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Intrinsic bioremediation of trichloroethylene and chlorobenzene: field and laboratory studies

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Abstract

Activities at a former fire training area at Robins Air Force Base in Georgia, USA resulted in contamination of groundwater with a mixture of trichloroethylene (TCE) and chlorobenzene (CB). Results from the field investigation suggest that intrinsic bioremediation process is occurring, which caused the decrease in TCE and CB concentrations, and increase in TCE degradation byproducts [e.g., dichloroethylene isomers (DCEs), vinyl chloride (VC)] concentrations. Contaminated groundwater samples collected from this site were used to conduct microbial enumeration tests, and used as the inocula for microcosm establishment. Results from the microbial enumeration study indicate that methanogenesis was the dominant biodegradation pattern within the source and mid-plume areas, and the aerobic biodegradation process dominated the downgradient area. Laboratory microcosm experiments were conducted to evaluate the feasibility of using CB as the primary substrate to enhance the intrinsic biodegradation of TCE. Microcosm results suggest that CB can serve as the primary substrate (electron donor), and enhance TCE biodegradation to less-chlorinated compounds under both aerobic cometabolism and reductive dechlorination conditions.

Keywords: Groundwater contamination; Intrinsic bioremediation; Trichloroethylene; Chlorobenzene; Microcosm; Cometabolism; Reductive dechlorination

1. Introduction

Groundwater at many existing and former industrial sites and disposal areas is contaminated by halogenated organic compounds that were released into the environ-

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ment. The chlorinated solvent trichloroethylene (TCE) is one of the most ubiquitous of these compounds. The maximum contaminant level (MCL) (the concentration established under the Safe Drinking Water Act as being protective of human health and the environment) is 5 μg/l for TCE [1].

Cleanup of sites to mandated concentrations using currently available technologies (e.g. pump-and-treat, air sparging) is extremely expensive and time-consuming [2–5]. Therefore, more economic and less expensive approaches are desirable for groundwater remediation to provide for long-term control of contaminated groundwater.

1.1. Intrinsic bioremediation

Natural attenuation, also known as intrinsic remediation or natural remediation, is a passive remedial approach that depends upon natural processes to degrade and dissipate contaminants in soil and groundwater. Natural attenuation processes include physical, chemical, and biological transformation (e.g. aerobic/anaerobic biodegradation, cometabolism, dispersion, volatilization, oxidation, reduction, and adsorption). Aerobic and anaerobic biodegradation (intrinsic bioremediation) are believed to be the major processes that account for both containment of the dissolved contaminant plume and reduction of the contaminant concentrations [6,7].

Aerobic biodegradation relies on dissolved oxygen (DO) as the electron acceptor used by the subsurface microorganisms. Anaerobic processes refer to a variety of biodegradation mechanisms that use nitrate, ferric iron [Fe(III)], sulfate, and carbon dioxide (CO_2) as terminal electron acceptors. Anaerobic biodegradation dominates the interior of a contaminant plume [8–10].

Environmental conditions and microbial competition will ultimately determine which anaerobic biodegradation processes would dominate, but in a typical aquifer that is devoid of DO, denitrification typically occurs first, followed by iron reduction, sulfate reduction, and methanogenesis. Advantages of intrinsic bioremediation over other engineered remediation technologies include the following:

1. contaminants are ultimately transformed to innocuous byproducts (e.g. carbon dioxide and water) most of the time, not just transferred to another phase or location within the environment;
2. intrinsic bioremediation is nonintrusive, and allows continuing use of infrastructure during the remediation;
3. intrinsic bioremediation is less costly; and
4. intrinsic bioremediation is not subject to limitations imposed by the use of mechanized remediation equipment.

To support remediation by intrinsic bioremediation, the proponent must scientifically demonstrate that degradation of site contaminants is occurring at rates sufficient to be protective of human health and the environment [11]. Three lines of evidence can be used to support intrinsic bioremediation of chlorinated aliphatic hydrocarbons, including:

1. observed reduction in contaminant concentrations along the groundwater flow path from the contaminant spill location;
2. documented loss of contaminant mass at the field scale; and
3. microbiological laboratory data that support the occurrence of biodegradation.

1.2. Biodegradation of TCE

Current evidence suggests that TCE can be degraded cometabolically by supplying an alternate electron donor under aerobic conditions [4,6]. Several aerobic microorganisms or microbial communities have the ability to synthesize oxygenase enzyme systems that cometabolize TCE and its degradation byproducts [e.g. dichloroethylene isomers (DCEs), vinyl chloride (VC)], when the organisms are grown on other substrates. DCE isomers contain 1,1-DCE, *cis*-1,2-DCE (*cis*-DCE), and *trans*-1,2-DCE (*trans*-DCE) [12–15]. Among these, *cis*-DCE is the dominant product. Moreover, VC has been shown to be available as a primary substrate [16,17].

Under anaerobic conditions, degradation of TCE has been observed in field studies, in continuous-flow fixed-film reactors, and in microcosms [18,19]. Previous investigation results indicate that TCE can be microbiologically transformed by sequential reductive dechlorination to less-chlorinated compounds [17].

A chlorine atom is replaced by a hydrogen atom at each step during reductive dechlorination [20]. In general, the reaction rates for each sequence decrease as chlorine is removed. Thus, reductive dechlorination is relatively facile for PCE, but relatively slow for VC. In fact, the rate-limiting step in the reductive dechlorination pathway appears to be the conversion of VC to ethylene (ETH) [21]. A number of organic compounds, including acetate, methanol, glucose, benzoate, phenol, methylamines, and alkylbenzenes, have been used as electron donors and carbon sources for reductive dechlorination under methanogenic conditions.

Therefore, intrinsic bioremediation (or in situ bioremediation) could become an alternative technology to restore TCE contaminated sites if primary substrates exist naturally (or can be provided to the subsurface economically).

1.3. Degradation of chlorobenzene

Biodegradation of chlorobenzene (CB) by aerobic organisms or by facultative bacterial under anaerobic conditions has been observed. Some of these species include commonly isolated genera such as *Arthrobacter* [22], *Pseudomonas* [23,24], and *Azotobacter* [25]. Degradation of CB generally involves conversion to chlorocatechols via dioxygenase and dehydrogenase reactions. The chlorocatechols are subsequently degraded to straight chain organic acid through *ortho* ring-cleavage [26]. Similar to other halogenated aromatic compounds, CB has been shown to undergo reductive dechlorination under anaerobic conditions [26]. Bosma [24] demonstrated reductive dechlorination of CB in anaerobic sediment columns. Fetzner [27] also observed reductive dechlorination of CB using enrichment cultures in the presence of lactate, glucose, ethanol, and isopropanol as electron acceptors.

1.4. Objectives

Activities at a former fire training area at Robins Air Force Base (AFB) in GA, USA resulted in contamination of groundwater with a mixture of TCE and CB. The objective

of this study was to (1) investigate the occurrence of intrinsic bioremediation at this study site, and (2) evaluate the potential for intrinsic biodegradation of TCE using CB as the primary substrate under aerobic and methanogenic conditions. In this study, field assessment and laboratory microbial enumeration and microcosm experiments were conducted within the investigation period.

2. Study site descriptions

This study site is a former fire training area located in the southeastern part of the Robins AFB between Luna Lake and Scout Lake. Fig. 1 presents a site map of the former fire training place and the surrounding area. This site has been used for a number of purposes throughout its history. Previous research on site activity reports that the site was used during the 1950s and 1960s for waste disposal and fire training exercises. Those activities have resulted in contamination of groundwater by organic solvents, mainly TCE and CB. Groundwater and dissolved contaminants within the surficial aquifer move from the spill area toward an unnamed creek located to the north and west. The fire training area occupies approximately 6 acres.

The topography in the vicinity of the site is characterized by a relatively flat upland area. The hydrogeology of the site is characterized by an unconfined surficial aquifer, a clay confining unit, and a confined alluvial unit. The surficial aquifer is a heterogeneous formation comprised of lenses and layers of sand, gravel, silt, and clay. The horizontal continuity of any material is limited. Groundwater flows more or less radically from the former fire training area toward the northwest, north, and northeast. The average hydraulic conductivity (K_s) of the surficial aquifer is approximately 12 m/day. The average hydraulic gradient is 0.017 m/m, and the calculated groundwater flow velocity is 0.8 m/day.

3. Materials and methods

3.1. Field investigations

A monitor well network was installed at this TCE/CB spill site to delineate the dissolved contaminant plume (Fig. 1). Groundwater levels varied from 3 to 8 ft below land surface (bls) within the study area. Groundwater samples were analyzed for geochemical parameters [e.g. DO, redox potential (Eh), chloride ion (Cl^-), Fe(II), CO_2, and pH] and organic/inorganic compounds [e.g. TCE, DECs, VC, ETH, methane (CH_4), total organic carbon (TOC), nitrate, nitrite, sulfide, sulfate]. Analysis of TCE and its daughter compounds were performed in accordance with US EPA Method 601, using a Tekmer Purge-and-Trap Model LSC 2000 with a Perkin-Elmer Model 9000 Auto System Gas Chromatograph (GC). CB was analyzed in accordance with EPA Method

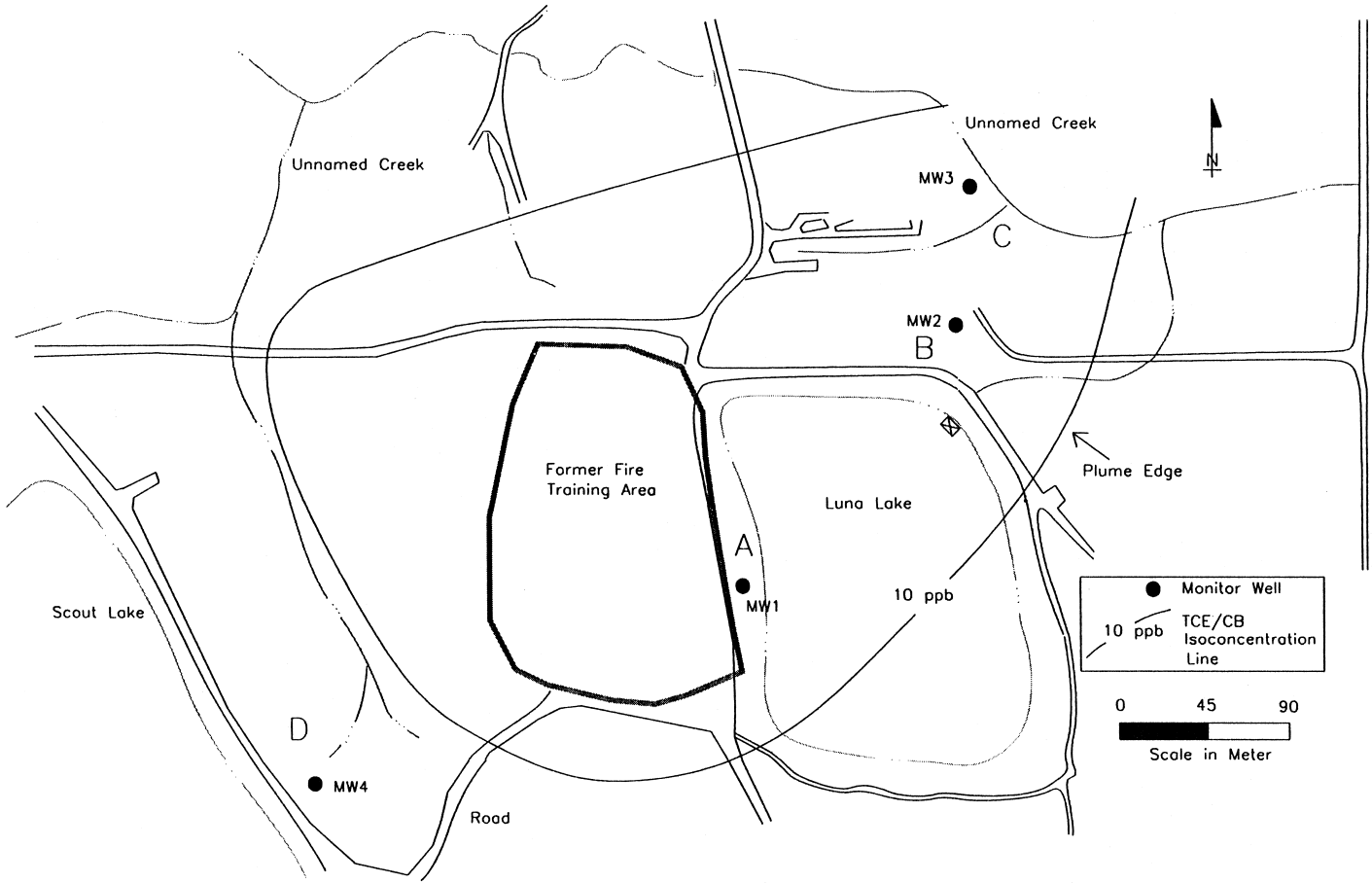

Fig. 1. Site map showing the former fire training area and four representative monitor wells.

8040 using a Hewlett Packard 6890 GC with a flame ionization detector. Methane was analyzed on a Shimadzu GC-9A GC using headspace techniques. Ion chromatography (Dionex) was used for inorganic nutrients and anions analyses. TOC was analyzed by Total Carbon Analyzer (Shimadzu). Chloride ion was analyzed using a Hach chloride titration kit (Model CD-51). An Accumet 1003 pH/Eh meter (Fisher Scientific) was used for pH/Eh measurements. An Orion DO meter (Model 840) was used for DO measurements, and a Hach digital titrator cartridge was used for CO_2 measurements. Instrument operating conditions and quality control measures are described by Kao et al. [8] and Kota [28].

For simplicity and ease of identification, the contaminant plume was divided into four zones, which were zone A (source area), zone B (mid-plume area), zone C (downgradient area), and zone D (background). The selected representative monitor wells for zones A, B, C, and D are MW1, MW2, MW3, and MW4, respectively. Monitor wells MW2 and MW3 are located approximately 180 and 270 m downgradient from monitor well MW1, respectively. Monitor well MW4 is located 260 ft upgradient from MW1. To confirm the occurrence of intrinsic bioremediation and evaluate the biodegradation of TCE and CB, groundwater samples collected from those four representative monitor wells were analyzed and compared.

3.2. Laboratory experiments

3.2.1. Microbial enumeration

To evaluate the trend of the degradation processes, groundwater samples collected from monitor wells MW1, MW2, and MW3 were analyzed for microbial population to define the distribution of microorganisms. Because DO concentrations in MW1 and MW2 were below 0.2 mg/l, total anaerobes, iron reducers, sulfate reducers, and methanogens in these two samples were enumerated. On the other hand, DO in MW3 was around 6 mg/l, and therefore, enumeration of aerobic heterotrophic bacterial population was performed.

Total iron reducers were determined by a 10-tube most probable number (MPN) assay, using a medium selective for iron reducers as described by Lovely and Phillips [28]. Media (9 ml) was first dispensed in anaerobic pressure tubes, sealed with black butyl rubber stoppers and aluminum crimp, and autoclaved at 121°C for 20 min. Serial dilutions of groundwater samples were prepared. A 1-ml portion from each dilution was inoculated into the MPN tubes and incubated at 25°C. After 2 months of incubation, an extract was removed from each tube, acidified, and analyzed for Fe(II) by reacting with phenanthroline for 15 min and reading adsorbance at 510 nm. Tubes were scored positive if the Fe(II) in the inoculated tubes was higher than in the uninoculated controls at the 95% level, based on a two-tailed *t*-test.

Total anaerobes and sulfate reducers were enumerated using a five-tube MPN assay and inoculated with the same inoculum used for iron reducers. All tubes were incubated at 25°C for 1 month. The total anaerobe tubes were scored positive based on optical density [29]. The sulfate reducer tubes contained media described by Chartrain and Zeikus [30], and were scored positive based on the appearance of black precipitate.

Methanogens were enumerated using a five-tube MPN assay and inoculated with the same inoculum used for iron reducers. All tubes were incubated at 25°C for 1 month. The methanogen tubes contained 20% H_2 and 80% CO_2 in the headspace, and were scored positive based on the production of methane [29].

Approximate size of the total heterotrophic bacterial population in the MW3 sample was determined by total plate counts using plate count agar (Difco) [31]. At several times, 1 ml subsamples were 10-fold serially diluted in sterile buffer solution. Triplicate 0.5 ml aliquots of 10^{-3} through 10^{-9} dilution levels were plated onto plate count agar using the spread plate method [31]. These plates were incubated inverted at 20°C for 2, 7, and 14 days. Longer into the acclimation time, visible colonies did not develop until 10 to 14 days of incubation.

3.2.2. Microcosm study

Two laboratory microcosm experiments were conducted to examine the feasibility of TCE biodegradation using CB as the primary substrate under (1) aerobic cometabolism and (2) reductive dechlorination conditions. Each microcosm was constructed with 20 ml sparged groundwater, 5 ml TCE solution, and 5 ml primary substrate (CB, phenol, or methanol) solution in a 70-ml serum bottle with 25 ml mineral medium solution [32] sealed with Teflon-lined rubber septa. The pH of the microcosm solution was approximately 7.2. Control bottles contained 250 mg/l mercuric chloride ($HgCl_2$) and sodium azide 500 mg/l (NaN_3).

In the first microcosm experiment, three groups of microcosms were constructed under aerobic conditions. Because groundwater from monitor well MW3 (zone C) was under aerobic conditions, it was used as the inocula for this aerobic microcosm test. Each microcosm in Group 1 contained groundwater, TCE solution, and phenol solution. Phenol was served as the primary substrate to enhance TCE biodegradation in the first group. Group 2 microcosm contained groundwater and TCE/CB solution. CB was served as the primary substrate in this group. Group 3 was a control group. Microcosms in Group 3 contained groundwater, TCE/CB solution, $HgCl_2$, and NaN_3. In the second microcosm experiment, three groups of microcosms were constructed under anaerobic conditions. Because groundwater from monitor well MW2 (zone B) was under anaerobic conditions, it was used as the inocula source for this anaerobic microcosm test. Each microcosm in Group 1 contained groundwater, TCE solution, and methanol solution. Methanol was served as the primary substrate to enhance TCE biodegradation. Group 2 microcosm contained groundwater and TCE/CB solution. Group 3 (control group) microcosms contained groundwater, TCE/CB solution, $HgCl_2$, and NaN_3.

Anaerobic microcosms were prepared in an anaerobic glovebox to preclude intrusion of oxygen. Hungate techniques were used to prepare anaerobic solutions [28,32]. A redox indicator (0.0002% resazurin) and reducing agent (1 mM sodium sulfide) were added to each bottle. Sodium sulfide was chosen because it would not serve as a carbon source, and it has a redox potential (-571 mV) low enough to reduce resazurin.

The initial TCE, CB, phenol, and methanol concentrations in microcosm bottles were approximately 2.5, 3.5, 20, and 20 mg/l, respectively. Triplicate microcosms were scarified at each time-point during the analysis. Samples were analyzed for TCE, CB, and TCE degradation byproducts (including *cis*-DCE and VC) concentrations. Because

cis-DCE is the dominant byproduct among DCE isomers during the TCE biodegradation process, only *cis*-DCE was analyzed in this study.

4. Results and discussions

4.1. Field investigations

Table 1 presents the analytical results for groundwater samples collected from four representative monitor wells (MW1, MW2, MW3, and MW4). Results are averages of the latest six quarterly monitoring events. The groundwater analytical results indicate that dissolved TCE and CB are being transported from the former fire training area to the farther downgradient area (zone C). Based on the analytical results, TCE concentration dropped from 5500 μg/l in MW1 to 150 μg/l in MW2, then dropped to 25 μg/l in MW3. CB concentration dropped from 3110 μg/l in MW1 to 752 μg/l in MW2, then dropped to 280 μg/l in MW3. Significant amounts of TCE degradation byproducts (e.g., DCEs, VC, and ETH) were observed in both MW1 and MW2. Moreover, chloride ions were detected in those three monitor wells (MW1, MW2, and MW3) within the plume. Results indicate that intrinsic bioremediation of TCE is occurring, which caused the decrease in TCE and increase in DCEs, VC, and ETH concentrations.

Lower pH measurements were observed in MW1 and MW2, compared to the background pH value (pH = 6.6 in MW4). This was due to the production of CO_2.

Table 1
Analytical results for groundwater samples collected from selected monitor wells (BQL = below quantitation limit)

	MW1	MW2	MW3	MW4
Representative zone	A	B	C	D
Distance to MW1 (m)	0	180	270	−260
TCE (μg/l)	5500	150	25	BQL
DCEs (μg/l)	4500	322	20	BQL
VC (μg/l)	960	340	33	BQL
ETH (μg/l)	160	37	BQL	BQL
Chloride ion (mg/l)	52	44	28	BQL
Chlorobenzene (μg/l)	3110	752	280	BQL
pH	5.2	5.4	6.4	6.6
DO (mg/l)	0.1	0.2	6	7.3
Eh (mV)	−96	−55	232	296
CO_2 (mg/l)	16	18	21	11
TOC (mg/l)	21	11	9	7
Nitrate as N (mg/l)	BQL	BQL	7	6
Nitrite as N (mg/l)	BQL	BQL	BQL	BQL
Fe(II) (mg/l)	15	26	BQL	BQL
Sulfate as S (mg/l)	BQL	BQL	11	9
Sulfide as S (mg/l)	6	8	2	BQL
CH_4 (mg/l)	220	150	24	BQL
Temperature (°C)	23	22	22	21

Table 2
Results of microbial enumeration from three groundwater samples (NA = not analyzed; CFU = colony forming unit)

Monitor well	Zone	Total anaerobes (cell/ml)	Methanogens (cell/ml)	Fe reducers (cell/ml)	Sulfate reducers (cell/ml)	Total heterotrophs (CFU/ml)
MW1	A	1.3×10^4	5.7×10^3	12	<2	NA
MW2	B	7.5×10^5	2.1×10^3	1.1×10^2	<2	NA
MW3	C	NA	NA	NA	NA	1.5×10^6

Lower Eh and DO values were also observed within the plume compared with the background well measurements. The depletion in DO indicates that the degradation pattern has been shifted from aerobic to anaerobic processes. After the depletion of oxygen, other electron acceptors (e.g. nitrate, ferric iron, sulfate) were used to oxidize dissolved contaminants. The decline in Eh within the plume reflects the change from oxidizing conditions in the background area (absence of dissolved hydrocarbons) to reducing conditions within the plume (presence of dissolved hydrocarbons). The decrease in TOC concentrations from MW1 to MW3 reveals the removal of contaminant concentrations. The complete consumption of nitrate as well as sulfate and the produc-

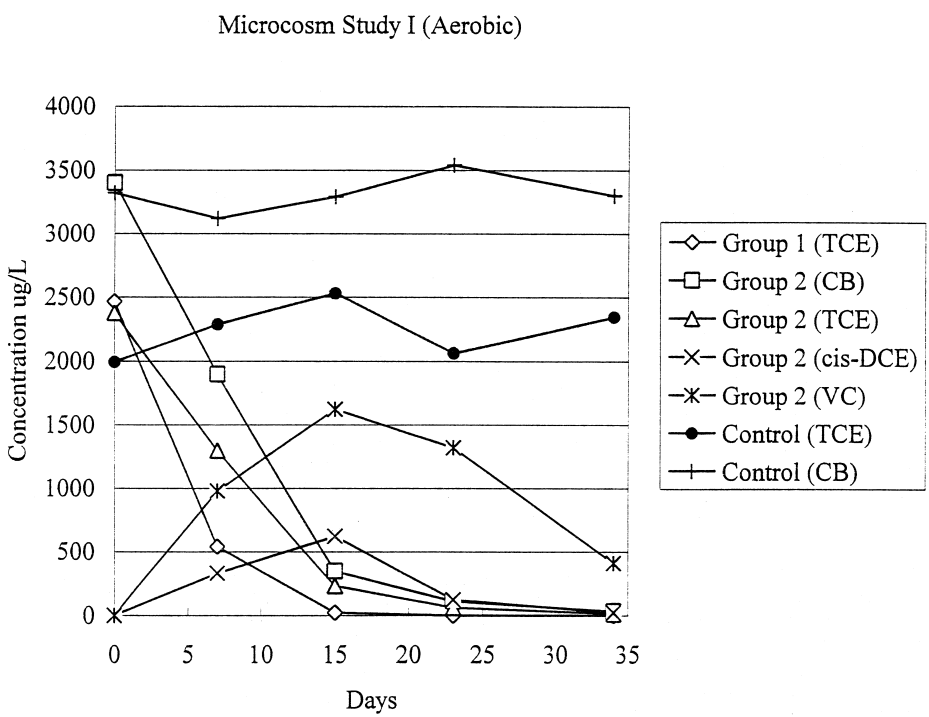

Fig. 2. Results from the aerobic microcosm experiments.

tion of Fe(II) and methane in MW1 and MW2 suggest that methanogenesis was the dominant biodegradation process within those areas.

4.2. Laboratory experiments

4.2.1. Microbial enumeration

Table 2 presents the results of the microbial enumeration tests. Results for MW1 and MW2 samples show that the population for sulfate reducers was below quantitation limit (BQL) (2 cells/ml of groundwater), and population for iron reducers was below 110 cells/ml of groundwater. Higher total anaerobes (1.3×10^4 and 7.5×10^5 cells/ml of groundwater for MW1 and MW2, respectively) and methanogens (5.7×10^3 and 2.1×10^3 cells/ml of groundwater for MW1 and MW2, respectively) were observed (Table 2). Results indicate that both sulfate reduction and iron reduction processes were not significant, and methanogenesis was the dominant biodegradation process in zones A and B. Total heterotrophic bacterial plate counts for MW3 sample indicates that significant amount of aerobic heterotrophs was observed (1.5×10^6 cell/ml of groundwater) after 14 days of incubation. This indicates that high microbial activity occurred in the downgradient area (zone C).

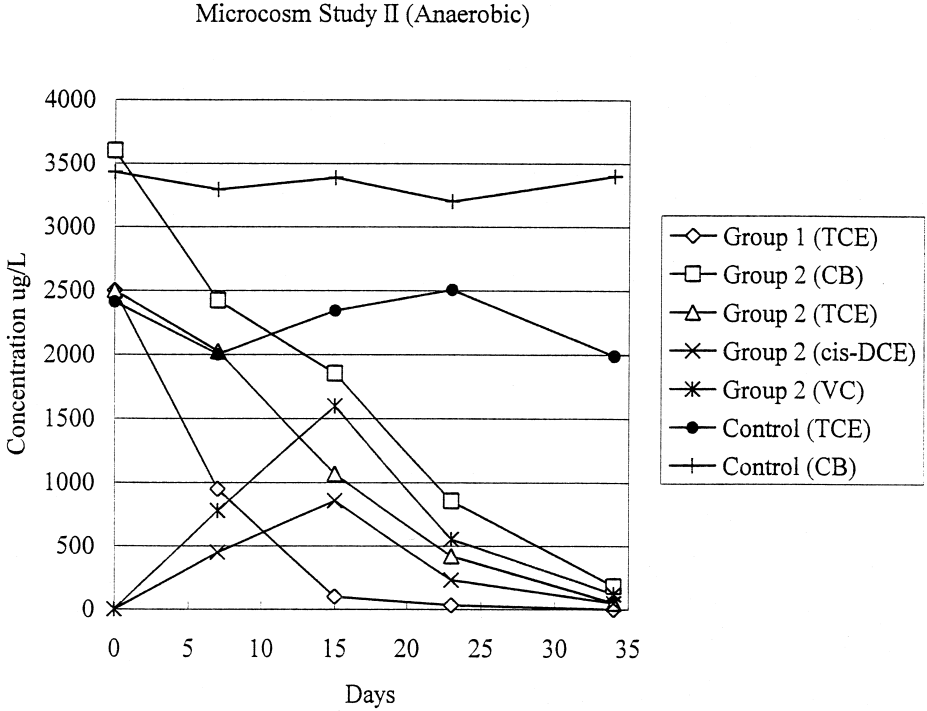

Fig. 3. Results from the anaerobic microcosm experiments.

4.2.2. Microcosm study

Fig. 2 presents the results from the first aerobic microcosm experiments. Results are averaged concentrations for triplicate samples. In Group 1 microcosms, complete TCE degradation was observed after approximately 15 days of incubation using phenol as the primary substrate. In Group 2 microcosms, decrease in CB and TCE concentrations was also detected, and almost complete TCE degradation was observed after 23 days of incubation. TCE biodegradation intermediates (*cis*-DCE and VC) were also observed (Fig. 2). However, the intermediates were not accumulated in the microcosms. No significant TCE and CB decrease was observed in the control group. Results indicate that both phenol and CB can serve as the primary substrates to enhance the aerobic cometabolism of TCE.

Fig. 3 presents the results from the second anaerobic microcosm experiments. In Group 1 microcosms, complete TCE removal was observed after approximately 23 days of incubation using methanol as the primary substrate. Decrease in CB and TCE concentrations was detected in Group 2 microcosms, and complete TCE degradation was observed after 34 days of incubation. TCE daughter compounds were detected, and the accumulations of those compounds were not observed (Fig. 3). No degradation of TCE and CB were observed in the control group. Results suggest that both methanol and CB can serve as the primary substrate to enhance the reductive dechlorination of TCE.

5. Conclusions

The field investigation activities and groundwater analytical results suggest that intrinsic bioremediation mechanisms are occurring at the former fire training site. Evidences for intrinsic bioremediation include:

1. depletion of DO, nitrate, and sulfate within the plume;
2. Fe(II) and CO_2 production within the plume;
3. decreased pH within the contaminated zones;
4. decreased TOC concentrations from upgradient to downgradient zones within the plume;
5. production of chloride ion within the plume; and
6. production of TCE degradation byproducts within the plume.

Five conclusions can be drawn based on the results of the laboratory microbial enumeration and microcosm experiments.

(1) Methanogenesis was the dominant biodegradation process within the upgradient and mid-plume areas (zones A and B), and iron reduction and sulfate reduction were not significant in both zones.

(2) The aerobic biodegradation was the dominant process in the downgradient area (zone C).

(3) CB can serve as the primary substrate and biodegrade under both aerobic and anaerobic conditions.

(4) TCE can be biodegraded to less-chlorinated compounds using phenol and methanol as the primary substrates under aerobic cometabolism and reductive dechlorination conditions, respectively.

(5) Enhanced TCE biodegradation to less-chlorinated compounds (e.g. *cis*-DCE, VC) using CB as the primary substrate was observed. The intermediates were not accumulated in the microcosms.

Results from the field and laboratory studies indicate that it is feasible to biodegrade TCE using CB as the primary substrate under both aerobic cometabolism and reductive dechlorination conditions. The observed decrease in TCE concentrations at the former fire training area may be due to the combination of aerobic and anaerobic processes enhanced by the CB biodegradation. Monitoring results indicate that the TCE/CB plume is not growing and has reached a pseudo-steady-state. Therefore, intrinsic bioremediation plays an important role in plume containment at this study site.

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