described.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field, Group
IN-SITU BIOREMEDIATION*	TCE	GROUNDWATER	
IN-SITU PROCESSES	DCE	BIODEGRADATION	
CHLORINATED SOLVENTS	VINYL CHLORIDE	BIORESTORATION	
AEROBIC TREATMENT		BIOTRANSFORMATION	
BIOLOGICAL DEGRADATION			
BIORESTORATION			
*Suggest Addition to List			

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