Journal of Hazardous Materials, 32 (1992) 145–162
Elsevier Science Publishers B.V., Amsterdam

Pilot scale field studies of *in situ* bioremediation of chlorinated solvents *

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Abstract

This paper discusses results from pilot scale field studies that evaluated enhanced *in situ* bioremediation of chlorinated solvents. A stimulus–response methodology for performing controlled field experiments is exemplified. The cometabolic transformation of chlorinated aliphatic compounds by methanotrophic bacteria is of primary focus.

Introduction

There is much interest in applying *in situ* biological processes for remediating aquifers contaminated with organic compounds. *In situ* bioremediation may serve as a means of (1) decreasing the time required for restoring contaminated aquifers; (2) degrading the compounds completely to circumvent the risk of transferring the contaminants elsewhere; and (3) using the subsurface as a bioreactor to eliminate the need for surface treatment processes. Controlled field studies are the most direct and convincing means of assessing different *in situ* biological treatment processes. Methods are being developed for performing studies that permit scientific evaluation of the treatment process. Questions that need to be addressed in these studies include the following:

- Are biological transformations actually observed?
- What are the extents of transformation?
- What are the transformation products?
- What are the transformation rates?
- Which factors limit the transformation rates?

Obtaining this information in the complex subsurface environment is a major challenge. Transport, physical, and chemical processes as well as biological

*Paper presented at the GCHSRC Fourth Annual Symposium: Ground Water — The Problem and Some Solutions, Lamar University, Beaumont, TX, U.S.A., April 2–3, 1992.

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processes must be considered. Thus, when designing field experiments, and determining the experimental protocol, it is important to consider whether the above questions can be answered given the complexity of the subsurface environment and the many processes that are occurring.

The *in situ* bioremediation of aquifers contaminated with organic compounds has been thoroughly reviewed by Lee et al. [1], McCarty [2] and Wilson et al. [3]. The *in situ* bioremediation of contaminated aquifers was first attempted systematically by Raymond et al. [4], who pioneered *in situ* reclamation of aquifers contaminated by petroleum products. Raymond's work showed that promoting the proper conditions in the subsurface (i.e., by adding oxygen and nutrients), stimulated a native population of microorganisms that degraded the hydrocarbon contaminants, whereby the bacteria used the hydrocarbons as primary substrates for growth.

Enhancing biological transformations in the subsurface will usually require the addition of nutrients. The addition may include growth substrates, electron acceptors such as oxygen, and minor nutrients such as nitrogen and phosphorus. Understanding transport and mixing processes is critical in determining how to effectively add the nutrients to enhance biological transformations. When nutrient addition and mixing are performed, it is often difficult to determine whether the observed decrease in contaminant concentration results from biological transformations or dilution. Experimental methodologies must therefore consider how advective and dispersive transport processes affect the spatial and temporal distribution of contaminants, nutrients, and microbial population in the subsurface. Thus obtaining information on transport in the treatment zone is critical.

Contaminant sorption onto the aquifer solids is a process that must also be considered. Sorption will determine the contaminant mass associated with the aquifer solids and the mass in solution. For strongly sorbed compounds, most of the contaminant mass will be associated with the aquifer solids. In order for biotransformation to proceed, desorption to the aqueous phase must occur. Slow desorption from the aquifer solids provides a continued source of contamination, and may bias estimates of the extents and the rates of transformation. Slow desorption may also limit the overall rates of clean-up. Thus, obtaining information on the sorption process through laboratory and field studies is needed in designing and evaluating *in situ* bioremediation tests.

Mathematical models that represent the key physical, transport, chemical and biological processes are powerful tools for evaluating field tests results, since they provide a means of simulating the combined processes. Models can also be used to compare results of laboratory studies with those obtained under different conditions in the field. Models that are validated through field studies might also be used as a tool in designing field scale *in situ* treatment.

This paper presents the results from pilot scale field studies that evaluated the enhanced *in situ* bioremediation of chlorinated solvents. Examples will be

presented to illustrate a stimulus–response methodology for performing controlled field experiments that has proven to be very useful. Model simulations will also be presented for simulating the concentration response to the experimental stimulus.

Background

In situ biorestoration of aquifers contaminated by halogenated aliphatic compounds (HACs) requires a different approach than that used for petroleum contamination, since in most cases HACs cannot be utilized by native microorganisms as primary substrates for growth. However, they can be degraded by cometabolism, a process in which microorganisms growing on one compound (primary substrate) produce an enzyme which fortuitously transforms another compound, from which they cannot obtain energy for growth [5].

In 1985, Wilson and Wilson [6] showed for the first time that trichloroethylene (TCE) may be susceptible to aerobic degradation (by soil microbial communities fed natural gas). Methanotrophs that grow on methane under aerobic conditions possess an enzyme, methane monooxygenase (MMO), that initiates the oxidation of TCE [7]. Scientific research aimed at exploiting this phenomenon has included numerous laboratory investigations [7–13] as well as directed field experiments [14,15].

Based on the findings with methanotrophs [7,17], it can be concluded that TCE is most likely oxygenated to TCE-epoxide, an unstable compound which is quickly rearranged nonenzymatically in aqueous solution to yield various products including carbon monoxide, formic acid, glyoxylic acid, and a range of chlorinated acids [18]. 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 [8–10].

Pilot scale field studies were performed that assessed, under field conditions, the capacity of native microorganisms, i.e., bacteria indigenous to the groundwater zone, to degrade HACs when proper conditions are 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 HACs, including *cis*- and *trans*-1,2-dichloroethylene (*c*-DCE and *t*-DCE), and vinyl chloride (VC), was assessed by means of controlled addition, frequent sampling, quantitative analysis, and mass balance comparisons.

The experimental approach taken was similar to that proposed for bioremediation in the field (Fig. 1). Extracted groundwater from the treatment zone is amended with methane and oxygen and reinjected to stimulate methanotrophic growth. HACs in the extracted groundwater are reinjected into the biostimulated zone. Bioremediation conducted in this manner will promote

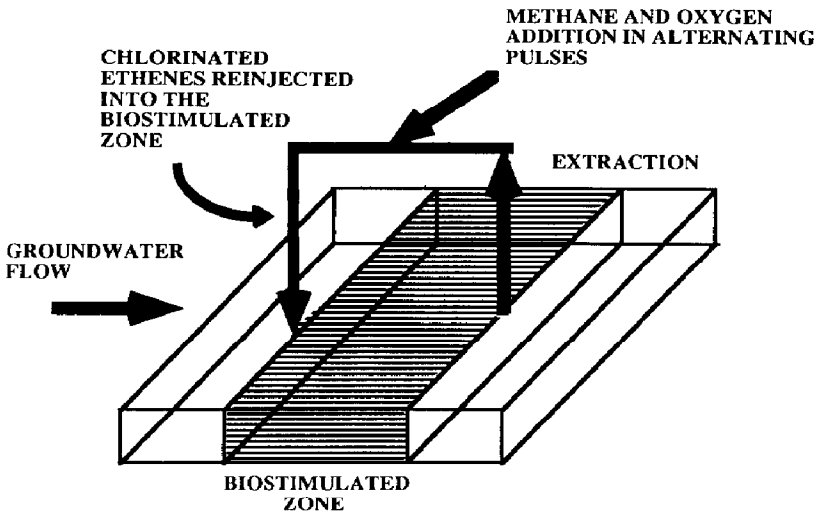

Fig. 1. Conceptual model for *in situ* bioremediation by methanotrophic bacteria (from McCarty et al. [31].

degradation of inplace contaminants as well as contaminants that are extracted and reinjected, thus obviating above ground treatment.

Site characterization

The test methodology was developed after detailed information on the pilot scale test zone was obtained through thorough site characterization. The site chosen for the field demonstration, at Moffett Naval Air Station, offered a near-ideal combination of characteristics [14]. The test drilling identified a shallow confined aquifer which is known as the "A" Aquifer, the shallowest of several in the region. 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 (Fig. 2). The aquifer, consisting of fine-to-coarse grained sand and gravels, appears poorly sorted in most cores. The site is 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.

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

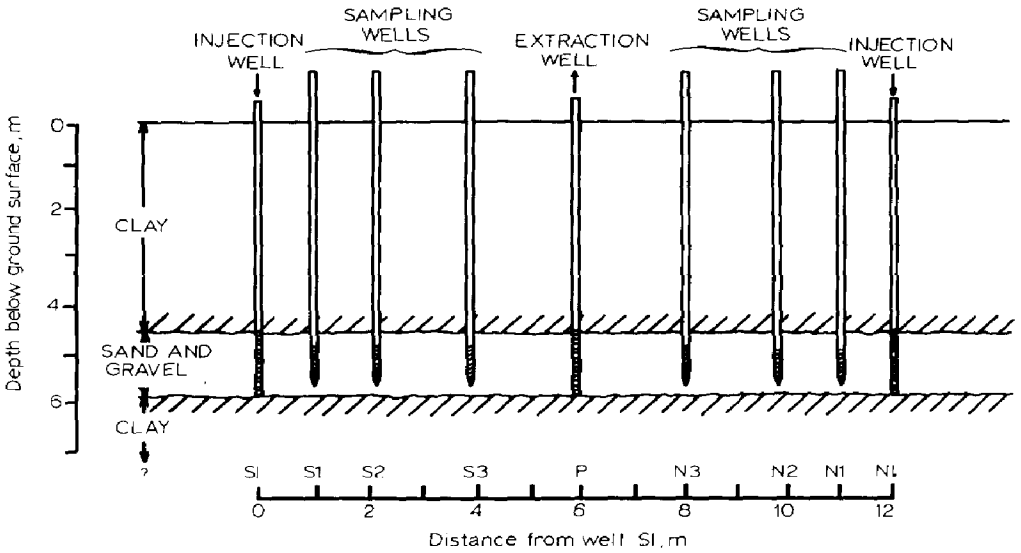

Fig. 2. Cross section of the test zone and the wellfield used in the experiments (from Roberts et al. [14]).

isomers, and VC – chosen as target compounds for this study. Thus, these compounds would have to be added in a controlled manner in the study.

There were no appreciable amounts of toxic metals [19]. 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 were probably 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, with a water-table aquitard model best fitting the pump test observations [20].

Bromide tracer tests under natural gradient conditions showed that the local groundwater velocity was approximately 2 m/day. Mathematical modeling of the flow field with RESSQ, [21], 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).

Microbiological studies

Microbial studies included mixed and pure culture studies on TCE transformation, and soil microcosm studies. These studies determined whether methane-utilizing populations were present and were capable of transforming TCE. Mixed and pure culture studies were performed in the laboratory using microbes enriched and isolated from the aquifer solids samples and from the formation groundwater. Details of these studies are provided by Henry and Grbić-Galić [9,17,22], and Henry [23]. These studies showed different types of methanotrophs were present in the test zone. They all had the ability to degrade TCE, but at different rates. The mixed cultures completely degraded the TCE to CO_2 and chloride. The rates of TCE transformation were also shown to depend on growth conditions, such as the mineral growth media used.

Details of the soil microcosm studies are presented by Mayer et al. [13] and Lanzarone and McCarty [11]. These studies also confirmed that methane-utilizing bacteria could be easily stimulated through methane and oxygen addition. Approximately 20% of the TCE added was degraded to CO_2 at TCE concentrations ranging from 20 to 40 μg/L.

Sorption studies

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 [14,24]. Figure 3 shows TCE sorption isotherms with Moffett core solids. The studies confirmed that sorption equilibrium was

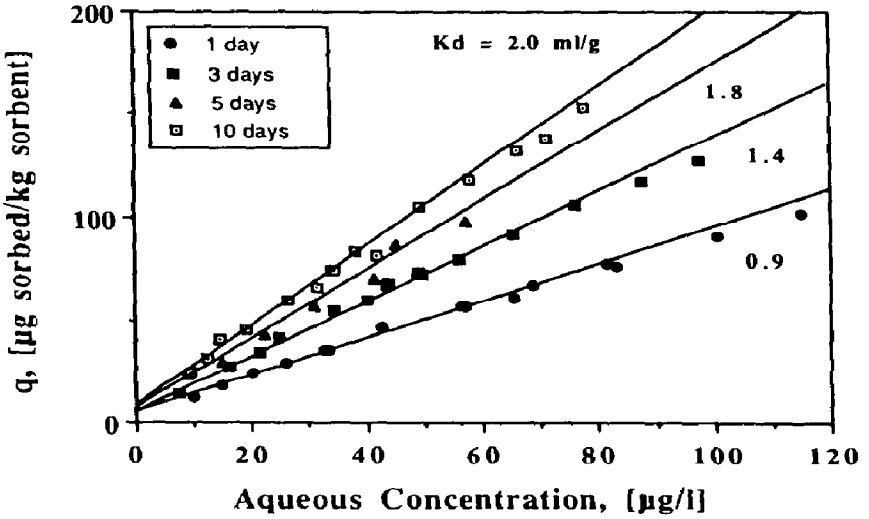

Fig. 3. Sorption isotherm at increasing times for Moffett bulk solids and TCE (from Roberts et al. [14]).

approximately linear, justifying the use of a distribution coefficient for interpreting and reporting the sorption equilibrium data. The studies also showed the apparent K_d increasing with the time of equilibration due to slow diffusion of TCE into the porous aquifer solids [24]. This finding points out that deviations from sorption equilibrium owing to rate limitations may be an important factor influencing bioremediation, since desorption from the solids to the aqueous phase would be required for transformation to occur. The studies also found that sorption was strongest for TCE and weakest for VC, among the compounds studied.

Mathematical modeling

A non-steady-state model [16,25] developed for simulating the results of the field experiments proved extraordinarily useful in interpreting the results and comparing with the laboratory data. The model incorporated advection, dispersion, sorption with and without rate limitation, and the microbial processes of substrate utilization, growth, and cometabolic transformation of the halogenated aliphatics using a competitive inhibition model. The transport was simplified by assuming one-dimensional, uniform flow, as a computational compromise to permit more rigorous representation of the biological processes. Input parameters were estimated based on the results of the laboratory research, or on values from the literature. The initial population of methane-utilizing bacteria was allowed to vary as an unconstrained fitting parameter. Transformation rate coefficients for the HACs were also fitted to the field observations, and compared with laboratory derived rates. Both the response to biostimulation (methane and dissolved oxygen (DO) uptake), and the biotransformation of the chlorinated aliphatics were simulated.

Experimental methodology

The detailed characterization of the test zone and early bromide tracer tests indicated several important factors had to be included in the experimental design. The absence of several of the HACs in the test zone required their continuous controlled addition to the test zone. The strong component of regional flow required the experiments to be conducted under induced gradient conditions created by pumping from a downgradient extraction well (P), while introducing solutes in known amounts at an injection well (SI) six meters upgradient, and measuring concentrations regularly at the injection, extraction, and intermediate observation points (Fig. 2). Interpretation of biotransformation behavior could then be made by quantitative examination of the solutes' concentration histories at the several monitoring points, comparing results under biostimulation conditions with results obtained under similar conditions in the absence of biostimulation measures.

A specially designed, automated data acquisition and control system constructed for this purpose, proved capable of providing continuous records of high-accuracy data over sustained periods that enabled the computation mass balances with relative errors of only a few percent. Details of the system design and operation are presented by Hopkins et al. [26]. The monitoring setup was capable of measuring key chemical components: bromide as a conservative tracer, the halogenated aliphatics (TCE, *c*-DCE, *t*-DCE, and VC), methane, and DO, with one sample processed hourly.

The frequency of analysis realized by the automated data acquisition system permitted stimulus–response experiments with rapid dynamic forcing functions. The stimulus is the injection of chemicals of interest, and the response being the measured concentration histories of the chemicals at observation locations. A systematic sequence of stimulus-response experiments was performed to (1) determine transport characteristics of the test zone; (2) demonstrate convincingly enhanced *in situ* biodegradation by methanotrophic bacteria; and (3) gain an understanding of complex processes effecting biodegradation, such as competitive inhibition. The series of experiments are outlined in Table 1.

Bromide was used as a conservative tracer during all phases of the experiments. Initially bromide was used to quantify advection and dispersion transport. During the latter stages of the experiments it was used to quantify the degree of breakthrough of the injected fluid at observation locations, thus helping to insure that contaminant concentration decreases were associated with transformation processes rather than changes in hydraulic conditions.

The experimental sequence provided strong evidence that biotransformation was occurring in response to biostimulation of methane-utilizing bacteria. The sequence included the following phases (Table 1): a pseudo-control (Phase 2) during which the contaminants were added in the absence of biostimulation;

TABLE 1

Sequence of field experiments

Phase	Injected chemicals	Phenomena investigated
1	Br^-	Advection/dispersion
2	Br^- + Organic + O_2	Retardation/transformation
3	CH_4 + 2	Biostimulation/biotransformation
4	(2) + Transient tests	
	a) Dynamic pulsing CH_4	Biotansformation competitive/inhibition
	b) CH_4 Stopped	Biotransformation
	c) Formate/methane	Competitive/inhibition biotransformation

an active biostimulation (Phase 3) with methane addition, where evidence of biotransformation in response to biostimulation was obtained; and a series of transient tests (phase 4) to provide additional evidence for biostimulation and to study complex process such a competitive inhibition. Examples will be presented that illustrate some of the stimulus–response tests that were performed in the different phases.

Results from the field experiments

Example of a bromide and organic transport experiment

Bromide tracer and organic transport experiments were undertaken to quantify transport velocities and residence times in the test zone, to determine how strongly the chlorinated organics were retarded due to sorption processes, as to serve as a pseudo control prior to active biostimulation. Figure 4 shows the normalized concentration breakthrough of bromide and TCE at the S1 observation well during an induced flow test. The arrival of TCE was retarded compared to that of bromide tracer due to TCE sorption onto the aquifer solids. Detailed modeling studies of Harmon et al. [24] simulated this breakthrough response using a transport model that included rate-limited diffusional sorption with input parameters derived from laboratory sorption studies (Fig. 3).

The bromide tracer tests confirmed that the aquifer was virtually completely permeated by the injected fluid in the observation zone, and the injected fluid was essentially completely recovered by the extraction well. The hydraulic residence times between the injection well and the two nearest observation wells (S1 and S2), ranged from 8 to 23 h, and 25 to 40 h to the extraction well (Table 2). 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 2). Retardation estimates were in the range of those based on the laboratory sorp-

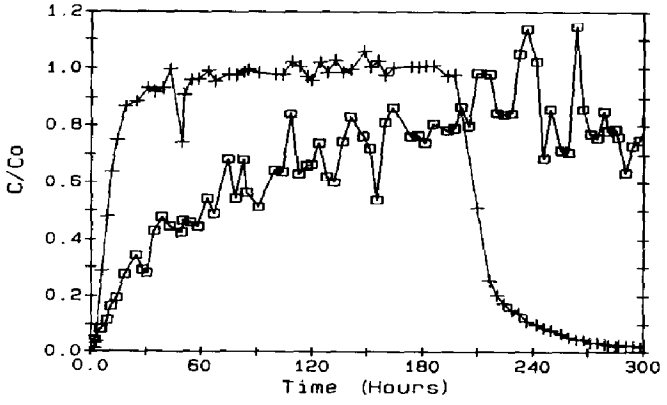

Fig. 4. Normalized breakthrough of bromide (+) and TCE (□) at the S1 observation well in an induce flow tracer test.

TABLE 2

Resident times ($t_{50\%}$ breakthrough) and retardation factors for the chlorinated organic compounds (from [14])

Experiment	Compound	Well S1 $t_{50\%}$ (h)	Well S2 $t_{50\%}$ (h)	R (S1)	R (S2)
Tracer 4	Br^-	8	20		
	1,1,1-TCA	10	30	1.3	1.5
Tracer 5	Br^-	9	17		
	TCE	40	160	5	9
	Br^-	7.5	16		
Tracer 8	TCE	60	150	8	9
	t-DCE	50	150	7	9
	c-DCE	30	70	4	4
	Br^-	9	23		
Tracer 11	TCE	50	175	6	8
	t-DCE	120	280	13	12
	c-DCE	45	90	5	4
Tracer 12	Br^-	8	21		
	VC	13	42	1.6	2.0

tion studies, with TCE being the most strongly sorbed and VC the most weakly sorbed. Breakthrough of the chlorinated organics to 90 to 95% of the injected concentration at the S1 and S2 observation wells indicated minimal losses before biostimulating the test zone. Details of the results of the tracer tests and modeling the tracer breakthroughs are given by Roberts et al. [14], and Chrysikopoulos et al. [27].

Example of biostimulation experiments

The experimental methodology used in the biostimulation and biotransformation experiments is discussed by Roberts et al. [14] and Semprini et al. [15,28]. Groundwater was saturated with methane or oxygen using two countercurrent 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 5 shows the concentration history of methane and DO at the S2 observation well during the initial biostimulation experiment along with model simulations. At early time, methane and DO behaved like the conservative bromide tracer, indicating no retardation and minimal consumption. During the period of 200 to 430 h methane and DO concentrations rapidly decreased, indicating the growth of methane-utilizers. The model simulations represented by the solid line matched the field observations using a reasonable set of biological input parameters. The model supports the conclusions that methanotrophic bacteria were stimulated in the test zone and that the processes can be well simulated when appropriate rate equations are used [16].

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, with a pulse cycle time of 4 and 8 h, respectively. The arrival of methane and DO pulses at the S2 well was observed at later time. Model simulations reproduced well the dynamic response due to pulsing and predicted a more distributed biomass [16]. Biofouling of the near well-bore region was thus limited by the pulsing methodology, as anticipated in the experimental design.

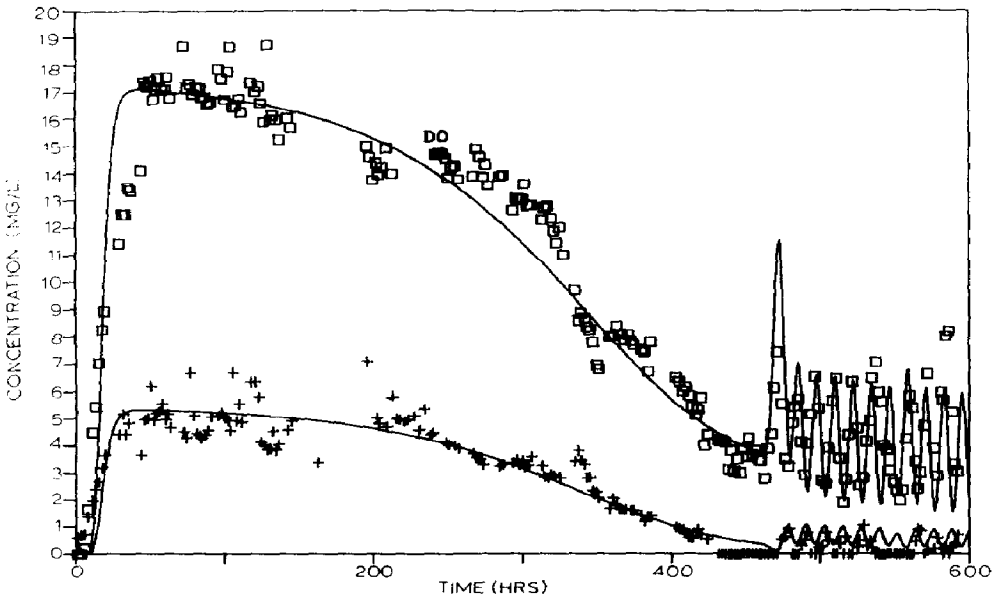

Fig. 5. Methane (+) and DO (□) response at the S2 well due to biostimulation of the test zonew during the first season of field testing. The solid lines are model simulations of Semprini and McCarty [16].

Example of biotransformation experiments

In order to evaluate biotransformation, the chlorinated compounds were added to the injected water at concentrations ranging from 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. Figure 6 shows the response at the S1 well of the target compounds in the third season and corresponding model simulations. Transformation of the organic target compounds ensued immediately following the introduction of methane at time zero, increasing with time as the bacterial population grew. Rapid transformations of VC and *t*-DCE were observed, followed by *c*-DCE and TCE (not shown). Competitive inhibition of VC and *t*-DCE transformation by methane was indicated in response to the dynamic pulsing of methane and oxygen that was initiated at 20 h. Model simulations [25] matched well the response observed in the field, with the effects of competitive inhibition more pronounced at the S1 well compared to the S2 well as indicated by greater oscillations in concentration at the S1 well. Both competitive inhibition kinetics and rate limited sorption were required to reproduce the field observations.

The effects of rate-limited sorption are illustrated by Fig. 7, where the response of VC at the S2 observation well is shown along with model simulations. The rate-limited sorption model provides a better match to the field observa-

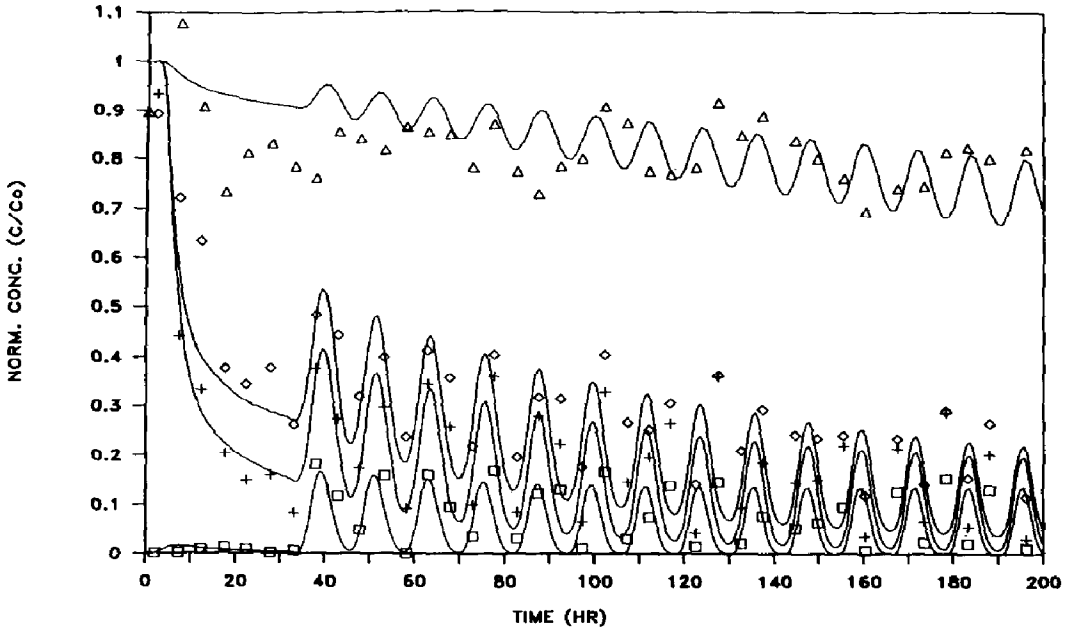

Fig. 6. Response of methane (□), VC (+), *trans*-DCE (◇), and *cis*-DCE (△) at the S1 well due to biostimulation in the third season of field testing and model stimulations (from Semprini and McCarty, [25].

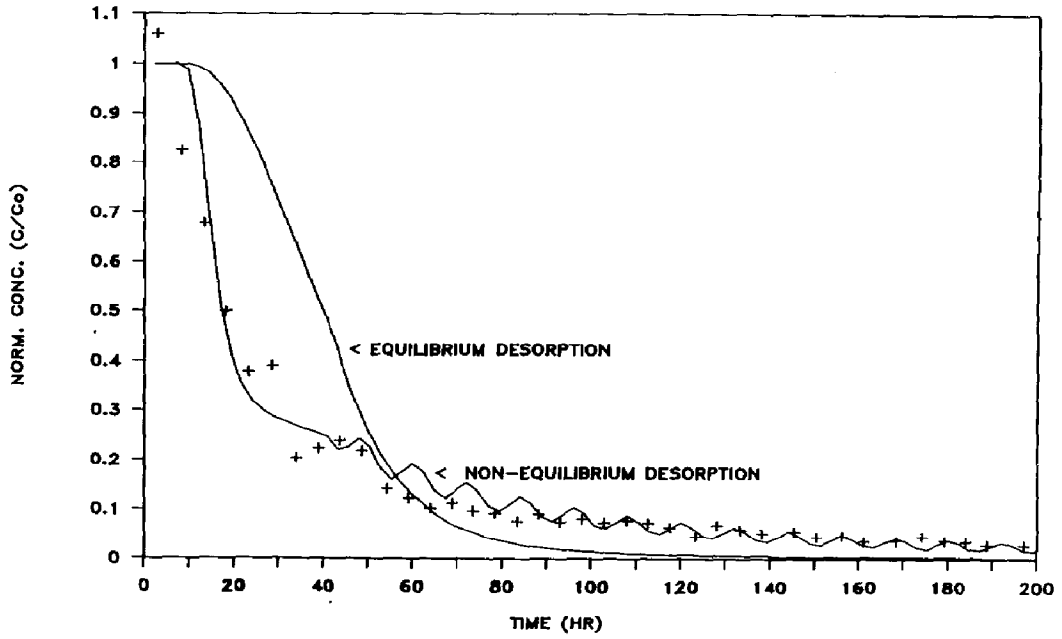

Fig. 7. Response of VC (+) at the S2 well and simulations based on rate-limited sorption and equilibrium sorption.

TABLE 3

Model parameters for sumulation of cometabolic transformations (from Semprini and McCarty [25])

Compound	K_d (1/mg)	α (d^{-1})	k (d^{-1})	K_s (mg/L)	k/K_s (L/mg-d)
Methane	0.0	0.00	2.0	1.0	2.0
VC	0.40	0.5	1.0	1.0	1.0
t-DCE	1.60	0.3	1.0	1.0	1.0
c-DCE	1.60	0.3	0.05	1.0	0.05
TCE	2.25	0.2	0.01	1.0	0.01

[a] K_d = sorption distribution coefficient, α = rate coefficient for sorption, k = maximum transformation rate, K_S = half-saturation coefficient.

tions than the equilibrium sorption model. The simulations indicate that the overall rate of decrease in VC concentration may have been limited by the rate of its desorption from the aquifer solids. The simulations indicated that physical processes such as desorption can limit times of clean-up of an enhanced microbial process.

The comparison of the rate parameters (Table 3) shows VC and *t*-DCE were transformed at rates similar to that of methane the substrate for growth, while *c*-DCE and TCE were transformed at rates one to two orders of magnitude

slower than methane. The simulations indicate that *t*-DCE concentrations decrease more slowly than VC, since it is more strongly sorbed (higher K_d) and thus a greater contaminant mass must be degraded. The difference in rates for *c*-DCE and *t*-DCE illustrates the effect that a small change of structure can exert on the cometabolic transformation rates.

Example of a transient to confirm biotransformation

Gas chromatographic analysis of water samples during active biotransformation of *t*-DCE provided evidence of an intermediate transformation product identified in laboratory studies to be the epoxide of *t*-DCE [29], which was present in amounts equivalent to a few percent of the parent compound. No other intermediate products were identified. The presence of the epoxide supported the biotransformation of *t*-DCE by methane-utilizing bacteria.

Figure 8 shows results of a transient test in which methane addition was terminated after active biostimulation. Details of this test are presented by Semprini et al. [15]. Termination of the methane feed at approximately 275 h was followed by cessation of transformation activity, as indicated by the increase in *t*-DCE concentration and the decrease in concentration of the epoxide transformation product. The increase occurs at approximately the same time scale as that of organic transport (i.e, one to ten days). Upon reintroduction of methane at 475 h, the *t*-DCE concentration decreased and the epoxide reappeared. Model simulations (not shown) indicate that the microbial population remained active in the absence of methane for only a short time before ceasing to transform the target organic compounds. The transient test confirmed that the transformation was strongly tied to methane-utilization.

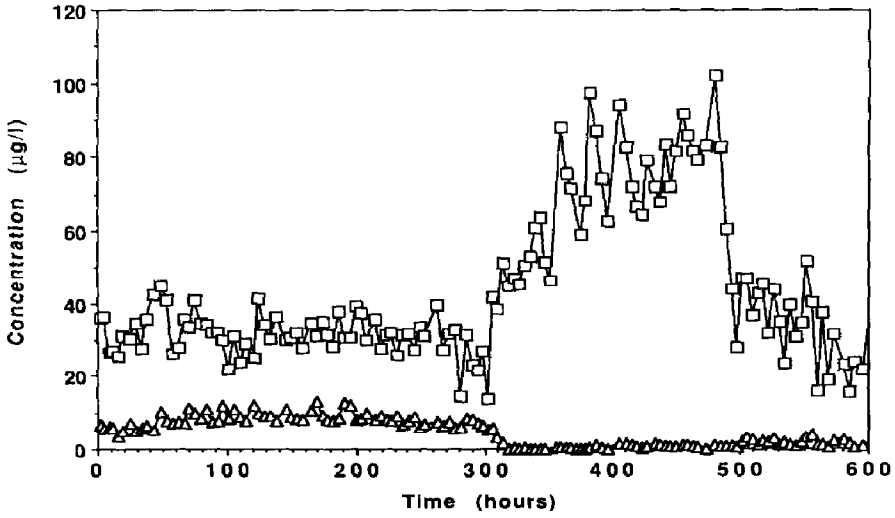

Fig. 8. Transient response of *t*-DCE (□) and *t*-DCE-epoxide (△) to stopping methane addition at 275 h and restarting methane addition at 475 h (from Semprini et al. [15]).

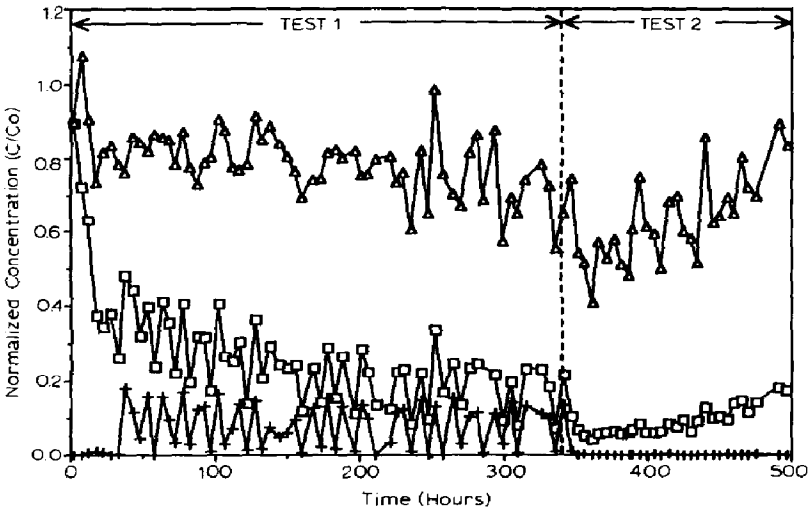

Fig. 9. Transient response of methane (+), *c*-DCE (△), and *t*-DCE (□) at the S2 well during the transition from methane (Test 1) to formate addition (Test 2) (from Semprini et al. [28]).

Example of a transient test to confirm competitive inhibition

A transient test was performed where formate was substituted for methane to confirm competitive inhibition of contaminant transformation by methane, and to determine whether enhanced transformation rates resulted. Details of this test are provided by Semprini et al. [28]. Formate provides an energy source for methanotrophs but does require the MMO enzyme to be assimilated [30]. Thus, formate should not inhibit the transformation, and may enhance it. The results of the transient test with formate are shown in Fig. 9. Upon switching to formate the concentration oscillations of *t*-DCE essentially stopped, indicating competitive inhibition by methane. Transformation was temporarily enhanced with formate addition, demonstrating the need of an energy source to drive the transformation.

Discussion

The examples presented illustrate that carefully planned stimulus-response tests constitute a powerful means of studying *in situ* bioremediation processes. The examples presented here focus on the cometabolic transformation of HACs by methanotrophic bacteria. A similar stimulus-response methodology, however, can be used to study other types of *in situ* treatment processes. While helping to demonstrate that transformations were occurring, they also provided a means of investigating more complex processes such as the competitive inhibition of cometabolic transformation, and the effects of rate limited desorption from the aquifer solids.

Careful monitoring is required for such studies. This study was aided by an

automated data acquisition system that provided frequent and reproducible analyses. Also of key importance is the ability to control transport in the subsurface and to apply a controlled stimulus which in these tests was the constant or pulsed controlled addition of chemicals. Design of stimulus–response tests also required adequate understanding of the key microbial and transport processes.

Mathematical modeling is a powerful tool for simulating the results of transient-response experiments. Modeling helps the study of complex kinetic and transport processes that are indicated by the stimulus-response tests. In developing appropriate models, it is essential to strike a judicious compromise between the competing goals of accurate process representation and computational feasibility. The mathematical model chosen for the present application stressed relatively complete representation of the relevant biological processes, and compensated with a highly simplified, one dimensional model for advective/dispersive transport.

The mathematical modeling supported the experimental evidence that a methanotrophic population was stimulated in the aquifer, using rate parameters expected for methanotrophic bacteria. The simulations also indicated that the chlorinated organics were transformed at different rates. The cometabolic transformation modeling required a competitive inhibition kinetic model, while sorption was best modeled as a rate-limited process. The simulation indicated that slow desorption probably limited the removal of the more rapidly transformed contaminants, and influenced the response due to competitive inhibition.

The results support incorporating experimental controls and quantitative mass balances to the fullest possible extent as an absolute prerequisite for meaningful experimentation, in the field as in the laboratory. Strong dynamic forcing is helpful in stimulating positive characteristic responses that aid in identifying mechanisms and in testing hypotheses and mathematical models. Only field experimentation of this kind can provide a reliable engineering scientific basis for evaluating and designing *in situ* biorestoration measures.

Acknowledgments

This study was funded by the U.S. Environmental Protection Agency under Assistance Agreement CR-812220 to Stanford University; it has not been subjected to the Agency's required peer and administrative review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. The preparation of this manuscript was supported by the Office of Research and Development, U.S. Environmental Protection Agency through the Western Region Hazardous Substance Research Center, under agreement R-815738.

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