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Project Summary

In-Situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria

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This project evaluated the potential of an innovative approach to aquifer restoration: enhanced in-situ biotransformation of chlorinated aliphatic solvents by a bacterial community grown on methane under aerobic conditions. The target chlorinated compounds were trichloroethene (TCE), cis- and trans-1,2-dichloroethene (DCE), and vinyl chloride (VC). Laboratory studies were conducted to improve understanding of the microbial growth and transformation rates and to characterize important transport properties. In the field experiments, biostimulation was accomplished by introducing methane and oxygen into a shallow, confined, sand and gravel aquifer to encourage the growth of a native bacterial community. Methane utilization commenced rapidly, within ten days in the first biostimulation attempt, and within one day in subsequent biostimulation episodes. Biotransformation of the target organic compounds ensued immediately after commencement of methane utilization, and reached steady-state values within three weeks. The approximate extents of transformation were as follows: VC, 95%; trans-DCE, 85%; cis-DCE, 40%; and TCE, 20%.These amounts of biotransformation were achieved in a relatively small biostimulated zone, with travel distances of 1 to 4 m and travel times of 8 to 25 hrs. Mathematical modeling of the transport and transformation process confirmed that the behavior observed in the field demonstration was consistent with the results of the laboratory research and theoretical expectations. This technology has been demonstrated to be effective in continuous operation under carefully controlled conditions in a real subsurface environment at small scale, and is a viable candidate for consideration at real contamination sites where conditions are favorable.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The in-situ remediation of aquifers contaminated with halogenated aliphatic contaminants is a promising alternative in efforts to protect and restore groundwater quality. Approaches based on extracting the contaminated groundwater by pumping and subsequently treating above ground have proven effective for the restoration of aquifers contaminated by these compounds, but often entail great expense as well as a risk of transferring the contaminants to another medium, e.g., the atmosphere. To circumvent these difficulties, in-situ treatment of the contaminants is being considered as a potentially favorable

alternative, with development efforts centering on promoting biotransformation of the contaminants.

This project has assessed under field conditions the ability of native microorganisms, i.e., bacteria indigenous to groundwater zone, to degrade halogenated organic contaminants when proper conditions are provided to enhance bacterial growth. Specifically, the growth of methane-utilizing microbial communities 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. To provide guidance for the field work as well as a firm foundation for interpretation, and to improve basic understanding of key microbial and physical processes, laboratory experiments were also performed.

Objectives

The specific objectives of this project were the following: 1) to demonstrate whether the proposed method is effective, by conducting controlled experiments in a regulated natural groundwater setting; 2) to quantify the rate of decomposition and to identify intermediate transformation products, if any; 3) to determine the factors that govern biodegradation rates; 4) to bracket the range of conditions under which the method is effective; 5) to quantify the sorption of the chlorinated compounds on the aquifer solids, and its effect on transport and exchange between porewater and solids; and 6) to simulate the in-situ biodegradation process using a mathematical model that incorporates the principal biological and transport processes, and to develop suitable models for that purpose.

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 populations 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.

Evidence of biotransformation can then be assessed 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. A specially designed, automated data acquisition and control system 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.

Site Characterization

The site chosen for the field demonstration, at Moffett Naval Air Station, offered a near-ideal combination of characteristics. The site was representative of a typical situation of groundwater contamination, in which a shallow sand-and-gravel aquifer is contaminated by chlorinated aliphatic compounds widely used as solvents. Drilling logs revealed that the shallow aquifer at the test site consisted of a layer of sand and gravel, approximately 5 m below the ground surface and 1.2 m thick, 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 average organic carbon content of the aquifer solids was 0.11% and the specific surface area was 5 m^2/g.

The formation groundwater was also of appropriate composition for the field experiments. The water was moderately saline 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. Both nitrate and phosphorus were naturally present in the subsurface in amounts adequate to support the anticipated biological growth.

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. Extensive tracer tests, conducted while extracting at 10 l/min, were undertaken to quantify transport velo and residence times in the test (Table 1). These tracer tests confi that the aquifer was virtually compl permeated by the injected fluid ir observation zone, as evidencec complete breakthrough of bromide t at the observation wells--S1, S2, under the chosen experime conditions. Further, the overall balances, comparing the amount tracer injected and extrac demonstrated that the tracer recove the extracted water was essen complete: after raising the injectior extraction rates in the second and years of field work, the amou bromide extracted agreed withir percent with the amount injected (1). This was necessary to assure validity of the experimental approac quantify the extent of biotransformat the organic solutes by comp instantaneous concentrations at injection and monitoring points, d steady-state periods after advection/sorption transients.

The hydraulic residence times (1) between the injection well and th nearest observation wells (S1 and quantified by the tracer tests forced-gradient conditions, were fou be in the range of 8 to 23 hrs. residence time between the injectio extraction well was 30 to 40 hrs. residence times were later found suitable for quantifying the transforn rates of interest in this work. retardation factors for the organic sc evaluated from relative mobility obtained in the field, were in the rar two to twelve (Table 2).

Laboratory Studies

Sorption

The retardation factors quantifiec the field data were consistent wit results of laboratory studies of soi The sorption of the organic solut aquifer core samples from the M site confirmed that sorption equili was approximately linear, justifyin use of a distribution coefficier interpreting and reporting the so equilibrium data. Sorption was str for TCE and weakest for VC, amor compounds studied. The retar factors calculated from the labo sorption data agreed closely with estimated from the transport experi conducted in the field. The ext sorption was approximately equal grain size fractions, but equilibriur

Table 1. Comparison of Bromide Tracer Tests Under Induced Gradient Conditions

	Monitoring Point[b]	TR8[a]	TR11	TR12
Injection Rate (l/min)		1.36	1.5	1.5
Extraction Rate (l/min)		10.0	10.0	10.0
Percent Steady-State Breakthrough	S1	100	102	100
	S2	98	100	99
	S3	84	96	95
	Ext	13	14	15
Time to 50% Breakthrough (hrs)	S1	8	9	8
	S2	16	23	21
	S3	20	27	27
	Ext	30	40	42
Percentage Recovered at the Extraction Well		105	94	ND

[a]TR8 = Tracer experiment, etc.
[b]Distances from injection well to monitoring wells: S1, 1.0 m; S2, 2.2 m; S3, 4 m; and Extraction well (Ext), 6 m.

Table 2. Residence Times and Retardation Factors for the Chlorinated Organic Compounds Based on the Time Required to Achieve 50% Fractional Breakthrough

Experiment	Compound	Well S1 $t_{50\%}$ (hrs)	Well S2 $t_{50\%}$ (hrs)	R (S1)	R (S2)
Tracer8	TCE	60	150	7	8
	trans-DCE	50	115	6	7
	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

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 and biotransformation behavior.

Growth and Transformation Rates

Biotransformation studies of several kinds were conducted in the laboratory to characterize the populations of methanotrophic bacteria at the field site. These included studies with enriched mixed cultures and isolated pure cultures grown on nutrient media, as well as experiments with the natural population grown on aquifer solids under conditions simulating the field experiments, in batch exchange soil columns and a continuously fed column.

The experiments with mixed cultures enriched from Moffett samples evaluated the ability of populations grown on several substrates--methane, propane, and ethylene--to transform TCE as the target compound. Methane oxidizers transformed TCE about one hundred times faster than ethylene oxidizers; propane oxidizers showed no ability to transform TCE. Pure cultures of both methane- and ethylene-oxidizing organisms were isolated from the corresponding mixed cultures, and were shown to be capable of transforming TCE. Acetylene inhibited both methane oxidation and TCE transformation, implying that the methane monooxygenase (MMO) enzyme was responsible for both processes.

Experiments with varying methane concentration revealed that high methane concentration slows or stops the transformation of TCE, presumably through the competition between methane and TCE for the MMO enzyme. The properties of the various cultures enriched from the Moffett aquifer material differed somewhat with respect to transformation rates and the effects of environmental variables on rates. In some, but not all, cultures, TCE concentrations above 10 mg/l were found to inhibit the rates of both methane oxidation and TCE transformation. Extremely high concentrations of oxygen (i.e. > 30 mg/l) also exercised a slight inhibitory effect. Cultures containing storage compounds (PHB granules) were able to transform TCE as rapidly in the absence of methane as in the presence of low methane concentrations; this finding illustrates the importance of the availability of reducing power in sustaining the normal function of MMO.

Batch soil column experiments with cultures grown on Moffett solids largely confirmed the results of the experiments with cultures grown on nutrient media, and served to demonstrate the applicability of the results to the aquifer at the Moffett site. The experiments showed conclusively that a native methanotrophic community could be

stimulated in a porous medium consisting of Moffett aquifer material, without the addition of microbes or nutrients. The natural system contained sufficient nitrate and phosphate as nutrient sources; the column experiments showed that transformation rates were not enhanced by supplying additional nitrogen and phosphorus.

Columns fed methane and oxygen began to utilize the methane within 7 days, and partial TCE transformation ensued within 80 days, reaching approximately 20% after a year. No significant amounts of intermediate transformation products of TCE were found. Mass balances on columns previously saturated with sorbed TCE and then purged with water for prolonged periods, with and without biostimulation, showed that the TCE was removed from the solids twice as fast by the combination of biodegradation and desorption as by desorption alone. Vinyl chloride (VC) degraded much more rapidly than TCE, being removed about one-half as fast as methane itself. Within two days, VC degradation was essentially complete.

The concentration observations from the column experiments generally supported the hypothesis of enzyme competition, and showed that methane should not be present at too high a concentration. It was further demonstrated that methane does not have to be added continuously for TCE degradation to proceed; TCE transformation persisted for several days after methane depletion, and indeed seemed to be more rapid at very low methane concentrations.

The continuous flow column experiments closely simulated the conditions of the field experiment. The experiments were conducted with continuous feed of methane and oxygen, with a hydraulic residence time of one day, corresponding approximately to the travel times between the injection well and the observation wells at the field site. In the initial biostimulation with methane and oxygen, substantial methane utilization commenced 20 days after beginning the methane feed, increasing rapidly over the next 5 days to the point where methane was completely utilized. Following attainment of complete methane utilization, transformation of TCE began, ultimately reaching approximately 20%. The transformation of TCE was not improved by raising the influent methane concentration from 4.5 to 6.5 mg/l. On the contrary, TCE transformation was improved substantially (from 22% to 29%) by temporarily ceasing the methane input for a period of up to 20 days. The transformation of trans-DCE under similar conditions was much greater than that of TCE (85% vs 22%). Transformation of trans-DCE in the continuous column persisted unabated for more than 40 days after the methane input was ceased.

Field Demonstration of Biostimulation and Biotransformation

The biostimulation and biotransformation evaluations conducted in the field were consistent in most major respects with expectations based on the laboratory results and theory.

It was confirmed that a native community of methane-oxidizing bacteria could be stimulated by introducing dissolved methane and oxygen into the aquifer in proper amounts, without any other supplementary nutrients. In the first year's biostimulation experiment, the population of methane utilizers had grown to the point of utilizing substantial amounts of methane within ten days, and within another five days methane utilization was complete (Figure 1). Clogging of the injection well and borehole could be controlled effectively by alternately pulsing methane and oxygen, e.g. for time periods of 4 and 8 hrs, a strategy which also served to spread the microbial growth more uniformly over a larger domain around the injection point. The ratio of oxygen consumption to methane consumption was 2.5 g/g, consistent with literature data and laboratory results on methanotrophic metabolism.

In order to evaluate transformations of the target chlorinated organics, they were added to the injection 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 as shown in Figure 2 for the third year's experimental results.

The steady-state transformations observed during the third year's field work (Table 3), quantified by normalization to the bromide fractional breakthrough, were as follows: TCE, 29%; cis-DCE, 33 to 45%; trans-DCI to 90%; and VC, 90 to 95%. Of values cited, the lower end of the r represents the nearest observation (1 m distant, 8 hr residence ti whereas the upper end of the r represents more distant observ points with longer residence times (2 m; 16 to 27 hr). A chlorinated al present as a background contami 1,1,1-trichloroethane (TCA), was degraded to any appreciable exter analysis of water samples during a biotransformation of trans-DCE pro evidence of an intermed transformation product identifie laboratory studies to be the epoxi trans-DCE, which was present in am equivalent to a few percent of the p compound. No other interme products were detected.

Termination of the methane feec followed by cessation of transform activity on approximately the same scale as that of organic trans suggesting that the microbial popu remained active in the absenc methane for only a short time b ceasing to transform the target o compounds. These results differ some of the laboratory evidence, suggests continued activity for l periods in the absence of methane.

The concentration oscillatio response to the alternate pulsi methane and oxygen did ma definite signs of methane inhibition: examination of the concentr variations showed that the or compounds were transformed mor when the methane concentratio lower.

Employing peroxide as a mea increasing the electron acceptor permitted operating at a higher r methane feed for increased biol growth, but did not enhance the r transformation of the target o compounds.

Transient experiments in formate and methanol were subs for methane, showed that me inhibition effects could be overcon higher transformation rates cou achieved temporarily, i.e., for s days.

Overall, the field results confirm existence of a natural commur methane oxidizers that cou stimulated by introducing metha oxygen, demonstrated that quan comparisons could confirm the e

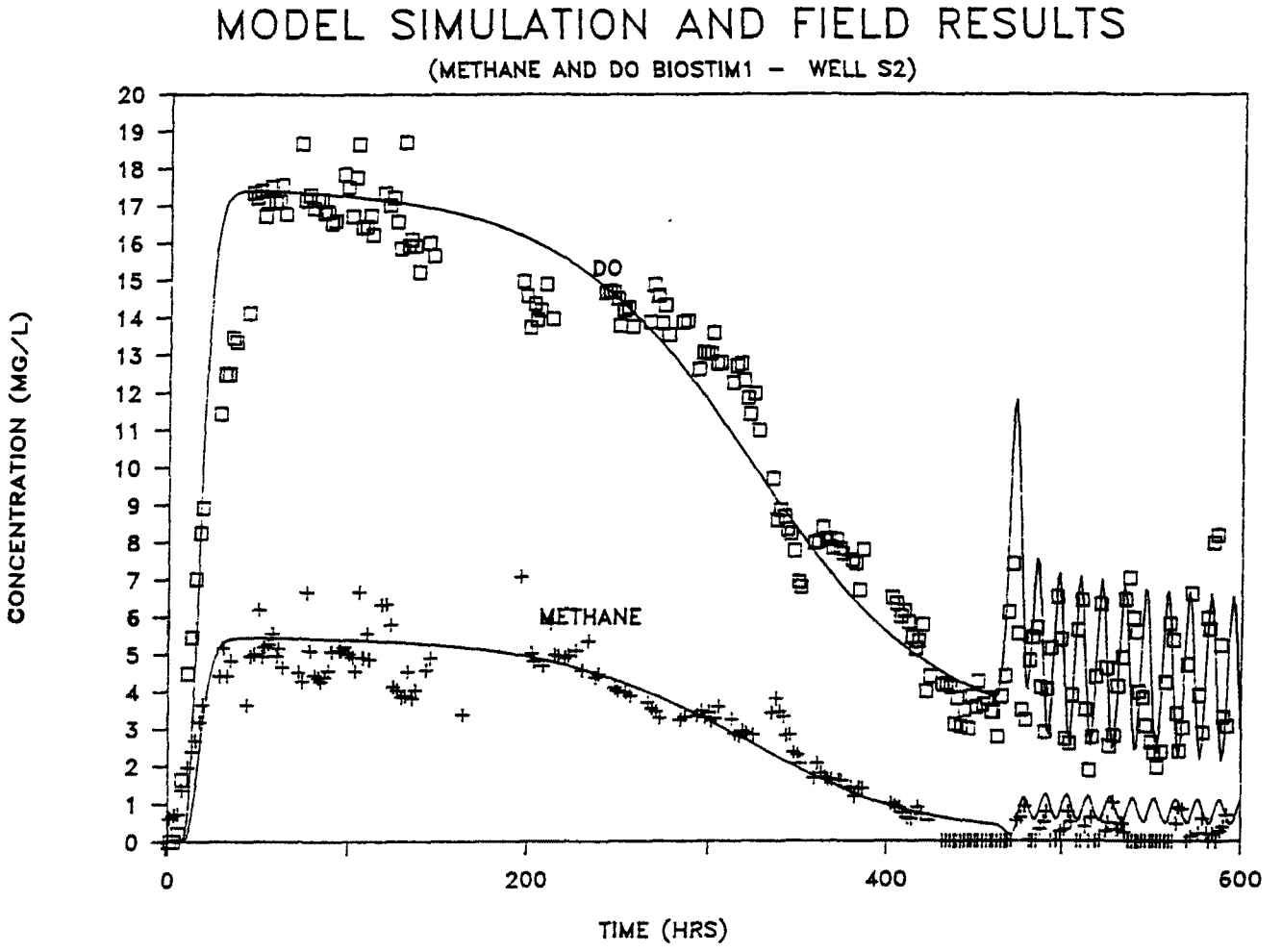

Figure 1. *Observed methane (+) and DO (□) responses at the S2 well due to biostimulation of methanotrophs in the first season of field testing and corresponding model simulations (solid lines). Four-hour and eight-hour alternate pulses of methane and DO were started at 454 hrs.*

transformation within five percent, and showed that substantial transformation of TCE, cis- and trans-DCE, and VC occurred within a distance of a few meters and residence times on the order of a few days.

Mathematical Modeling

A non-steady-state model 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, halogenated aliphatic transformation, and competitive inhibition. 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. Only the initial biomass of methane-utilizing bacteria was allowed to vary as an unconstrained fitting parameter.

The model was able to simulate the dynamic behavior of the biostimulated system very closely (Figure 1). The observed transient responses of the target organic compounds also were closely matched by the model simulations (Figure 2), using rate parameters (Table 4) that were consistent with the values inferred from rate experiments conducted in the laboratory. The transformation rate parameter values suggest that vinyl chloride and trans-DCE were transformed about as rapidly as methane, whereas cis-DCE and TCE were transformed one and two orders of magnitude less rapidly, respectively. Model simulations of the effects of competitive inhibition and rate-limited sorption-desorption also agreed well with the observed dynamic behavior in response to the pulsed injection of methane and oxygen, showing substantial attenuation of the organic solute concentrations due to both these processes.

Conclusions and Recommendations

This project demonstrated conclusively the efficacy of enhanced in-situ biotransformation of chlorinated

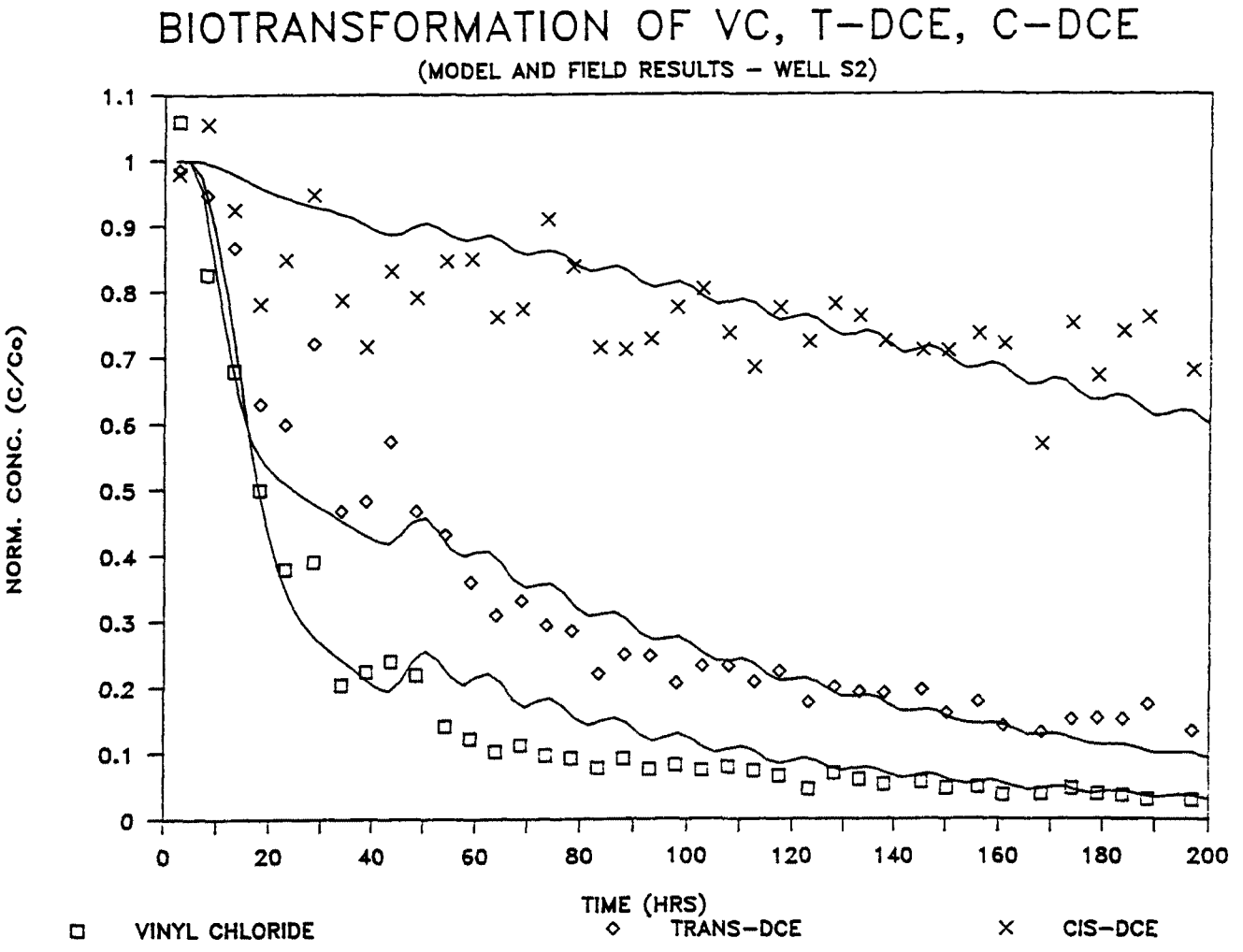

Figure 2. *The biotransformation response of vinyl chloride, trans-DCE, and cis-DCE to biostimulation in the third test year. Model simulations include the processes of growth, competitive inhibition transformation of kinetics, and rate-limited sorption-desorption of the chlorinated organics.*

Table 3. *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.

alkenes by microbial communities comprising methanotrophic and heterotrophic bacteria. It proved easy to stimulate the growth of the native population of methanotrophic bacteria by providing oxygen and methane in the proper amounts. Once stimulated, the mixed methane-grown communities metabolized the target chlorinated compounds at rates that ranged from moderately rapid (one to two orders of magnitude less than the primary substrate) to very rapid (same order as the primary substrate). The transformations appeared to progress completely to stable, harmless end products, for the most part, although in one case a transitory intermediate product was identified.

Incorporating experimental controls and quantitative mass balances to the extent possible is essential for meaningful experimentation, in the field as in the laboratory. Strong dyn forcing is helpful in stimulating po characteristic responses that ai identifying mechanisms and in te hypotheses and mathematical mo Moreover, the laboratory research field work reinforced one another t extent that the results and conclu were consonant, and hence perm stronger statements regarding governing mechanisms and rel processes than otherwise would been possible. This kind of syne expressed itself throughout the reported here, as the overall picture one of general agreement betwee results of the field and the labor work. The combination of f laboratory, and modeling studies o kind can provide a reliable engine

Table 4. Model Parameters for Simulation of Chlorinated Organics in Biostim3 (Figure 2)

Compound	K_d (l/mg)	α (d^{-1})	k (d^{-1})	K_s (mg/l)	k/K_s (l/mg-d)
Methane	0.0	0.00	2.0	1.0	2.0
VC	0.40	0.33	2.0	2.0	1.0
trans-DCE	1.60	0.33	2.0	1.0	2.0
cis-DCE	1.90	0.33	0.10	1.0	0.1
TCE	2.25	0.33	0.025	1.0	0.025

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].

scientific basis for evaluating and designing in-situ biorestoration measures.

This innovative biorestoration technology, premised on the ability of methane-oxidizing bacteria to cometabolize targeted chlorinated compounds as secondary substrates, merits full consideration for application to real aquifer remediation cases. This technology should be considered as an alternative where the contamination consists in large part of the compounds for which methanotrophic transformation has been shown effective in the demonstration phase of the present work: namely, VC, trans- and cis-DCE, and TCE.

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The complete report, entitled "In-Situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria," (Order No. PB 89-219 992; Cost: $28.95, subject to change) will be available only from:
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