Intrinsic Remediation of Trichloroethene Driven by Tetraalkoxysilanes as Co-contaminants: Results of Microcosm and Field Studies

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Biological reduction of trichloroethene (TCE), driven by the transformation products of tetraalkoxysilanes, was investigated in seasonal field monitorings and anaerobic groundwater microcosms. Under anaerobic conditions, tetraalkoxysilanes such as tetrabutoxysilane (TBOS) and tetrakis(2-ethylbutoxy)silane (TKEBS) are abiotically hydrolyzed to their corresponding alcohols, 1-butanol and 2-ethylbutanol, respectively, and silicic acid. These alcohols are then fermented by subsurface microorganisms to their corresponding acids and hydrogen, which likely serves as the ultimate electron donor facilitating reductive dechlorination of TCE. At Site 300, Lawrence Livermore National Laboratory (LLNL), California, tetraalkoxysilanes are present along with TCE as subsurface contaminants. Intrinsic transformation of TCE to cis-dichloroethene (c-DCE) was observed at this site, and this was promoted by the transformation products of these tetraalkoxysilanes. Efficient transformation of high concentrations of TCE (150–300 mg/l aqueous concentration) was observed in anaerobic microcosms constructed with groundwater from this site. The lack of transformation of c-DCE to vinyl chloride (VC) and ethene in the microcosms is consistent with studies of the microbial community in the site groundwater reported by Lowe et al. (2002). © 2003 Wiley Periodicals, Inc.

INTRODUCTION

Trichloroethene (TCE) is a common groundwater contaminant in aquifers throughout the United States (Westrick et al., 1984). TCE ranks in the top ten priority pollutants listed by the U.S. Environmental Protection Agency (EPA) (Federal Register, 1989). Over the past decade, microbial degradation of TCE has been extensively studied (Hopkins et al., 1993; Mars et al., 1996; Alvarez-Cohen & McCarty, 1991; Gibson & Sewell, 1992; Maymo-Gatell et al., 1997; Distefano et al., 1992; Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997; Sharma & McCarty, 1996; Semprini et al., 1995). Reductive dechlorination under anaerobic conditions and aerobic cometabolic processes are the predominant pathways for TCE transformation. In aerobic cometabolic processes, fortuitous oxidation of TCE is catalyzed by the enzymes induced and expressed for the initial oxidation of the growth substrates (Hopkins et al., 1993; Mars et al., 1996; Alvarez-Cohen & McCarty, 1991). In the reductive dechlorination process, TCE serves as an electron acceptor and chlorine atoms are replaced by hydrogen (Gibson & Sewell, 1992; Maymo-Gatell et al., 1997; Distefano et al., 1992; Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997). Electron donors such as lactate, benzoate, and

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Published online in Wiley Interscience (www.interscience.wiley.com). DOI: 10.1002/rem.10062

ethanol (Gibson & Sewell, 1992; Distefano et al., 1992; Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997) have been shown to support the dechlorination reaction. A range of dechlorinating cultures have been isolated and studied to determine the extent of dechlorination (Distefano et al., 1992; Holliger et al., 1993; Neumann et al., 1994; Smatlak et al., 1996; Maymo-Gatell et al., 1997; Fennell et al., 1997; Ballapragada et al., 1997; Holliger et al., 1998; Löffer et al., 2000). Incomplete dechlorination to *cis*-dichlorethene (*c*-DCE) or vinyl chloride (VC) is often observed. However, some cultures are reported to dechlorinate completely to ethene (Distefano et al., 1992; Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997).

The potential for enhancing anaerobic transformation processes for bioremediation is currently being tested and several recent studies have focussed on the role of H_2 (Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997) in the reductive dechlorination process. It has been shown that H_2, generated from more complex organic substrates via fermentation, can serve as the ultimate electron donor in the dechlorination of TCE (Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997). In natural systems, H_2-utilizing microorganisms include methanogens, acetogens, sulfidogens, and dechlorinators. The dechlorinators must compete with these other hydrogenotrophs for the evolved H_2 (Smatlak et al., 1996; Ballapragada et al., 1997). A significant advantage for the dechlorinators can be obtained by maintaining a steady source of H_2 at low H_2 partial pressures (Fennell et al., 1997; Smatlak et al., 1996; Ballapragada et al., 1997).

Building 834 at Lawrence Livermore National Laboratory (LLNL) Site 300, in California, is located near a shallow aquifer contaminated with TCE and tetraalkoxysilanes. TCE along with tetraalkoxysilanes such as tetrakis(2-ethylbutoxy)silane (TKEBS) ($C_{24}H_{52}O_4Si$) and tetrabutoxysilane (TBOS) ($C_{16}H_{36}O_4Si$), likely present as a trace component of the TKEBS, were used as a heat-exchange fluid in the materials testing facility with contamination resulting from leaking pipes and other components. The building complex is located in the vicinity of well W-834-D3 (D3) (Exhibit 1). Mixtures of TCE (100–30 percent) and TKEBS (0–70 percent) were periodically released from the facility (Lowe et al., 2002). The source area at the site is located on a hilltop and is underlain by a shallow (~ 15 m) perched water-bearing zone of variable thickness (<0.1–4 m) (Lowe et al., 2002).

Tetraalkoxysilanes are known to hydrolyze to their corresponding alcohols in the presence of water (Hasegawa & Sakka, 1998; Aelion et al., 1950; McNeil et al., 1980; Eaborn, 1960). Both TBOS and TKEBS hydrolyze to 1-butanol and 2-ethylbutanol, respectively. In our laboratory, it was determined that both TBOS and TKEBS have low aqueous solubility (0.5–1.0 mg/l) and that the hydrolysis was acid- and base-catalyzed, and independent of temperature (Vancheeswaran et al., 1999). Typical rates of hydrolysis of TBOS and TKEBS, observed at pH 7, 30 °C and an initial concentration of 28 μM (10 mg/l) were 0.32 μM/day and 0.048 μM/day, respectively. The rates of hydrolysis increased with the concentration of TBOS or TKEBS above their solubility limits. Biologically mediated degradation of TBOS and TKEBS under aerobic conditions has also been studied in our laboratory (Vancheeswaran et al., 1999), and rates of hydrolysis were enhanced. TBOS was also determined to be an effective growth substrate for the aerobic cometabolism of TCE and *c*-DCE (Vancheeswaran et al., 1999).

The rates of hydrolysis increased with the concentration of TBOS or TKEBS above their solubility limits.

The primary objective of this research was to investigate the role of TKEBS in promoting the biologically mediated reductive dechlorination of TCE. Groundwater microcosms were constructed with water collected from well D3 located in the source

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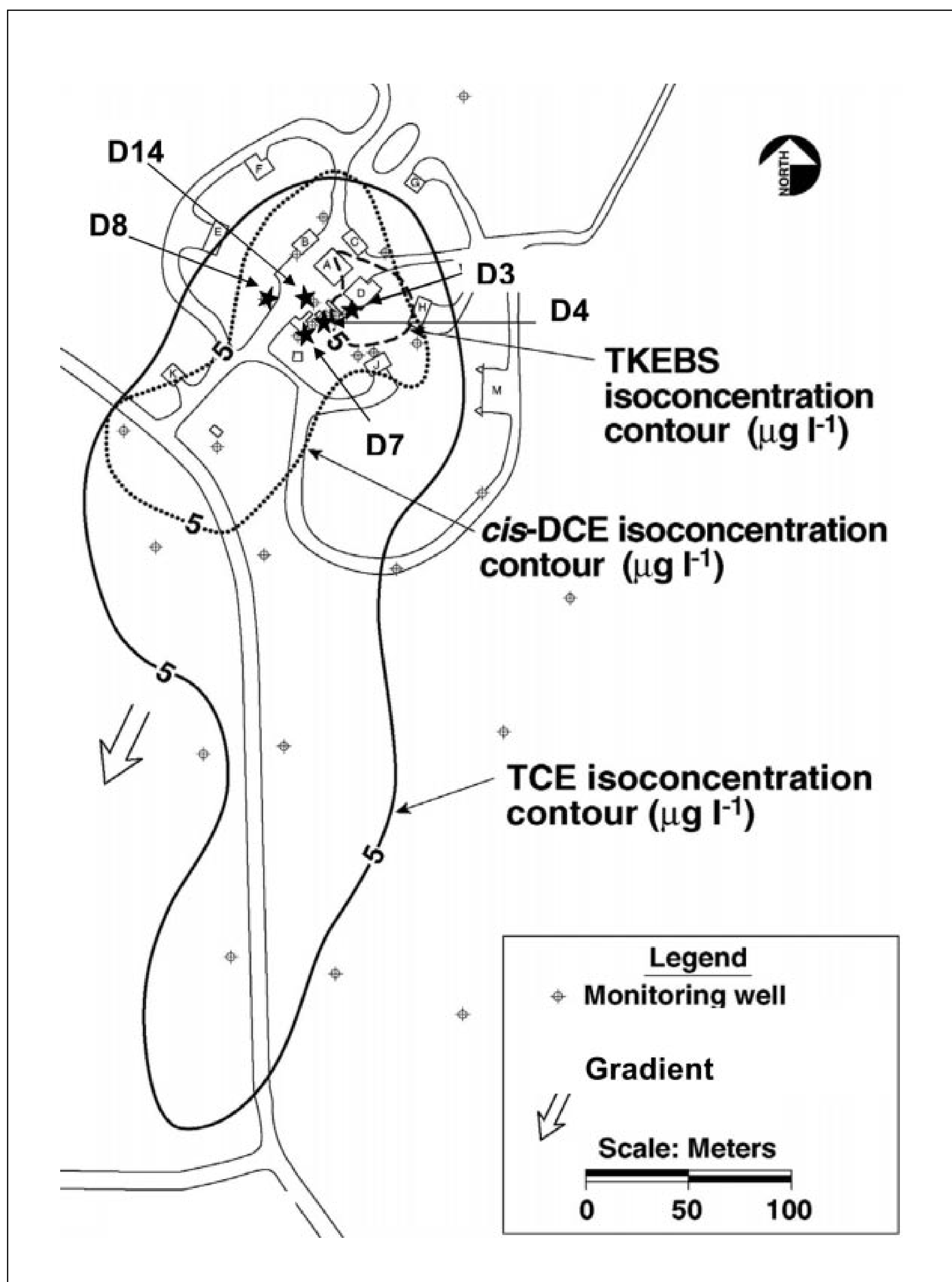

Exhibit 1. Site 300 and the locations of wells from which groundwater samples were obtained. The map and groundwater concentration contours were adapted from Lowe et al. (2002).

area of contamination (Exhibit 1), to study the transformation of TBOS and TKEBS and their hydrolysis products under anaerobic conditions. Laboratory microcosm evidence and field evidence from analysis of groundwater samples is presented to demonstrate the role of TKEBS as an effective co-substrate promoting the anaerobic transformation of TCE.

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MATERIALS AND METHODS

Chemicals and Stock Solutions

TCE (99.9 percent purity), *c*-DCE (99.9 percent), HPLC-grade dichloromethane, 1-butanol (99.9 percent) and 2-ethylbutanol (96 percent purity) were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). TKEBS (United Chemical Technologies Inc., 97 percent, Bristol, Pennsylvania), TBOS (Aldrich Chemical Co., 97 percent, Milwaukee, Wisconsin), and tetrapropoxysilane (TPOS) (Gelest Inc., 98 percent, Tullytown, Pennsylvania) were obtained for analytical standards. Glacial acetic acid (Mallinckrodt, Inc., 99.5 percent), sodium butyrate (Fisher Scientific Co.), and 2-ethylbutyric acid (Aldrich Chemical Co., 97 percent) were used in the preparation of analytical standards. H_3PO_4 (Mallinckrodt, Inc., 95 percent) was used to prepare 8-M aqueous solutions for preservation of liquid samples.

TKEBS concentrations in the groundwater were above its solubility limit in water, thus it was present as a neat phase.

Analytical Methods

The same analytical methods were used for both groundwater analysis and the aqueous samples from the microcosms. TBOS, TKEBS, 1-butanol, and 2-ethylbutanol concentrations were extracted from aqueous samples (1.0 ml) with dichloromethane (0.5 ml) and agitated for 5 minutes on a vortex mixer. TKEBS concentrations in the groundwater were above its solubility limit in water, thus it was present as a neat phase. In order to obtain representative aqueous samples of TKEBS, the microcosms were rigorously shaken by hand prior to sampling. All the TKEBS present in the aqueous sample was then extracted into the dichloromethane. After complete separation of the two immiscible phases, 2 µl of the dichloromethane extract was introduced into a Gas Chromatograph/Mass Spectrometer (GC/MS). Analyte concentrations in the extract samples were determined with a HP-5890 GC connected to a HP-5971 mass selective detector. The chromatographic separation was carried out with an Rtx-20 column (30 m × 0.25 mm, 1.0 µm film) from Restek, Inc. (Bellefonte, Pennsylvania). The mass spectrometer was operated in the selective ion monitoring (SIM) mode for the quantitative analysis of the compounds. The ions monitored were m/z 56 for 1-butanol, m/z 70 for 2-ethylbutanol, m/z 235 for TPOS (internal standard), m/z 277 for TBOS, and m/z 361 for TKEBS (fragmentation achieved by electron-ionization [EI]). The concentrations were normalized using TPOS (10 mg/l) as an internal standard. Solid Phase Micro Extraction (SPME) (Arthur et al., 1992; Pawliszyn, 1994) was employed for the extraction and GC/MS analysis of the groundwater and microcosm samples to obtain qualitative information on the nature of contamination at the site (results represented in Exhibit 2). An 85-µm polyacrylate fiber (Supelco Inc.) was employed for the SPME extraction of 1-ml aqueous samples.

Gas phase TCE, *c*-DCE, ethene, and vinyl chloride concentrations were measured by injecting 10–100 µl of a gaseous microcosm sample into a HP-5890 GC connected to a photoionization detector (PID) followed by a flame ionization detector (FID). Chromatographic separation was carried out with a 30-m megabore GSQ-PLOT column from J&W Scientific (Folsom, California). The gases nitrogen, hydrogen, methane, oxygen, and carbon dioxide in the microcosm headspace were measured with a HP-5890 GC connected to a thermal conductivity detector (TCD). The method involved direct injection of a 0.1-ml gas sample from the headspace of the microcosm into the GC with a gas-tight syringe (Hamilton Co., Reno). Chromatographic separation was carried out

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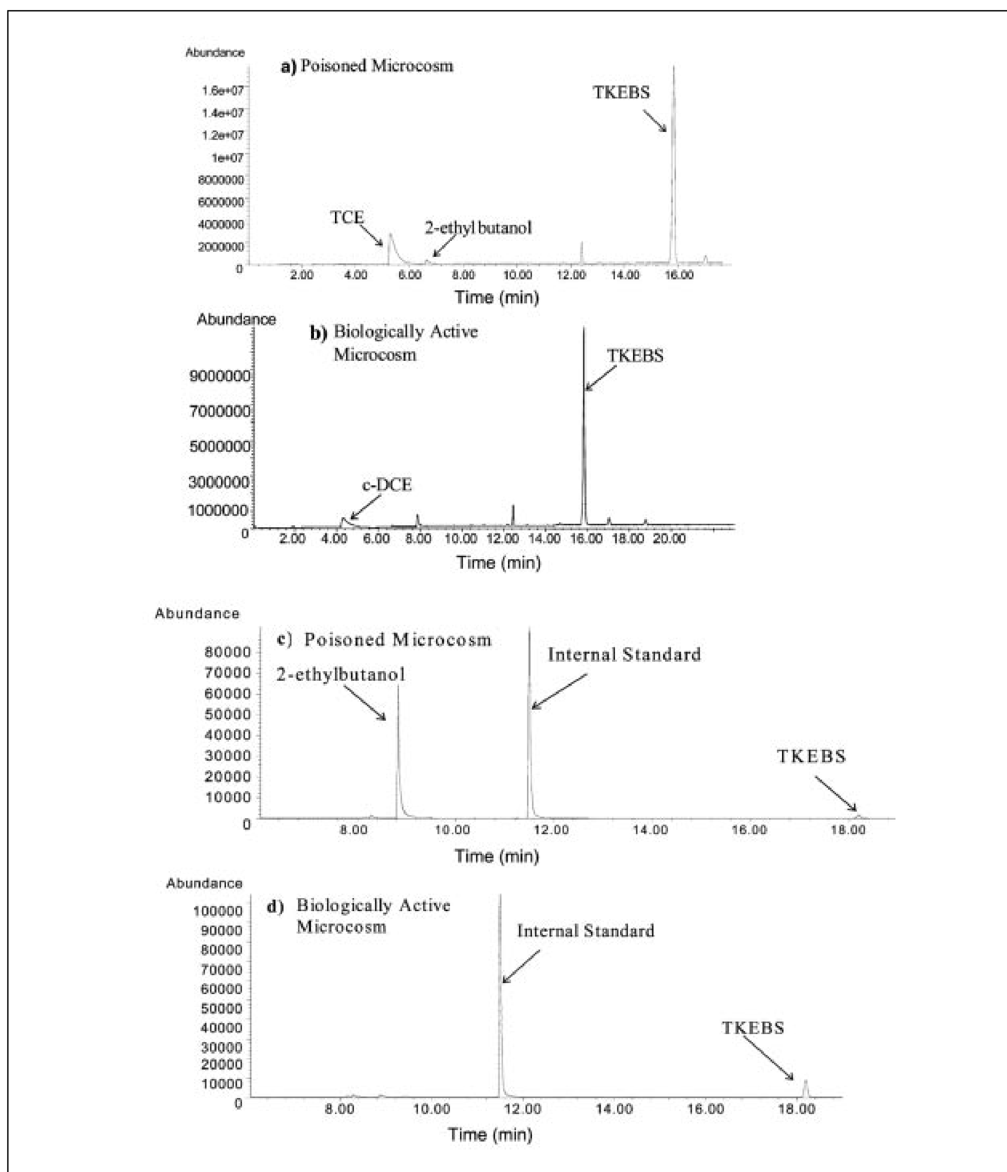

Exhibit 2. GC analysis of biologically active and poisoned microcosms. SPME was used for qualitative analysis of the volatile compounds in solution after 60 days of incubation in a poisoned (a) and biologically active (b) microcosm. Extraction with dichloromethane was used for the quantitative determination of non-volatile compounds in solution after 60 days of incubation in a poisoned (c) and biologically active (d) microcosm. For c and d, the SIM mode was used and the *m/z* for TCE and *c*-DCE were not monitored. PID measurements were used for TCE and *c*-DCE quantification.

with a Carboxen™ 1000 packed column (15 ft × 0.125 in, S.S Support) from Supelco (Bellefonte, Pennsylvania) with argon as the carrier gas. The hydrogen detection limit was 4 nM (liquid concentration basis) with the TCD measurement. The volatile acids were measured by injecting 0.5–2 µl of acidified and prefiltered aqueous samples into a HP-5890 GC connected to a FID detector. Chromatographic separation was carried out with a HP-Innowax column (30 m × 0.25 mm, 1.0-µm film) from Hewlett-Packard

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(Wilmington, Delaware). Samples were preserved by adding 10 µl of 8 M H_3PO_4 acid to 0.5-ml aqueous solution and then centrifuged prior to analysis.

Microcosm Preparation

Microcosms were constructed with 310-ml and 1,050-ml serum bottles (Wheaton Industries, Inc., Seattle, Washington) with rubber-lined caps and butyl rubber septa. Groundwater for the microcosm preparation was collected in glass bottles with rubber-lined screw caps and no headspace to maintain anaerobic conditions during storage. The groundwater was stored at 4 ºC until it was used in constructing the microcosms. Groundwater was collected from the W-834-D3 well (D3 well) in the center of the Building 834 plume where high concentrations of *c*-DCE were observed. The geochemistry of groundwater from the D3 well for three samples obtained from July 28 to September 23, 1998 is summarized in Exhibit 3. The groundwater contained high concentrations of TCE and *c*-DCE, but VC was below detection. As will be discussed later, TKEBS, its hydrolysis product 2-ethylbutanol, as well as 2-ethylbutyrate and acetate, which are potential fermentation products of ethylbutyrate, were present in the groundwater. The groundwater pH was around neutral, and the Eh levels indicated that it was anoxic. Sulfate was present as the main soluble inorganic electron acceptor.

Microcosms were prepared by transferring groundwater to the serum bottles in an anaerobic glove box. Aquifer solids were not available, so the tests relied on

Analyte	Contaminated Well W-834-D3	Unit
Primary contaminants and metabolites		
TCE	56 ± 13	mg/l
Cis-DCE	16 ± 11	mg/l
Vinyl chloride	BD	mg/l
Ethene	ND	mg/l
Ethane	ND	mg/l
Secondary contaminants and metabolites		
TKEBS	11 ± 8	mg/l
2-Ethylbutanol	7 ± 9	mg/l
2-Ethylbutyrate	94 ± 141	mg/l
Acetate	21 ± 32	mg/l
Geochemical indicator		
pH	7.2 ± 0.1	
Eh	–121 ± 87	mV
Dissolved oxygen	< 2	mg/l
Nitrate	< 5	mg/l
Nitrite	< 0.5	mg/l
Sulfate	13 ± 5	mg/l
Sulfide	< 1	mg/l

[a]Unless otherwise noted, reported values represent average concentrations (± 1 S.D.) of three measurements made between July 28 and September 23, 1998. BD, below detection; ND, not determined

Exhibit 3. Chemistry and geochemistry of perched groundwater of the W-834-D3 well (adapted from Lowe et al., 2002).

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microorganisms present in the groundwater. The 316-ml microcosm bottles contained 230 ml of groundwater and 86 ml of headspace (microcosm bottles of Exhibits 4 and 5) while the 1,050-ml microcosm bottles contained 950 ml of groundwater and 100 ml of headspace (microcosm bottles of Exhibit 6). Due to the fact that hydrogen was introduced into the microcosms from the glove box atmosphere (90 percent nitrogen and 10 percent hydrogen) and the presence of residual TCE and *c*-DCE in groundwater, the microcosms were purged with nitrogen that was treated in a tube furnace (Thermolyne, Inc., Dubuque, Iowa) to remove any traces of oxygen. Appropriate volumes of pure liquid TCE were added to achieve the desired TCE concentrations. Control microcosms were prepared by chemical poisoning with mercuric chloride ($HgCl_2$) at a concentration of 25 mg/l. The microcosms were incubated in an environmental chamber at 30 °C with periodic shaking.

The total mass of TCE, *c*-DCE, or H_2 in the microcosms was determined by measuring gas phase concentrations and calculating aqueous phase concentrations assuming equilibrium Henry's law partitioning (Henry's law constants were obtained from several sources [Smatlak et al., 1996; Neumann et al., 1994]). From the volumes of liquid and headspace in the microcosms and the concentrations of TCE and *c*-DCE in these two compartments, the total mass balances in the microcosms were verified.

RESULTS AND DISCUSSION

Five monitoring wells–W-834-D3 (D3), W-834-D4 (D4), W-834-D7 (D7), W-834-D8 (D8), and W-834-D14 (D14)–were selected from the existing monitoring network around Building 834, Site 300, to characterize the nature of contamination and to study the transformation processes involving TCE, TBOS, and TKEBS. Quantitative and qualitative analyses of groundwater in these selected monitoring wells were conducted. Groundwater in wells D3 and D4 contained high concentrations of TCE (> 50 mg/l), TKEBS (10–80 mg/l), and traces of TBOS. The concentration of TKEBS was above its solubility limit in water, thus it was present as a separate phase at this location. This result is consistent with the location of the D3 and D4 wells being close to the source zone of contamination (Exhibit 1). TBOS and TKEBS contamination was limited to the wells D3 and D4, while TCE was detected in all the selected monitoring wells. The TCE plume extends across all the selected wells and into a much larger area (about 1,500 feet long and 600 feet wide) with concentrations near the source ranging from 20 to over 100 mg/l (Exhibit 1).

Analysis of groundwater from well D3 by SPME followed by GC/MS indicated that TKEBS was present as the primary co-contaminant along with traces of TBOS. The hydrolysis product of TKEBS, 2-ethylbutanol, was also present in significant amounts. 1-Butanol was not detected.

Analysis of groundwater from well D3 by SPME followed by GC/MS indicated that TKEBS was present as the primary cocontaminant along with traces of TBOS.

Preliminary Evidence of TCE Reduction Driven by the Transformation Products of TKEBS

Preliminary evidence of TKEBS transformation and its role in driving reductive dechlorination of TCE was obtained by constructing and incubating live and poisoned anaerobic microcosms containing groundwater from well D3 (Exhibit 2). After approximately 60 days of incubation, analysis of the aqueous phase in the poisoned control indicated the accumulation of 2-ethylbutanol, which is the hydrolysis product of TKEBS, while in the

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live microcosm, 2-ethylbutanol was not observed. This suggested the abiotic hydrolysis of TKEBS to 2-ethylbutanol, and the biologically mediated transformation of 2-ethylbutanol. Analysis of the aqueous phase of these microcosms using SPME followed by GC/MS also indicated that TCE was dechlorinated to *c*-DCE in the live microcosm; whereas no transformation was observed in the poisoned control. These results provided the preliminary insight on the origin of the organic substrate (2-ethylbutanol, from the hydrolysis of TKEBS) that supported the reductive dechlorination reaction.

Subsequently, additional pilot microcosm experiments were designed to better understand the reductive dechlorination of TCE and to identify the substrates driving the dechlorination reaction. Results from the TCE transformation study conducted with D3 well groundwater are shown in Exhibit 6. TCE at an initial aqueous concentration of 750 µM (100 mg/l) was rapidly dechlorinated to *c*-DCE within 14 days. During the initial lag period of about 3 to 5 days after purging the residual hydrogen and addition of TCE, a steady build up of hydrogen occurred while TCE transformation was minimal. The eventual decrease in hydrogen concentration correlates with the increase in the rate of TCE transformation to *c*-DCE. The initial lag period may be related to the time required for: microbial growth, the production of hydrogen, or to develop the needed redox conditions in the microcosms. *c*-DCE persisted in the microcosms and no further transformation was observed over a 3-month incubation (data not shown). Neither vinyl chloride nor ethene was detected, and methane production as an indication of methanogenesis was not observed.

Electron mass balances indicate that the amount of 2-ethylbutanol that fermented to 2-ethylbutanol was in the range of that needed to produce the hydrogen required for the dehalogenation reactions.

Analysis of Exhibit 6b indicates a decrease in 2-ethylbutanol concentration and a corresponding increase in the concentration of 2-ethylbutyric acid. High background concentrations limit accurate mass balances of the fermentation of 2-ethylbutanol to 2-ethylbutyric acid. The production of hydrogen indicated that fermentation reactions were proceeding. TKEBS concentration (80 mg/l, 185 µM) was observed to remain relatively constant during the course of this experiment (data not shown), indicating the hydrolysis to 2-ethylbutanol was slow. Electron mass balances indicate that the amount of 2-ethylbutanol that fermented to 2-ethylbutanol was in the range of that needed to produce the hydrogen required for the dehalogenation reactions. During one other similar experiment, the addition of hydrogen gas to the microcosm was observed to improve the TCE dechlorination rates (data not shown).

Long-Term Microcosm Study for TKEBS Hydrolysis and Its Role in Reductive Dechlorination of TCE

Long-term experiments were designed to study in more detail the transformation pathway of TKEBS and its hydrolysis product under anaerobic conditions (Exhibits 4 and 5). Live and poisoned microcosms containing groundwater from well D3, collected in April 1998, were used for this study. Background concentrations of the compounds present in the groundwater were: TKEBS 65 µM (28 mg/l), 2-ethylbutyrate 2,672 µM (310 mg/l), butyrate 182 µM (16 mg/l), and acetic acid 1,017 µM (61 mg/l). TBOS was not detected in the groundwater during this sampling event. Background TCE and *c*-DCE were purged according to the methods described in the experimental section. Neat TCE was added to achieve the desired aqueous TCE concentrations of 304 µM (40 mg/l).

The transformation processes observed in the duplicate poisoned anaerobic microcosms over a period of 6 months are shown in Exhibit 4. Duplicate microcosms showed nearly identical results. The formation and accumulation of 2-ethylbutanol during the

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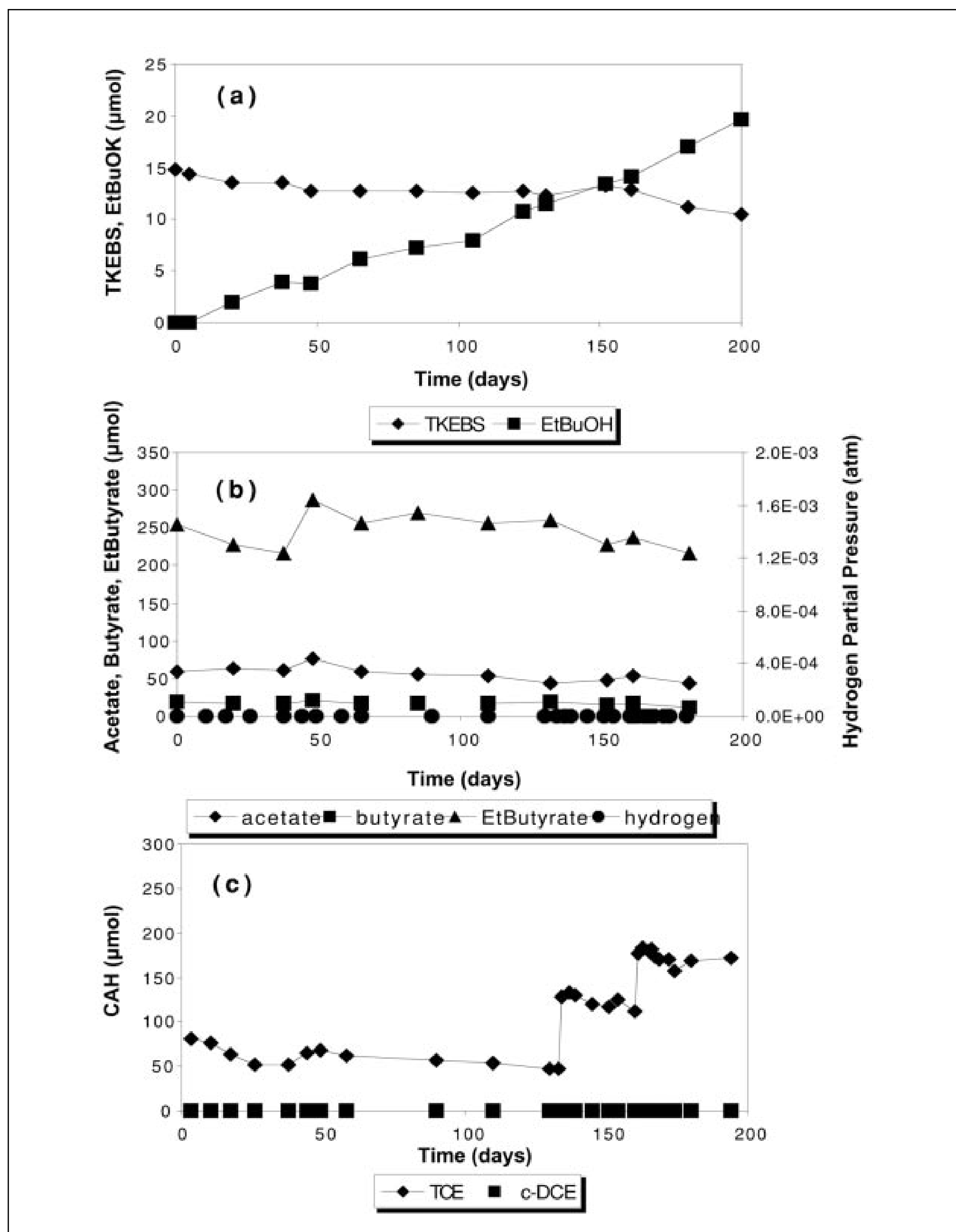

Exhibit 4. Poison control microcosm containing groundwater from Site 300 of LLNL sampled in April of 1998. (a) TKEBS and 2-ethylbutanol. (b) Acids from fermentation process and hydrogen. (c) TCE and *c*-DCE. Studies were conducted in 316-ml serum bottles with a 230-ml liquid volume and a 86-ml headspace.

hydrolysis of TKEBS is shown in Exhibit 4a. The formation of 2-ethylbutanol was more easily tracked than TKEBS disappearance. (The analytical method employed for the measurement of TKEBS concentration has a low precision [~85–115 percent], which precludes reliable measurements of small changes [<15 percent] in TKEBS concentration). The rate of 2-ethylbutanol formation appeared to be zero order consistent with observations of Vancheeswaran et al. (1998). Based on linear regression analysis of 2-ethylbutanol concentration data, the TKEBS hydrolysis rates was 0.11 μM/day at an initial TKEBS concentration of 65 μM (28 mg/l). This rate is comparable to the abiotic

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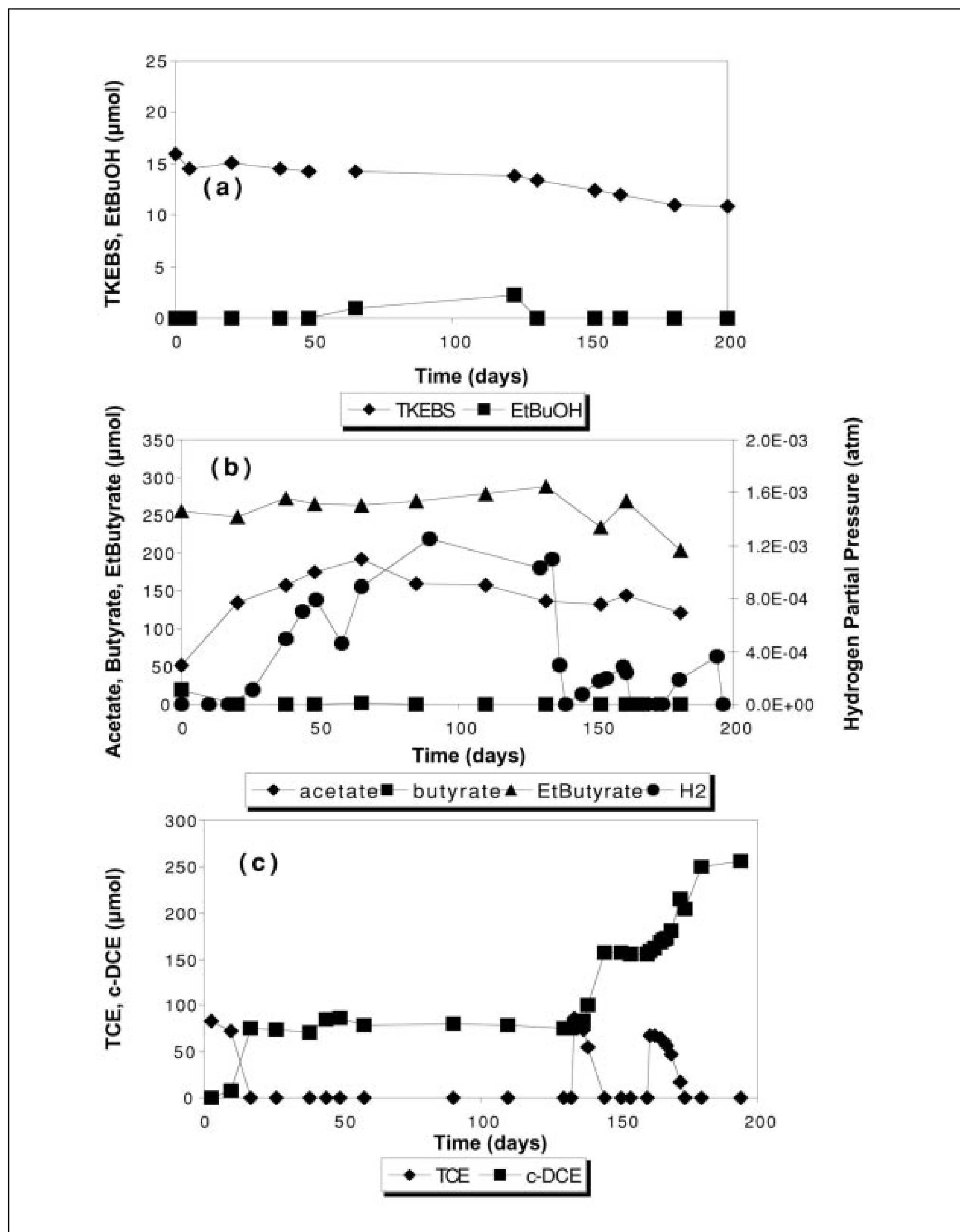

Figure 5. Live microcosm containing groundwater from Site 300 of LLNL sampled in April of 1998. (a) TKEBS and 2-ethylbutanol. (b) Acids from fermentation process and hydrogen. (c) TCE and *c*-DCE. Studies were conducted in 316-ml serum bottles with a 230-ml liquid volume and a 86-ml headspace.

hydrolysis rates determined previously for TKEBS (0.13 µM/day, initial concentration of 24 µM (10 mg/l)) (Vancheeswaran et al., 1999). The slower rate in the current study likely resulted from the less vigorous shaking of the microcosms.

The concentrations of the fermentation products of 2-ethylbutanol–2-ethylbutyrate and acetate–remained constant in the poisoned control (Exhibit 4b) and no H_2 was detected. TCE was added in the beginning of the experiment, and again after 133 and 160 days after incubation (Exhibit 4c). No transformation of TCE was observed based on

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the constant TCE concentrations and the lack of formation of *c*-DCE and vinyl chloride as dehalogenation products. These observations demonstrated the inhibition of biological fermentation and dehalogenation reactions in the poisoned controls.

Results of TKEBS and TCE transformation in live anaerobic microcosms over a 6-month period are shown in Exhibit 5. Duplicate microcosms showed nearly identical trends, but data from only one microcosm is shown. The formation and subsequent utilization of 2-ethylbutanol is shown in Exhibit 5a. The formation and utilization of 2-ethylbutyrate, acetate, and hydrogen are shown in Exhibit 5b. Rapid transformation of TCE to *c*-DCE (Exhibit 5c) was observed within the first 18 days of the experiment, and following repeated TCE additions after 134 and 160 days of incubation. During the transformation of TCE to *c*-DCE in the first 18 days of incubation, butyrate concentrations decreased and

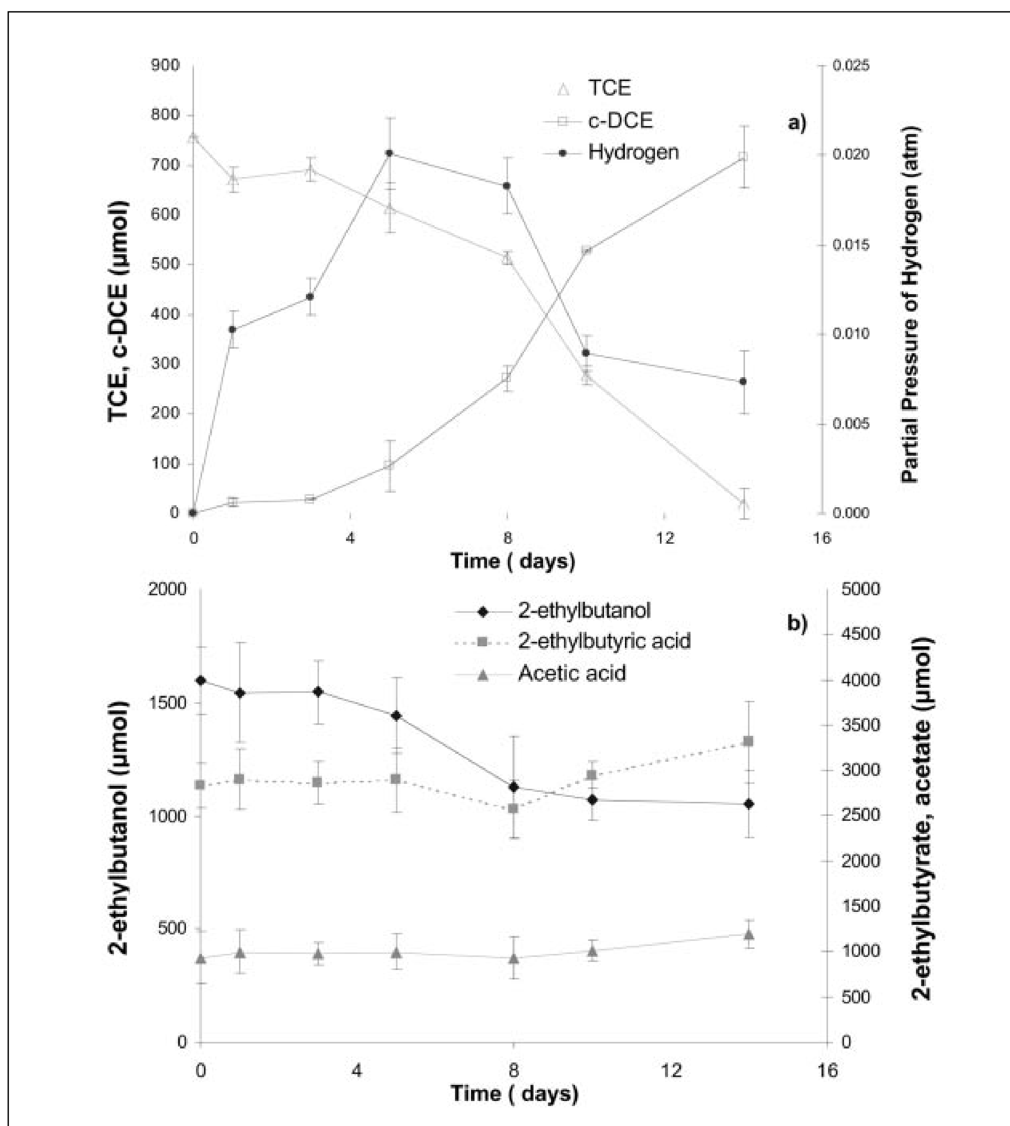

Exhibit 6. Reductive dechlorination of TCE in anaerobic microcosms constructed with groundwater sampled in September 1997. (a) TCE, *c*-DCE, and hydrogen concentrations in the microcosms. (b) Profiles of 2-ethylbutanol, 2-ethylbutyrate, and acetate concentrations. Studies were conducted in 1.05-l serum bottles with a 950-ml liquid volume and a 100-ml headspace. The average results from three microcosms and standard deviations are presented.

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acetate concentrations increased. After TCE was transformed to *c*-DCE, 2-ethylbutyrate and hydrogen increased with time. Butyrate, which was initially present in the groundwater, was not detected during the rest of the experiment. When TCE was again added to the microcosms at 134 and 160 days of incubation, a rapid decrease in hydrogen concentrations and a simultaneous decrease in 2-ethylbutyrate were observed. After the transformation of TCE to *c*-DCE, an increase in hydrogen concentration was observed. *c*-DCE persisted in the microcosms and no further transformation to vinyl chloride and ethene was observed over the 6-month incubation. No methanogenesis was observed in these microcosms, even at the high hydrogen partial pressures and the high acetate concentrations.

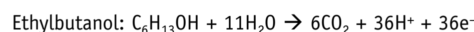
Electron mass balances were performed based on the results presented in Exhibit 5, following the procedures described by Yu and Semprini (2002). Electron equivalents were compared based on the concentrations of TKEBS, alcohols, acids, and CAHs before and after dechlorination (Exhibit 7). A good electron mass balance of about 91 percent was achieved. The dehalogenation reaction of TCE to *c*-DCE accounts for a significant fraction of the electrons transferred, and is in the same range of that observed for the production of acetate. Approximately 20 to 40 percent of the electrons transferred were associated with the dehalogenation reaction.

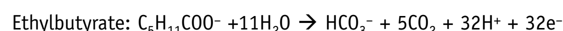
The absence of dehalogenation of *c*-DCE to VC and ethene observed in the microcosms is supported by an analysis of the microbial diversity of the groundwater from the D3 well performed by Lowe et al. (2003). Using 16S rDNA methods, they found that bacteria were present that are closely related to genus *Dehalobacter*, which are capable of transforming TCE to *c*-DCE. However, the *Dehalococcoides* species that can transform TCE to ethene were absent in the groundwater. In microcosm studies performed with well D3 groundwater with additional TBOS added, ethene and methane production were observed when a mixed culture was added that can transform TCE to ethene (Yu & Semprini, 2002). These results further support the observation that microbes are

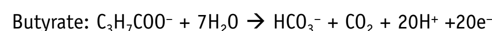
	e^- equiv. per mole	e^- μ equivalents	
		Day 0	Day 181
TKEBS	144[a]	2160	1584
Ethylbutanol	36	0	0
Ethylbutyrate	32	7840	6752
Butyrate	20	380	0
Acetate	8	416	1008
c-DCE	2	0	498
Total		10796	9842
Recovery (%)			91

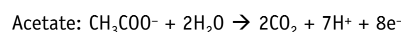
[a]Based on the assumption that 1 mol of TKEBS hydrolyzes to 4 mol of ethylbutanol.

Based on the following equations:


Ethylbutanol: $C_6H_{13}OH + 11H_2O \rightarrow 6CO_2 + 36H^+ + 36e^-$


Ethylbutyrate: $C_5H_{11}COO^- + 11H_2O \rightarrow HCO_3^- + 5CO_2 + 32H^+ + 32e^-$


Butyrate: $C_3H_7COO^- + 7H_2O \rightarrow HCO_3^- + CO_2 + 20H^+ + 20e^-$


Acetate: $CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$

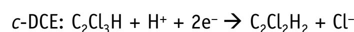

c-DCE: $C_2Cl_3H + H^+ + 2e^- \rightarrow C_2Cl_2H_2 + Cl^-$

Exhibit 7. Electron mass balances based on the microcosm results presented in Exhibit 5.

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absent or not present in high enough number to promote the dehalogenation of TCE beyond *c*-DCE. Methanogens also appear to be absent in the site D3 groundwater, since methane production was observed when the mixed culture was added.

These results indicate that 2-ethylbutanol was fermented to 2-ethylbutyrate, which was further fermented to acetate. Hydrogen formed in these fermentation reactions likely served as an electron donor for the biological reduction of TCE to *c*-DCE. This conclusion is based on decrease in hydrogen concentration during the transformation of TCE to *c*-DCE, and its accumulation after the transformation stopped. The fermentation process may have been driven by the availability of TCE as an electron acceptor and the utilization of hydrogen as the electron donor. Enhanced hydrogen consumption and organic substrate fermentation has been observed previously in the presence of a chlorinated electron acceptor (Dolfing & Tiedje, 1991). Depletion of hydrogen should permit more rapid and complete fermentation of alcohol and acids (Thauer et al., 1977).

In the poisoned microcosm, the rate of TKEBS hydrolysis was determined by the formation of the 2-ethylbutanol. This method could not be used in the live microcosms since 2-ethylbutanol ferments to 2-ethylbutyrate. Based on a simple linear regression fit of the TKEBS data (Exhibit 5a), it was estimated that the TKEBS concentration decreased during the course of the experiment by about 30 percent of its initial concentration, which is comparable to the abiotic rate (Exhibit 4). Thus, TKEBS hydrolysis to 2-ethylbutanol was mainly abiotically mediated under the anaerobic conditions of the microcosms. A biotic process may have contributed to TKEBS hydrolysis, but only to a minor extent.

Based on these results, the pathway for the anaerobic transformation of TKEBS is

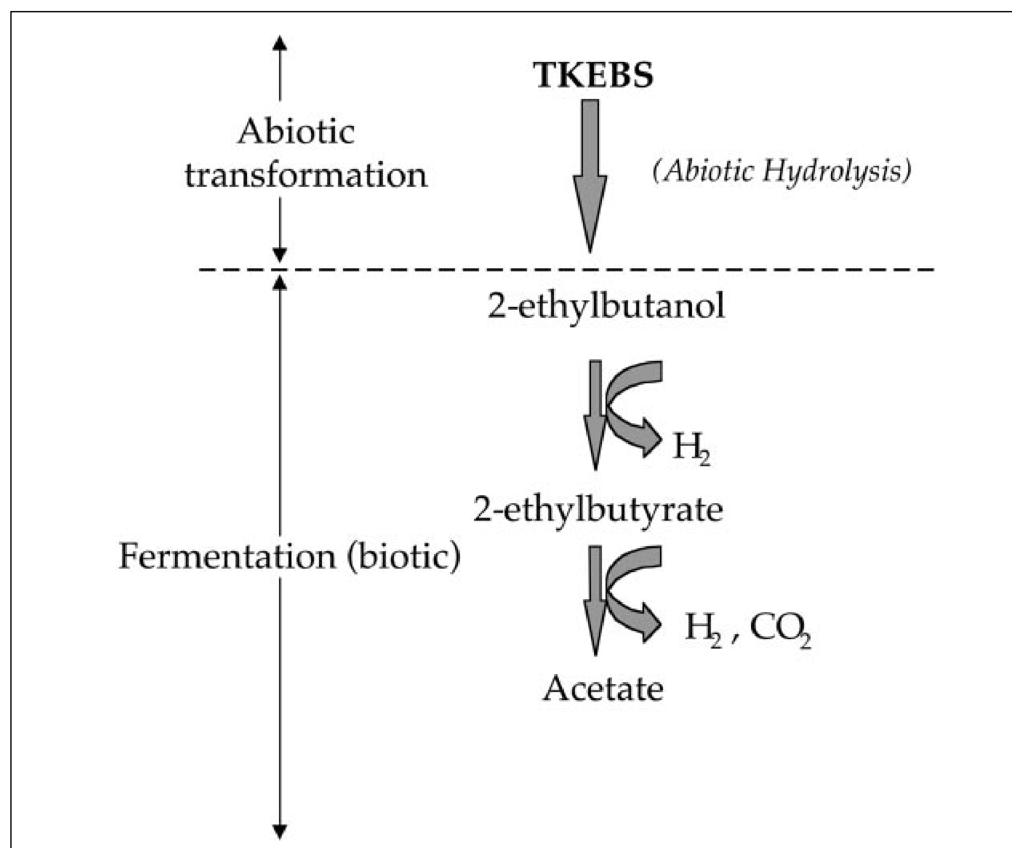

Exhibit 8. Proposed anaerobic transformation pathway for TKEBS.

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proposed (Exhibit 8). TKEBS is abiotically hydrolyzed to 2-ethylbutanol, which is then fermented to 2-ethylbutyrate, and subsequently to acetate. Hydrogen is produced during these fermentation reactions. Although the products in all the proposed steps are observed, we do not have direct evidence for the pathway for acetic acid formation. Butyrate was present initially in the microcosms as a background, and was not observed during the course of the experiment. Butyrate may have been associated with the hydrolysis of TBOS present at the site and the fermentation of butanol. Consistent with fermentation pathways of McInerney et al. (1981) and Stieb and Schink (1985), 2-ethylbutyric acid (a fatty acid with even number of carbon atoms) should ferment directly to acetic acid and hydrogen. The rate of the initial hydrolysis step is expected to equal the abiotic hydrolysis rates; however, this rate could possibly be enhanced by the continuous biological removal of the hydrolysis product, 2-ethylbutanol. Within our ability to measure the decrease in TKEBS concentration, the abiotic process could account for the observed hydrolysis rates. Biologically mediated hydrolysis, however, cannot be ruled out due to the limited accuracy of the TKEBS measurement.

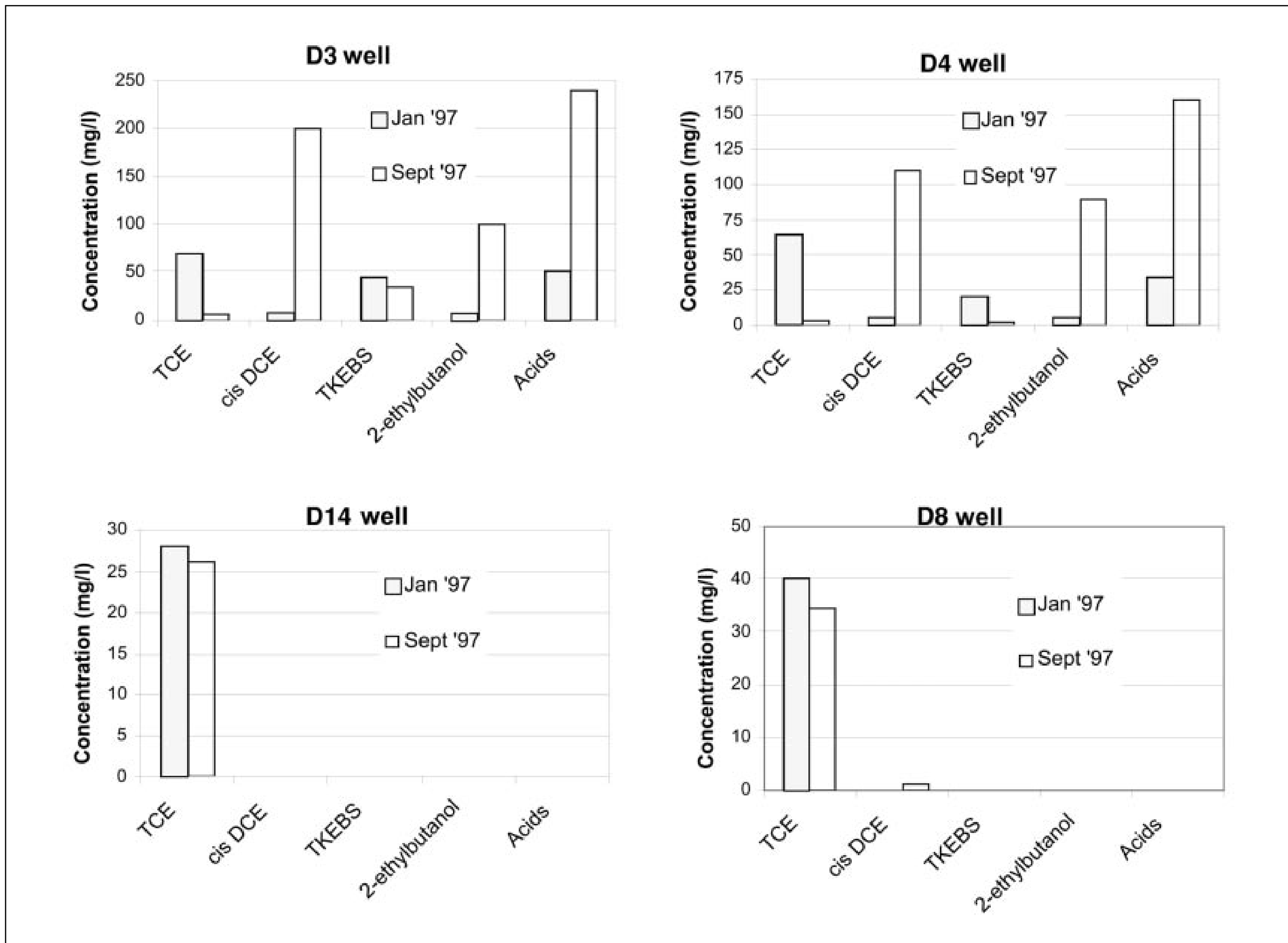

Exhibit 9. Groundwater chemistry data of 4 wells at Site 300 suggesting enhanced TCE dechlorination in the presence of TKEBS contamination. Acids formed during the fermentation of tetraalkoxysilane hydrolysis products include 2-ethylbutyrate, butyrate, and acetate.

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Evidence of In Situ TCE Transformation from Field Sampling

The results from the analysis of chlorinated ethenes, TKEBS, alcohols, and fermentation products at Site 300 in wells D3, D4, D8, and D14 shown in Exhibit 9, provide supporting evidence of the role played by TKEBS in promoting TCE transformation to *c*-DCE. Groundwater from monitoring wells D3 and D4 sampled in January and September 1997, demonstrate high in situ TCE transformation, whereas those near D8 and D14 show low TCE transformation potential. The TCE transformation activity at wells D3 and D4 (Exhibit 9a, b) can be attributed primarily to the presence of TKEBS and its hydrolysis product (2-ethylbutanol) and the fermentation products (2-ethylbutyrate and acetate). Though TBOS was present in wells D3 and D4, it was observed in trace quantities. It is believed that TBOS may have also contributed to the reductive dechlorination of TCE and the presence of butyrate in the groundwater, as previously discussed. The apparent persistence of *c*-DCE at high aqueous concentrations (about 200 mg/l) and the lack of further transformation to VC or ethene at the site are consistent with the microcosm data. There was evidence of sulfides and low levels of sulfates at locations around well D3, indicating sulfate reduction. Sulfate levels in the D3 groundwater 13 mg/l (Exhibit 3), while measureable sulfide concentrations were less than 0.1 mg/l. No TCE transformation, based on the lack of detection of *c*-DCE, was observed near wells D14 and D8 (Exhibit 9c, d) and this is likely due to the absence of TKEBS, TBOS, or their transformation products. These wells are located lateral to the source zone of contamination, while wells D3 and D4 are located directly down gradient of the source of contamination (Exhibit 1). The field results also indicate a one-unit decrease in the pH of the groundwater sampled at wells D3 and D4 in September. These results were also consistent with our microcosm observation of lower pH resulting from the hydrogen ion released in the TCE dechlorination reaction (data not shown).

The apparent persistence of *c*-DCE at high aqueous concentrations (about 200 mg/l) and the lack of further transformation to VC or ethene at the site are consistent with the microcosm data.

Long-Term Field Monitoring

The microcosm studies and the field data collected during two separate sampling events (shown in Exhibit 9) suggested a natural attenuation pathway for TCE at Site 300. In order to obtain a better understanding of the natural processes occurring in situ, a more detailed groundwater monitoring program was conducted from August 1998 to October 1999. The results of the long-term field monitoring on D3 well are presented in Exhibit 10.

The concentration of TCE and *c*-DCE was observed to vary quite significantly between August 1998 and 1999 (Exhibit 10a). The TCE and *c*-DCE concentration histories indicate that as TCE concentrations decrease, *c*-DCE concentrations increase, and vice versa. It is interesting to note that *c*-DCE formed during the reduction process did not accumulate, as was observed in the microcosm studies, but was rapidly attenuated (Exhibit 10a). One possibility is the existence of oxidative transformation pathways that convert *c*-DCE to carbon dioxide and chloride ion. The fact that vinyl chloride and ethene were not detected during these sampling events further supports this possibility.

During periods when the TCE transformation rates were high and *c*-DCE concentrations increased, the concentrations of 2-ethylbutanol, 2-ethylbutyrate, and acetate were observed to reach their maxima (Exhibit 10b), as observed in September 1998 and July 1999. Similarly, when *c*-DCE disappeared, the concentrations of TKEBS, 2-ethylbutanol, 2-ethylbutyrate, and acetate were also observed to decrease (Exhibit 10b, c). Though no direct evidence has been gathered to date, it is possible that aerobic cometabolism of *c*-

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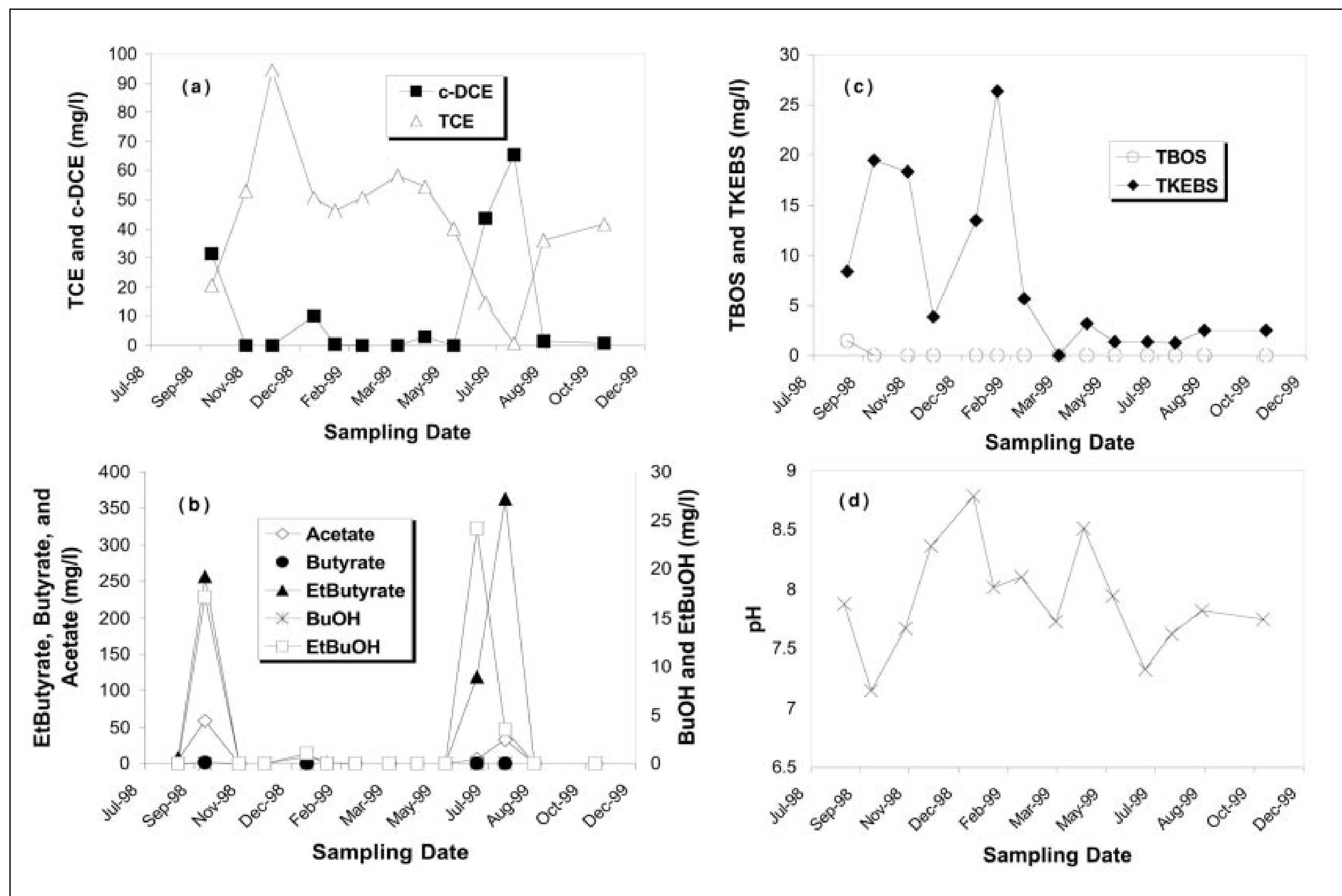

Exhibit 10. Long-term monitoring data of groundwater sampled from the D3 well.

DCE by microorganisms that degrade TBOS or TKEBS occurs in transitional aerobic zones in the aquifer. Vancheeswaran et al. (1999) found that aerobic microorganisms enriched from a wastewater treatment plant could cometabolize *c*-DCE and TCE when grown on TBOS and TKEBS. This enrichment culture also cometabolizes *c*-DCE when grown on butanol, acetate, and butyrate (Tesajen & Semprini, 2002). Gao and Skeen (1999) reported the aerobic cometabolism of *c*-DCE in riverbed sediments that were fed glucose. Attempts to enrich aerobic microorganisms from Site 300 groundwater samples that grow on TKEBS, 2-ethylbutanol, 2-ethylbutyrate, and acetate, and that can cometabolize *c*-DCE, however, have been unsuccessful. Recently, a microorganism has been isolated that can grow on *c*-DCE as an electron donor under aerobic conditions (Coleman et al., 2002). As shown in Exhibit 1, the *c*-DCE plume is much shorter than the TCE plume, indicating natural attenuation processes are likely removing *c*-DCE. The process(es) causing the attenuation of *c*-DCE need to be investigated in future work.

CONCLUSION

This study has provided insight into the anaerobic transformation processes that occur at LLNL Site 300. Based on the understanding of the system that emerged from the microcosm studies and field analyses, it was determined that the hydrolysis and fermentation reactions are occurring as a result of TKEBS (and TBOS) contamination. The fermentation

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reactions produce hydrogen that appears to be serving as the ultimate electron donor in supporting reductive dechlorination of TCE. The fermentations of alcohols and organic acids result in a steady and sustained supply of hydrogen. The slow abiotic hydrolysis of TBOS and TKEBS provides a continuous source of alcohols for the fermentation reactions.

The microcosm results are consistent with the analysis of the microbial diversity in the site groundwater that indicates that microbes are present that can transform TCE to *c*-DCE, but not TCE to ethene (Lowe et al., 2002). The seasonal production of the acid fermentation products along with *c*-DCE and their subsequent disappearance needs further investigation. Processes that are potentially oxidizing *c*-DCE under aerobic and possibly anaerobic conditions need to be studied.

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-Eng-48. The authors would like to acknowledge Rick Landgraf for his assistance with field sampling. Also, we thank Lutz Friedrich and George Pon for their contributions in preliminary microcosm studies.

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