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Scale-up issues for *in situ* anaerobic tetrachloroethene bioremediation

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For the full scale implementation of *in situ* anaerobic bioremediation of tetrachloroethene (PCE) in groundwater, the following issues must be addressed: which organic substrates at which concentration would be most effective in promoting dechlorination and are economical; how far the substrate, electron acceptor, and nutrients can be transported in the aquifer; and the placement of delivery and recovery wells for distributing these amendments. In a microcosm study, almost all of the tested inexpensive substrates supported reductive dechlorination of PCE through vinyl chloride (VC) under methanogenic conditions. A minimum of about 60 mg L^{-1} of organic carbon was needed to dechlorinate 23 μM PCE with a single feeding. In a second microcosm study dechlorination stopped at 1,2-dichloroethene (DCE) in microcosms fed higher concentrations of several substrates. At the highest concentrations the substrates inhibited DCE production. Three field tracer tests were conducted to evaluate methods to distribute the amendments across the aquifer. The natural groundwater gradient is not sufficient to distribute substrate evenly. Groundwater injection at 60 times the natural flux rate increased the distribution of substrate. A mixing strategy of cross-gradient injection further increased the distribution of the substrate. Ammonia-nitrogen, sulfate, and phosphate were retarded relative to the substrate and inorganic tracer.

Keywords: tetrachloroethene; PCE; *in situ* bioremediation; anaerobic; reductive dechlorination

Introduction

Scale-up of *in situ* anaerobic bioremediation for chlorinated ethene-contaminated aquifers is being considered [3,4,19]. The complete reductive dechlorination of tetrachloroethene (PCE) to trichloroethene (TCE), dichloroethene, vinyl chloride, and finally ethene has been demonstrated in the field for the West Landfill of the DuPont Victoria, Texas Plant during an initial pilot project [3]. Sodium benzoate at a concentration of 38 mg L^{-1} was used as the substrate to promote the growth of sulfate-reducing and methanogenic bacteria and to support the breakdown of the chlorinated ethenes.

A number of organic substrates have been used in the laboratory to promote anaerobic dechlorination of PCE. The optimal substrate to support dechlorination may be site-dependent, as no universally applicable substrate has been yet identified. Complex substrates such as yeast extract, molasses, or corn steep liquor will promote growth of fermentative, sulfate-reducing, acetogenic, and methanogenic bacteria as these substrates are reduced to simpler compounds [16]. Yeast extract generally provided higher rates of PCE conversion to DCE than benzoate or acetate in the six sites investigated by Odom *et al* [16]. Complex substrates may also provide trace minerals and vitamins that may be important for the dechlorinating bacteria [7,13]. The drawback to using these complex substrates is that they foster the growth of a wide range of bacteria, but only a small fraction of the population that develops may be capable of carrying out the dechlorination reactions.

Catabolic intermediates from the degradation of complex substrates such as benzoate, lactate, and butyrate will likely promote the growth of a narrower subset of the population [16]. Of six substrates screened by Haston *et al* [12], benzoate gave the most extensive conversion of PCE to vinyl chloride (VC) and ethene during a 6-week incubation of soil from a St Joseph, MI site. At high concentrations, benzoate can also be used as a microbial inhibitor to control growth and potential plugging near an injection well [15]. Gibson *et al* [11] linked butyrate oxidation to PCE dechlorination when subsurface soil microcosms were fed a mixture of fatty acids. Of the substrates evaluated by Fennell *et al* [7], butyrate sustained dechlorination the best, possibly because butyrate may serve as a slow release form of hydrogen that allows the dechlorinators to more effectively compete for available hydrogen. However, the butyrate-fed culture required addition of a vitamin solution to overcome an apparent nutrient limitation.

Alcohols such as ethanol and methanol also support dechlorination in some laboratory studies. Freedman and Gossett [8] isolated a high-rate PCE dechlorinating culture on methanol. This culture could degrade concentrations of PCE as high as 550 μM at a maximum rate of 4.6 μmol of PCE transformed per mg of volatile suspended solids per day [20]. Haston *et al* [12] found incomplete dechlorination in 6 weeks with methanol and ethanol using soils from the St Joseph, MI site. Gibson and Sewell [10] found rapid dechlorination with ethanol, but little PCE dechlorination with methanol or isopropanol in incubations of Traverse City, MI soil with these substrates. Alcohols offer the advantages of being relatively inexpensive and are easy to

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deliver because of their high solubility in water. However, methanol addition may be prohibited because of regulatory concerns over its potential for human toxicity at relatively low levels.

The last class of substrates that should be considered are the final catabolic intermediates such as acetate or hydrogen. Acetate targets a relatively narrow group of sulfate-reducers and methanogens and in some cases has promoted complete dechlorination of PCE [16]. However, both Odom *et al* [16] and Rasmussen *et al* [17] reported that complex substrates gave more complete dechlorination than acetate alone. Hydrogen is one of the key materials needed for dechlorination and may be the ultimate electron donor regardless of the initial substrate. Some studies have shown that hydrogen gas can support dechlorination [5,14], but other laboratory studies with hydrogen gas alone have shown it to support incomplete dechlorination with enrichment cultures, perhaps due to nutrient or carbon limitations [6,17]. Delivery of hydrogen in gaseous form to aquifers is possible, but there would be safety concerns around the use of a potentially explosive gas such as hydrogen, and a carbon source would probably still be required.

Another issue for scale-up of *in situ* anaerobic bioremediation is which electron-accepting process will provide the most rapid and complete dechlorination of the chlorinated solvents. Several investigators [2,12,18] have shown that concentrations of sulfate in excess of 2 mM or approximately 200 mg L^{-1} may inhibit the complete dechlorination of PCE. Numerous methanogenic cultures have been found with the ability to completely dechlorinate PCE [5,8,14,17,19].

The mechanism for delivery of the substrate, nutrients, and electron acceptor to the subsurface is another important issue for scale-up of the *in situ* anaerobic bioremediation of PCE. The number and placement of injection and recovery wells will be controlled by the amount of mixing that can be achieved in the aquifer to distribute the amendments across the contaminant plume. The dispersivity of the aquifer, or the amount of spreading that occurs as a result of inhomogeneities in the aquifer, is a key measure of the mixing that will occur in the aquifer. Dispersivity is a function of the fluid velocity. Delivery can be accomplished with a barrier system that intercepts the flow of groundwater or with a recirculation system that captures and then treats the plume. Amendments are added to the barrier system and the groundwater is treated as it is transported downgradient. A barrier system using the natural groundwater gradient to carry the amendments may require fewer wells and will minimize the costs for pumping water than an induced gradient system. However, a barrier system using the natural groundwater gradient will be slower to deliver the amendments than an induced gradient system. The barrier system with the natural groundwater gradient relies on native dispersivity of the aquifer and will not be as efficient in delivery of amendments evenly across the injection plane as an induced gradient system where more mixing can be achieved. A barrier system can also be operated with recirculation to give more mixing. If the rate of dechlorination is slow or the microbes require a high dosage of substrate, additions of high concentrations of substrate may be necessary in a barrier system. In a recirculation system, the treatment time for the contaminated groundwater can be easily adjusted, lower quantities of substrate added over time, and the groundwater flow rates increased with an induced gradient. Typically, a recirculation system will require a longer time to treat a plume than a barrier system. The recirculation system may be easier to control and monitor than a barrier system.

As substrate is delivered to the aquifer, microbial growth around the injection points may lead to plugging and a subsequent decrease in the flow rate of the material injected [4]. One strategy to avoid plugging problems is to inject high concentrations of an inhibitory substrate, for example sodium benzoate [15]. The inhibitory substrate is diluted to levels that can be used by the microbes further out in the aquifer. Another method to avoid biofouling is to deliver alternately substrate, electron acceptors, and nutrients so that conditions for optimal growth of the microbes are not realized until the substrates, nutrients, and electron acceptors mix in the aquifer away from the point of injection [3]. Reduced substrate loadings and increased pumping rates may minimize or delay aquifer biofouling [21]. Once well plugging has occurred, the wells can be treated with acid to remove mineral precipitates or with biocides such as sodium hypochlorite (bleach) to control microbial growth.

In this work, laboratory studies using microcosms were performed to evaluate cost-effective substrates and substrate requirements for dechlorination of PCE. Field tracer studies were performed to evaluate delivery mechanisms to allow scale-up of the anaerobic bioremediation process.

Site characteristics

Figure 1 shows a cross section of the aquifer at the Victoria site. The West Landfill is primarily in a surficial clay unit and extends into the A sand. The A sand is generally unsaturated, but can contain perched groundwater. A discontinuous clay unit known as the A clay lies beneath portions of the A sand. A continuous clay layer, designated as the intermediate clay, separates the A unit from the B sand, the primary aquifer at the site. The B sand is upwardly fining and extends from 22 m below land surface to a depth of about 32 m. The B sand is underlain by a low permeability clay layer. The upper portion of the B sand aquifer is a medium-grained sand with a hydraulic conductivity of 9.9×10^{-3} cm s^{-1}. The middle portion is slightly more permeable with a hydraulic conductivity of

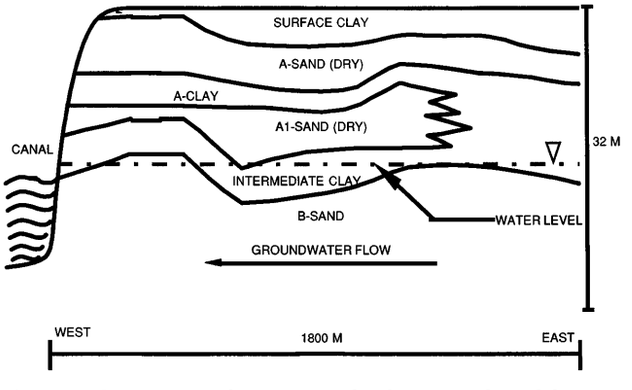

Figure 1 Cross-section of Victoria aquifer showing geological features.

1.4×10^{-2} cm s^{-1}. The lower portion of the aquifer contains coarse sand and gravel and has a hydraulic conductivity of 7.1×10^{-2} cm s^{-1}. The B sand is a confined aquifer with the hydrostatic surface located 21 m below land surface. Groundwater flow is from east to west toward a man-made canal. Groundwater moves at approximately 0.3 m per day. The aquifer matrix has a low total organic matter content of 0.1% (range 0.07–0.71%) and contains between 6 and 30% by weight clay, primarily smectite.

A pump and treat system has been in operation at the West Landfill for about 15 years. It controls movement of contaminated groundwater to the canal. A clay cap has also been installed over the landfill to limit infiltration.

An array of wells was installed for the tracer tests into the B sand aquifer including a row of one injection well and two withdrawal wells and two rows of clustered monitoring wells at three depths (see Figure 2 for well locations). Injection well 221 was flanked by two withdrawal wells, 220 and 222, that were 15 m on either side of the injection well. The injection well and withdrawal wells were constructed of 12.5-cm diameter polyvinyl chloride (PVC) and were screened from 21.7 to 27.8 m below land surface. Bentonite and cement/bentonite seals were placed above the sand pack. A row of three clustered monitoring wells (228, 229, and 230) was installed 8.3 m downgradient of the injection well at 1.7-m intervals perpendicular to the direction of groundwater flow. The clustered monitoring wells were screened at 24.5 to 26 m, 27.5 to 29 m, and 30.5 to 32 m below land surface. To install the clustered monitoring wells, the following procedure was used:

- a 30-cm diameter borehole was drilled to 32 m;
- a 5-cm diameter PVC well with a 1.5-m screen was inserted;
- a sand pack was added followed by a bentonite seal and fine sand to reach the next monitoring interval of 29 m;
- the next monitoring point was placed into the same borehole;
- the procedure was repeated for next monitoring point.

Another row of six clustered monitoring wells was placed 18 m downgradient of the injection well. Monitoring wells 226, 225, 224, and 231 were placed in the middle of the second row at a distance of 3.7 m from each other. Well 227 was installed to the south of well 226 at a distance of 11 m. Well 223 was located 7.4 m to the north of well 231. All wells were developed with the air lift method.

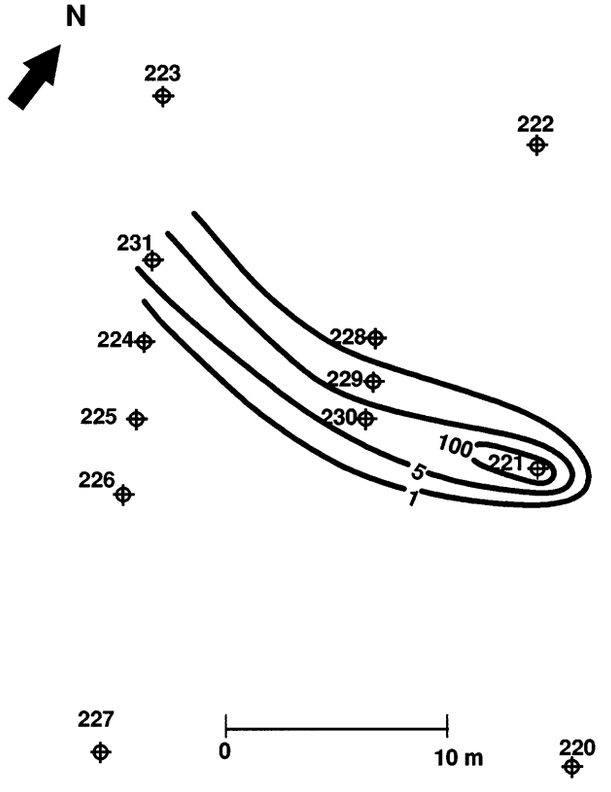

Figure 2 Isocontour interval map of bromide concentrations in tracer test 1 under natural groundwater flux conditions; 900 mg L^{-1} bromide injected at 0.11 L min^{-1} into well 221.

Materials and methods

Microcosms

In the first study involving microcosms, several inexpensive substrates were evaluated for their potential to promote dechlorination and to determine the minimum carbon concentration required. The electron yield for a particular substrate is a better measure of the overall efficiency of dechlorination than carbon content. Unfortunately, molecular formulas and electron yields are not available for these inexpensive substrates such as molasses or yeast extract.

The first study was conducted using single 160- or duplicate 280-ml serum bottles that were subsampled periodically over 56 or 91 days. The microcosms contained 25% by volume soil from the site and 75% groundwater. The soil and groundwater were collected in August 1994, from within the PCE-contaminated plume at a depth of 24–27 m, but in an area of the Victoria West Landfill that was not impacted by the pilot demonstration conducted by Beeman *et al* [3]. The groundwater spiking solution was prepared by saturating 1 L of sterile groundwater with PCE (Aldrich, Milwaukee, WI, USA). The following average concentrations of chlorinated ethenes were found in the initial samples from the microcosms: 23 μM PCE, 0.6 μM TCE, and 3.3 μM 1,2-DCE. TCE and DCE were also present in the non-sterile groundwater used to prepare the microcosms. The groundwater contained 140 mg L^{-1} sulfate. Each substrate-amended microcosm received 25 mg L^{-1} nitrogen from ammonium chloride (Fisher Scientific, Fair Lawn, NJ, USA) and 5 mg L^{-1} phosphorus from disodium phosphate (Fisher Scientific). Controls were prepared without substrate (unamended), autoclaved soil and groundwater (sterile), and a blank with sterile groundwater and no soil. The following substrates were added to the microcosms at organic loadings of between 20 and 400 mg carbon per L (mg C L^{-1}):

- Yeast extract (Difco, Detroit, MI, USA) is a water-soluble extract from yeast that contains a large number of organic materials including proteins, vitamins, and trace minerals. Yeast extract contained 38% carbon.

- A wastewater stream from the Victoria Plant contains formate, acetate, and propionate as well as other fatty acids, but only 4.5% carbon.
- Cheese whey permeate (Ecological Chemical Products Company, Adell, WI, USA) includes waste liquids and solids from the production of cheese. Lactose and proteins are the primary components. The cheese whey permeate used for this test contained 26% soluble carbon.
- Corn steep liquor (AE Staley Manufacturing Company, Decatur, IL, USA) is produced by steeping corn in water. The resulting liquid contained 17% soluble carbon. Corn steep liquor has 47% protein and 26% lactic acid on a dry weight basis.
- Molasses (Imperial Sugar, Sugarland, TX, USA) is a byproduct of the production of sugar that contains 36% sucrose, 5% glucose, and 6% fructose plus 0.6% sulfate. Analysis of the molasses determined that it contained 29% soluble carbon.
- A suspension of chicken manure (University of Delaware Agricultural Department, Newark, DE, USA) was autoclaved and filtered to form a tea; the water-soluble extract contained 3% carbon.

Subsamples were collected from these microcosms over time through Teflon®-faced silicon septa. The microcosms were prepared and incubated inverted in an anaerobic glovebox at ambient temperature, 22°C. Approximately 4 ml of groundwater and 1 g of sediment were removed at each sampling point. The quantity of headspace in the serum bottles increased over time. Any methane, ethene, or chlorinated ethenes that volatilized into the headspace were lost during the sampling procedure and did not accumulate in the bottles.

The objectives of the second study using microcosms were to evaluate dechlorination at high concentrations of substrates and to determine whether there were inhibitory concentrations of substrates that could reduce the amount of microbial growth near the injection point. The second study was set up in 26-ml serum bottles that were completely filled with 50% soil by volume and 50% groundwater. The soil and groundwater were collected in December 1994 at a depth of 24–27 m below surface from the same area where the soil was collected for the first study. A saturated solution of PCE in groundwater was added to give an average of 26 μM PCE in the initial samples. The groundwater contained 140 mg L^{-1} sulfate. Each substrate-amended microcosm received 50 mg L^{-1} nitrogen from ammonium chloride and 10 mg L^{-1} phosphorus from disodium phosphate. The microcosms were supplied with substrates at organic loadings of between 200 and 23 000 mg C L^{-1}. The substrates evaluated were: sodium benzoate (DuCoa, Highlands, IL, USA) with 58% carbon, sodium acetate (EM Science, Cherry Hill, NJ, USA) with 29% carbon, molasses (Imperial Sugar, Sugarland, TX, USA) with 29% carbon, and corn steep liquor (AE Staley Manufacturing Company, Decatur, IL, USA) with 17% carbon.

Controls for the second study consisted of an unamended treatment that did not receive any substrate, and a blank treatment with sterile water, but no soil. All bottles were sealed with Teflon®-lined butyl rubber septa and crimp tops. The microcosms were prepared and incubated inverted in an anaerobic glovebox at ambient temperature, 22°C. Triplicate samples were killed periodically for up to 98 days.

Analyses conducted on the samples from both microcosm studies were: volatile chlorinated organics by purge and trap gas chromatography/mass spectrometry; dissolved-phase ethene, ethane, and methane by gas chromatography; and total organic carbon (TOC) using a TOC analyzer [16]. Acridine orange direct microbial counts (AODC) were performed on at least one subsample of each replicate using the method described by Ghiorse and Balkwill [9].

Field tracer tests

Three tracer tests were performed to determine: 1) the natural dispersion under existing groundwater flux rates; 2) the induced dispersion from injection at a higher groundwater flow rate; and 3) the dispersion induced by a mixing strategy where groundwater was withdrawn from two outer wells and injected cross-gradient with the tracer into the center well.

The tracer tests were conducted in an area up-gradient of the PCE plume at the West Landfill of the Victoria Plant. The first tracer test used 955 mg L^{-1} sodium bromide as a tracer at a flow rate of 0.11 L min^{-1}. The first tracer test was conducted for 58 days. The second tracer test employed a 400 mg L^{-1} potassium iodide solution injected into well 221 at a flow rate of 6.8 L min^{-1} for 24 days followed by clean water for 116 days. Iodide was used instead of bromide so there would be no confusion in the interpretation of the results of the second tracer test due to residual bromide in the groundwater. For the third tracer test, groundwater was recovered at 3.8 L min^{-1} from wells 220 and 222 and the recovered groundwater injected along with 3.8 L min^{-1} of a tracer solution of 900 mg L^{-1} bromide, 206 mg L^{-1} TOC, 81 mg L^{-1} sulfate, 12.8 mg L^{-1} phosphate, and 4.3 mg L^{-1} ammonia-nitrogen into well 221. The tracer solution was prepared with sodium bromide, sodium benzoate, ammonium sulfate, and dipotassium phosphate. Although a total of 11.4 L min^{-1} was injected for the third tracer test; the net addition of water to the aquifer was only 3.8 L min^{-1}. The third tracer test was conducted for 146 days. Bromide, iodide, ammonia-nitrogen, sulfate, and phosphate analyses were performed by ion chromatography according to Standard Method 4110B [1]. The TOC was analyzed according to EPA Method 415.1 protocols [22].

Results

Microcosms

The initial TOC, the percent of the initial TOC removed, the maximum dissolved phase methane concentration detected, the percentage of the initial chlorinated ethenes degraded to the final dechlorination endproduct, and the maximum AODC counts for the first microcosm study are presented in Table 1. The first study evaluated different organic loadings of the unamended, control, yeast extract, Victoria wastewater, cheese whey permeate, molasses, corn steep liquor, and manure tea. Some treatments were sampled over 56 days while others were sampled over 91 days. The TOC removals were greater than 60% with

Table 1 Summary of first microcosm study results

Treatment	Days	Initial TOC (mg L^{-1})	% TOC removed	Maximum methane (mg L^{-1})	Final daughter product[a] and % initial CE[b]	Maximum AODC (c g^{-1}) $\times 10^7$
Sterile control	91	23	35	0.0	DCE 1	NA[c]
Unamended	91	18	33	1.5	DCE 1	2.9
Blank	91	44	88	0.0	DCE 2	NA
Yeast extract	56	23	74	0.1	DCE 8	NA
	56	34	68	0.1	DCE 8	NA
	56	46	72	0.3	VC 4	NA
	56	83	81	1.6	VC 19	NA
	56	330	77	27.0	VC 24	NA
	91	373	94	18.0	VC 2	24
Victoria wastewater	56	23	87	0.1	DCE 22	NA
	56	54	78	0.1	DCE 8	NA
	56	76	62	1.4	VC 25	NA
	91	404	81	13.0	VC 23	20
Cheese whey permeate	56	23	48	12.0	DCE 11	NA
	56	43	91	0.1	VC 12	NA
	56	64	80	3.9	VC 23	NA
	56	300	90	6.0	VC 13	34
Molasses	56	22	100	0.1	DCE 10	NA
	56	42	76	0.1	DCE 10	NA
	91	300	90	9.4	VC 1	30
Corn steep liquor	56	23	100	0.8	DCE 12	NA
	56	37	100	0.1	DCE 14	NA
	56	69	77	2.9	VC 24	NA
	91	260	93	4.6	VC 4	12
Manure tea	56	22	100	0.0	DCE 9	NA
	56	37	84	0.1	DCE 9	NA
	56	66	74	1.1	VC 28	NA
	56	210	63	6.9	VC 1	19

[a]Final daughter product detected during study.
[b]CE = Initial chloroethene concentration = PCE + TCE + DCE; VC = vinyl chloride.
[c]NA = not analyzed.

almost all substrates and substrate loadings, indicating that the microbes could readily utilize these concentrations of substrates during the 56 to 91-day incubation period. The pH was not controlled in the microcosms, but generally remained neutral (data not shown). Methane production was elevated at the higher substrate loadings. Less than 1 mg L^{-1} of methane was produced below 83 mg C L^{-1} of yeast extract, 76 mg C L^{-1} for the Victoria wastewater, 64 mg C L^{-1} for cheese whey permeate (the 23 mg C L^{-1} loading also had a high methane production), 240 mg C L^{-1} of molasses, 69 mg C L^{-1} of the corn steep liquor, and 66 mg C L^{-1} for the manure tea. One of the intermediate loadings for molasses was inadvertently omitted from the study. Partial dechlorination to DCE was generally found in the treatments with the low substrate loadings, but no vinyl chloride was produced. Organic loadings of all substrates in excess of 60 mg L^{-1} supported dechlorination to vinyl chloride. Almost all of the organic loadings that resulted in methane accumulations in excess of 1 mg L^{-1} also supported dechlorination of PCE to TCE, DCE, and VC. At the highest substrate loadings, VC was first produced with corn steep liquor and yeast extract followed by the Victoria wastewater, cheese whey permeate, and molasses, and then the manure tea (Figure 3). Relatively little VC was detected with the manure tea and molasses treatments either because degradation of VC was rapid or little VC was produced. No VC was produced in the unamended, autoclaved controls, or blank treatments. The VC concentrations fell with all substrates. Ethene was not detected in these studies; the failure to detect ethene may be due to the volatility of ethene or the parent compounds into the headspace or the relatively high detection limit for ethene of 2.2 μM in the dissolved phase.

Direct microbial counts were lowest in the unamended treatment (Table 1), with a maximum of 2.9×10^7 counts per g soil (c g^{-1}). The highest loadings of the substrates yielded maximum microbial counts of between 1.2 to 3.4×10^8 c g^{-1}. For the highest organic loadings of the yeast extract, cheese whey permeate, corn steep liquor, and molasses treatments, the highest microbial counts were produced within the first 4 weeks, and AODC counts declined thereafter (data not shown). The decline in AODC counts was presumably due to death of the cells. The Victoria wastewater and manure tea treatments supported a slower microbial growth rate with the highest counts after day 56. Microbial plugging of the injection wells could become a problem at these high cell densities.

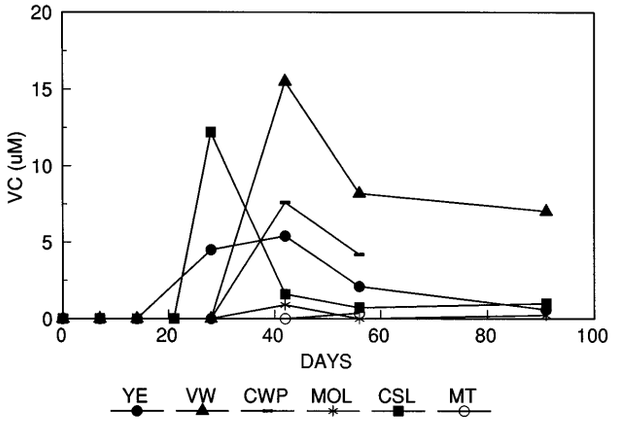

Figure 3 Vinyl chloride production in the treatments with the highest substrate loadings in the first microcosm study. YE = yeast extract, VW = Victoria wastewater, CWP = cheese whey permeate, MOL = molasses, CSL = corn steep liquor, and MT = manure tea.

Table 2 presents the initial TOC, the percent of the initial TOC removed, the maximum methane concentration detected, the percentage of the initial chlorinated ethenes degraded to the final dechlorination endproduct, and the maximum AODC counts for the second study with the higher substrate loadings. The TOC removals generally declined with increasing substrate concentrations. The microbes could not completely utilize substrate loadings in excess of 3000 mg C L^{-1} during the 98-day incubation period. The pH was not controlled in the microcosms, but remained neutral (data not shown). All of the treatments except the unamended, blank, and the 3500 to 23 000 mg C L^{-1} loadings of benzoate resulted in the formation of greater than 1.0 mg L^{-1} of methane. The higher quantities of benzoate inhibited methanogenesis. The higher loadings of molasses and corn steep liquor produced so much gas that the septa were displaced and the studies could not be completed for these treatments. Several other treatments contained methane concentrations in excess of methane's solubility of 25 mg L^{-1} at room temperature; these microcosms may have become pressurized or bubbles of methane were collected with the liquid samples for the methane analyses. Benzoate loadings of 7500 mg C L^{-1} and 23 000 mg C L^{-1} inhibited dechlorination of PCE to TCE and DCE. The 4200 mg C L^{-1} loading of acetate and 6200 mg C L^{-1} of corn steep liquor also inhibited dechlorination of PCE to DCE. Up to 1800 mg C L^{-1} of molasses supported PCE transformation to DCE. However, in none of the microcosms with high substrate loading was vinyl chloride or ethene observed. Limited biotransformation of PCE to DCE was observed in the unamended treatment and no transformation was observed in the blank treatments.

In the second microcosm study, the maximum acridine direct microbial counts of greater than 1×10^8 cells g^{-1} were found for the fermentable substrates: corn steep liquor and molasses. Maximum counts of 5.2 to 8.5×10^7 c g^{-1} were lower in the treatments with acetate and benzoate. The unamended treatment had a gradual increase in microbial numbers from 2×10^7 c g^{-1} to 3×10^7 c g^{-1} (data not shown). After a rapid increase in the microbial numbers to greater than 10^8 c g^{-1} in the treatments with corn steep liquor and molasses, the counts began a gradual decline.

Table 2 Summary of second microcosm study results

Treatment	Days	TOC (mg L^{-1})	% TOC removed	Maximum methane (mg L^{-1})	Final daughter product[a] and % initial PCE	Maximum AODC $\times 10^7$ c g^{-1}
Unamended	84	46	19	0.1	DCE 1	4.5
Blank	98	14	100	0.0	PCE[b] 0	NA[c]
Benzoate	98	240	86	6.6	DCE 100	5.7
	98	350	85	15.0	DCE 100	7.7
	98	770	94	22.0	DCE 100	6.3
	98	1900	5	1.3	DCE 100	NA
	98	3500	0	0.3	DCE 100	NA
	98	7500	7	0.0	DCE 4	NA
	98	23000	11	0.0	PCE 0	NA
Acetate	98	190	78	31.0	DCE 100	8.5
	98	1500	99	71.0	DCE 100	4.4
	84	2300	98	120.0	DCE 100	5.2
	70	4200	77	42.0	DCE 51	11
Corn steep liquor	98	320	86	24.0	DCE 100	12
	98	900	95	62.0	DCE 100	15
	84	1600	95	76.0	DCE 100	34
	56	2700	69	18.0	DCE 100	NA
	70	6200	37	13.0	TCE 3	NA
Molasses	98	400	91	16.0	DCE 100	16
	98	870	93	18.0	DCE 100	29
	84	1800	94	41.0	DCE 100	30

[a]Final daughter product detected during study.
[b]No degradation of PCE observed.
[c]NA = not analyzed.

Similar numbers of bacteria of between 4.5×10^7 to 1.2×10^8 c g^{-1} were found at the end of the study in all treatments.

Tracer tests

The first tracer test was run with the injection of 955 mg L^{-1} of bromide into well 221 at a flow rate of 0.11 L min^{-1} or the equivalent of the natural flux rate of groundwater through the well screen. Figure 2 shows the results of the first tracer test. The contour intervals are based on the maximum concentrations of tracer that were detected in any of the three zones of the monitoring wells. The tracer plume was very narrow: only wells 230 and 231 contained more than 5 mg L^{-1} bromide. The overall groundwater flow direction was deflected northward by a nearby pumping well. Under natural flow conditions of about 0.3 m day^{-1}, dispersion is very limited and the tracer was diluted by over 99%. Insufficient data were available from the first tracer test to calculate dispersion.

The second tracer test was designed to induce dispersion both transversely and longitudinally at the point of injection by creating a groundwater mound at the injection point. The second tracer test was performed with the injection of iodide tracer into well 221 at a flow rate of 6.8 L min^{-1} or approximately 60 times the natural groundwater flux rate. The 400 mg L^{-1} iodide solution was prepared with potassium iodide in potable water. Dispersion of the tracer was much greater with the induced flux than the natural flux as wells 228, 229, and 230 all received greater than 100 mg L^{-1} of the iodide tracer (Figure 4). The plume continued out to wells 224 and 231 at over 50 mg L^{-1}. Most of the tracer moved from the upper interval, where it was injected, into the middle interval (data not shown). Low concentrations of the tracer were detected in the upper, finer-grained interval and no tracer was detected in the lower interval. Calculated groundwater velocities in the middle interval ranged from 0.30 m day^{-1} for well 228 to 0.64 m day^{-1} for well 224. The longitudinal dispersion ranged from 0.2 to 20 ft in the upper, fine-grained interval. Longitudinal dispersion ranged from 1.2 to 3.7 m in the middle, coarse-grained interval. The transverse dispersion was estimated to be around one-tenth of the longitudinal dispersion, or 0.03 to 0.61 m. The tracer was diluted by only 18% under the conditions employed in the second test.

For the third test, groundwater was withdrawn from wells 220 and 222 at a rate of 3.8 L min^{-1}. The withdrawn water was mixed with 3.8 L min^{-1} of tracer solution containing bromide, benzoate, ammonium sulfate, and potassium phosphate and injected into well 221. Figure 5 depicts the results of the non-reactive bromide tracer for the third tracer test. The maximum concentration of tracer detected at any of the three depth intervals was used to calculate the contour intervals. Modeling estimates were used for the southern portion of the contour maps because of problems with the accumulation of silt in monitoring wells 226 and 230. The distribution of the tracer was greater in this test than the second tracer test. The mixing strategy of cross-gradient injection resulted in a greater dispersion than the induced

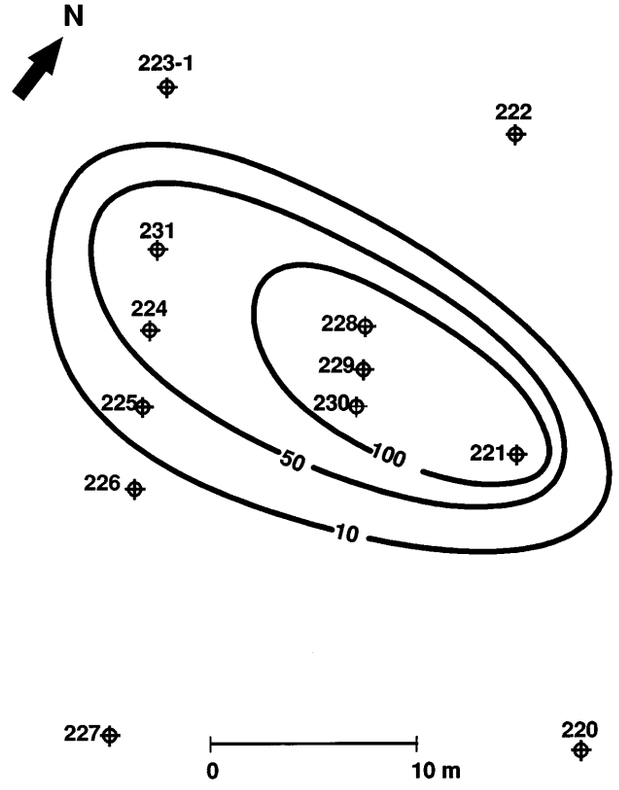

Figure 4 Isocontour interval map of iodide concentrations in tracer test 2 with induced gradient; 400 mg L^{-1} iodide injected at 6.8 L min^{-1} into well 221.

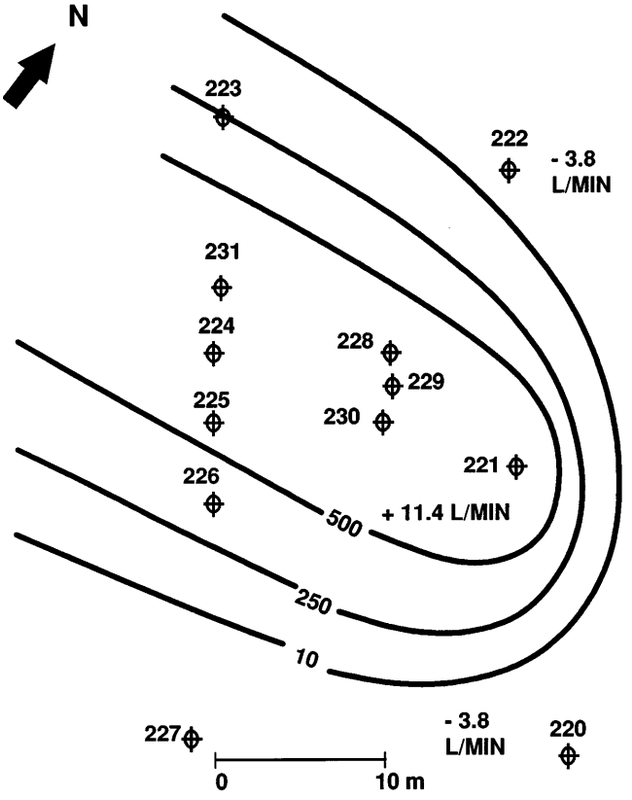

Figure 5 Isocontour interval map of bromide concentrations in tracer test 3 with forced dispersion from cross-gradient injection; 3.8 L min^{-1} was withdrawn from each of wells 222 and 220 and injected along with 3.8 L min^{-1} of a 900 mg L^{-1} bromide tracer into well 221.

gradient from the second test. The longitudinal dispersion for the third test was 0.12 to 0.27 m, while the transverse dispersion was higher, 0.67 to 0.73 m. Not only would the increased transverse dispersion reduce the number of injection wells required, but this mixing strategy would minimize the displacement of contaminated groundwater by the injection of clean water containing the substrate. This mixing strategy reduced the dilution of the tracer to only 9%.

Figure 6 shows the concentrations of TOC (a measure of the benzoate), bromide tracer, sulfate, ammonia-nitrogen, and phosphate in well 229 relative to the average input concentrations. Low concentrations of the TOC arrived about 15 days after the inorganic tracer bromide had reached well 229 on day 15, but the breakthrough curves for bromide and TOC matched closely until day 48. The delayed arrival of the TOC demonstrates that there was some retardation of benzoate due to biodegradation or sorption. The relative bromide concentrations became higher than the TOC from day 48 to 77, presumably due to biodegradation of the substrate. After day 77, the relative TOC concentrations were greater or equivalent to the bromide. Sulfate concentrations increased from the background concentrations of 37 mg L^{-1} to a maximum of 85 mg L^{-1} on day 43, but began to decline after the substrate had reached well 229 and fell to below 1.0 mg L^{-1}. The sulfate was consumed by sulfate-reducing bacteria degrading the benzoate. Dissolved oxygen concentrations in the groundwater in the tracer area were below 1 mg L^{-1}. Ammonia-nitrogen was first detected in well 229 on day 43, approximately 28 days after the bromide. The delayed arrival and low maximum relative concentration of ammonia-nitrogen of 62% were a result of sorption and biodegradation. Phosphate was delayed even more, with the first detection in well 229 on day 55 and the maximum concentration only 32% of the input concentration. The second row of monitoring wells contained low levels of ammonia-nitrogen, but no detectable phosphorus. The ammonia-nitrogen and phosphate were either adsorbed onto the aquifer matrix or removed by microbial consumption. The following retardation factors to well 229 were calculated based upon the breakthrough curves relative to bromide: TOC 1.0, sulfate 1.27, phosphate 1.81 and ammonia-nitrogen 1.19.

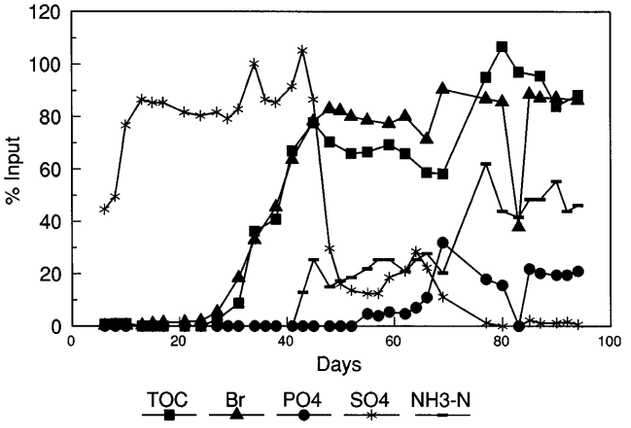

Figure 6 Relative concentrations of TOC, bromide, sulfate, ammonia-nitrogen, and phosphate for well 229 during tracer test 3 as percentage of input concentrations.

Discussion

The minimum concentration of substrate necessary to support dechlorination is an important issue for scale-up of *in situ* anaerobic bioremediation of chlorinated ethenes. Approximately 2.4 mg C L^{-1} was the minimum concentration of substrate necessary to support dechlorination of 1 μM PCE to vinyl chloride in the first study using microcosms. The substrate requirements for dechlorination in these studies are in the range reported by other researchers. Gibson *et al* [11] observed that a 0.1-mM mixture of butyric, propionic, lactic, and acetate acids and 1 mM methanol or the equivalent of 16.3 mg C L^{-1} supported dechlorination of 30 μM PCE to DCE in microcosms with Traverse City, MI soils; this is equivalent to a ratio of 0.54 mg C μM^{-1} PCE. Bouwer [4] estimated a substrate requirement of 970 kg lactate for 16.3 kg of PCE based upon a stoichiometry of 110.67 mole lactate per mole PCE degraded or the equivalent of 4 mg carbon μM^{-1} PCE. This stoichiometry was based on studies which employed columns conducted by de Bruin *et al* [5]. Using a more favorable stoichiometry of 4.33 mole methanol per mole PCE degraded for an enrichment culture [6], Bouwer [4] estimated the substrate requirements of 13.6 kg methanol for 16.3 kg PCE or the equivalent of 0.052 mg C μM^{-1} PCE. The stoichiometries for the carbon requirements for degradation of PCE may be more favorable than these calculated values because the substrate to PCE ratio was not optimized in these studies [4]. Extrapolating from data provided by Skeen *et al* [19], a stoichiometric requirement of 2200 g C L^{-1} from methanol per μM PCE can be estimated and so much biomass would be produced that the void space of the aquifer would be filled.

The expense of the substrate for an *in situ* anaerobic bioremediation project can vary greatly depending upon the quantity of the substrate required and its cost. The costs for substrate to treat 1 L PCE (1.63 kg or 9 800 000 μmoles) can be estimated based upon the range of organic loadings projected by Bouwer [4] of 0.31 to 4 mg C μM^{-1} PCE. Molasses, sodium benzoate, and yeast extract are representative substrates to illustrate the differences in costs. Molasses can be purchased at 22 cents per kg or 77 cents per kg of carbon. The range of substrate costs for molasses to treat 1 L of PCE would be \$2.30 to \$30, depending upon the loading. Benzoate is of intermediate cost, \$1.69 per kg, or the equivalent of \$2.90 per kg C. At the lowest substrate loading of 0.31 mg C mg^{-1} PCE considered, sodium benzoate would cost \$8.80. Substrate costs would be \$110 if the loading of 4 mg C μM^{-1} PCE was required. Yeast extract is the most costly of the three substrates considered, at \$6.60 per kg or \$17.10 per kg of carbon. Yeast extract would cost between \$52 to \$670 to treat the 1 L of PCE. Not only must the cost of the substrates be considered, but the effectiveness of the substrates in supporting dechlorination is critical. In some sites, yeast extract may be a more effective substrate than cheaper substrates that do not give as rapid or complete dechlorination.

With the Victoria samples, all of the inexpensive substrates stimulated dechlorination of PCE to DCE under methanogenic conditions. It is postulated that any substrate that will yield hydrogen under fermentative and/or methan-

ogenic conditions will serve as a substrate to support dechlorination of PCE to DCE if the microbial population is capable of carrying out the dechlorination reaction. Further biotransformation of DCE to VC and ethene does not appear to be as universal and may require specific substrates or enrichment strategies.

The highest concentration of substrate that can be injected is important at the injection point. In the second study using microcosms, PCE was only dechlorinated to DCE at the high substrate concentrations. Several possible explanations were hypothesized for the limited dechlorination observed in the second study. The high organic loadings may have shifted the microbial population away from dechlorination. The sample used in the second microcosm study was collected from the same general location and depth as the sample used for the first study, but at a different time and may not have contained the same consortia of dechlorinating microbes. Inhibitory concentrations of several substrates were observed. Concentrations of sodium benzoate greater than 3500 mg C L^{-1} inhibited methanogenesis and dechlorination of PCE to DCE. High concentrations of sodium acetate and corn steep liquor also reduced the transformation of PCE to DCE.

Production of up to 3×10^8 cells per g was observed with fermentable substrates such as molasses and corn steep liquor. These high microbial numbers would likely lead to plugging of the injection points. High concentrations of sodium benzoate inhibited microbial growth and activity could thus serve as a mechanism to control growth at the injection point. Leethem *et al* [15] successfully demonstrated this technique in a field study.

The tests with tracers addressed the issue of how to distribute effectively the substrate, electron acceptors, and nutrients in the aquifer. The natural dispersion of the aquifer was not sufficient to distribute substrate evenly across the injection plane. Injection of groundwater at 60 times the natural flux increases dispersion and may be sufficient to support full-scale bioremediation. The mixing strategy of injecting cross-gradient that was evaluated increased dispersion further. The mixing strategy would reduce the number of injection wells that would have to be installed at the site. Another benefit of this mixing strategy is that it reduces the amount of water to be injected. Injection of too much water will cause contaminated groundwater to go around the treatment cell and not be treated.

The third test with tracers demonstrated that sodium benzoate and inorganic nutrients can be transmitted through this formation without being consumed immediately adjacent to the injection well. However, the sulfate, ammonia-nitrogen, and phosphate were retarded in these sandy soils relative to the bromide tracer and substrate. The ammonia-nitrogen and phosphate did not reach the second set of monitoring wells either due to adsorption or microbial consumption. Distribution of inorganic nutrients such as ammonia-nitrogen and phosphate will likely be more difficult in soils having a high clay content. Leethem *et al* [15] observed delayed arrival times for ammonia-nitrogen (165–170 days) and phosphate (266–408 days) compared to bromide (73–77 days) and TOC (112–180 days) at a site with a mixture of fluvial sand, silt, and interbedded clay.

Further evaluation of the economics and the optimal delivery mechanisms are warranted to be able to implement full scale *in situ* anaerobic bioremediation of PCE. The number of delivery and recovery wells and their placement is determined by the rate of microbial utilization of the substrate, the groundwater flow rate, and the amount of lateral dispersion that can be induced by mixing.

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