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Bioremediation of unsaturated soils contaminated with chlorinated solvents*

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Abstract

One- and two-carbon chlorinated aliphatic solvents are common environmental contaminants, as a result of the large quantities produced, their widespread use and their mobility in the environment. Chlorinated solvents are regularly identified in unsaturated soils, saturated soils, ground water and landfill leachates. This research focused on *in situ* bioremediation as one possible treatment process for unsaturated soil contaminated with chlorinated solvents. Biodegradation of chlorinated solvents in unsaturated soils is feasible through the stimulation of indigenous methanotrophic organisms by the addition of methane and oxygen. At the concentrations studied on our experiments bioremediation would require weeks to several months to accomplish. The experiments also provided a clear indication of toxicity effects as the concentration of chlorinated hydrocarbons becomes too large. Likewise, extended absence of methane from the soil environment greatly reduced microbial activity.

Introduction

One- and two-carbon chlorinated aliphatic solvents are common environmental contaminants, as a result of the large quantities produced, their widespread use and their mobility in the environment. Chlorinated solvents are regularly identified in unsaturated soils, saturated soils, ground water and landfill leachates. This research focused on *in situ* bioremediation as one possible treatment process for unsaturated soil contaminated with chlorinated solvents.

A number of recent studies [1–6] have shown that methylotrophic bacteria can degrade chlorinated solvents. Methylotrophs are aerobic organisms that grow on reduced carbon compounds containing one or more carbon atoms, but no carbon–carbon bonds. Methane is a prime example of such a substrate, and methylotrophs that grow on methane are known as methanotrophs. Methanotrophs are ubiquitous in both aquatic and soil environments because of the widespread occurrence of methane [7]. Therefore, *in situ* bioremediation may

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be possible by adding sufficient methane and oxygen to the soil to stimulate growth of the indigenous organisms. For unsaturated soil, provision of methane and oxygen would be possible through a series of injection and withdraw wells.

Provision of methane is essential because, although methanotrophs can degrade chlorinated solvents, the chlorinated solvents do not serve as a carbon and energy source for the organisms. Furthermore, the chlorinated solvents do not induce the production of the enzyme necessary for their degradation. The degradation of chlorinated solvents essentially is fortuitous with the organism gaining little or no benefit. This phenomenon is known as cometabolism and is mediated by nonspecific enzymes. In methanotrophs, methane monooxygenase, the enzyme that catalyzes the oxidation of methane to methanol, frequently is nonspecific and is capable of catalyzing the oxidation of a variety of other chemicals in addition to methane. Thus, addition of methane stimulates the growth of methanotrophs and the production of the enzyme necessary for the oxidation of chlorinated solvents.

With a few exceptions, studies of chlorinated solvent biodegradation by methanotrophs have been conducted in aqueous or saturated soil environments. Wilson and Wilson [6] reported the biodegradation of TCE in an unsaturated, sandy soil after acclimating the soil organisms for 3 weeks to a stream of air containing 0.6% by volume natural gas. Strand and Shippert [4] achieved significant biodegradation of chloroform in batch studies on sandy soil that had been exposed to natural gas and air for 3 to 8 weeks. These two studies established that biodegradation of chlorinated solvents in unsaturated soils is possible. Speitel and Closmann [8,9] expanded upon this previous work to examine three chlorinated solvents, trichloroethylene (TCE), chloroform and 1,2-dichloroethane (DCA); several different soil types and the effect of nutrient availability. The main objective of the work was the measurement of biodegradation rates and characterization of factors affecting the rate, as the next step in assessing the feasibility of *in situ* bioremediation of unsaturated soils. Batch kinetic experiments were conducted in small vials to study biodegradation in three unsaturated soils in which indigenous methanotrophs had been stimulated by extended exposure to a 1 vol.% methane in air atmosphere.

The experimental results are summarized in Table 1, which reports the zero-order conversion rates to CO_2. These are the most conservative estimates of degradation rates, as they only account for complete mineralization of the parent chlorinated solvent. Disappearance of the chlorinated solvent through conversion to nonvolatile products, however, was significant in several experiments. Of the three chemicals, DCA clearly was the most biodegradable and chloroform the least biodegradable.

The experiments demonstrated that methanotrophs capable of chlorinated solvent degradation are widespread in the environment; however, the rates of degradation observed in some experiments were relatively slow, suggesting that

TABLE 1

Summary of batch kinetic experiments [9]

Exp.	Soil type	Chemical	Nutrient addition (Y/N)[a]	Initial solvent (μg/g soil)	Mineralization[b] rate (μg/g-day)
1	Sandy clay	DCA	Y	60	1.31
2	Sandy clay	DCA	N	60	0.04
3	Sand	DCA	Y	60	0.83
4	Silty loam	DCA	Y	60	0.22
5	Sandy clay	TCE	Y	7	0.35
6	Sandy clay	Chloroform	Y	100	0.08

[a]Y = yes; N = no.
[b]Zero order rate of conversion to CO_2 (μg solvent/g dry soil-day).

bioremediation could require a period of years, instead of months, in some cases. Two steps may be appropriate to address slow rates. The first is to increase the methane concentration in the air passing through the soil. In this research 1% methane was used to stimulate growth of indigenous organisms; however, recent work in aqueous systems indicated that higher concentrations of 4 to 20% methane may provide better results [10]. Concentrations above 5% methane probably should not be applied continuously, however, to avoid enzyme competition between methane and the chlorinated solvents. A second way to improve rates is through nutrient addition, as illustrated by a comparison of the rates between experiments Exp. 1 and Exp. 2 in Table 1 for the nutrient-poor sandy clay. Although nitrogen was added in this research, many methanotrophs are able to fix nitrogen concurrently [7]. Therefore, application of phosphorus alone may be sufficient, which would eliminate the possibility of nitrogen contamination of underlying ground water.

The research reported here extends the batch kinetic work to laboratory-scale soil columns. Concentration, type of chlorinated solvent and availability of methane were tested for their effect on the degradation rate of chlorinated solvents. Also, comparisons were made between measured degradation rates in the soil columns versus the batch experiments.

Materials and methods

Recirculating batch reactors

The degradation of chlorinated solvents was studied using two recirculating batch reactors containing soil with indigenous methanotrophic bacteria. Each recirculating batch reactor consisted of a glass column (3 feet by 2 inches I.D. or 90×5 cm) to contain the soil and a capped 45-liter glass reservoir with a valved sampling port. Each reactor also contained a recirculation pump, flow meter, and the necessary tubing to regulate the gas flow. During experiments,

gas was continuously recirculated through the closed system. One reactor received methane to stimulate microbial growth, while the other served as a control, receiving no methane.

Methane was added to one of the reactors as natural gas until gas analysis showed the mole fraction of the methane to be approximately 4%. The chlorinated solvent, DCA or TCE, was added to both reactors by direct injection through the sampling port. The amount of the chlorinated solvent to be added was determined by establishing either a target gas concentration or a desired soil loading. Soil adsorption isotherms were used to determine the total amount of solvent required.

Experimental procedure

Both gas and soil phase contaminant concentrations were measured in the experiments to verify biodegradation and to calculate degradation rates. The concentration of the constituent gases (carbon dioxide, oxygen, and methane) in the reactors was also monitored. Gas phase sampling typically occurred three times a week, while the soil was sampled at the conclusion of each experiment.

Gas samples were taken from the sampling port in the reservoir. A 1-mL or 0.25-mL gas-tight syringe with an 8-inch side port needle was used to take the gas samples. The samples were injected into a gas chromatograph equipped with megabore column and flame ionization detector. The constituent gases were analyzed on a gas partitioner equipped with a thermal conductivity detector. Soil samples were collected directly from the columns through built-in sampling ports. The soil was extracted with methanol, and the extract was analyzed on a gas chromatograph equipped with a megabore column and an electron capture detector. The experiments typically continued until a minimum of 50% of the chlorinated solvent disappeared in the reactor that received methane.

The microbial populations of the soil columns were determined by an acridine orange direct count (AODC). The acridine orange solution stained the microorganisms in the sample, which were then visible with an epifluorescence microscope. The microorganisms in twenty representative grids in each sample were counted. The AODC counts were correlated to standard plate counts performed on samples that had been cultured in a 10 vol.% methane atmosphere.

Experimental conditions

The experimental conditions for the microbially active reactor are summarized in Table 2 for each of the five experiments with DCA. The experimental conditions for the control reactor were similar to those for the active reactor. The initial chlorinated solvent loading on the soil was increased in each experiment to determine the effect of soil loading on microbial degradation.

The first experiment was conducted with an initial soil loading of 2.0 μg/g

TABLE 2

Initial conditions for dichloroethane (DCA) experiments

Experiment	Duration (weeks)	DCA (μg/g soil)	DCA (μg/L air)	Methane (vol.%)
1	2	2.0	500	3.37
2	3	4.4	1,110	4.28
3	3	61	15,300	3.93
4	2	61	15,300	0.0
5	2	613	153,000	3.98

DCA, corresponding to a gas concentration of 500 μg/L at equilibrium. The initial soil loading was approximately 2% of the primary loading rate used in the previous work [9], shown in Table 1. The amount of DCA added was sufficient to allow both the testing of the procedures and the measurement of gas contaminant concentrations, while not overloading the microbial population. The initial soil loading was increased to 4.4 μg/g for the second experiment to determine the effect of increased concentration on degradation. In the third experiment, the initial soil loading was increased by about a factor of ten to 61 μg/g to more closely match loadings used in previous work.

The fourth experiment was conducted to study the effect of the absence of methane on the degradation of DCA. The initial soil loading, 61 μg/g, was identical to that of the third experiment. The initial soil loading of DCA was increased by a factor of ten again for the fifth experiment to 613 μg/g. Methane was reintroduced to the reactor to study the recovery of the methanotrophic population in the column.

Results and discussion

Typical profiles of gas phase concentration of the chlorinated solvent are shown in Fig. 1 for both reactors in Exp. 3. DCA losses occurred in the control reactor, but DCA disappeared to a much greater extent in the reactor receiving methane. DCA losses in the control reactor might be attributable to both abiotic and biotic processes, since the soil was not sterilized. The corresponding profiles of methane and carbon dioxide are shown in Fig. 2. The reactor that received methane showed a steady decrease in methane and increase in carbon dioxide concentration (mol.%) throughout the experiment, which indicates the presence of an active population of methanotrophs. Although not shown, the oxygen concentration likewise decreased over time. The control reactor showed no production of carbon dioxide during the experiment, as would be expected in the absence of any significant source of substrate for the organisms.

Performance of the reactors receiving methane is presented for all five DCA

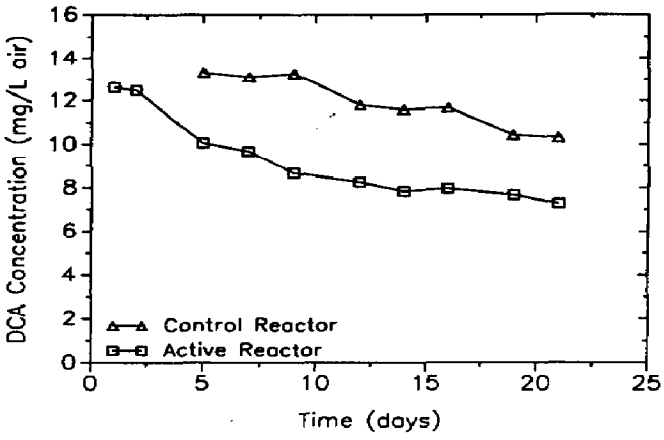

Fig. 1. Gas phase concentrations of DCA in Exp. 3.

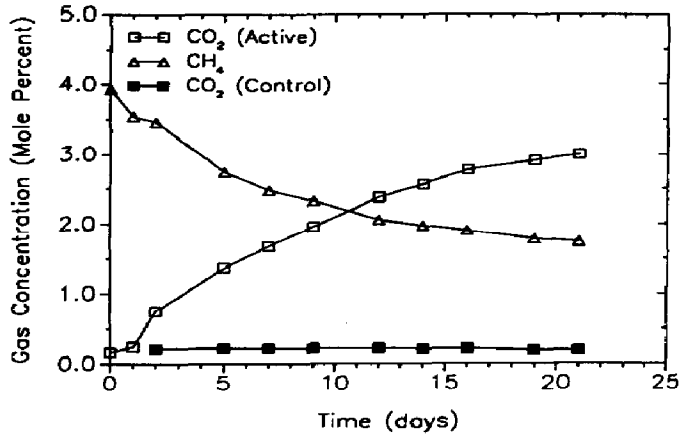

Fig. 2. Methane and carbon dioxide concentrations in DCA Exp. 3.

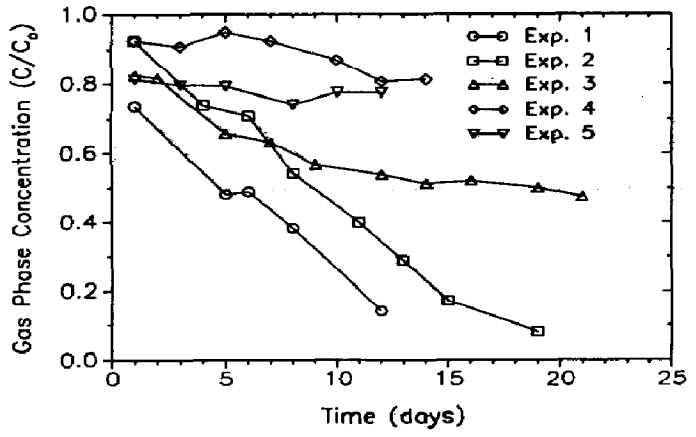

Fig. 3. Gas phase concentrations in microbially active reactor for all DCA experiments.

experiments in Fig. 3, which shows the fraction of DCA remaining over time. Several observation can be made directly from the figure. In comparing experiments 3 and 4, which are virtually identical except for the absence of methane in Exp. 4, the important role of methane in the degradation of DCA becomes obvious. The degradation rate in Exp. 4 was substantially smaller than in Exp. 3, which suggests that methane is needed, if not constantly, at least on a fairly regular basis to maintain maximum microbial activity. In Exp. 5, essentially no degradation of DCA was observed, nor did any degradation of methane occur. The results suggest that the elevated concentration of DCA in Exp. 5 was toxic to the methanotrophs.

The concentration data from Fig. 3 and the corresponding soil loadings, as calculated from the adsorption isotherm, were analyzed by linear regression analysis to estimate biodegradation rate constants. Both zero and first order rate constants were calculated, because the order of the reaction was unclear. Similar analyses also were performed on the data from the control reactor.

The zero order rate constants were estimated by a linear regression on total mass of chlorinated solvent in the reactor versus time. The total mass includes that adsorbed to the soil and that present in the gas phase. This total mass was subsequently normalized by dividing by the mass of soil to provide a rate constant of practical use, μg chlorinated solvent/g soil-day.

First order rate constants were estimated by assuming that degradation was proportional to the chlorinated solvent loading on the soil. Furthermore, instantaneous equilibrium between the gas and soil phases was assumed. This equilibrium was described by a linear adsorption isotherm, which was experimentally measured. Estimation of the rate constant must account for resupply of substrate from the gas phase; therefore, the rate equation becomes:

$$(1 + V_g/WK)\ dq/dt = -k_1\ q$$

where V_g is the volume of the gas phase (L), W is the mass of soil (g), K is the adsorption isotherm constant (μg-L/g-μg), q is the soil loading (μg/g), t is time (days) and k_1 is the first order rate constant (day^{-1}).

The rate constant attributable to methanotrophic activity was taken as the difference between that of the control reactor and that of the reactor receiving methane. This provides the most conservative estimate of the rate constants. The rate constants for each reactor and the difference between the two are tabulated in Table 3. Both the zero and first order rate constants for experiments 4 and 5 were larger in the control reactor than in the active reactor, supporting the observations from Fig. 3 that little degradation occurred through methanotrophic activity in these experiments. The rate constants for experiments 1 and 2 were approximately equal for both the zero order and first order kinetic analyses. In Exp. 3, which was run at a much larger concentration, the zero order rate constant was larger than observed in experiments 1 and 2, while the first order rate constant was smaller. The increase in the zero order rate

TABLE 3

Rate constants for dichloroethane (DCA) experiments

Experiment No.	Zero order (μg/g soil-day)			First order (day^{-1})		
	Methane	Control	Difference	Methane	Control	Difference
1	0.75	0.35	0.40	1.04	0.272	0.768
2	1.55	1.06	0.49	0.957	0.240	0.717
3	7.37	5.78	1.59	0.192	0.122	0.070
4	4.50	7.74		0.0844	0.161	
5	16.2	55.1		0.0334	0.131	

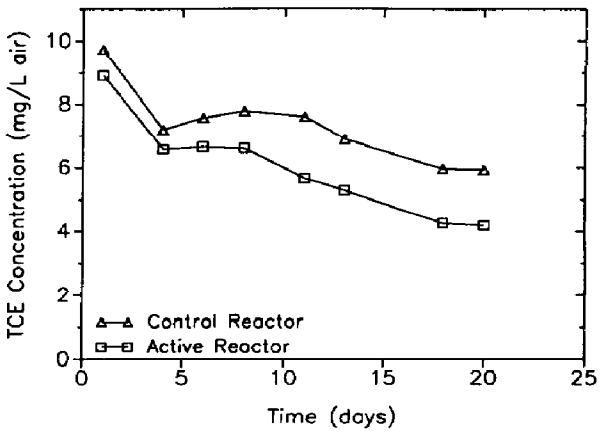

Fig. 4. Gas phase concentrations in TCE experiment.

constant with increasing concentration suggests that the kinetics are not zero order over a broad range of concentration, even though the zero order model fit individual experiments as well as the first order model. The decrease in the first order rate constant with increasing concentration suggests that DCA was inhibiting microbial activity to some extent. Inhibition and toxicity effects are expected from the chlorinated solvents at sufficiently high concentrations and have been observed most widely with TCE.

The zero order rate constant from Exp. 3 is quite similar to that measured previously at the same concentration in small-scale batch tests (Table 1, Exp. 1). As an example, the rate constants measured in Experiment 3 imply that 35 to 40 days would be required for 95% degradation of the DCA, once the indigenous methanotrophs were stimulated with methane. At the smaller concentrations of Experiments 1 and 2, 95% degradation would occur within the order of a week. Thus, as concentration increases, a longer time will be needed for complete bioremediation. If the concentration is too large, however, toxicity effects may drastically reduce the potential for bioremediation. In such situa-

tions, a two-step treatment may be needed in which soil vapor extraction is employed initially to reduce concentrations to levels that are amenable to biodegradation.

The results of the first experiment with TCE are shown in Fig. 4. The initial soil loading was 4 μg/g, and the resulting rate constants were 1.76 μg/g-day^{-1} for zero order kinetics and 0.884 day^{-1} for first order kinetics. The rate constants are larger than those measured for comparable initial loadings of DCA. As shown in Table 1, earlier small-scale batch experiments yielded a significantly smaller zero order rate constant at a larger concentration. Again, this may be indicative of an inhibitory effect as concentration increases.

Conclusions

These experiments provide a further indication that biodegradation of chlorinated solvents in unsaturated soils is feasible through the stimulation of indigenous methanotrophic organisms by the addition of methane and oxygen. At the concentrations studied in these experiments bioremediation would require weeks to several months to accomplish. However, the experiments also provide a clear indication of toxicity effects as the concentration of chlorinated hydrocarbons becomes too large. Likewise, extended absence of methane from the soil environment greatly reduced microbial activity.

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