AN ABSTRACT OF THE THESIS OF

Sanjay Vancheeswaran for the degree of Master of Science in Chemistry and
Civil Engineering presented on June 10, 1998.

Title: Abiotic and Biological Transformation of TBOS and TKEBS, and their Role in
the Biological Transformation of TCE and c-DCE.

Redacted for Privacy

Abstract approved: _____

James D. Ingle, Jr

Redacted for Privacy

Abstract approved: _____

Lewis Semprini

At Site-300, Lawrence Livermore National Laboratory (LLNL), CA,
trichloroethene (TCE) is present along with tetraalkoxysilanes such as tetrabutoxysilane
(TBOS) and tetrakis(2-ethylbutoxy) silane (TKEBS), as subsurface contaminants.
Intrinsic transformation of TCE to cis-dichloroethene (c-DCE) was observed in the
groundwater at locations co-contaminated with TBOS or TKEBS. Attenuation of TBOS
and TKEBS by abiotic hydrolysis and biological mineralization and the role played by
TBOS and TKEBS in driving the TCE transformation were investigated.

Under abiotic conditions, TBOS and TKEBS were found to slowly hydrolyze to
1-butanol and 2-ethylbutanol, respectively, and silicic acid. Hydrogen was produced as
a result of the fermentation of the alcohols to the corresponding acids, and then
subsequently to carbon dioxide. The hydrogen likely served as the electron donor for the
microbially-mediated reductive dechlorination of TCE.

The rates of hydrolysis of TBOS and TKEBS were determined and typical rates at pH 7, 30 °C and 28 μM initial concentration, were 0.32 and 0.048 μM/day, respectively. The TBOS hydrolysis reaction was observed to be acid and base catalyzed and independent of temperature from 15 to 30 °C. All hydrolysis experiments were conducted at concentrations above the solubility limit of TBOS and TKEBS and the rate of hydrolysis increased with concentration of TBOS or TKEBS. An aerobic microbial culture from the local wastewater treatment plant that could grow and mineralize the alkoxysilanes was enriched. The enriched culture rapidly hydrolyzed TBOS and TKEBS and grew on the hydrolysis products. The microorganisms grown on TBOS cometabolized TCE and c-DCE. TCE and c-DCE degradation was inhibited by acetylene indicating the stimulation of a monooxygenase enzyme. Acetylene did not inhibit the hydrolysis of TBOS.

Abiotic and Biological Transformation of TBOS and TKEBS,
and their Role in the Biological Transformation of TCE and c-DCE.

by

Sanjay Vancheeswaran

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed June 10, 1998
Commencement June 1999

Master of Science thesis of <u>Sanjay Vancheeswaran</u> presented on <u>June 10, 1998</u>

APPROVED:

Redacted for Privacy

_____

Co-Major Professor, representing Chemistry

Redacted for Privacy

_____

Co-Major Professor, representing Civil, Construction and Environmental Engineering

Redacted for Privacy

_____

Chair of the Department of Chemistry

Redacted for Privacy

_____

Head of the Department of Civil, Construction and Environmental Engineering

Redacted for Privacy

_____

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

_____

Sanjay Vancheeswaran, Author

ACKNOWLEDGEMENT

CONTRIBUTION OF AUTHORS

Dr. Lewis Semprini was the main principal investigator for the research project and he was involved in the design of experiments, analysis of data and revision of this manuscript. Dr. James Ingle Jr. was involved in revision of this manuscript and also provided assistance with development of analytical methodology. Dr. Kenneth Williamson was also a principal investigator for the project and he provided technical support and helped in the interpretation of data.

Lutz Friedrich was involved with some of the laboratory work related to the anaerobic microcosm studies. Dr. Paul Daley from Lawrence Livermore National Laboratory, CA, assisted by providing information regarding the field site and also in collecting field samples.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDICES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

ABIOTIC AND BIOLOGICAL TRANSFORMATION OF TBOS AND TKEBS, AND THEIR ROLE IN THE BIOLOGICAL TRANSFORMATION OF TCE AND c-DCE

CHAPTER 1
INTRODUCTION

Trichloroethene (TCE) is a common groundwater contaminant in aquifers throughout the United States (*1*). Currently TCE ranks in the top ten priority pollutants listed by the US EPA. TCE has been widely used as a degreasing agent and as a solvent in industry. Improper handling and leakage of TCE has resulted in its widespread contamination of aquifers. The compound cis-1,2 dichloroethylene (c-DCE) is also a major contaminant in many aquifers and its presence is often attributed to the incomplete transformation of TCE under anaerobic conditions.

Alkoxysilanes are a group of silicon based compounds that contain an oxygen bridge from the central silicon atom to an organic group. Compounds that contain four such oxygen bridges are termed as tetraalkoxysilanes and are also often named as derivatives of orthosilicic acid, $Si(OH)_4$. Tetraalkoxysilanes possess excellent thermal properties and are therefore commonly used as lubricants and heat-exchange fluids (*2*). The thermal properties improve with length and branching of the organic substituents (*2*). Tetrabutoxysilane ($C_{16}H_{36}O_4Si$) (TBOS) and tetrakis(2-ethylbutoxy)silane ($C_{24}H_{52}O_4Si$) (TKEBS) are some examples of tetraalkoxysilanes used primarily as heat exchange fluids and lubricants.

At Site-300, Lawrence Livermore National Labs (LLNL), CA, mixtures of TBOS and TKEBS have been used as a sealant in heat exchanger pipes. TBOS and TKEBS

contamination in the subsurface has resulted from leaking heat exchanger pipes. TCE that is used as a heat exchange fluid is also found as a contaminant at the site. TBOS and TKEBS being lighter than water are light non-aqueous phase liquids (LNAPL) that float on top of the water in the aquifer. TCE being denser than water is a dense non-aqueous phase liquid (DNAPL) and sinks to the bottom of the aquifer. A sizable plume of TBOS, TKEBS and TCE exists at the site and it was of interest to understand the fate of these compounds in the subsurface.

Numerous studies have been carried out on the lower homologues of the tetraalkoxysilanes, such as tetramethoxysilane and tetraethoxysilane, which transform abiotically under a hydrolytic pathway to give the corresponding alcohols (3-6). Both TBOS and TKEBS have been studied minimally. To our best knowledge, no prior work relating to the hydrolysis, biodegradation or environmental analytical chemistry of TBOS and TKEBS has been published.

TCE on the other hand has been extensively studied to understand its transport and fate in the subsurface due to its toxicity and possible carcinogenic effects. Over the past decade, microbial degradation of TCE has been the primary focus of many researchers (7-15). Reductive dechlorination under anaerobic conditions and aerobic cometabolic processes are the predominant pathways for TCE transformation. In aerobic cometabolic processes (7-9), fortuitous oxidation of TCE, catalyzed by the enzymes initiated for oxidation of the growth substrates occurs. In the reductive dechlorination process (10-17), TCE serves as the respiratory electron acceptor and chlorine atoms are replaced by hydrogen. An electron donor is required to complete this reaction by providing the two electrons required for each dechlorination step. Electron

donors such as lactate, benzoate and ethanol (*10, 12-15*) have been shown to support the dechlorination reaction. A range of dechlorinating cultures have been isolated and studied to determine the extent of dechlorination. Most studies report incomplete dechlorination to cis-dichloroethylene (c-DCE) or vinyl chloride (VC). However, some cultures are reported to dechlorinate completely to ethene (*11-15*).

The potential for enhancing anaerobic transformation processes for bioremediation is just being realized and several recent studies have focussed attention on the role played by hydrogen (*11-15*) in the reductive dechlorination. Evidence suggests that H_2 serves as the primary electron donor in the dechlorination of TCE, and substrates that are more complex act primarily as precursors to hydrogen via fermentation (*12,13*).

1.1 Objectives of the Research

The purpose of this research was to investigate the fate of TBOS, TKEBS and TCE at Site 300, LLNL, CA. The detailed objectives of this study were as follows:

1) To study the rates of abiotic hydrolysis of TBOS and TKEBS under conditions of pH, temperature and concentration encountered in natural environments.

2) To investigate the biodegradability of TBOS and TKEBS under aerobic conditions.

3) To investigate the biodegradability of TBOS and TKEBS under anaerobic conditions.

4) To evaluate the potential for in-situ remediation of TBOS and TKEBS.

5) To evaluate the intrinsic anaerobic transformation of TCE driven by TBOS and TKEBS.

6) To investigate TBOS and TKEBS as a potential substrate for the aerobic cometabolism of TCE and cis-DCE.

1.2 Outline of Thesis

The thesis research presented in the subsequent chapters cover a broad range of disciplines including microbiology, biochemistry, environmental engineering and analytical chemistry. Chapter 2 provides a brief historical review of previous work related to the various aspects of the project. It addresses the available literature regarding the properties and synthesis of alkoxysilanes, transformation of alkoxysilanes, and the transformation pathways for TCE including aerobic cometabolism and anaerobic reductive dechlorination. The role of H_2 in the reductive dechlorination of TCE is also discussed. Solid Phase Micro Extraction (SPME) is also introduced.

Studies of the rates of hydrolysis of TBOS and TKEBS, the aerobic biodegradation of TBOS and TKEBS, and the aerobic cometabolism of TCE and c-DCE by microorganisms grown on TBOS are discussed in Chapter 3. Most studies were conducted in microcosms. The effect of pH, temperature and concentration on the rates of hydrolysis were determined by monitoring the disappearance of TBOS and TKEBS, and the formation of the corresponding alcohol. The biodegradation experiments focussed on the mineralization of TBOS and TKEBS, which was evaluated by monitoring the O_2, CO_2 in the headspace of the microcosms. Cometabolic oxidation of TCE and c-DCE by microorganisms grown on TBOS is also discussed.

Chapter 4 deals with anaerobic reductive dechlorination of TCE and evidence for intrinsic TCE transformation in groundwater at Site-300. Numerous anaerobic microcosm studies were conducted to understand the role of TBOS and TKEBS in driving the transformation of TCE. The transformation of TCE and the processes supporting it were studied by monitoring TCE, c-DCE, H_2, TKEBS, 2-ethylbutanol, 2-

ethylbutyric acid and acetic acid in the microcosms. A conceptual model illustrating the various intrinsic transformation processes that occur at sites co-contaminated with TBOS and TKEBS, developed from the analysis of lab and field observations, is also presented.

Chapter 5 is the conclusion chapter. It includes a summary of laboratory results that support the observations of natural attenuation of TCE occurring in-situ. Possible remediation strategies that could be applied in the field are discussed. Future laboratory and pilot-scale studies that could add substantial insight for proper selection of a suitable remediation approach are suggested.

Several appendices include supplementary material such as supporting data and sample calculations that were not included in Chapters 3 and 4. Appendix A provides explicit directions for the preparation of standards and samples, calibration and operation of instrumentation and sample calculations. Appendix B includes instructions for measurement of aqueous solubility of TBOS in water. Gas chromatograms and mass spectra obtained from the analyses are also included. Appendix C contains all the tables and figures that support the results discussed in Chapter 3 and 4.

1.3 Reference

(1)   Westrick, J. J.; Mello, J. W.; Thomas, R. F., *J. Am. Water Works Assoc.*, **1984**, *76* (5), 52-59.

(2)   Alagar, A.; Krishnasamy, V., *Chem. Tech. Biotechnol.*, **1988**, *36*, 577-582.

(3)   Hasegawa, H.; Sakka, S., *J. Non-crystalline solids*, **1988**, *100*, 201-205.

(4)   Aelion, R.; Loebel, A.; Eirich, F., *J. Am. Chem. Soc.*, **1950**, *72*, 5705-5712.

(5)   McNeil, K. J.; DiCaprio, J. A.; Walsh, D. A., *J. Am. Chem. Soc.*, **1980**, *102:6*, 1859-1864.

(6)   Eaborn, C., *Organo-silicon Compounds*, Buttersworth publications limited: Edinburgh, **1960**.

(7)   Semprini, L.; Hopkins, G. D.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1993**, *59* (7), 2277-2285.

(8)   Mars, A. E.; Houwing, J.; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 886-891.

(9)   Johan, E. T.; Vlieg, H. V.; Koning, W. D.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 3304-3312.

(10) Gibson, S. A.; Sewell, G. W., *Appl. Environ. Microbiol.*, **1992**, *58*, 1392-1393.

(11) Maymo-Gatell, X.; Chien, Y.; Gossett, J. M.; Zinder, S. H., *Science*, **1997**, *276*, 1568-1571.

(12) Distefano, T. D.; Gossett, J. M.; Zinder, S. H., *Appl. Environ. Microbiol.*, **1992**, *58*, 3622-3629.

(13) Fennell, D. E.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1997**, *31*, 918-926.

(14) Smatlak, C.R.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1996**, *30*, 2850-2858.

(15) Ballapragada, B. S.; Stensel, D. H.; Puhakka, J. A.; Ferguson, J. F., *Environ. Sci. Technol.*, **1997**, *31*, 1728-1734.

CHAPTER 2
HISTORICAL REVIEW

The literature review in this chapter summarizes information on prior art and research related to the general characteristics and transformation reactions of TBOS, TKEBS and TCE. In specific, the biological transformation of TCE under aerobic and anaerobic conditions is addressed.

2.1 General Characteristics and Transformations of TBOS and TKEBS

Tetrabutoxysilane (TBOS) and tetrakis(2-ethylbutoxy)silane (TKEBS) are non-volatile silicon-based organic compounds that are primarily used as heat-transfer fluids, hydraulic fluids and lubricants. No known literature is available about the contamination of subsurface by these compounds and LLNL Site-300 is probably unique as a TBOS and TKEBS-contaminated site. To our best knowledge, no prior research has been conducted on biological transformation of these compounds. Literature about the abiotic hydrolysis and polycondensation of alkoxysilanes is available but does not address the rates of hydrolysis under natural environmental conditions. Available literature is focused primarily on abiotic hydrolysis at low pH (pH 2) conditions at high molar ratios (about 1:1) with water (*2-5*), which are not normal groundwater conditions. Substantial work has also been carried out to investigate the thermal properties (*6, 7*) of these compounds and data for their enthalpy of vaporization and decomposition temperature are available. A brief listing of their properties is provided in the following section.

2.1.1 Physical Properties of TBOS and TKEBS

The physical properties of TBOS and TKEBS and the products of hydrolysis, 1-butanol and 2-ethylbutanol, obtained from literature and experimental data are listed in Table 2.1.

Table 2.1   Physical properties of tetraalkoxysilanes[a] and the corresponding alcohols[b]

Compound Name	Tetrabutoxy silane (TBOS) $C_{16}H_{36}O_4Si$	Tetrakis (2-ethylbutoxy silane) (TKEBS) $C_{24}H_{52}O_4Si$	1-Butanol C_4H_9OH	2-Ethyl butanol $C_6H_{13}OH$
Mol. Wt. (g/ mol)	320.4	432.1	74	102
Boiling Pt. (°C)	275	340	117.7	146
Melting Pt. (°C)	-145 [c]	NA	-90	-15
Density (g/ mL)	0.899	0.836	0.810	0.830
Refractive Index	1.4282	1.4298	1.3990	1.4220
Solubility (mg/L) @ 20 °C	< than 0.5-1 [c]	< than 0.5 [c]	74,000	---
Viscosity @ 38 °C (cSt)	2.0	4.35	---	---
$(\Delta H)_{vap}$ (kcal/mol)	14.8	16.9	--	--
Surface Tension (dynes/cm)	22.8	22.8	---	---

[a] Barry Arkles, A Survey of Properties and Chemistry, Gelest, **1995**
[b] The Merck Index
[c] Determined in this work (See Appendix A.5)

TBOS and TKEBS have very low aqueous solubility and high boiling points and are non-volatile. These properties suggest that the transport of TBOS and TKEBS in the subsurface would be limited to the transport of the water-soluble portion. TBOS and TKEBS, being lighter than water, are described as light non-aqueous phase liquids (LNAPL). They float on top of the water in the aquifer.

2.1.2. Synthesis of Alkoxysilanes

The principal method for the synthesis of alkoxysilanes is described by Von Ebelman (8). It involves the reaction of stochiometric quantities of silicon tetrachloride ($SiCl_4$) with the corresponding alcohol. The reaction is carried out in benzene solvent in the presence of pyridine as an acid acceptor. The hydrogen chloride produced during the reaction is neutralized by the pyridine added to form pyridinium chloride.

a)

$$SiCl_4 \; + \; 4\, C_4H_9OH \quad \xrightarrow[\text{Benzene}]{\text{Pyridine}} \quad Si\,(OC_4H_9)_4 \; + \; 4\,HCl$$

(TBOS)

b)

$$SiCl_4 \; + \; 4\, \underset{C_2H_5}{C_4H_9OH} \quad \xrightarrow[\text{Benzene}]{\text{Pyridine}} \quad Si\,\underset{(C_2H_5)_4}{(OC_4H_9)_4} \; + \; 4\,HCl$$
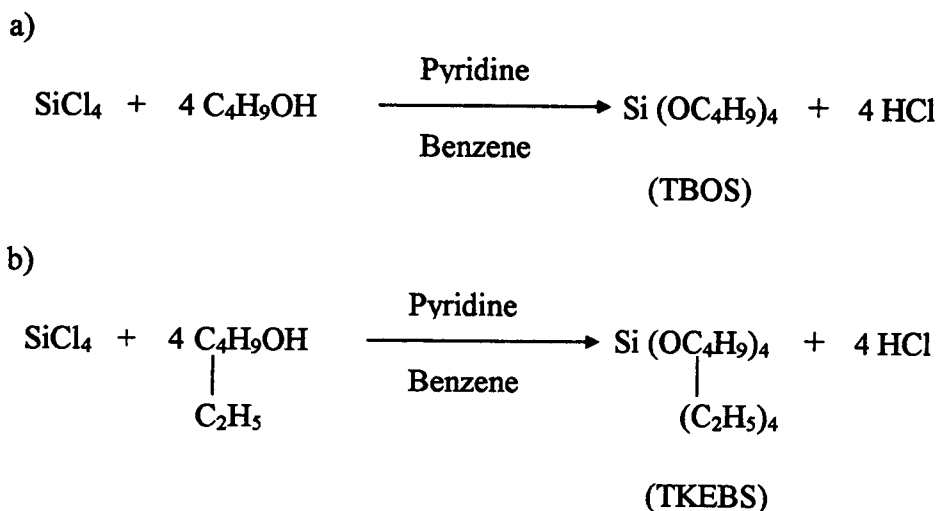
(TKEBS)

Figure 2.1. Synthesis of a)TBOS and b)TKEBS by Von Ebelman's method

A slight excess of the alcohol is added to avoid the formation of trialkoxy and lower substituted silanes. After the reaction is completed, the solid pyridinium chloride is filtered out and the filtrate is vacuum distilled to obtain >95% purity tetraalkoxysilane. This method was adopted for the preparation of TKEBS.

2.1.3 Abiotic Hydrolysis and Polymerization of TBOS and TKEBS

Lower homologues of tetraalkoxysilanes are known to hydrolyze to the corresponding alcohols and silicic acid (3, 4). Hydrolysis of tetraalkoxysilanes involves stepwise substitution of the alkoxy group by water (5, 9) as shown in the schematic below.
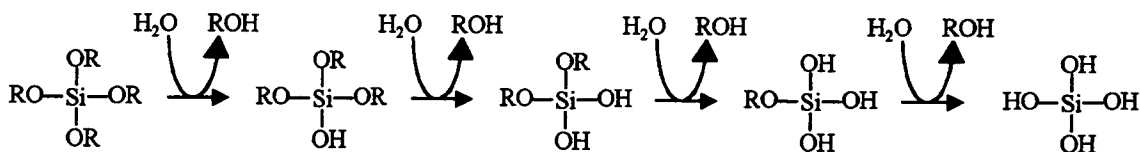
The corresponding alcohol, ROH, is produced during each hydrolysis step. The first step of this hydrolysis reaction is the rate-limiting step (5, 9). The rest of the hydrolysis steps are rapid and do not limit the overall rate of hydrolysis except when the alkoxy groups are considerably large.

The hydrolysis reaction is both acid and base catalyzed and the rates of hydrolysis are lowest at neutral pH (5). At high molar ratios of the alkoxysilane to water, polycondensation products begin to form as hydrolysis progresses (9).

$$
\equiv SiOH\ ROH \qquad\qquad \equiv SiOH\ ROH \qquad\qquad \equiv SiOH\ ROH \qquad\qquad \equiv SiOH\ ROH
$$

$$
\begin{array}{c}
OR \\
| \\
RO-Si-OR \\
| \\
OR
\end{array}
\longrightarrow
\begin{array}{c}
OR \\
| \\
RO-Si-OR \\
| \\
OSi\equiv
\end{array}
\longrightarrow
\begin{array}{c}
OR \\
| \\
RO-Si-OSi\equiv \\
| \\
OSi\equiv
\end{array}
\longrightarrow
\begin{array}{c}
OSi\equiv \\
| \\
RO-Si-OSi\equiv \\
| \\
OSi\equiv
\end{array}
\longrightarrow
\begin{array}{c}
OSi\equiv \\
| \\
\equiv SiO-Si-OSi\equiv \\
| \\
OSi\equiv
\end{array}
$$
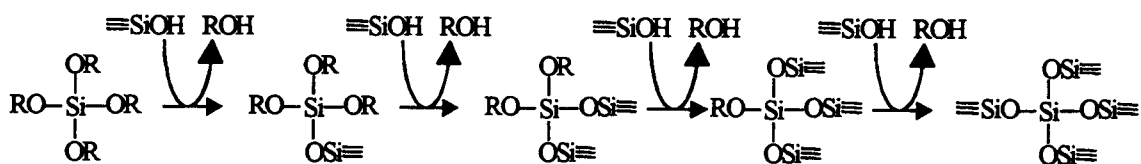
where $\equiv Si$ represents the silicon atom attached to three organic groups.

These polymerization products grow in molecular weight until most of the alkoxy groups are removed and a non-linear network of Si-O-Si gels out. Higher rates of condensation are observed when the reaction is OH^- catalyzed; whereas, higher rates of hydrolysis are observed when it is H^+ catalyzed. Hydrolysis rates are correlated to the steric bulk of the alkoxy group (*9*). The rates of hydrolysis vary with the alkoxy group as follows: $CH_3O > C_2H_5O > C_3H_7O > C_4H_9O >$ branched alkoxy groups (2-ethylbutyl, t-butyl groups, etc).

It is expected that TBOS and TKEBS would hydrolyze to 1-butanol and 2-ethylbutanol, respectively, as illustrated in Figure 2.2.

a)

$$CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O \underset{\substack{|\\O\\|\\CH_2\\|\\CH_2\\|\\CH_2\\|\\CH_3}}{\overset{\substack{CH_3\\|\\CH_2\\|\\CH_2\\|\\CH_2\\|\\O\\|}}{Si}} O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3 \quad + \quad 4\ H_2O \xrightarrow{\text{Hydrolysis}} 4\ C_4H_9OH \quad + \quad Si(OH)_4$$

1 - butanol   Silicic acid

b)

$$CH_3\text{-}CH_2\text{-}CH\text{-}CH_2\text{-}O \underset{}{\overset{}{Si}} O\text{-}CH_2\text{-}CH\text{-}CH_2\text{-}CH_3 \quad + \quad 4\ H_2O \xrightarrow{\text{Hydrolysis}} CH_3\text{-}CH_2\text{-}CH_2\text{-}CH\text{-}OH \quad + \quad Si(OH)_4$$

2 - ethyl butanol   Silicic acid
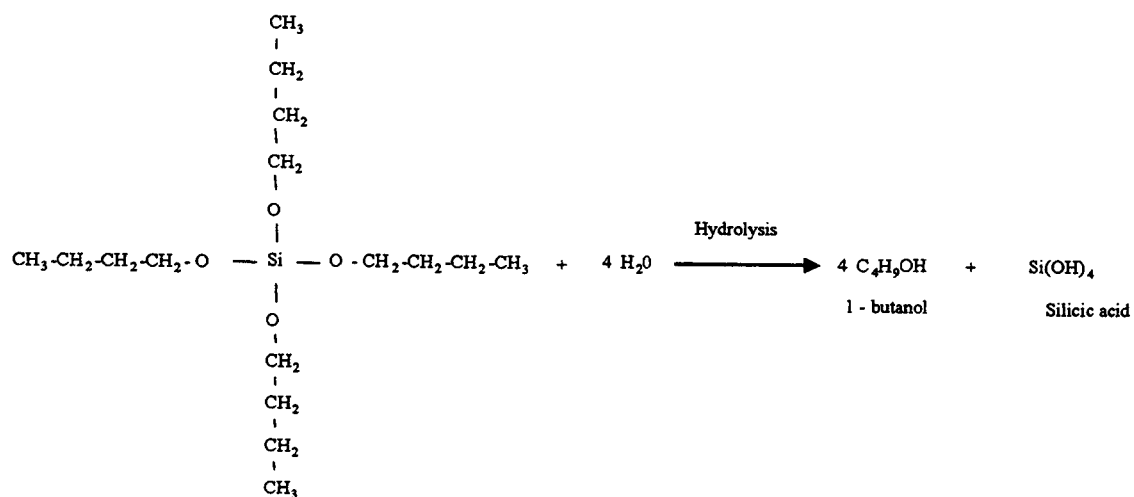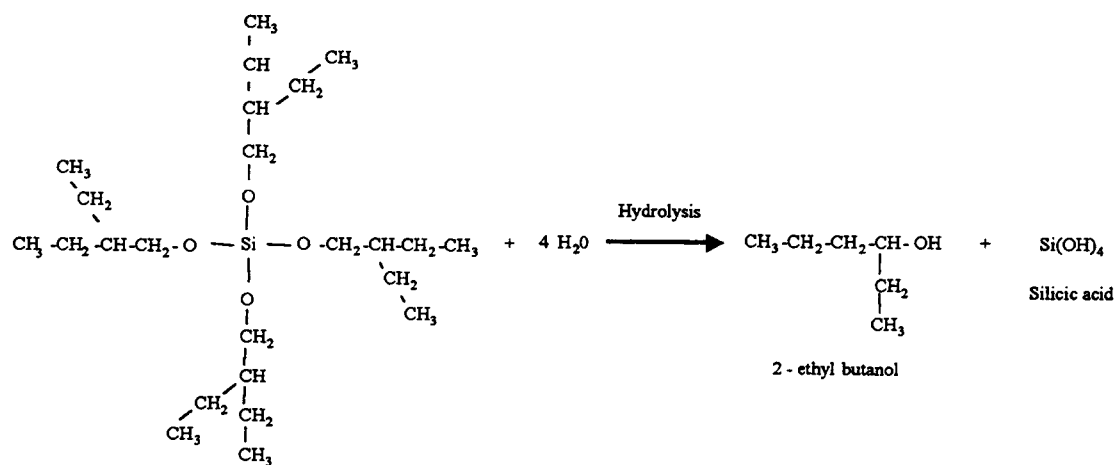
Figure 2.2. Abiotic hydrolysis reaction for a) TBOS and b) TKEBS

2.2 Properties and Transformations of TCE

TCE has been widely used in industry as a degreasing agent and solvent. The erroneous belief about its safety (*1*) led to its widespread use, indiscriminate disposal, and in widespread contamination of aquifers throughout the United States. The slow transformation of TCE in the subsurface environment contributed to accumulation of high concentrations of TCE in the groundwater (*10*). Serious public health concerns over TCE contaminated sites arose when TCE was suspected as a possible carcinogen (*11*). The maximum contaminant level (MCL) in drinking water is regulated at 5 µg/L (*Federal Register, 1989*) (*12*).

Due to its high toxicity, TCE has been extensively studied and characterized (*13*). The fate and transport of TCE in the subsurface determines its distribution and persistence in the subsurface. TCE is only moderately soluble in water (1300 mg/L) and also has a density greater than water (1.46 g/mL). Hence, TCE is described as a dense non-aqueous phase liquid (DNAPL). Its high density causes it to sink under gravity to the bottom of aquifers forming pools of liquid TCE. The pools of TCE would then act as a continuous source of TCE in water as it slowly diffuses and dissolves in water. The octanol-water partitioning coefficient (K_{ow} = 240) is moderately high causing sorption of TCE into the organic matter in the soil. The air-water partitioning coefficient (Dimensionless Henry's constant, H, 0.47) for TCE is also high. The processes influencing the transport, distribution and fate of TCE in groundwater are advection, dispersion, sorption, transformation, immiscible transport and diffusion transport as discussed by McCarty et al.(*13*).

The possible pathways of transformation of TCE include: a) substitution reactions such as hydrolysis, b) oxidation by strong oxidizing agents such as permanganate, c) abiotic reduction reactions, d) reductive dechlorination by microorganisms under anaerobic conditions and e) cometabolic oxidation of TCE by aerobic microorganisms grown on special growth substrates.

The reductive dechlorination reaction involves the replacement of the chlorine atoms by hydrogen and could be abiotic or microbially-mediated. Abiotic reductive dechlorination of chlorinated compounds has been achieved by the use of granular iron (*14*) and by corrinoid-mediated reactions (*15*). Microbial dechlorination of chlorinated ethenes has also been extensively studied over the past decade and several microorganisms that use them as respiratory electron acceptors have been reported (*28*). In the environment, dechlorination of TCE and tetrachloroethene (PCE) has been reported to occur naturally at some sites (*10*). However, natural attenuation may require several months or years to reach the MCLs. Enhancing natural reductive dechlorination of chlorinated ethenes has received wide acceptance as a remedial alternative and may depend on several factors such as nutrient limitations, site characteristics and extent of contamination.

2.3 Oxidation and Reduction Reactions in Microorganisms

Microorganisms obtain energy from chemicals through oxidation–reduction processes or redox reactions, extracting the energy present in molecules through the transfer of electrons. Oxidation refers to a substance losing electrons whereas reduction implies gaining electrons. First an electron donor must be present to act as the source of reducing power and secondly there must exist an electron acceptor to oxidize the reducing agent to provide the means of releasing energy stored in the molecules. The electron donor is oxidized while the electron acceptor is reduced. This is the fundamental way by which cells transform and consume the energy present in molecules and perform work (*30*).

In microorganisms, chemical energy released in redox reactions is most commonly transferred to phosphate bearing compounds such as ATP, in the form of high-energy phosphate bonds. These compounds function as the energy source to drive energy – requiring reactions in the cell. The transfer of electrons from donor to acceptor in a cell involves the participation of one or more electron carriers. Some electron carriers are fixed in membranes structures whereas others diffuse freely, transferring electrons from one place to another in the cell. Two of the most common electron-carriers are nicotinamide-adenine (NAD) and nicotinamide-adeninephosphate (NADP).

The electron acceptors used in the metabolism define the type of organisms and its characteristics. When the metabolism occurs in the presence of molecular O_2 as an electron acceptor, it is termed as *aerobic respiration*. Metabolism that occurs only in the absence of molecular O_2 may be subdivided into *anaerobic respiration* and

fermentation. Anaerobic respiration uses oxidized inorganic or organic compounds such as nitrate (NO_3^-), sulfate (SO_4^{2-}), ferric iron (Fe^{3+}) or CO_2 as the electron acceptor.

In general, the choice of an electron acceptor by microorganisms is based on availability, presence of alternate electron acceptors, and the related oxidation-reduction potential of the surrounding environment. The energy yield for microorganisms from hydrocarbon metabolism varies greatly and depends mainly on the electron acceptor. The preferred sequence of electron acceptors based on energetic considerations is as follows: $O_2 > NO_3^- > MnO_2 > Fe^{3+} > SO_4^{2-} > CO_2$. Microorganisms gain the most energy with O_2 and it is the preferred electron acceptor for aerobic microorganisms due to its high reduction potential.

Fermentation occurs when an organic compound serves as both the electron donor and the electron acceptor (*20, 30*). Fermentation can proceed only under strictly anaerobic conditions and yields a mixture of products some more oxidized than the substrate and others more reduced. However, the average oxidation-state of the oxidized and the reduced products remain identical to that of the substrate fermented. In most fermentation reactions, the electron-balance is maintained by the production of molecular H_2. In H_2 production, protons (H^+) derived from water serve as electron acceptors. H_2 production functions primarily to maintain redox balance

For example, the reactions for butyrate and acetate are shown below:

$$\text{Butyrate}^- + 2\,H_2O \longrightarrow 2\,\text{Acetate}^- + 5\,H^+ + 5\,e^- \quad \text{(Fermentation)}$$

$$\text{Acetate}^- + 4\,H_2O \longrightarrow 2\,HCO_3^- + 9\,H^+ + 9\,e^- \quad \text{(Fermentation)}$$

$$2\,H^+ + 2\,e^- \longrightarrow H_2$$

$$4\,H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + 3\,H_2O \qquad \text{(Methanogenesis)}$$
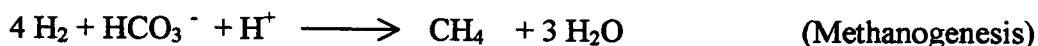
The energetics of H_2 production are somewhat unfavorable and so most fermentative organisms produce only small amounts of H_2. However, the fermentation process is made feasible by the phenomenon of *syntrophy*. Favorable energetics are obtained when the H_2 produced is continuously utilized by other energetically favorable reactions. A favorable association occurs when H_2-producing fermentation of alcohols and fatty acids that have positive free energies (ΔG) are coupled with H_2-utilizing reactions that possess negative ΔG's. If the sum of the free energies of these two reactions yield a negative ΔG, the overall reaction is favorable energetically. This phenomenon is called *interspecies H_2 transfer* (*30*). The principles of free energy indicate that the actual concentration of reactants and products in a given reaction can drastically change the available free energy of a reaction. This implies that the presence or absence of H_2 affects the energetics of the reaction. Therefore depending on ΔG of a particular reaction, the H_2 produced must be maintained at appropriate levels to drive the reaction forward. Constant removal by H_2-utilizing microorganisms such as methanogens, sulfate-reducers, or dechlorinators is necessary.

In the natural environment, H_2 produced from the fermentation of organic compounds is continuously consumed by microorganisms that use sulfate, Fe(III) or CO_2 as electron acceptors (*16*). These H_2-utilizing microorganisms exhibit different affinities for H_2. Fe(III) reducers are relatively more efficient and they can maintain lower steady-state H_2 concentrations (0.2-0.8 nM) than either sulfate reducers (1-4 nM) or methanogens (5-15 nM). Lovley, Chapelle and coworkers (*16, 17*) have suggested that H_2 levels can be used to determine the terminal electron acceptor and redox potential.

Fatty acids with even number of carbon atoms undergo sequential beta-oxidation to give acetate and H_2 as products (*18, 19*). Fatty acids with odd number of carbon atoms undergo sequential beta-oxidation to give acetate, propionate and H_2 as products. Propionate is further oxidized to acetate, H_2 and CO_2. These fatty acid oxidation reactions yield free-energy changes that are positive (*20*). Therefore, the H_2 levels must be maintained low enough (typically less than 1×10^{-4} atm or 76.9 nM) to provide an overall favorable reaction.

Alcohols such as 1-butanol and 1-propanol are oxidized to the corresponding carboxylic acid forms, which further undergo conversions as described above. The oxidation of alcohols is a favorable reaction and has a negative ΔG (*20*). Here the H_2 levels can remain substantially higher for providing a overall favorable reaction.

2.4 Reductive Dechlorination of TCE

Chlorinated ethenes such as PCE and TCE do not serve as electron donors (primary substrate) and support growth in aerobic microorganisms. However, they can be utilized as electron acceptors and reductively dechlorinated by natural anaerobic microbial communities and mixed microbial enrichment cultures under anaerobic conditions. The reductive dechlorination of TCE occurs by sequential replacement of the chlorine atoms with hydrogen. The reduction products of TCE are cis-dichloroethylene (c-DCE), vinyl chloride (VC) and a non-toxic product, ethene. The pathway for sequential dechlorination of TCE is presented in Figure 2.3.
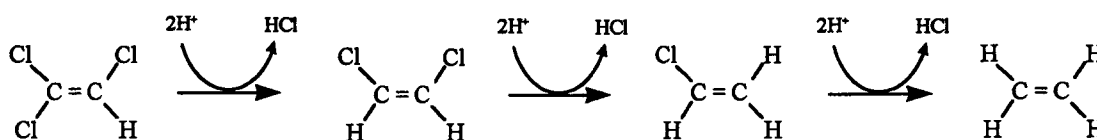
Figure 2.3. TCE dechlorination pathway

TCE serve as the respiratory electron acceptors in reductive dechlorination reaction. Each dechlorination step requires two electrons, and therefore an electron donor is necessary to complete the reaction. A number of organic compounds have been successfully used as electron donors for the anaerobic dechlorination of TCE. Electron donors such as lactate, benzoate and ethanol (24-28) have been shown to support the dechlorination reaction. A range of dechlorinating cultures have been isolated and studied to determine the extent of dechlorination. Gibson and Sewell et al (24) showed

in soil microcosms that TCE could be converted to c-DCE with addition of lactate, propionate, crotonate, butyrate, or ethanol as electron donor. Most studies report incomplete dechlorination of TCE to c-DCE or VC. However, some cultures are reported to dechlorinate TCE completely to ethene (*25-28*).

The potential for enhancing anaerobic transformation processes for bioremediation is just being realized and several recent studies have focussed attention on the role played by H_2 (*25, 26, 28*) in the reductive dechlorination. Even though a wide range of electron donors support dechlorination, H_2 produced from the fermentation of these electron donors may be the ultimate electron donor. Evidence suggests that substrates that are more complex act primarily as precursors to H_2 via fermentation (*26*). The maximum H_2 partial pressures typically attained by fermentation of lactate and ethanol (0.5 mM) exceed 1×10^{-3} atm (770 nM in solution); whereas, butyrate and propionate (0.5 mM) ferment at H_2 levels lower than 1×10^{-4} atm. The cultures enriched by Freedman and Gossett with methanol were shown to dechlorinate with H_2 as the sole electron donor (*25-27*). Though H_2 is not always the only electron donor, it is in many cases the electron donor used by dechlorinators.

In natural systems, H_2-utilizing microorganisms include methanogens, acetogens, sulfidogens and dechlorinators. The dechlorinators must then compete with these other hydrogenotrophs for the evolved H_2 (*26, 27*). Several studies, conducted by Ferguson et al (*28*) and Gossett et al (*27*), confirmed that the half-velocity constants (substrate concentration when the rate of substrate utilization is half of maximum rate) with respect to H_2 for dechlorinators are approximately an order of magnitude lower than that of the methanogenic organisms in the culture (See Table 2.2). Though the threshold H_2 level

(lowest observed limit for use of H_2 as an electron donor) for dechlorinators has not been reported, dechlorination has been observed at levels lower than that for methanogens (28). These observations suggest a strategy for selective enhancement of dechlorination by managing the H_2 delivery to maintain lower levels of H_2 that impart a competitive advantage to dechlorinators (28).

Measured H_2 levels in anaerobic subsurface environments are typically lower than the half-velocity constants for the dechlorinators. Lovley et al (15) measured H_2 levels to be typically in the range of 5-10, 1-1.5, 0.2, and 0.05 nM for methanogenic, sulfate reducing, iron-reducing and nitrate-reducing environments, respectively. These threshold values indicate that while dechlorinators might out-compete methanogens, the significantly lower threshold H_2 levels for other microorganisms might make the dechlorinators less competitive.

Table 2.2. Half-velocity constants reported for dechlorinators, methanogens and sulfate-reducers

Half-Velocity Constants (K_s)	Lovley et al (16) atm (nM) RSD	Gossett et al (26) atm (nM) RSD	Ferguson et al (28) atm (nM)	Tiedje et al (28) atm (nM)
Dechlorinating Micro organisms	---	1.3×10^{-4} (100) $\pm 50\%$	$(1\text{-}3) \times 10^{-5}$ (8-10)	---
Methanogens	5.7×10^{-3} (4350) $\pm 23\%$	1.3×10^{-3} (960) $\pm 20\%$	---	$(3\text{-}16) \times 10^{-3}$ (2200-12,000)
Sulfate Reducers	1.4×10^{-3} (1060) $\pm 31\%$	---	---	---

$K_H = 1300$ M^{-1}atm, conversions: 1 ppm ≈ 1.3 nM, 1 nM $= 1.3 \times 10^{-6}$ atm

Enhancing anaerobic reductive dechlorination may be an economical choice for in-situ treatment of TCE contaminated sites. It requires injection of a carbon and energy source into the aquifer to stimulate indigenous dechlorinating microorganisms and to support the dechlorination reaction. The choice of the substrate added is based on the several factors such as a) toxicity of the substrate and its transformation products, b) ease of obtaining regulatory approval and c) demonstrated success in efficiently supporting the dechlorination reaction. A major drawback in the application of reductive dechlorination processes for TCE remediation is the potential for the formation of more toxic transformation products such as VC and 1,1-DCE.

2.5 Aerobic Cometabolism of TCE and c-DCE

Organic compounds can be transformed by microorganisms through either metabolic or by cometabolic processes. *Metabolism* implies both *catabolism* (break down of organic compound) and *anabolism* (synthesis of cells from the available nutrients and energy source). An organic compound serves as a metabolic primary substrate when it serves as both the energy and carbon source and also supports growth. This is the usual process for decomposition of most organic compounds in nature.

Cometabolism on the other hand is the fortuitous oxidation of an organic compound by enzymes or cofactors produced by microorganisms for the oxidation of the growth substrates. In cometabolic processes, only breakdown of the organic compound (catabolism) occurs whereas there is no biosynthesis (anabolism). The microorganisms obtain no obvious or direct benefit from the oxidation reaction. Cometabolism is also a natural process but it has not been extensively practiced for treatment of organic waste, due to limited knowledge of these processes. It is however the process by which most of the chlorinated aliphatic hydrocarbons (CAH) are degraded.

No microorganism that can grow on TCE or c-DCE is known, but cometabolic oxidation of c-DCE and TCE has been reported by many researchers. Several growth substrates such as methane, propane, butane, phenol and toluene have been successfully shown to induce oxygenase enzymes that cometabolize TCE (*21-23*). Some of these compounds have been shown to induce characteristic enzymes in microorganisms such as methane monooxygenase, phenol hydrolase, propane monooxygenase and butane monooxygenase that are responsible for the oxidation of the TCE (*21-23,31*). The

aerobic cometabolism of c-DCE is observed in most microorganisms that cometabolize TCE and the pathways of transformation of these compounds are similar.

The aerobic organisms that are capable of oxidizing c-DCE and TCE possess an oxygenase enzyme for the initial oxidation with the growth substrates such as methane or propane. The mechanisms of monooxygenase catalyzed oxidation of c-DCE and TCE are presented in Figure 2.4.

a)
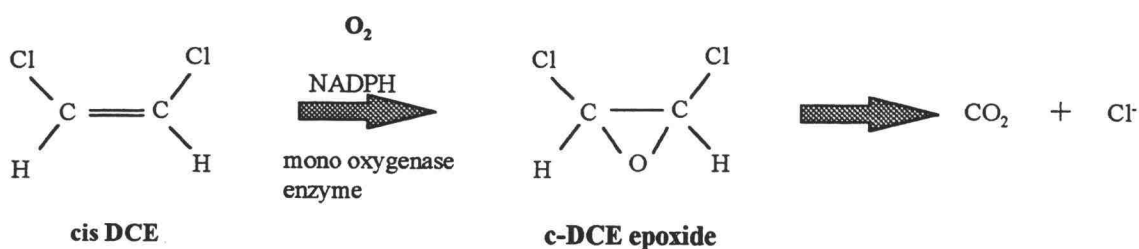
b)
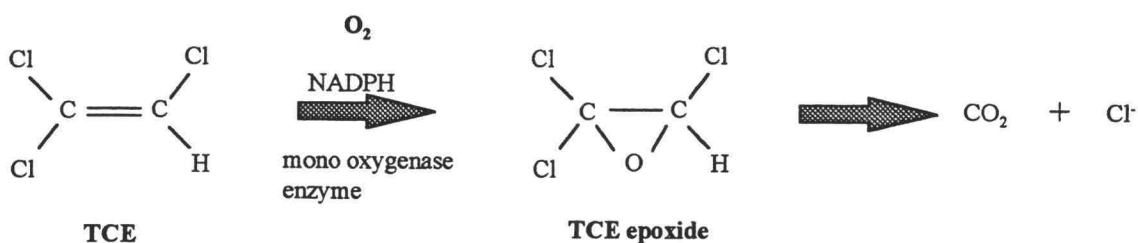
Figure 2.4. Aerobic oxidative transformation pathway for a) cis-DCE and b)TCE

The oxidative dechlorination of c-DCE and TCE requires NADH as the reducing energy source and molecular oxygen as an electron acceptor. The oxidation of chlorinated ethenes cause depletion of NADH in cells because NADH is not produced

by the oxidation of the chlorinated ethenes. Therefore, NADH has to be supplied via cometabolic substrate oxidation and endogenous energy reserves. The oxidation of c-DCE and TCE lead to the formation of the corresponding epoxides (*23*) which are highly unstable with half lives of 70 hr and a few seconds, respectively. The TCE epoxide and c-DCE epoxide are further transformed to CO_2 and chloride ions. The c-DCE epoxide transformation can be an abiotic reaction or a biologically-mediated reaction (*23*).

For cometabolism to occur, an active population of microorganisms that induce the enzymes or cofactors responsible for cometabolism must be present. This means that the appropriate primary substrate for growth and maintenance of these organisms must also be present. This aspect adds greater complexity and cost to cometabolic transformation. The major advantage of applying aerobic cometabolism for remediation of TCE is that complete transformation to CO_2 is obtained and no intermediate toxic products are formed.

2.6 Solid Phase Micro Extraction

Solid Phase Micro Extraction (SPME) is an unique solvent-free extraction technique that employs a coated fiber to simultaneous extract and concentrate analytes of interest from a matrix (32, 33). The silica fiber is coated with a liquid polymer such as polydimethylsiloxane (DPMS) into which the analytes are partitioned. SPME (Appendix A.2.2) integrates sampling, extraction, concentration and sample introduction into a single step resulting in high sample throughput.

The principle behind SPME is the partitioning of analytes between the sample matrix and the coating on the fiber (33). The mass of analyte partitioning into the fiber is given by the equation 2-1

$$n = \frac{K_{fs} V_f C_o V_s}{K_{fs} V_f + V_s} \qquad (2\text{-}1)$$

where m is the mass of the analyte partitioned, V_f and V_s are the volumes of film coating and sample, K_{fs} is the partitioning coefficient and, C_o is the initial concentration of the analyte in the sample.

The K_{fs} values for some organic compounds with commonly available coatings are large, such that the extraction is exhaustive. This results in a very high concentrating effect and improves the detection limit of analysis. In cases where the K_{fs} value is lower, the extraction is an equilibrium process. In all cases, SPME can be calibrated with standards and used to accurately determine the concentration of target analytes in a sample matrix. If the sample volume, V_s, is large, the mass of analyte concentrated (see equation 2-1) becomes independent of sample volume. This method is ideally suited for field sampling and analysis.

SPME can be performed on liquid samples or the headspace of the liquid samples depending on the characteristics of the target analytes. The kinetics of extraction of analytes from a sample matrix are dependent on the mass transport of analytes from the sample matrix to the coating. The amount of analyte absorbed is hence a function of time. Depending on the needs of the analysis, a choice between the speed of extraction and sensitivity should be made. Exhaustive extraction and high sensitivity is obtained when the duration of the sample extraction is long. The mass transfer limitations in extraction can be overcome by vigorous agitation methods such as sonication or stirring of the solution.

Use of SPME involves two important steps: extracting the analytes and desorbing them into the chromatograph. The speed of extraction, sensitivity, accuracy and precision are determined largely by the extraction step. The desorption step controls the efficiency of chromatographic separation and the precision of the quantitation and impacts the quality of the data obtained. The several parameters to be considered and optimized for efficient extraction and desorption are discussed in Appendix A.

Depending on the type of analytes to be extracted several types of SPME coatings are available. Non-polar compounds are effectively extracted by non-polar fibers such as polydimethysiloxane (PDMS). Polar compounds on the other hand are effectively extracted by polar coatings such as polyacrylate and carbowax. The fibers are also available with varying thickness of film to achieve optimum sensitivity and retention characteristics.

In this project, SPME was employed as a screening tool for qualitative analysis of groundwater and other aqueous samples. It provides several advantages over the

traditional liquid-liquid extraction (LLE). The elimination of the solvent can be valuable if the unknown analytes co-elute with the solvent preventing identification. The SPME technique is a single-step sampling, extraction, pre-concentration and sample introduction technique that makes it less labor intensive.

Despite the advantages, there are several limitations in the use SPME, which are as follows:

1. Calibration and quantification is a laborious process.

2. Very sensitive to changes in stirring rate and temperature which affect the mass transfer of analytes into the polymer coating.

3. The partitioning constants can be affected by pH and ionic strength of the sample.

4. It is a one-shot analytical technique. The analysis of a particular sample can be made only once because the total amount extracted on the fiber is desorbed completely into the GC inlet port. If an operator or instrumental error is made, a completely new sample has to be extracted. On the other hand with LLE, the volume of solvent extract is sufficiently large to allow several chromatographic injections.

2.7 References

(1) Westrick, J. J.; Mello, J. W.; Thomas, R. F., *J. Am. Water Works Assoc.*, **1984**, *76* (*5*), 52-59.

(2) Hasegawa, H.; Sakka, S., *J. Non-crystalline solids*, **1988**, *100*, 201-205.

(3) Aelion, R.; Loebel, A.; Eirich, F., *J. Am. Chem. Soc.*, **1950**, *72*, 5705-5712.

(4) McNeil, K. J.; DiCaprio, J. A.; Walsh, D. A., *J. Am. Chem. Soc.*, **1980**, *102:6*, 1859-1864.

(5) Eaborn, C., *Organo-silicon Compounds*, Buttersworth publications limited: Edinburgh, **1960**.

(6) Alagar, A.; Krishnasamy, V., *Chem. Tech. Biotechnol.*, 1988, *36*, 577-582.

(7) Alagar, A.; Krishnasamy, V., *Chem. Eng. Comm.*, 1989, *80*, 1-9.

(8) Von Ebelman, J., *Ann.Chem.*, **1845**, *57*, 319.

(9) Barry Arkles, *A Survey of Properties and Chemistry*, Gelest, **1995**.

(10) Wilson, J. T.; Noonan, M.J.; McNabb, J. F., *Groundwater. Qual.*, **1985**, Wiley & Sons, New York.

(11) Infante, P.F.; Tsongas, T.A, *Envir. Sci. Res.*, **1982**, *25*, 301-327.

(12) Federal Register, **1989**, *National Primary and Secondary Drinking Water Regulation*, *54*, 22062-22160.

(13) McCarty, P.L.; Robert, P.V.; Reinhard, S.M; Hopkins, G, Fate of Pesticides and Chemicals in the Environment, John-Wiley & Sons, Inc., NewYork, **1992**,191-209.

(14) Matheson, L.J; Tratnyek, P. G., *Environ. Sci. Technol*, **1994**, *28*, 2045-2053.

(15) Glod, G.; Angst, W.; Holliger, C.; Schwarzenbach, R. P., *Environ. Sci. Technol*, **1997**, *31*, 253-260.

(16) Lovley, D.R; Chapelle, F.H; Woodward, J.C, *ES&T Research*, 1994, *28*, 1205-1209.

(17) Chapelle, F.H; Haack, S.K; Adriaens, P; Henry, M.A; Bradley, P.M, *Environ.Sci.Technol*, **1996**, *30*, 3565-3569.

(18) McInerney, M. J.; Bryant, M. P.; Hespell, R. B.; Costerton, J. W., *Appl. Environ. Microbiol.*, **1981**, *41*, 1029-1039.

(19) Stieb, M.; Schink, B., *Arch. Microbiol.*, **1993**, *140*, 2991-2997.

(20) Thauer, R. K.; Jungermann, K.; Decker, K., *Bacteriol. Rev.*, **1977**, *41*, 100-180.

(21) Hopkins, G. D.; Semprini, L.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1993**, *59* (7), 2277-2285.

(22) Mars, A. E.; Houwing, J.; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 886-891.

(23) Johan, E. T.; Vlieg, H. V.; Koning, W. D.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 3304-3312.

(24) Gibson, S. A.; Sewell, G. W., *Appl. Environ. Microbiol.*, **1992**, *58*, 1392-1393.

(25) Distefano, T. D.; Gossett, J. M.; Zinder, S. H., *Appl. Environ. Microbiol.*, **1992**, *58*, 3622-3629.

(26) Fennell, D. E.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1997**, 31, 918-926.

(27) Smatlak, C.R.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1996**, *30*, 2850-2858.

(28) Ballapragada, B. S.; Stensel, D. H.; Puhakka, J. A.; Ferguson, J. F., *Environ. Sci. Technol.*, **1997**, *31*, 1728-1734.

(29) Maymo-Gatell, X.; Chien, Y.; Gossett, J. M.; Zinder, S. H., *Science*, **1997**, *276*, 1568-1571.

(30) Madigan, M.T; Martinko, J.M; Parker, J., *Brock Biology of Microorganisms*, **1997**, 8th ed., Prentice hall, NJ.

(31) Hamamura, N.; Page, C.; Long, T.; Semprini, L.; Arp, D. J., *Appl. Environ. Microbiol.*, **1997**, *63* (*9*), 3607-3613.

(32) Arthur, C. L.; Killam, L. M.; Buchholz, K. D.; Pawliszyn, J., *Anal. Chem.*, **1992**, *64*, 1960-1966.

(33) Zhang, Z.; Yang, M. J.; Pawliszyn, J., *Anal. Chem.*, **1994**, *66*, 844A-853A.

CHAPTER 3
ABIOTIC AND BIOLOGICAL TRANSFORMATION OF TETRAALKOXYSILANES AND TCE, c-DCE COMETABOLISM DRIVEN BY TBOS-DEGRADING MICROORGANISMS

Sanjay Vancheeswaran,

Kenneth J. Williamson[t], James D. Ingle, Jr.[a] and Lewis Semprini[*t]

[t]Department of Civil, Construction and Environmental Engineering

and

[a]Department of Chemistry

Oregon State University,

Corvallis, OR 97330, USA.

3.1 Introduction

Tetraalkoxysilanes are a group of silicon based compounds that contain four oxygen bridges from the central silicon atom to the corresponding organic (alkoxy) group. Tetraalkoxysilanes are also often named as derivatives of orthosilicic acid $(Si(OH)_4)$. These compounds are widely used as heat exchange fluids and lubricants because they possess excellent thermal properties, which improve with length and branching of the organic substituent (1). The tetraalkoxysilanes of interest for this study are tetrabutoxysilane (TBOS)$(C_{16}H_{36}O_4Si)$ and tetrakis(2-ethylbutoxy)silane (TKEBS) $(C_{24}H_{52}O_4Si)$.

The site under consideration has a shallow aquifer contaminated with TBOS and TKEBS. The contamination in the subsurface has resulted from leaking heat-exchanger pipes. Trichloroethylene (TCE) is also a major contaminant at the site. TBOS and TKEBS, being lighter than water, are present as light non-aqueous phase liquids (LNAPL) in the groundwater, and TCE, being denser than water, is present as a dense non-aqueous phase liquid (DNAPL) in the groundwater. It was of interest to study the abiotic hydrolysis and biological transformation of these compounds.

Literature related to TBOS and TKEBS is mainly focussed on their thermal properties (1). Specific research work related to the transformation of these compounds under environmental conditions is limited. To our best knowledge, biological degradation of these compounds has not been investigated. However, numerous hydrolysis studies have been conducted on the lower homologues of the tetraalkoxysilanes such as tetramethoxysilane and tetraethoxysilane (2-5). These compounds transform abiotically under a hydrolytic pathway to give the corresponding

alcohols (2-5, 7). It was expected that TBOS would hydrolyze to 1-butanol and TKEBS would hydrolyze to 2-ethylbutanol. The general equation for the hydrolysis of tetralkoxysilanes is as follows:

$$Si\,(OR)_4 \ + \ H_2O \ \longrightarrow \ 4\,ROH \ + \ Si\,(OH)_4 \qquad (1)$$
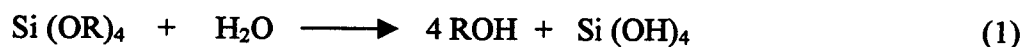
where 'R' is the substituent organic group such as $-C_4H_7$, $-C_6H_{13}$, or an aromatic ring.

Trichloroethylene (TCE) is the other contaminant at the site and is a common groundwater contaminant in aquifers throughout the United States (8,9). Since TCE is a suspected carcinogen, the fate and transport of TCE in the environment and microbial degradation of TCE have been extensively studied (10-17). Reductive dechlorination under anaerobic conditions and aerobic cometabolic processes are the predominant pathways for TCE transformation. In aerobic cometabolic processes (10-14), fortuitous oxidation of TCE, catalyzed by the enzymes initiated for oxidation of the growth substrates occurs. Several growth substrates such as methane, propane, butane, phenol and toluene have been shown to induce oxygenase enzymes that cometabolize TCE (10-12).

The objective of this research was to investigate the abiotic and biological transformation of TBOS and TKEBS. The rates of hydrolysis under environmental conditions were determined to evaluate the significance of the hydrolytic attenuation pathway. The biological transformation of TBOS and TKEBS under aerobic conditions was studied with indigenous microorganisms from a site contaminated with TBOS, TKEBS, and TCE, and also from a wastewater treatment plant activated sludge. Also tested was the ability of the aerobic microorganisms grown on TBOS to cometabolize TCE and c-DCE.

3.2 Materials and Methods

3.2.1 Chemicals and Stock Solutions

Trichloroethylene (TCE) (99.9% purity), cis-1,2 dichloroethylene (99.9%), HPLC-grade dichloromethane, 1-butanol (99.9%) and 2-ethylbutanol (96% purity) were obtained from Aldrich Chemical Co. (Milwaukee, WI). TBOS (98 % purity) and tetrapropoxysilane (TPOS) (98 % purity) was obtained from Gelest, Inc. (Tullytown, PA.). TKEBS was synthesized in the lab by adopting Von Ebelman's synthesis for tetraalkoxysilanes (1, 6). Stoichiometric quantities of silicon tetrachloride (semiconductor grade, Aldrich Co.) were reacted with a slight excess of 2-ethylbutanol, in the presence of pyridine as an acid acceptor in benzene solvent. The products were vacuum distilled to yield approximately 95% pure TKEBS. The above compounds were used as both culture substrates and in the preparation of analytical standards. Glacial acetic acid (Mallinckrodt, Inc., 99.5%), sodium butyrate (Fisher scientific Co.) and 2-ethylbutyric acid (Aldrich Chemical Co., 97%) were used in the preparation of analytical standards.

3.2.2 Analytical Methods

TBOS, TKEBS, 1-butanol and 2-ethylbutanol concentrations were quantitatively determined by liquid-liquid extraction of 1-mL aqueous samples with 0.5 mL of dichloromethane and agitation for 5 min on a vortex mixer. After complete separation of the two immiscible phases, 2 µL of the dichloromethane extract was injected into the

GC/MS, a HP-5890 gas chromatograph connected to a HP-5971 mass-selective detector. The chromatographic separation was carried out with a Rtx-20 column (30 m x 0.25 mm, 1.0-μm film) from Restek, Inc. (Bellefonte, PA). 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. The concentrations were normalized with TPOS (10 mg/L) as an internal standard. Because TBOS and TKEBS have low aqueous solubility (less than 0.5 mg/L) (19), samples that contain higher than soluble amounts of TBOS and TKEBS form an emulsion with water. The bulk solution must be appropriately mixed to maintain homogeneity of the compounds in solution for consistent results. The relative standard deviations of the TBOS and TKEBS measurements were approximately 10 and 15%, respectively.

Gas phase TCE and cis-DCE concentrations were measured by injecting 100 μL of the headspace sample into a HP-5890 gas chromatograph 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, CA). The gases O_2 and CO_2 in the batch bottle headspace were measured with a HP-5890 gas chromatograph connected to a thermal conductivity detector. The method involved direct injection of a 0.1-mL gas sample from the headspace of the batch bottle into the GC with a gas-tight syringe (Hamilton Co., Reno). Chromatographic separation was carried out with a Supelco Carboxen column.

The dichloromethane extract prepared for the TBOS determination was also used for the determination of c-DCE epoxide with TPOS as the internal standard. The

GC/MS was operated in the SIM mode and c-DCE epoxide was measured by monitoring the ions m/z 48 and m/z 112 (*14*).

3.2.3 Preparation of Batch Bottles

Batch bottles were constructed with 310-mL serum bottles (Wheaton Industries, Millville, NJ) with rubber-lined caps and butyl rubber septa. Abiotic hydrolysis studies were conducted in batch bottles with 250 mL of pH-buffered water and 60 mL of headspace. The pH-buffered solutions were prepared by mixing varying quantities of $NaHPO_4$ and KH_2PO_4 (approximately 22 mM total PO_4^{3-}) to obtain a range of pH between 5 and 9. These experiments were conducted under sterile conditions by chemical poisoning with mercuric chloride ($HgCl_2$) at a concentration of 25 mg/L.

Aerobic biodegradation experiments were conducted in batch bottles prepared under aseptic conditions by autoclaving all implements used for the construction of the Batch bottles. The batch bottles were operated under a laminar flow hood to avoid contamination. Autoclaved synthetic buffered medium (198 mL) and nutrient mixture (2 mL) were added to the batch bottles and the remaining 111-mL of headspace was purged with helium to remove nitrogen prior to the addition of 22 mL of pure O_2. This procedure facilitated accurate measurement of O_2 due to incomplete separation of O_2 and N_2 by the GC column.

A buffer medium designed to maintain a constant pH 7 was used as the aqueous matrix for the aerobic biodegradation studies. The buffer medium was prepared by adding KH_2PO_4 (15 g) and Na_2HPO_4 (20 g) to 1 L of de-ionized (DI) water and adjusting the pH to 7 with 6 M NaOH. The nutrient medium used was prepared by combining

concentrated stock solutions of major nutrients with a concentrated stock of trace nutrients at a 10:1 ratio. The major nutrient stock solution was comprised of 9 g of $NaNO_3$ and 0.5 g of $MgSO_4$ in 1 L of DI water and the minor nutrient stock was comprised of the following: $ZnSO_4 \cdot H_2O$, 0.303 g; $FeSO_4 \cdot 7H_2O$, 1.2 g; $CoSO_4 \cdot 7H_2O$, 0.102 g; $MnSO_4 \cdot H_2O$, 0.302 g; in 100 mL of DI water.

The TCE and c-DCE cometabolism experiments were conducted in batch bottles prepared in a manner similar to that explained above. The desired solution concentrations of TCE and c-DCE were obtained by spiking appropriate volumes of distilled water saturated with TCE and c-DCE. Control batch bottles were prepared by autoclaving and/or chemical poisoning with mercuric chloride ($HgCl_2$). The batch bottles were incubated in an environmental chamber at 30 °C with continuous shaking at 150 rpm. The aqueous phase concentration of TCE or c-DCE in the batch bottles was calculated from the measured gas phase concentration based on Henry's law. From the volumes of liquid and headspace in the batch bottles and the concentrations of TCE and c-DCE in these two compartments, the total mass balance in the batch bottle was calculated.

3.2.4 Bacterial Cultures

The microbial culture used for the aerobic biodegradation studies and the cometabolism experiments was stimulated from activated aerobic sludge obtained from the Corvallis, OR, wastewater treatment plant. The activated sludge was acclimated to high concentrations of TBOS (500 mg/L) in a 125-mL batch bottle for a period of over 10 months before the stimulation of the culture occurred. This microbial culture was

enriched by repeatedly centrifuging the cells and transferring them into a different batch bottle with buffered medium with re-growth on TBOS. The enriched culture was subsequently used as an inoculum for a 2-L (liquid volume) continuous-feed batch reactor that was continuously fed TBOS. The reactor was operated with the same nutrient media as described for the batch bottles. The reactor was continuously fed neat TBOS at a rate of 80 µL/day with a syringe pump. Everyday 200 mL of solution with cells was wasted from the reactor and 200 mL of fresh buffer medium and nutrients were added. The mean cell residence time in the reactor was approximately 5 days. The microbial culture from this reactor was ultimately used for the subsequent experiments.

Inhibition experiments were carried out by addition of acetylene, which is a universal inhibitor of the monooxygenase enzyme (*14*). Pure acetylene gas (20% of headspace volume) was added to the batch bottles to ensure maximum inactivation.

3.3 Results

3.3.1 Abiotic Hydrolysis Experiments

The effect of pH, temperature and tetraalkoxysilane concentration on the abiotic hydrolysis of TBOS and TKEBS was evaluated. The disappearance of TBOS and TKEBS and the subsequent formation of their transformation products were monitored. The effect of pH on the hydrolysis of TBOS at 30 °C and 10 mg/L (32 μM) aqueous concentration is shown in Figure 3.1.

At the beginning of the experiment, an almost immediate loss of a small fraction (about 3-5%) of the TBOS added and instantaneous release of stoichiometric amounts of 1-butanol were observed in all the batch-bottle experiments. The cause of this behavior is not known, but it was confirmed not to be a contamination of TBOS with 1-butanol. Subsequently the rates of hydrolysis decreased. The rates of formation of 1-butanol correlated well with the rates of hydrolysis of TBOS. On a molar basis, the amount of 1-butanol formed was about 3.7 - 4.1 times the amount of TBOS hydrolyzed, suggesting that 1-butanol is the main transformation product of TBOS hydrolysis. The rates of TBOS hydrolysis were about a factor of 2-3 times higher at pH 5 and 9, and at the site groundwater pH (pH 7.8) relative to rates at neutral pH. An acceptable linear regression fit was obtained with the data for the disappearance of TBOS and formation of 1-butanol. At neutral pH the rates of hydrolysis were about 0.32 μM/day (based on 1-butanol formation) as compared to 0.76 μM/day and 0.88 μM/day at pH 5 and 9, respectively (Table 3.1).
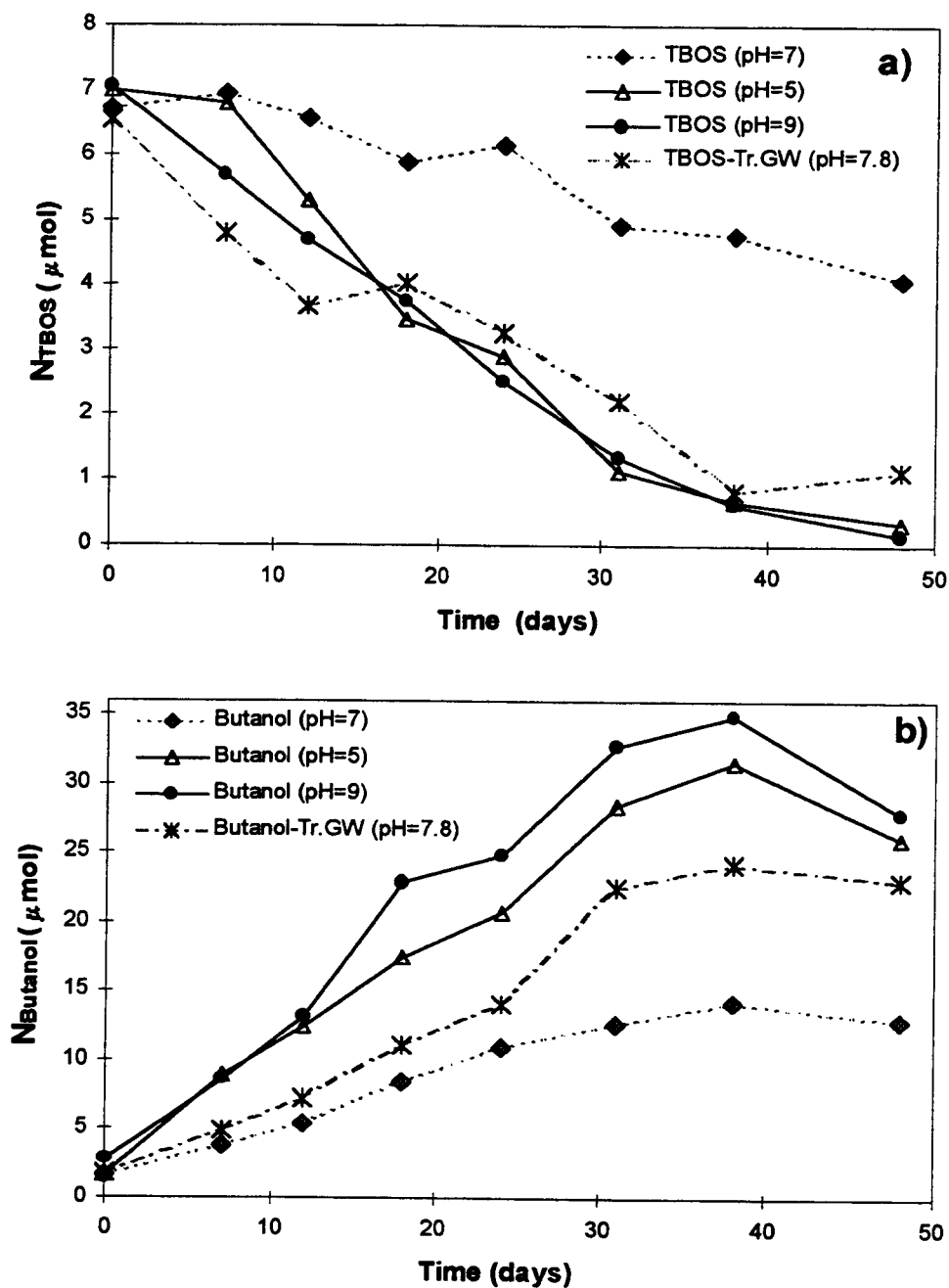
Figure 3.1. Effect of pH on the rate of hydrolysis of TBOS (above solubility limit) at 32 μM initial concentration and 30 °C. The rates of hydrolysis at pH 5, 7, 7.8 (Groundwater pH) and 9 were monitored. The rates were calculated based on the degradation rate of TBOS (a) or by rate of formation of the hydrolysis product, 1-butanol (b). The solution volume was 0.25 L.

Similar experiments were conducted with TKEBS which also hydrolyzes to stoichiometric quantities of 2-ethylbutanol. However, TKEBS hydrolyzed at a rate about an order of magnitude slower than TBOS.

The hydrolysis of TBOS and TKEBS at constant pH 7 and 10 mg/L aqueous concentration were studied at 15 and 30 °C (data not shown). Hydrolysis rates were not significantly different over this temperature range. Due to precision of the TBOS and TKEBS measurements (10 to 15 % RSD), a difference of less than 15 % is considered insignificant.

The hydrolysis of TBOS and TKEBS were monitored at two concentrations above the solubility limit of the compounds and the results of this experiment are shown in Figure 3.2. At pH 7 and 30 °C, a ten-fold increase in concentration of TBOS from 10 to 100 mg/L resulted in a factor of 4 increase in hydrolysis rates. Similarly for TKEBS, a ten-fold increase in concentration resulted in a factor of 8 increase in the hydrolysis rates.
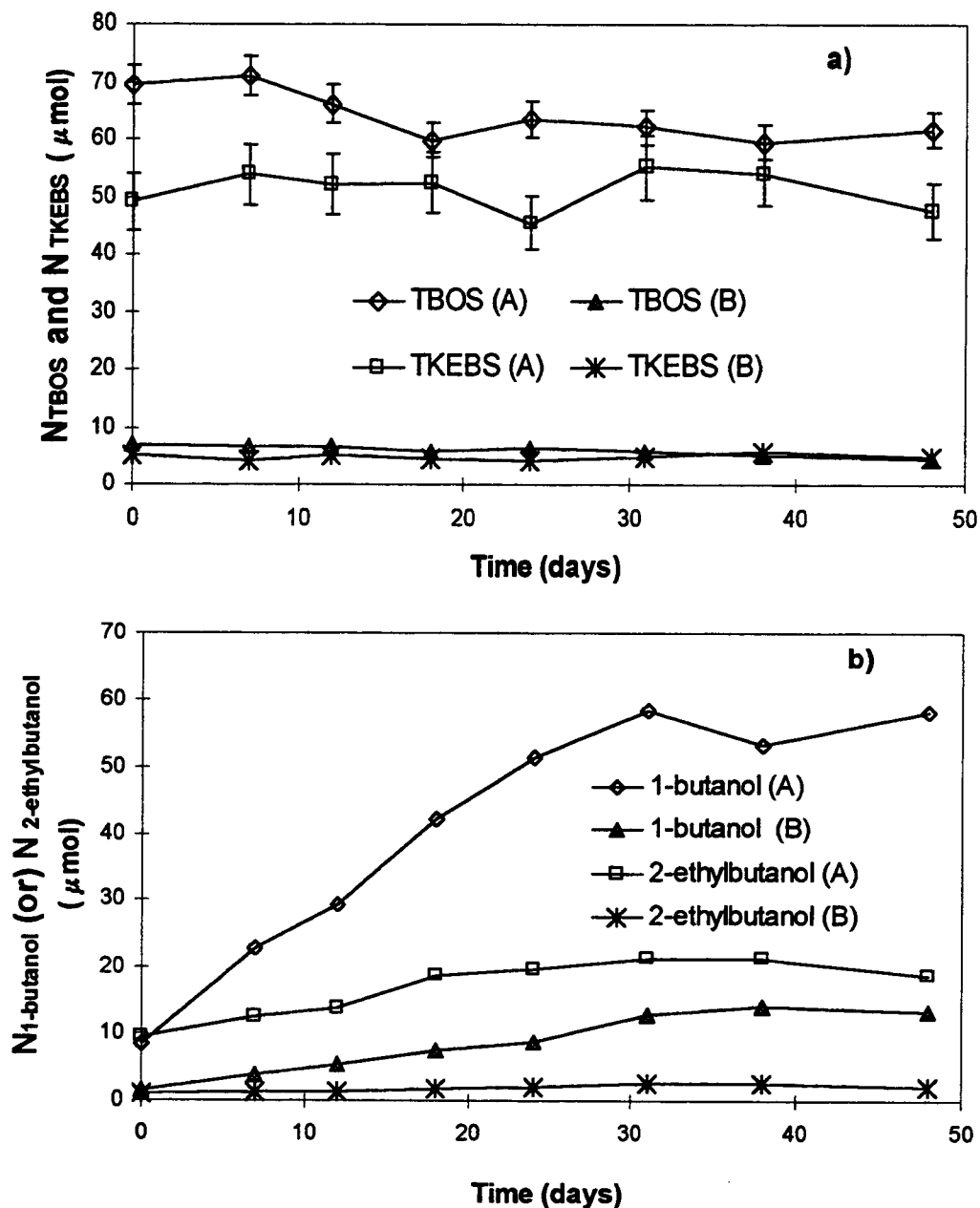
Figure 3.2. Effect of concentration (above solubility limits) on the rates of hydrolysis of TBOS or TKEBS at initial concentrations of 320 μM (A) and 32 μM (B), pH 7 and 30 °C. The rates were calculated based on the disappearance of TBOS or TKEBS (a) or by rate of formation of the hydrolysis products, 1-butanol or 2-ethylbutanol (b). The solution volume was 0.25 L.

Rates of hydrolysis under the different conditions studied are summarized in Table 3.1. The rates obtained from a simple linear regression fit are presented. The rates of transformation of TBOS are calculated based on both the rate of disappearance of TBOS and on the formation of 1-butanol. The rates of transformation for TKEBS are calculated based solely on the rate of formation of 2-ethylbutanol because the relative decrease in the TKEBS concentration is small compared to the precision of the measurements.

Table 3.1. Rates of hydrolysis of TBOS and TKEBS based on a linear regression fit

Environmental Variables			Rate of Hydrolysis (μM/day)		
pH	Temp (°C)	Initial concentration (mg/L)	TBOS based on disappearance of TBOS	TBOS based on 1-butanol production	TKEBS based on 2-ethylbutanol production
5.0	30	10	0.64	0.76	0.09
9.0	30	10	0.60	0.88	0.09
7.8	30	10	0.44	0.64	0.05
7.0	30	10	0.25	0.32	0.05
7.0	15	10	0.24	0.32	0.04
7.0	30	100	0.83	1.04	0.40

3.3.2 Aerobic Biological Degradation of TBOS and TKEBS

Initial studies focussed on stimulating a microbial population that could aerobically degrade TBOS and TKEBS. After incubation for about 1 year, a microbial culture from a sample of activated sludge grown on TBOS was stimulated.

The history of stimulation by addition of TBOS and the evidence for the enrichment of the microorganisms that could degrade TBOS under aerobic conditions are shown in Figure 3.3. The rate of transformation of TBOS in the presence of the microbial culture is much faster than that of the mercury poisoned-control. Within 30 days, 180 μmol of TBOS (from 3 separate additions of 60 μmol) were utilized in the biologically-active batch bottle, while in the poisoned-control, only a small amount of the initial 60 μmol of TBOS was hydrolyzed. The rate of hydrolysis of TBOS in the poisoned-control was in the same range of that achieved in the abiotic hydrolysis studies for similar initial TBOS concentrations. The rate of degradation of TBOS also increased with each successive addition of TBOS indicating growth and enrichment of a microbial culture that degrades TBOS. Cell growth was also indicated by an increase in optical density. The 1-butanol that was formed was subsequently utilized in the biologically-active culture, while 1-butanol accumulated in the poisoned-control (Figure 3.3c). These results demonstrate the microbially accelerated hydrolysis of TBOS and the aerobic metabolism of TBOS. The O_2 consumption data and the carbon dioxide production data (Figure 3.3b) are consistent with the TBOS utilization, and formation and disappearance of 1-butanol.
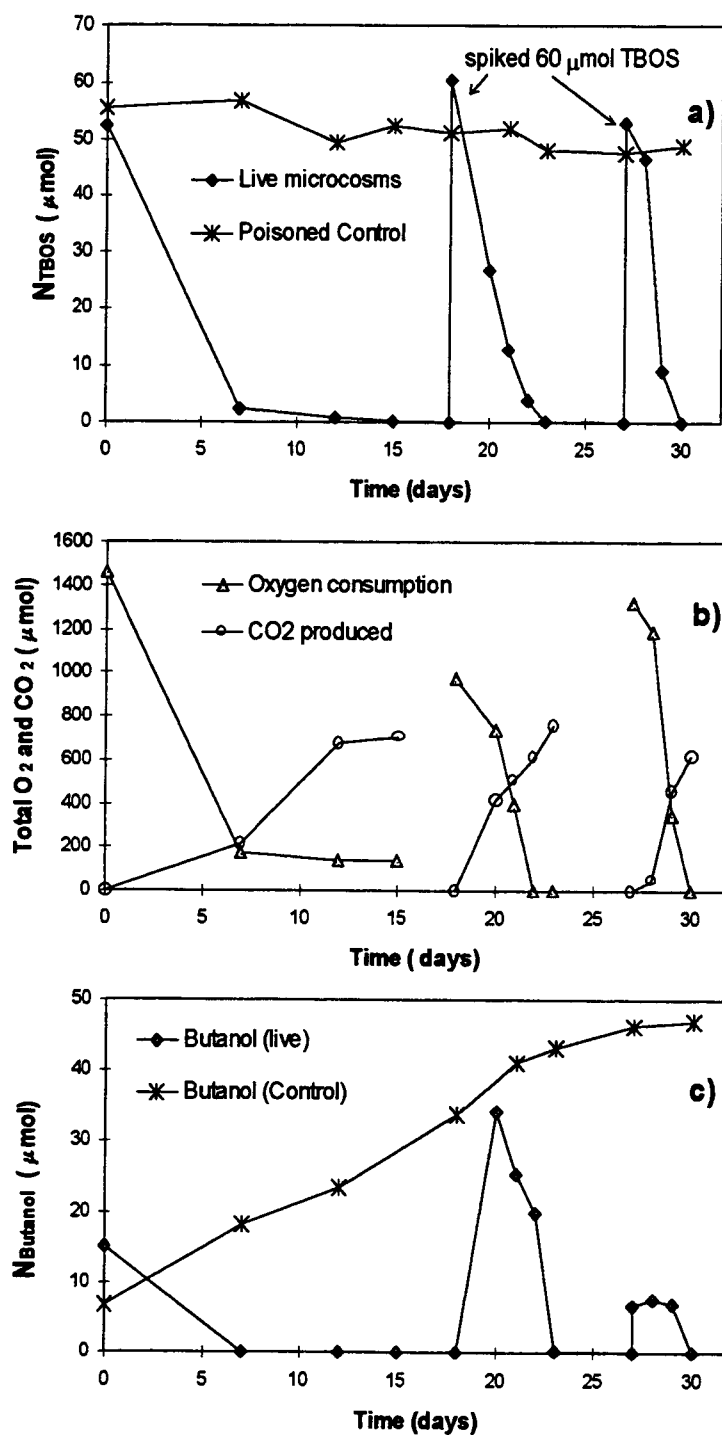
Figure 3.3. History of TBOS degradation providing evidence of enrichment and mineralization. TBOS degradation by the microorganisms from the activated sludge (a), the utilization of O_2 and production of CO_2 (b) and the formation of 1-butanol (c) as an intermediary product.

The moles of CO_2 produced were estimated from the headspace CO_2 concentration by assuming Henry's law equilibrium with the aqueous phase and the equilibrium speciation of CO_2 in solution. The equilibrium speciation of CO_2 between H_2CO_3, HCO_3^- and CO_3^{2-} was based on the measured pH. Approximately 700-750 μmol of CO_2 were produced during each addition of TBOS which corresponds to about 70% of the theoretical maximum (16 mole of CO_2 per mole of TBOS degraded). The incorporation of carbon from TBOS into cell mass could account for the remaining 30% of the carbon. These results indicate that TBOS is mineralized to carbon dioxide under aerobic conditions.

Further mineralization experiments were conducted with the enrichment culture obtained from the continuous-feed batch reactor. The enrichment culture could grow on TBOS or TKEBS as the carbon and energy source. The degradation experiments conducted with 10 mg (dry cell weight) of the microbial culture are shown in Figure 3.4. The microbial culture used for these tests were initially grown on TBOS.

Figure 3.4a shows that TBOS at aqueous concentrations of 20 mg/L (12 μmol total) was rapidly degraded within 12 hr. The concentration of 1-butanol increased during the first few hours due to accelerated hydrolysis of TBOS, and then decreased as it was gradually utilized by the microorganisms. The rate of TBOS degradation in the batch bottles with acetylene was similar to that observed in the live batch bottles. Acetylene did not inhibit the degradation of TBOS. Thus, a monooxygenase enzyme does not appear to be involved in TBOS utilization. No significant transformation of TBOS was observed in the autoclaved batch bottles containing cells. However, for the control batch bottles prepared by the addition of mercuric chloride, about 60% of the

TBOS was hydrolyzed to 1-butanol. Stoichiometric amount of 1-butanol, equivalent to the amount of TBOS degraded was formed.
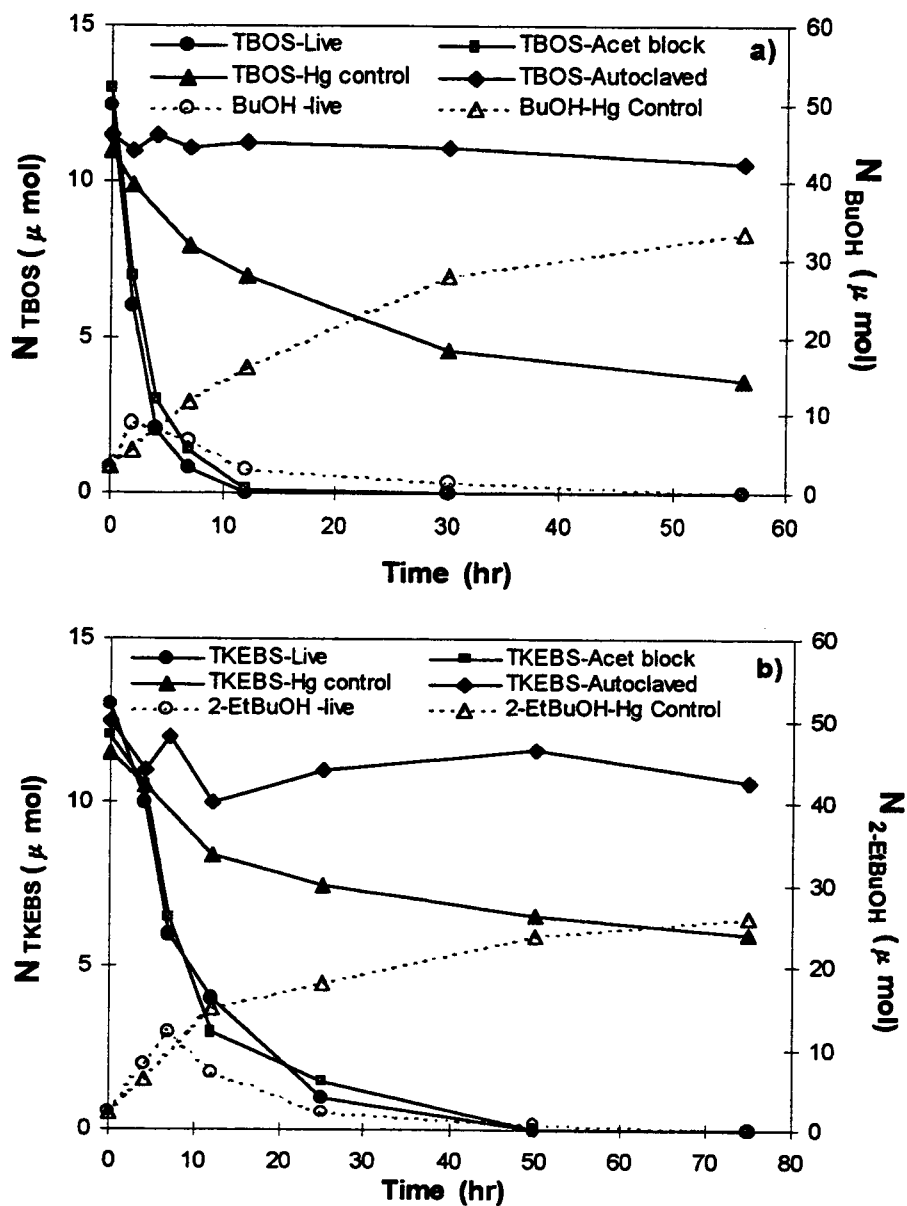
Figure 3.4. Aerobic degradation of 20 mg/L of TBOS (a) or TKEBS (b) by the enriched microbial culture. 9.0 mg cells (dry weight) were added to each batch bottle for the degradation study. Acetylene at 20% (v/v) was used as a block for the monooxygenase enzyme. Two controls were prepared, one by autoclaving and the other by addition of HgCl₂.

The rapid degradation of TKEBS within a matter of 50 hr with the same enriched culture that was initially grown on TBOS is clear from the data in Figure 3.4b. Also, complete mineralization of TKEBS and the formation of 2-ethylbutanol as an intermediate were observed. No inhibition of the degradation of TKEBS was observed by the addition of acetylene. Hydrolysis of about 40% of TKEBS to equivalent amounts of 2-ethylbutanol occurred in the batch bottles with mercuric chloride. No degradation of TKEBS was observed in the autoclaved batch bottles. Consistent with the TBOS results, heat treatment upon autoclaving destroyed the activity of the enzymes that promoted the hydrolysis.

Abiotic hydrolysis of TBOS and TKEBS observed in the poisoned-controls provides some evidence that the release of enzymes during the lysis of the killed cells maintain some activity for hydrolysis (*18*). The formation of stoichiometric amounts of the alcohol supports the conclusion that the cells were effectively poisoned and that this observed activity was entirely abiotic.

3.3.3 Degradation of TKEBS by Aerobic Microorganisms Stimulated from the Groundwater

Several unsuccessful attempts were made at stimulating indigenous site microorganisms with TBOS and TKEBS. Therefore, aerobic microorganisms were stimulated by the addition of 2-ethylbutanol as a growth substrate to the groundwater. This microbial culture was subsequently tested for its ability to degrade TKEBS. The results of these experiments are presented in Figure 3.5. O_2 consumption and CO_2 production rates are presented along with TKEBS degradation data (Figure 3.5b).

TKEBS was transformed at a much faster rate in the biologically-active batch bottles than in the mercury poisoned-control. Note that 2-ethylbutanol accumulated in the poisoned-control, while it is degraded in the biologically-active batch bottle. The initial rate of TKEBS transformation (about 10 μM/day) was much faster than that predicted by an abiotic transformation pathway (about 0.4 μM/day). However, after a period of 30 days the degradation of TKEBS stopped completely.

Oxygen consumption data were consistent with the aerobic degradation of TKEBS (Figure 3.5b). About 360 μmol of CO_2 were formed from the degradation of 30 μmol of TKEBS representing a CO_2/TKEBS ratio of 12. A ratio of 24 is expected with complete mineralization of TKEBS. The lower observed ratio may be attributed to incorporation of carbon from TKEBS into cell mass during the incubation.
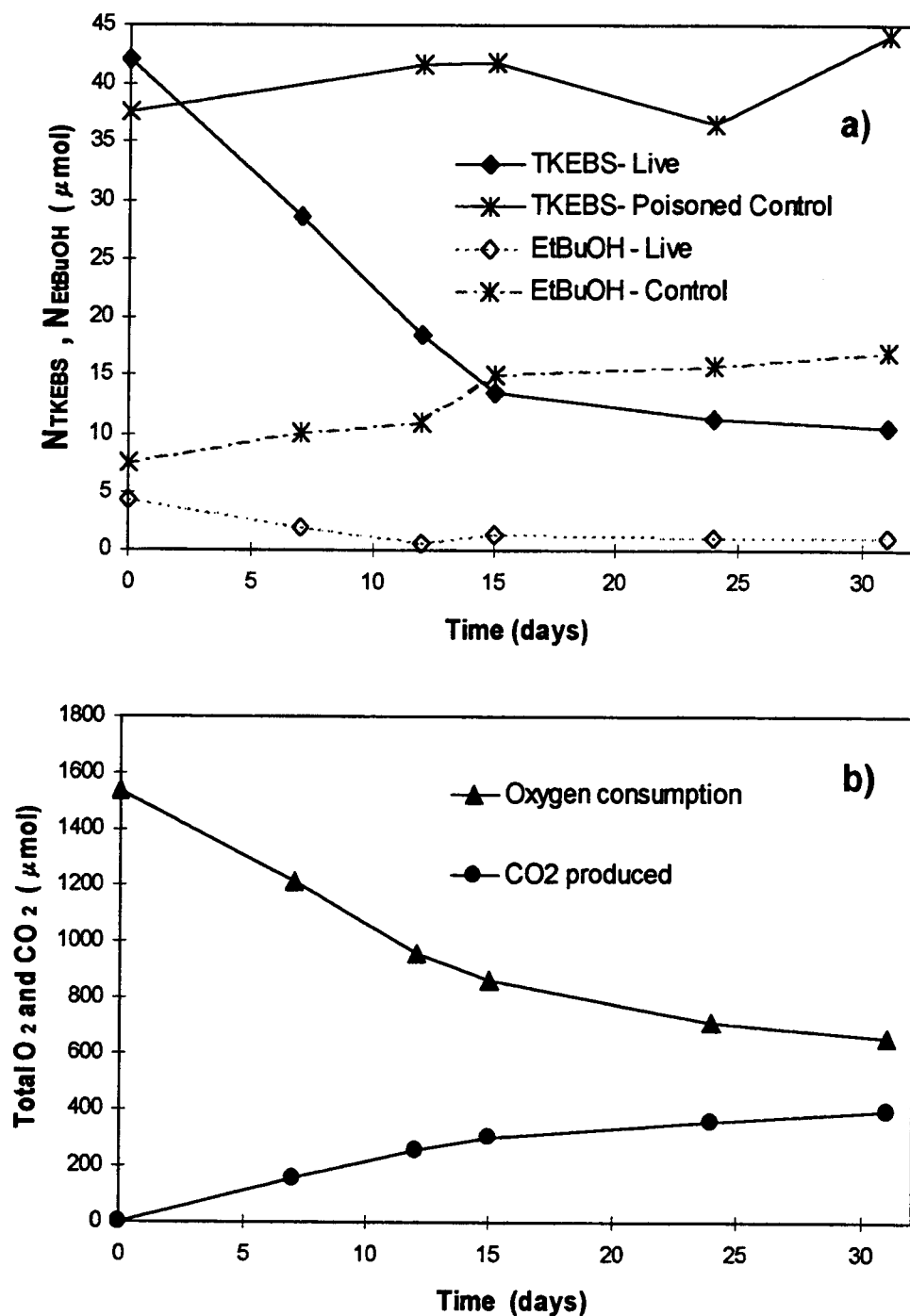
Figure 3.5. Aerobic degradation of TKEBS by indigenous microorganisms stimulated by the addition of 2-ethylbutanol as a growth substrate. TKEBS degradation (a) and the subsequent utilization of O_2 and production of CO_2 (b). The solution volume was 0.20 L.

3.3.4 TCE Transformation by Microorganisms Grown on TBOS

The enriched microbial culture (from the activated sludge) that grows on TBOS was tested for its ability to cometabolize TCE and c-DCE. Batch reaction kinetic tests were performed with 4.0 mg (dry weight) of enriched culture from the continuous-feed batch reactor. Five batch bottles were operated: a poisoned-control with TBOS and TCE, a biologically-active batch bottle with TBOS and TCE, two resting-cell batch bottles with only TCE (one with and the other without O_2), and an acetylene-block with TBOS and TCE. The acetylene-block was used to investigate the presence of a monooxygenase enzyme. The results of the experiments are shown in Figure 3.6.

In the batch bottle with TBOS added, TCE (0.89 mg/L or 1.6 μmol total) was completely cometabolized by the microbial culture within 65 hr. TBOS (20 mg/L or 12 μmol total) was also completely degraded but only after the TCE transformation was complete. With no TBOS added (resting-cell batch bottle with O_2), some TCE cometabolism occurred but at a slower rate and the total amount of TCE transformed was about 15 - 20% less than that achieved with TBOS addition. The transformation capacity calculated was about 0.04 g TCE/ g cells. No TCE transformation was observed in the poisoned-control. The results indicate that the degradation of TBOS helped drive TCE cometabolism.
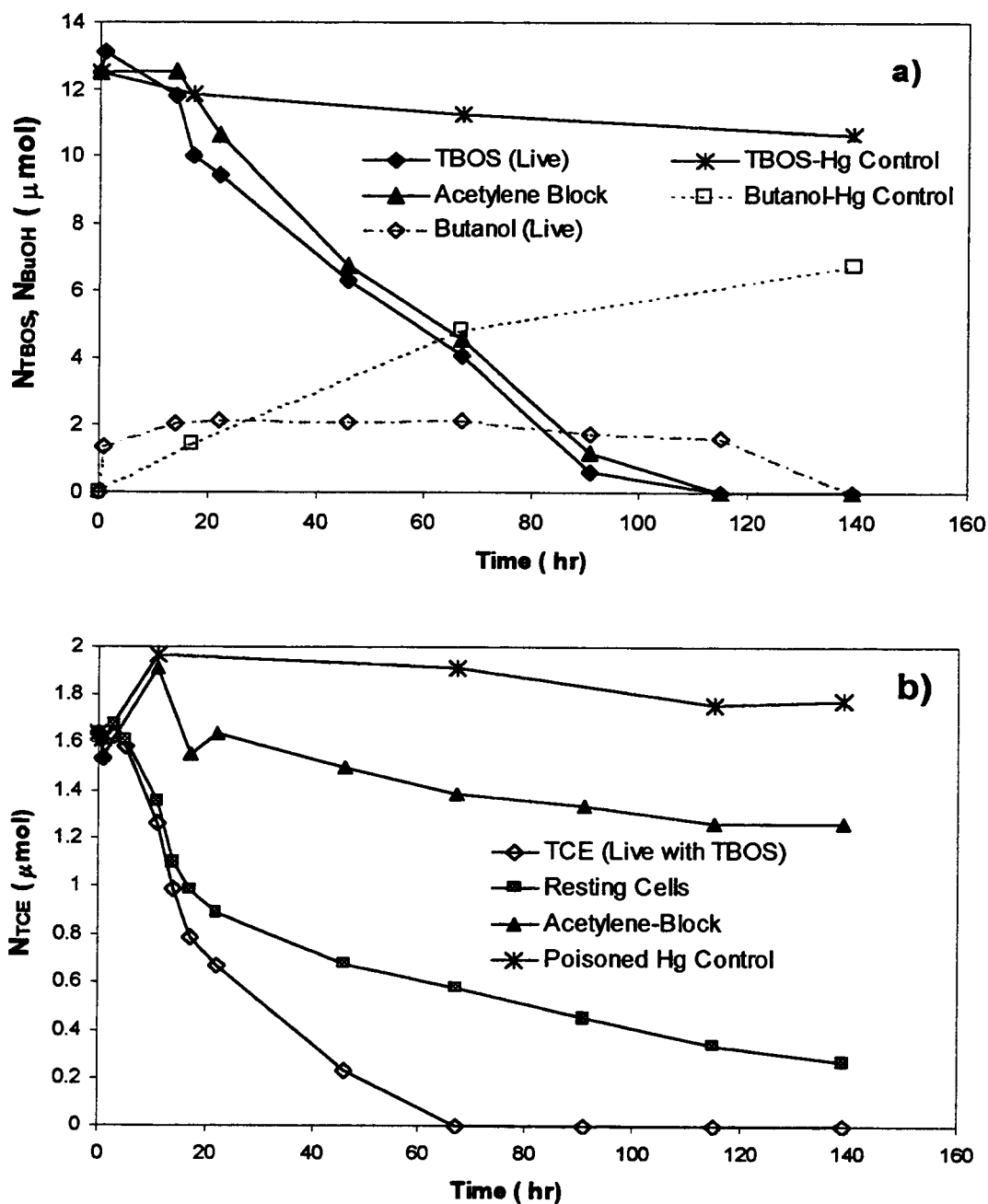
Figure 3.6. Aerobic cometabolism of TCE by microorganisms grown on TBOS. Disappearance of TBOS (a) and the degradation of TCE (b) by live microbes in the presence of TBOS and absence of TBOS (resting cells) as compared to the poisoned-control and the acetylene-block batch bottles. The solution volume was 0.20 L.

In the resting-cell batch bottle with TCE and no O_2, TCE was not transformed. This result indicates that O_2 is required for TCE transformation. In the acetylene-blocked batch bottle, TCE transformation was significantly reduced (about 20% degraded in 140 hr) but TBOS degradation was not inhibited. This difference suggests that different enzymes are responsible for the degradation of TBOS and the cometabolism of TCE. It is possible that the enzyme responsible for the cometabolism of TCE was induced by the presence of TBOS.

3.3.5 c-DCE Transformation by Microorganisms Grown on TBOS

The microbial culture grown on TBOS was tested for its ability to cometabolize c-DCE. Batch reaction kinetic tests were performed with 9.0 mg (dry cell weight) of enriched culture from the continuous-feed batch reactor. Six batch bottles were operated: a poisoned-control with TBOS and c-DCE, an autoclaved-control with TBOS and c-DCE, two biologically-active batch bottles with TBOS and c-DCE, a resting-cell batch bottle with only c-DCE, and an acetylene block with TBOS and c-DCE. The results of the experiments are shown in Figure 3.7. In the biologically-active batch bottles (in duplicate), TBOS and c-DCE degraded rapidly. TBOS and c-DCE at aqueous concentrations of 20 and 2 mg/L, respectively, were transformed within 15 hr of incubation. The degradation of c-DCE is well correlated to the formation of the unstable intermediate, c-DCE epoxide. The c-DCE epoxide was subsequently transformed at a slower rate (half-life of about 35-40 hr) which was slightly faster than the abiotic transformation rates (half-life of 70 hr) (14). This behavior is consistent with c-DCE cometabolism slowing after about 12 hr. Thus, the biotic transformation of the epoxide

is minimal. c-DCE cometabolism also appears to be highly correlated with TBOS utilization.

Complete transformation of TBOS was observed in the acetylene-blocked batch bottle with no suppression of the reaction rate while acetylene significantly reduced the rate of c-DCE transformation and the corresponding production of the epoxide. No c-DCE transformation occurred in the autoclaved or the Hg poisoned-control batch bottle. In the resting-cell batch bottle, an inherent capacity to degrade c-DCE was exhibited, but the rate of c-DCE transformation was less than that observed with TBOS addition. The transformation capacity calculated from the resting-cell batch bottle studies was 0.032 g c-DCE/ g cells. No c-DCE transformation was observed when acetylene was added to the resting cells (data not shown). These results suggest that TBOS or a product from TBOS transformation possibly 1-butanol, induces c-DCE cometabolism. These results are consistent with those obtained with TCE.
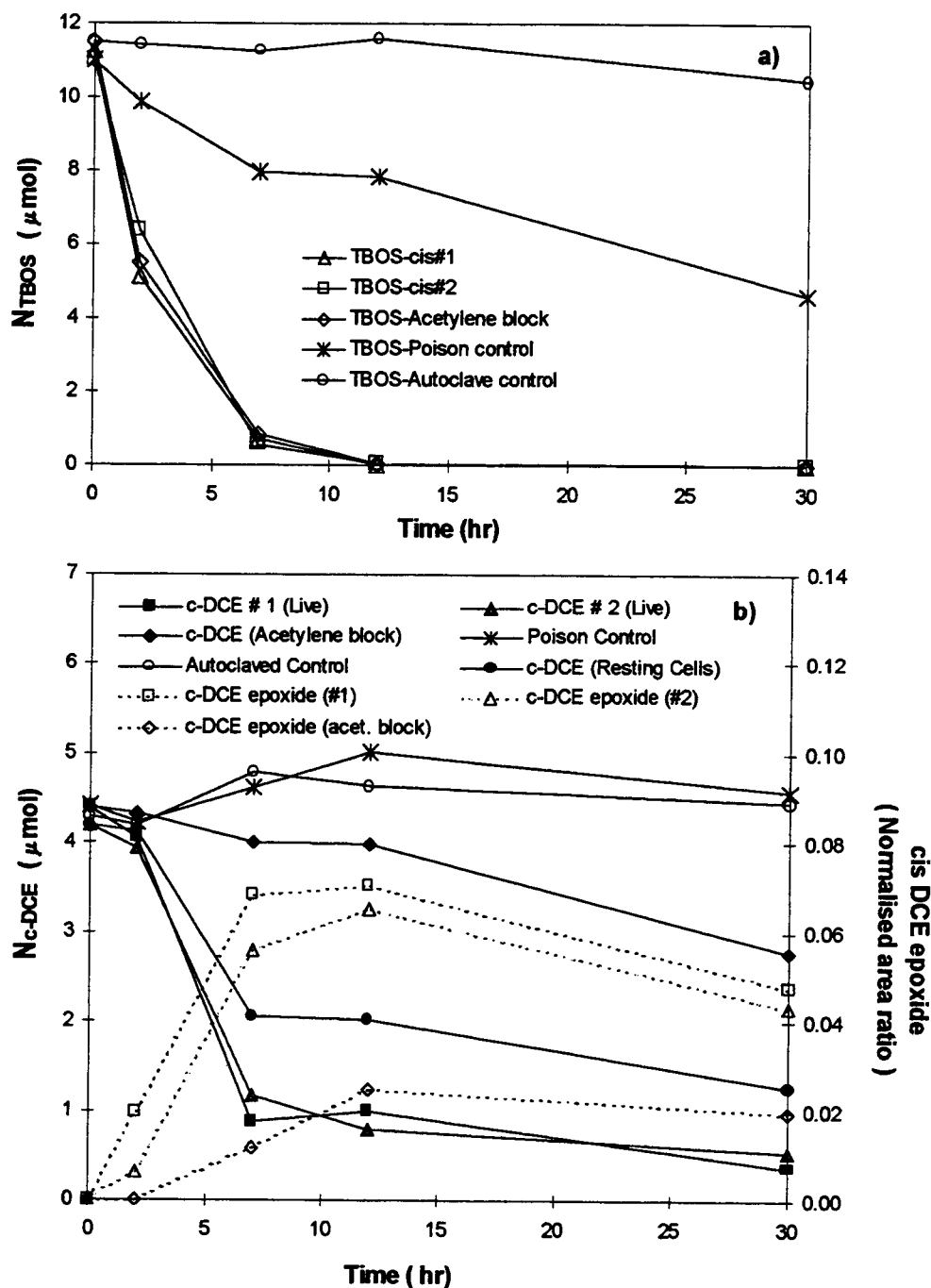
Figure 3.7. Aerobic cometabolism of c-DCE by microorganisms grown on TBOS. a) TBOS degradation profile in the batch bottles, b) the degradation of c-DCE by live microbes in the presence of TBOS and absence of TBOS (resting cells) as compared to the poisoned-control, autoclaved-control and the acetylene-block batch bottles. The normalized area ratio is the peak area of c-DCE epoxide with respect to the peak area of the internal standard.

Subsequent experiments were carried out with the duplicate biologically-active batch bottles. c-DCE was added to these batch bottles to achieve a 2 mg/L aqueous concentration. The batch-bottles were incubated for about 2 days but no c-DCE transformation was observed. At this time, TBOS (20 mg/L) was added to one of the batch-bottles and 1-butanol (20 mg/L) was added to the other. c-DCE was completely transformed in the batch-bottle with TBOS; whereas, no c-DCE transformation was observed in 1-butanol-fed batch bottle (data not shown). These results suggest that 1-butanol did not support the cometabolism of c-DCE. Similar experiments were conducted with TCE and the results obtained were consistent with that obtained for c-DCE. Also, based on these experiments estimates for the transformation yields (amount of chlorinated ethene degraded / amount of TBOS degraded) of 0.11 g c-DCE/ g TBOS and 0.04 g TCE/ g TBOS were obtained.

To evaluate the effect of O_2 and cometabolism at high concentrations of TCE or c-DCE (about 25 mg/L aqueous concentration), several batch-bottle experiments were operated so that O_2 was completely utilized during the course of the experiments. Two batch bottles were constructed with 9.0 mg cells (dry weight), high amounts of TBOS and either 25 mg/L of TCE or c-DCE. The data for these experiments are not shown but the results are discussed.

The TBOS concentration in the batch bottle dropped from 250 mg/L to about 110 mg/L in about 22 hr and simultaneously c-DCE (completely degraded) was transformed by the microorganisms to c-DCE epoxide. Upon depletion of O_2 in the batch bottle, biologically-mediated hydrolysis of TBOS remained unhindered, but the degradation of 1-butanol slowed. Almost stoichiometric amounts of 1-butanol accumulated. These

results suggest that O_2 was not required for the hydrolysis reaction and that the monooxygenase was not responsible for TBOS transformation. Active sulfate reduction was observed following this transition period. In the absence of O_2, c-DCE epoxide that had built-up in the batch bottles was transformed at rates (half-life of 65 hr) similar to the abiotic transformation rates.

Similar results were obtained with the batch bottle containing TCE. In this batch bottle, O_2 depletion occurred before TCE was completely cometabolized. TCE degraded from 25 mg/L to about 10 mg/L within 20 hr of incubation after which the transformation stopped completely due to lack of O_2. However, TBOS hydrolysis continued even in the absence of O_2, and almost equivalent amounts of 1-butanol accumulated in the batch bottle.

The oxygen data supports the acetylene-block data, which indicates that different enzyme systems are involved with the hydrolysis of TBOS and the cometabolism of TCE and c-DCE. The cometabolism of TCE and c-DCE are initiated by a monooxygenase enzyme; whereas, the hydrolysis reaction does not require O_2 and is not inhibited by acetylene.

3.4 Discussion

The transformation of alkoxysilanes occurred under both biotic and abiotic conditions. The rates of abiotic hydrolysis of TBOS and TKEBS to the corresponding alcohols are fairly slow under environmentally relevant conditions of pH and temperature. The hydrolysis reaction appears to be both acid and base catalyzed with rates of TBOS transformation at pH 5 and 9 about a factor of two higher than that at neutral pH. The rate of TKEBS transformation is about an order of magnitude lower than that of TBOS and was not substantially affected by change in pH (within 5 to 9). The lower hydrolysis rate might be attributed to the increased steric hindrance by the branching on the butoxy group which is consistent with theory (5, 7). The hydrolysis also showed very little temperature dependence.

At concentrations of TBOS and TKEBS above the solubility limit, the rate of hydrolysis increased with concentration (a factor of 4 and 8, respectively). These results suggest that the transformation of both TBOS and TKEBS involves both homogeneous and heterogeneous kinetics. When the solubility limit is exceeded and TBOS/TKEBS droplets form, the hydrolysis may be controlled by the dissolution rate of TBOS/TKEBS or direct hydrolysis may occur at the surface of the suspended droplets. In either case, the total rate of hydrolysis would be expected to increase with surface area.

Aerobic biological degradation experiments demonstrated the microbially-enhanced hydrolysis of TBOS and TKEBS to 1-butanol and 2-ethylbutanol. Mineralization of TBOS and TKEBS to CO_2 was observed. $HgCl_2$ did not inhibit the hydrolysis but did prevent the mineralization which suggests the presence of a hydrolyzing enzyme. The cometabolism of TCE and c-DCE also supports the

production of a monooxygenase enzyme, which introduces complexity to the mechanism of degradation (Figure B.5). TBOS hydrolysis was not inhibited by the presence of acetylene or the temporary lack of O_2 indicating that the monooxygenase was not involved in TBOS transformation. The utilization of 1-butanol was also not inhibited by acetylene, but lack of O_2 inhibited 1-butanol utilization.

The results also indicated that under aerobic conditions, TBOS and TKEBS at the contaminated site could be transformed by microorganisms that grow on the alcohols. This is a significant observation with regards to understanding the natural biological processes that occur at the site. Aerobic bioremediation as a remedial alternative for the clean-up of TBOS and TKEBS could potentially be explored. The aerobic bioremediation of TBOS and TKEBS could also be accelerated by the addition of either 1-butanol or 2-ethylbutanol.

The aerobic cometabolism studies demonstrated that TBOS can be used as a potential cometabolic substrate for c-DCE and TCE degradation. To our knowledge, these are the first observations that TBOS serves as a cometabolic substrate for chlorinated ethene transformations. Results from the acetylene-block experiments suggest that a monooxygenase enzyme was involved in the TCE and c-DCE degradation but not for the degradation of TBOS. One possibility is that the monooxygenase enzyme was induced as a side-reaction that did not directly support the degradation of TBOS. It is possible that the metabolism of the products of TBOS degradation could initiate the monooxygenase enzyme. However, separate experiments indicated that the cometabolism was not related to 1-butanol degradation and TBOS was required for cometabolism. It may be that the TBOS molecule with the straight-chain aliphatic

groups itself induces the monooxygenase enzyme. Competitive inhibition of the enzymes responsible for TCE and c-DCE cometabolism by TBOS could not be evaluated because of the low solubility of TBOS in water (less than 1 mg/L).

The transformation yields (0.11 g c-DCE/ g TBOS and 0.04 g TCE/ g TBOS) were comparable to other known cometabolic substrates such as methane, propane, butane, etc (*10-12*). The high capacity for the TBOS degraders to cometabolize c-DCE and TCE provide an opportunity to exploit the contamination of TBOS and TKEBS at Site-300 and take advantage of it as a pre-existing electron donor. It may also be possible to add TBOS as a primary substrate to cometabolize chlorinated ethenes at other contaminated sites.

3.5 References

(1)   Alagar, A.; Krishnasamy, V., *Chem. Tech. Biotechnol.*, **1988**, *36*, 577-582.

(2)   Hasegawa, H.; Sakka, S., *J. Non-crystalline solids*, **1988**, *100*, 201-205.

(3)   Aelion, R.; Loebel, A.; Eirich, F., *J. Am. Chem. Soc.*, **1950**, *72*, 5705-5712.

(4)   McNeil, K. J.; DiCaprio, J. A.; Walsh, D. A., *J. Am. Chem. Soc.*, **1980**, *102:6*, 1859-1864.

(5)   Eaborn, C., *Organo-silicon Compounds*, Buttersworth publications limited: Edinburgh, **1960**.

(6)   Von Ebelman, J., *Ann. Chem.*, **1845**, *57*, 319.

(7)   Barry Arkles, A Survey of Properties and Chemistry, Gelest, **1995**.

(8)   Wilson, J. T.; Noonan, M.J.; McNabb, J. F., *Groundwater. Qual.*, **1985**, Wiley & Sons, New York.

(9)   Westrick, J. J.; Mello, J. W.; Thomas, R. F., *J. Am. Water Works Assoc.*, **1984**, *76* (*5*), 52-59.

(10) Semprini, L.; Hopkins, G. D.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1993**, *59* (*7*), 2277-2285.

(11) Hamamura, N.; Page, C.; Long, T.; Semprini, L.; Arp, D. J., *Appl. Environ. Microbiol.*, **1997**, *63* (*9*), 3607-3613.

(12) Landa, A. S.; Sipkema, E. M.; Weijma, J.; Beenackers, A. A. C. M.; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1994**, *60*, 3368-3374.

(13) Mars, A. E.; Houwing, J.; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 886-891.

(14) Johan, E. T.; Vlieg, H. V.; Koning, W. D.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 3304-3312.

(15) Gibson, S. A.; Sewell, G. W., *Appl. Environ. Microbiol.*, **1992**, *58*, 1392-1393.

(16) Fennell, D. E.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1997**, *31*, 918-926.

(17) Ballapragada, B. S.; Stensel, D. H.; Puhakka, J. A.; Ferguson, J. F., *Environ. Sci. Technol.*, **1997**, *31*, 1728-1734.

(18) Madigan, M.T; Martinko, J.M; Parker, J., *Brock Biology of Microorganisms*, **1997**, 8th ed., Prentice hall, NJ.

(19) Vancheeswaran, S.; M. S. Thesis, *Abiotic and Biological Transformation of TBOS and TKEBS, and their Role in the Biological Transformation of TCE and c-DCE,* **1997**.

(20) Vancheeswaran, S.; Semprini, L.; Williamson, K. J.; Ingle, Jr. J. D.; Daley, P., Proceedings of the First International Conference on " Remediation of Chlorinated and Recalcitrant compounds", Monterey, CA., May 8th, **1998**.

(21) Thauer, R. K.; Jungermann, K.; Decker, K., *Bacteriol. Rev.*, **1977**, *41*, 100-180.

CHAPTER 4
INTRINSIC TRANSFORMATION OF TRICHLOROETHYLENE DRIVEN BY ORGANO-SILICON COMPOUNDS AT SITE-300, LAWRENCE LIVERMORE NATIONAL LABORATORY, CA

Sanjay Vancheeswaran [a†], Lutz Friedrich[†]

Kenneth J. Williamson[†], James D. Ingle, Jr.[a] and Lewis Semprini[*†]

[†]Department of Civil, Construction and Environmental Engineering

and

[a]Department of Chemistry

Oregon State University,

Corvallis, OR 97330, USA.

Paul Daley, Lawrence Livermore National Laboratory, CA.

4.1 Introduction

Trichloroethylene (TCE) is a common groundwater contaminant in aquifers throughout the United States (*1*). TCE ranks in the top ten priority pollutants listed by the US EPA (*2*). TCE has been widely used as a degreasing agent and as a solvent in industry. Improper handling and leakage of TCE has resulted in its widespread contamination of aquifers. Over the past decade, microbial degradation of TCE has been extensively studied (*3-11, 28, 29*). Reductive dechlorination under anaerobic conditions and aerobic cometabolic processes are the predominant pathways for TCE transformation. In aerobic cometabolic processes fortuitous oxidation of TCE, catalyzed by the enzymes initiated for oxidation of the growth substrates occurs (*3-5*). In the reductive dechlorination process, TCE serves as an electron acceptor and chlorine atoms are replaced by hydrogen (*6-11*). An electron donor is required to complete this reaction by providing the two electrons required for each dechlorination step. Electron donors such as lactate, benzoate and ethanol (*6, 8-11*) have been shown to support the dechlorination reaction. A range of dechlorinating cultures have been isolated and studied to determine the extent of dechlorination (*7-11, 28, 29*). Incomplete dechlorination to cis-dichloroethylene (c-DCE) or vinyl chloride (VC) is often observed. However, some cultures are reported to dechlorinate completely to ethene (*8-11*).

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 (*10, 11*) in the reductive dechlorination. Evidence suggests that H_2 serves as the primary electron donor in the dechlorination of TCE, and substrates that are more complex act primarily as precursors to generate hydrogen via fermentation (*9-11*). 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 (*10, 11*). A significant advantage for the dechlorinators can be obtained by managing H_2 delivery at a low concentration (*9-11*). This could be achieved by using electron donors that ferment at a low partial pressures of H_2 (*9*).

Site-300, Lawrence Livermore National lab (LLNL), CA, is a shallow aquifer contaminated with TCE. TCE was used in a materials testing facility as a heat-exchange fluid, and contamination has resulted from leaking pipes. Compounds, such as tetrabutoxysilane (TBOS)($C_{16}H_{36}O_4Si$) and tetrakis(2-ethylbutoxy)silane (TKEBS) ($C_{24}H_{52}O_4Si$) are also used as heat-exchange fluids (*15, 16*). Use of TBOS and TKEBS in the heat exchanger pipes at the site also resulted in contamination of the groundwater at Site-300, LLNL, CA.

Numerous hydrolysis studies have been carried out on the lower homologues of the tetraalkoxysilanes such as tetramethoxysilane and tetraethoxysilane (*12-15*), which transform abiotically under a hydrolytic pathway to give the corresponding alcohols (*15*). Although TBOS and TKEBS have been studied minimally, it was expected that TBOS would hydrolyze to 1-butanol and TKEBS would hydrolyze to 2-ethylbutanol. Separate studies in our laboratory were conducted to determine the solubility and the rate of hydrolysis (*20*) of these compounds as a function of pH, temperature and concentration. TBOS and TKEBS were found to have low aqueous solubility in the range of 0.5 - 1 mg/L. Hydrolysis rate experiments were conducted at concentrations above the solubility limit of TBOS and TKEBS. The hydrolysis was observed to be acid

and base-catalyzed and independent of temperature (*20*). 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 (*20*), 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 (*20*).

The objectives of this research were to investigate the potential for intrinsic bioremediation of TCE and to study the transformation of TBOS and TKEBS. Laboratory and field evidence is presented for the intrinsic anaerobic transformations of TCE and the role of TBOS and TKEBS as effective co-substrates supporting this transformation. The laboratory work involved detailed anaerobic microcosm studies with groundwater to better understand the processes occurring in-situ. Chemical analysis of the groundwater was performed half-yearly to understand the seasonal fluctuations in the field.

4.2 Materials and Methods

4.2.1 Chemicals and Stock Solutions.

Trichloroethylene (TCE) (99.9% purity), cis-1,2 dichloroethylene (99.9%), HPLC-grade dichloromethane, 1-butanol (99.9%) and 2-ethylbutanol (96% purity) were obtained from Aldrich Chemical Co. (Milwaukee, WI). TBOS (98 % purity) and tetrapropoxysilane (TPOS) (98 % purity) was obtained from Gelest, Inc (Tullytown, PA). TKEBS was synthesized in the lab by adopting Von Ebelman's synthesis for tetraalkoxysilanes (16, 17). Stoichiometric quantities of silicon tetrachloride (semiconductor grade, Aldrich Co.) were reacted with a slight excess of 2-ethylbutanol, in the presence of pyridine as an acid acceptor in benzene solvent. The products were vacuum distilled to yield approximately 95% pure TKEBS. The above compounds were used as both culture substrates and in the preparation of analytical standards. Glacial acetic acid (Mallinckrodt, Inc., 99.5%), sodium butyrate (Fisher scientific Co.) and 2-ethylbutyric acid (Aldrich Chemical Co., 97%) were used in the preparation of analytical standards. H_3PO_4 (Mallinckrodt, Inc., 95%) was used to prepare 8 M aqueous solutions to preserve liquid samples.

4.2.2 Analytical Methods

TBOS, TKEBS, 1-butanol and 2-ethylbutanol concentrations were quantitatively determined by liquid-liquid extraction of 1-mL aqueous samples with 0.5 mL of dichloromethane and agitation for five minutes on a vortex mixer. After complete separation of the two immiscible phases, 2 µL of the dichloromethane extract was

introduced into a GC/MS. Analyte concentrations in the extract samples were

determined with a HP-5890 gas chromatograph connected to a HP-5971 mass-selective

detector. The chromatographic separation was carried out with a Rtx-20 column (30 m x

0.25 mm, 1.0-μm film) from Restek, Inc. (Bellefonte, PA). 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. The

concentrations were normalized with TPOS (10 mg/L) as an internal standard. Solid

Phase Micro Extraction (SPME) (*18, 19*) was used for the analysis of the groundwater

and microcosm samples to obtain qualitative information on the nature of contamination

at the site. An 85-μm polyacrylate fiber (Supelco Inc.) was employed for the SPME

extraction of 1-mL aqueous samples.

Gas phase TCE and c-DCE concentrations were measured by injecting 10-100

μL of a gaseous sample into a HP-5890 gas chromatograph 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, CA). The gases N_2, H_2, CH_4, O_2 and CO_2 in the

microcosm headspace were measured with a HP-5890 gas chromatograph connected to a

thermal conductivity detector. 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 with a Carboxen™

1000 packed column (15 ft x 0.125 in, S.S Support) from Supelco (Bellefonte, PA). The

volatile acids were measured by injecting 0.5-2 μL of acidified and pre-filtered aqueous

samples into a HP-5890 gas chromatograph connected to a FID detector.

Chromatographic separation was carried out with a HP-Innowax column (30 m x 0.25

mm, 1.0-μm film) from Hewlett-Packard (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.

4.2.3   Microcosm Preparation

Microcosms were constructed with 310-mL and 1050-mL serum bottles

(Wheaton Industries, Inc., Seattle, WA) with rubber-lined caps and butyl rubber septa.

Groundwater for the microcosm preparation was collected in glass bottles capped with

stoppers and no headspace to maintain anaerobic conditions during storage. The

groundwater was stored at 4 °C till until it was used in constructing the microcosms.

Groundwater was collected from the D3 well in the center of the plume where high

concentrations of c-DCE were observed. Microcosms were prepared by transferring

groundwater to the serum bottles in an anaerobic glove compartment. The 310-mL

microcosm bottles contained 250 mL of groundwater and 60 mL of headspace while the

1050-mL microcosm bottles contained 950 mL of groundwater and 100 mL of

headspace. H_2 that was introduced into the microcosms from the glove box atmosphere

and the residual TCE and c-DCE in the groundwater was purged with nitrogen that was

treated in a tube furnace (Thermolyne, Inc., Dubuque, IA) to remove any traces of

oxygen. Appropriate volumes of pure liquid TCE were added, to achieve the desired

TCE concentrations. The microorganisms present in the groundwater served as the

microbial inoculum. 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 (*10, 27*)). 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 balance in the microcosms were verified.

4.3 Results

The TCE plume at Site-300, LLNL, CA, is extensive and concentrations ranged between 20-100 mg/L. The site map of the TCE plume is shown in Figure 4.1. The TCE plume was approximately 1500 feet long and 600 feet wide. The monitoring wells of particular interest and chosen for the groundwater analysis were wells D3, D4, D7, D8 and D14. The quantitative and qualitative analysis of these select monitoring wells was conducted to study the nature of contamination and the transformation products of TBOS and TKEBS. The hotspots of contamination were located around the wells D3 and D4 and groundwater at these wells contained high concentrations of TCE (> 50 mg/L), TKEBS (10-80 mg/L) and traces of TBOS. The TBOS and TKEBS contamination was limited to the wells D3 and D4 and was not observed in the other monitoring wells. However, the TCE plume extended into a larger area.

A gas chromatogram and the mass spectra of the non-volatile components of interest in the groundwater from D3 well are shown in Figure 4.2. The results of the GC analysis of the groundwater from D3 well indicate 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. It was also determined that the mass spectrum of TBOS, available from standard NIST library, was incorrect.
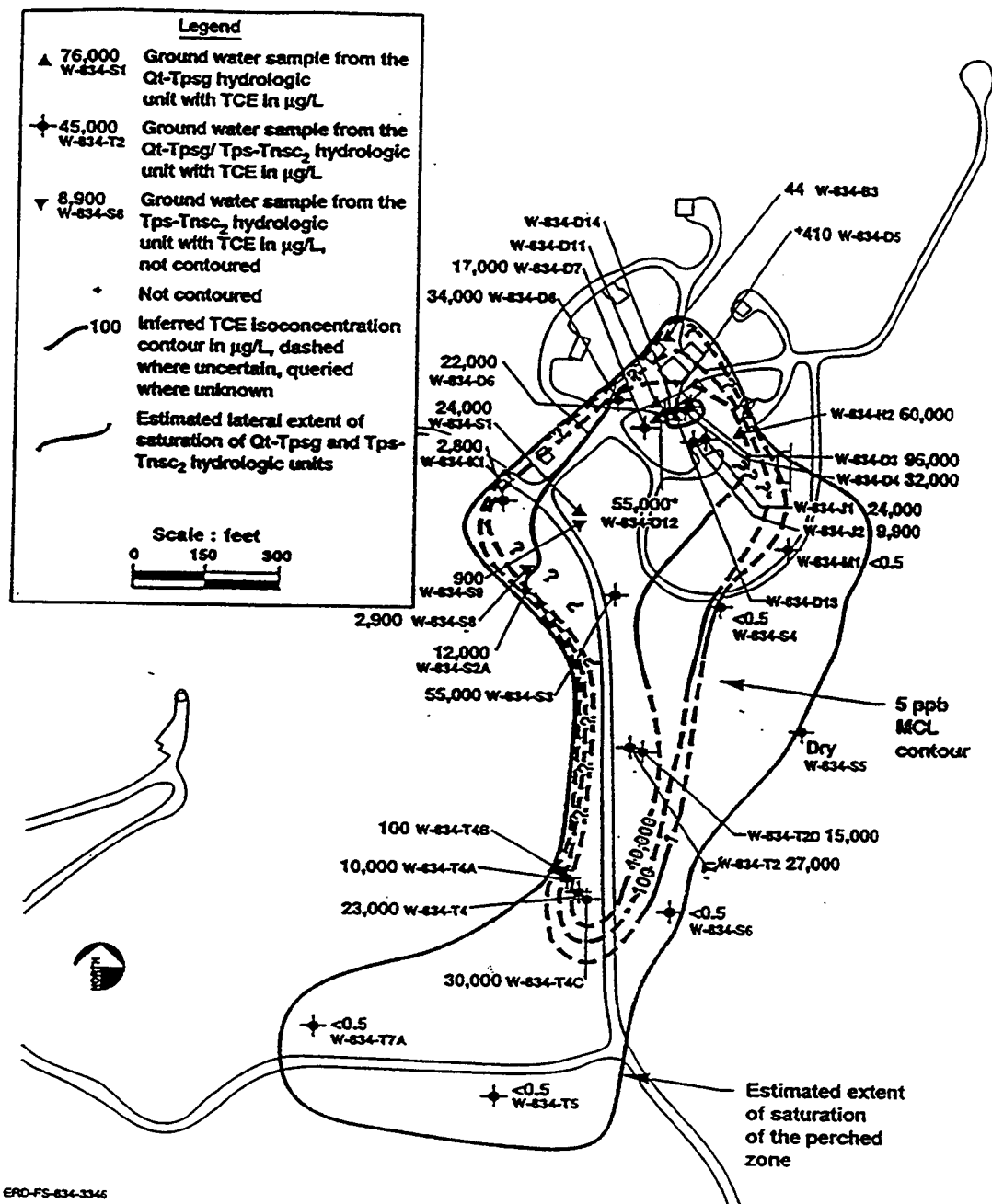
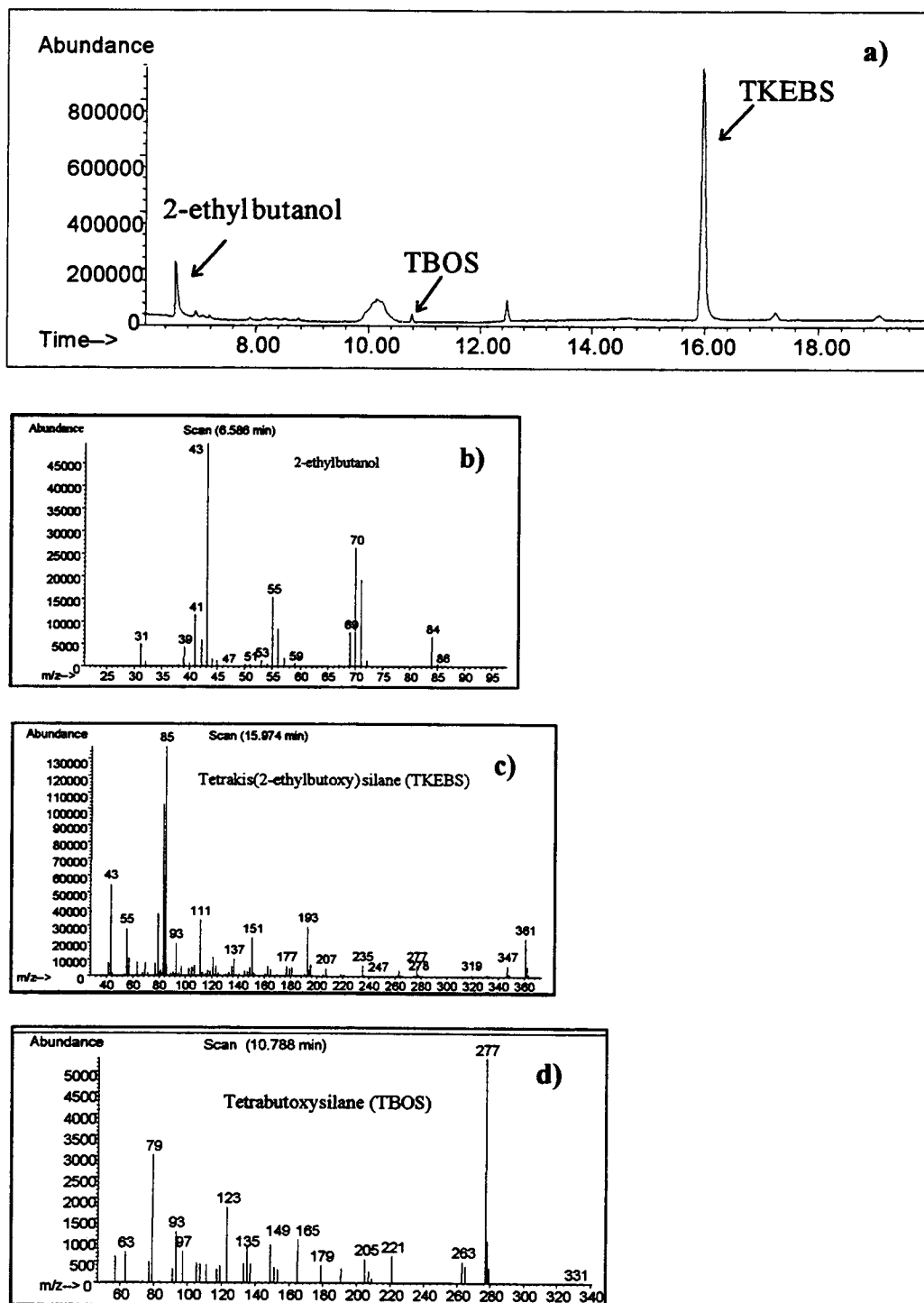Figure 4.1. TCE plume at Site 300, Lawrence Livermore National Labs, CA.

Figure 4.2. Analytical results from the dichloromethane extract of groundwater from well D3 showing the major non-volatile contaminants present. a) Gas Chromatogram, b) Mass Spectrum of 2-ethylbutanol, c) Mass Spectrum of TBOS, d) Mass Spectrum of TKEBS.

4.3.1 Preliminary Investigation Indicating the Role of TKEBS in Driving TCE Transformation

Initial experiments were conducted to evaluate the potential for anaerobic transformation of TCE at Site-300. Six microcosms were constructed with D3 groundwater and TCE concentrations in the range of 60-80 mg/L. Two microcosms (duplicates) were turned into controls one by autoclaving and the other by chemical poisoning. Two microcosms were amended with benzoate (200 mg/L) as an additional carbon source. The remaining two were left untouched (no electron donor added). The microcosms were incubated for over a period of 100-120 days. Active dechlorination was observed in microcosms with no electron donor added, and the dechlorination of TCE to c-DCE was complete in 60 days. The microcosms amended with benzoate exhibited dechlorinating activity, but at a slower rate. No TCE transformation was observed in the sterile controls showed.

Representative chromatograms from the GC/MS analyses of liquid samples from the microcosms after 60 days of incubation are shown in Figure 4.3. The absence of c-DCE in the poisoned microcosm (Figure 4.3b) clearly indicates that the transformation of TCE to c-DCE was biologically mediated. Also, 2-ethylbutanol formed by the hydrolysis of TKEBS was not observed in the biologically-active microcosms in which TCE was completely transformed to c-DCE, but it was present in the poisoned microcosms. The results suggest that the TCE transformation to c-DCE was driven by the 2-ethylbutanol and that TKEBS was the "apparent" electron donor. The subsequent discussion focuses on experiments that were conducted to investigate the fermentation pathway of the alcohol and TCE dechlorination rates.
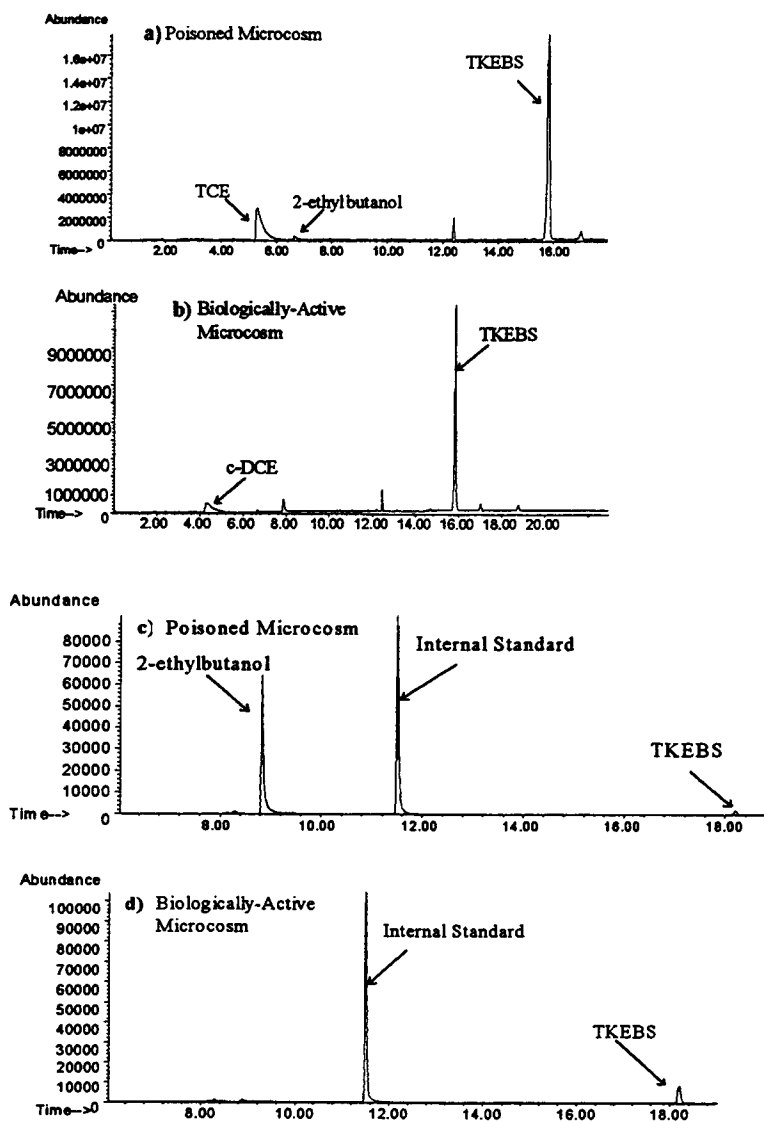
Figure 4.3. GC analysis of biologically-active and poisoned microcosms. SPME was used for qualitative analysis of the volatile compounds in solution after 60 days of incubation in a poisoned (a) and biologically-active (b) microcosm. Extraction with dichloromethane was used for the quantitative determination 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.

4.3.2   Reductive Dechlorination of TCE

Groundwater from monitoring well D3 at Site-300 was used to prepare anaerobic microcosms to study the fermentation and the dechlorination reactions occurring in-situ. The background concentrations of the various compounds present in the groundwater during the months of January and September 1997 are listed in Table 4.1.

Table 4.1. Background concentrations of compounds in the anaerobic microcosms

Compound	Concentration Range in January 1997 (mg/L)	Concentration Range in September 1997 (mg/L)
TKEBS	10 - 80	40- 60
TBOS	2-5	Not Detected
2-ethylbutanol	5 - 8	150 - 200
2-ethylbutyric acid	50 - 60	300 - 400
Acetic acid	30 - 40	40 - 60

The concentrations of 2-ethylbutanol and 2-ethylbutyric acid increased drastically from January to September 1997 and TBOS decreased to non-detectable levels. These results indicate that the hydrolysis of TKEBS over several months resulted in higher concentrations of 2-ethylbutanol and its subsequent fermentation products. Microcosms constructed with groundwater sampled at these different dates were used to identify the change in intrinsic TCE remediation rate with time.

Results from the TCE transformation study conducted with groundwater obtained in September 1997 are shown in Figure 4.4. These experiments were conducted in triplicate and profiles from the other experiments are similar to those shown. 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 3-5 days after purging the residual H_2 and addition of TCE, a steady build up of H_2 occurred while TCE transformation was minimal. The eventual decrease in H_2 concentration correlates with the increase in the rate of TCE transformation to c-DCE. c-DCE persisted in the microcosms and no further transformation was observed over a 3-month incubation. Vinyl chloride or ethene was not detected. No methanogenesis was observed in these microcosms even at the high H_2 partial pressures. The high levels of H_2, corresponding to about 0.017 atm as seen in Figure 4a, are attributed to the fermentation of 2-ethylbutanol. Thermodynamic arguments indicate that alcohols should ferment at high partial pressures of H_2 (*9, 22*).
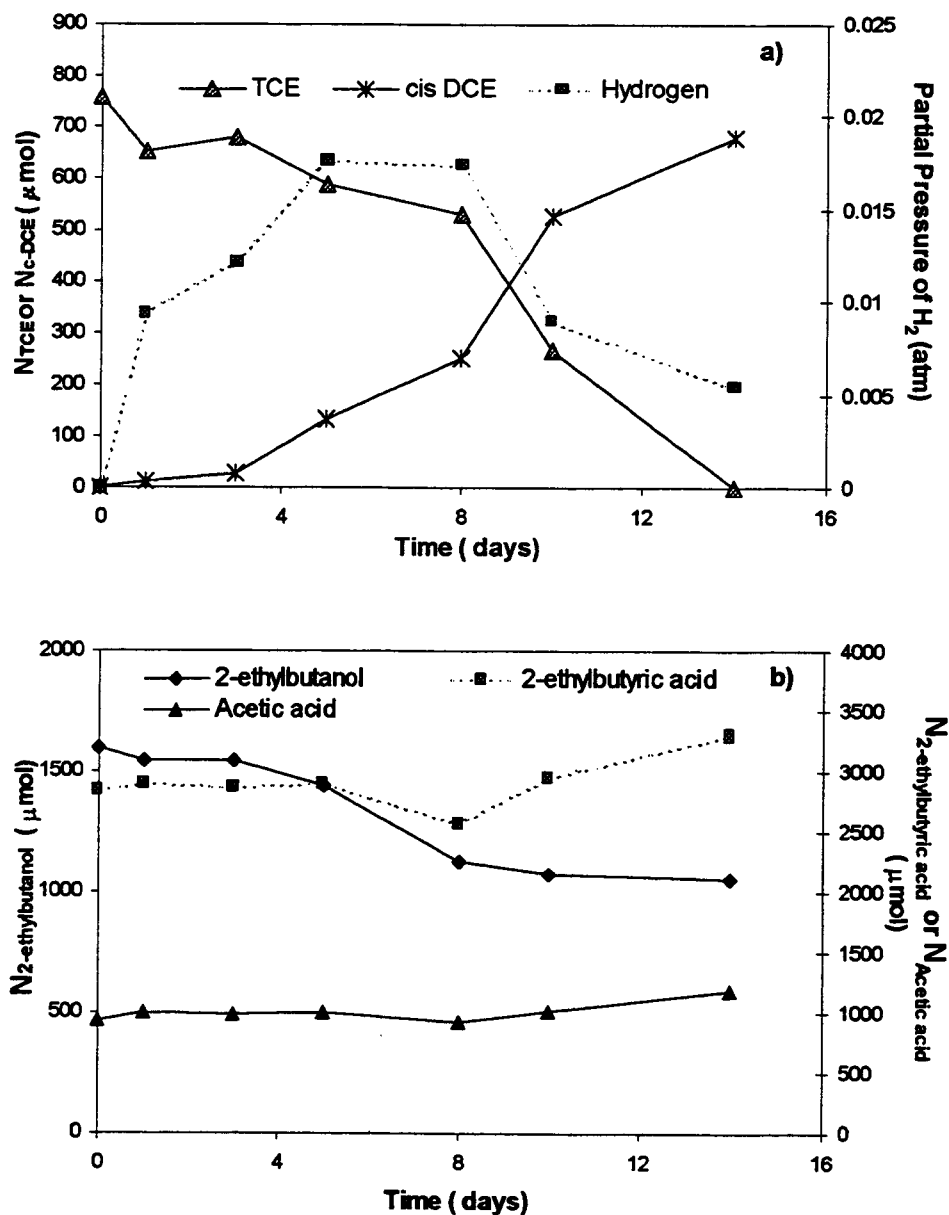
Figure 4.4 Reductive dechlorination of TCE in anaerobic microcosms constructed with groundwater sampled in September 1997. a) TCE, c-DCE and hydrogen data in the microcosms, b) Profiles of 2-ethylbutanol, 2-ethylbutyric acid and acetic acid concentrations. The studies were conducted in 1.05-L media bottles with a 950-mL liquid volume and a 100-mL headspace.

Analysis of Figure 4.4b indicates a decrease in 2-ethylbutanol concentration and a corresponding increase in the concentration of 2-ethylbutyric acid. The amount of acetic acid in the microcosm increased slightly. The amount of 2-ethylbutanol depleted was about 10-20% more than the amount of 2-ethylbutyric acid formed. The difference may be due to the 2-ethylbutanol that ferments all the way to carbon dioxide and hydrogen. The fermentation of 2-ethylbutanol was also accelerated by the TCE dechlorination and was likely promoted by the depletion of H_2 during TCE dechlorination. Depletion of H_2 would permit the fermentation reaction to proceed favorably and produce more H_2 (22). A mole balance of all the electron donors and acceptors in the microcosms, based on the amounts of 2-ethylbutanol and acids fermented and the carbon dioxide produced, suggests a high efficiency for H_2 utilization. It is estimated that about 20-30% of the H_2 produced is used in the dechlorination reaction. This estimate carries high uncertainty because the change in concentrations of 2-ethylbutanol, 2-ethylbutyric and acetic acid were small relative to the total concentrations already present in the groundwater.

Results of the dechlorination studies conducted with groundwater obtained in January 1997 indicate slower TCE transformation rates and are shown in Figure 4.5. These microcosms contained trace amounts of 2-ethylbutanol, about 60 mg/L of 2-ethylbutyric acid, and 80 mg/L of TKEBS. The headspace H_2 concentrations remained below the detection limit (1×10^{-5} atm) which indicates that the fermentation of the 2-ethylbutyric and acetic acid occurred at low partial pressures of H_2. Lower H_2 partial pressures are expected during the fermentation of acids (14, 16). However, the dechlorination continued to progress at a slow but steady rate. After about 75 days of

incubation, H₂ was spiked into the microcosm to yield a partial pressure of about 0.05

atm. Addition of H₂ as an external electron donor stimulated a significant increase in the

TCE transformation rate. The efficiency of H₂-utilization (moles of TCE transformed/

mole of H₂ added) was calculated to be greater than 100%. However, it is possible that

some of the H₂ added stimulated the activity of the dechlorinating microorganisms

enhancing the rates of transformation. These results strongly suggest that H₂ played a
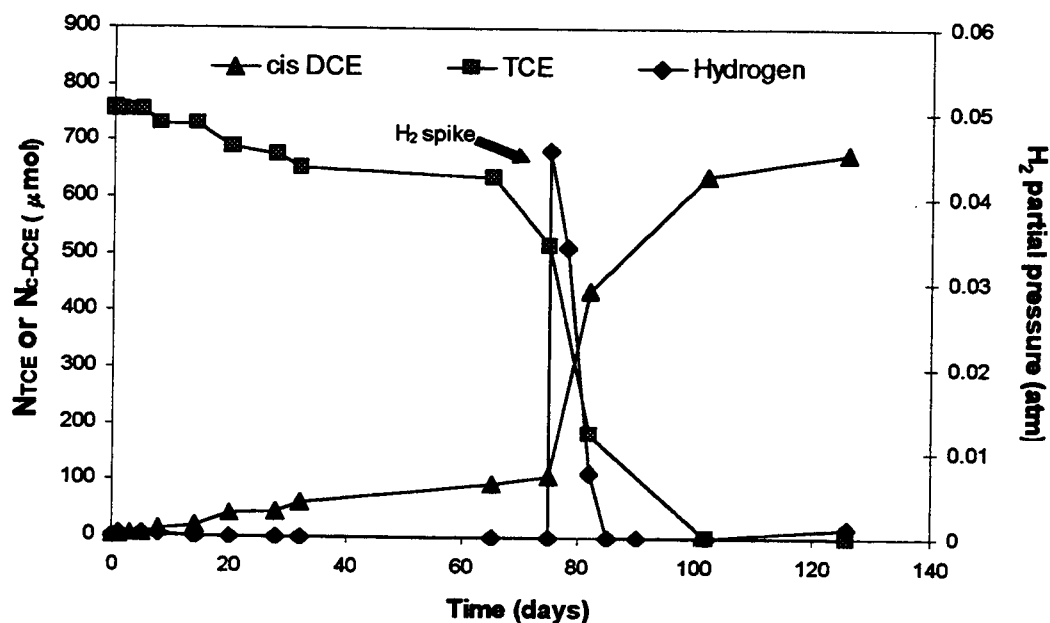
critical role in the dechlorination reaction.

Figure 4.5. Reductive dechlorination of TCE in anaerobic microcosms constructed with groundwater sampled in January 1997. The studies were conducted in 1.05-L media bottles with a 950-mL liquid volume and a 100-mL headspace. After 75 days, H₂ was spiked into the microcosm to achieve 5% H₂ by volume in the headspace.

Experiments were conducted to evaluate the effect of TCE concentrations on the dechlorination activity. Microcosms with aqueous TCE concentrations of 20 mg/L (150 μM), 40 mg/L (310 μM), 100 mg/L (770 μM), 150 mg/L (1150 μM) and 300 mg/L (2200 μM) were constructed with groundwater from D3 well at the site obtained in September 1997. Duplicate microcosms with no TCE added were used as controls, along with poisoned controls.

The results shown in Figure 4.6 indicate that at TCE concentrations below 100 mg/L (770 μM), the dechlorination rates were fast and the lag periods were short. When the TCE concentration was increased, longer lag periods in TCE transformation were observed (Figure 4.6a). After the initial lag period for all the microcosms, the rates of transformation of TCE were similar and in the range of 50-110 μM/day. These results indicate that the rates of transformation of TCE follow zero-order kinetics at substantially high concentrations of TCE. It is possible that the concentrations of TCE studied are well above the half-velocity constants (K_s) for the dechlorination reaction and in the saturation region of the Michaelis-Menton relationship model. The build up of H_2 was very rapid in microcosms without TCE added and took longer in microcosms with TCE (Figure 4.6 c). At high TCE concentrations, the H_2 partial pressures were markedly lower and often below the detection limit. Possibly, the fermentation activity was suppressed by high concentrations of TCE. The longer lag periods in TCE dechlorination are probably related to the lower concentrations of H_2 in the microcosms. After the complete transformation of TCE to c-DCE, in the microcosms with an initial high TCE concentration, the H_2 concentrations increased. Apparently other electron acceptors for H_2 were not present after TCE is transformed.
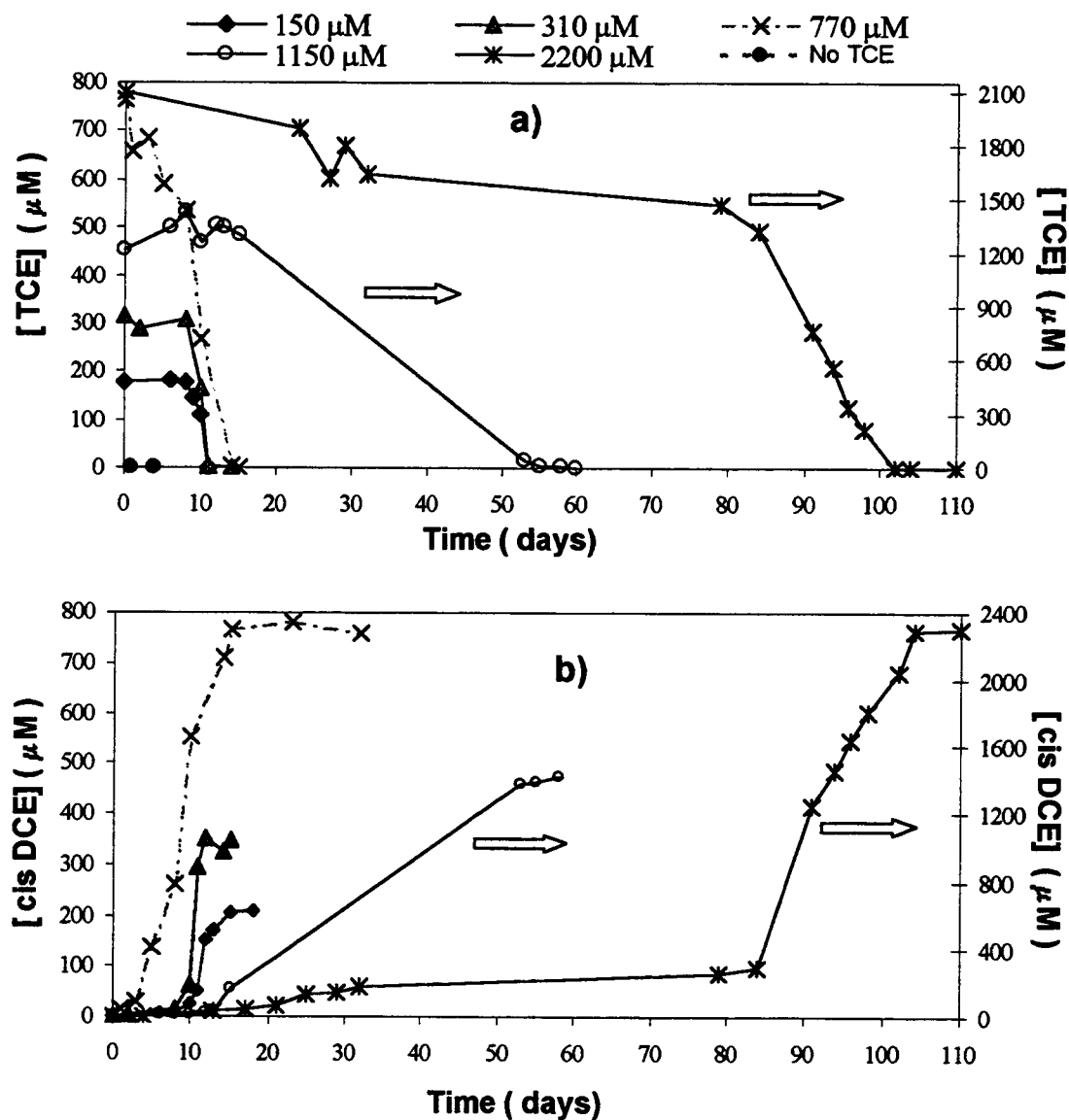
Figure 4.6. Effect of TCE concentration on the dechlorination in anaerobic microcosms constructed with groundwater sampled in September 1997. a) TCE transformation data, b) c-DCE formation, c) H_2 partial pressure in the headspace of the microcosms, d) Final CO_2 concentration in the headspace of the microcosms (No data was obtained for the 710 μM TCE microcosm). All studies were conducted in 310-mL media bottles with a 250-mL liquid volume and a 60-mL headspace, except for the study with 710 μM initial TCE study which was conducted in 1.05-L media bottles with a 950-mL liquid volume and a 100-mL headspace.
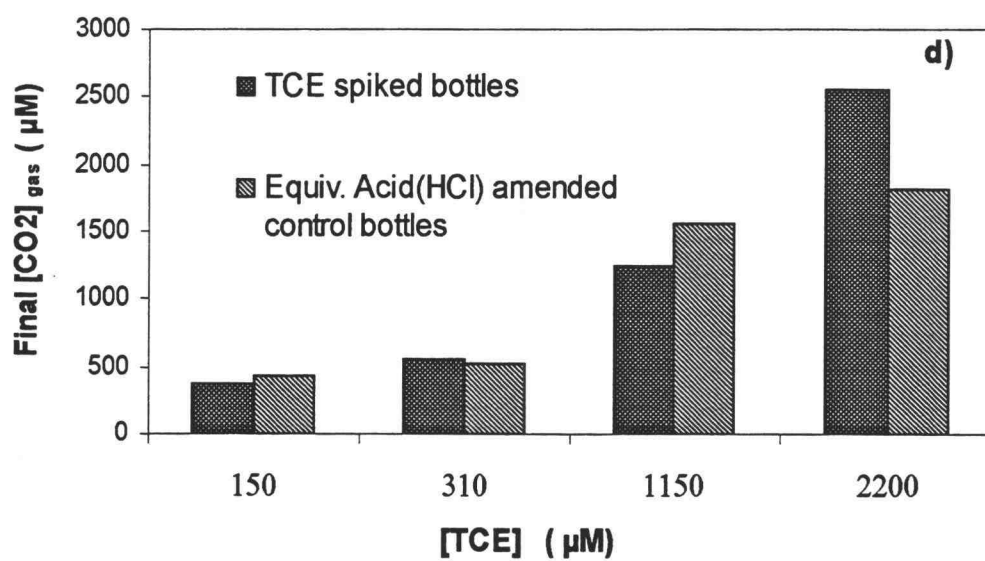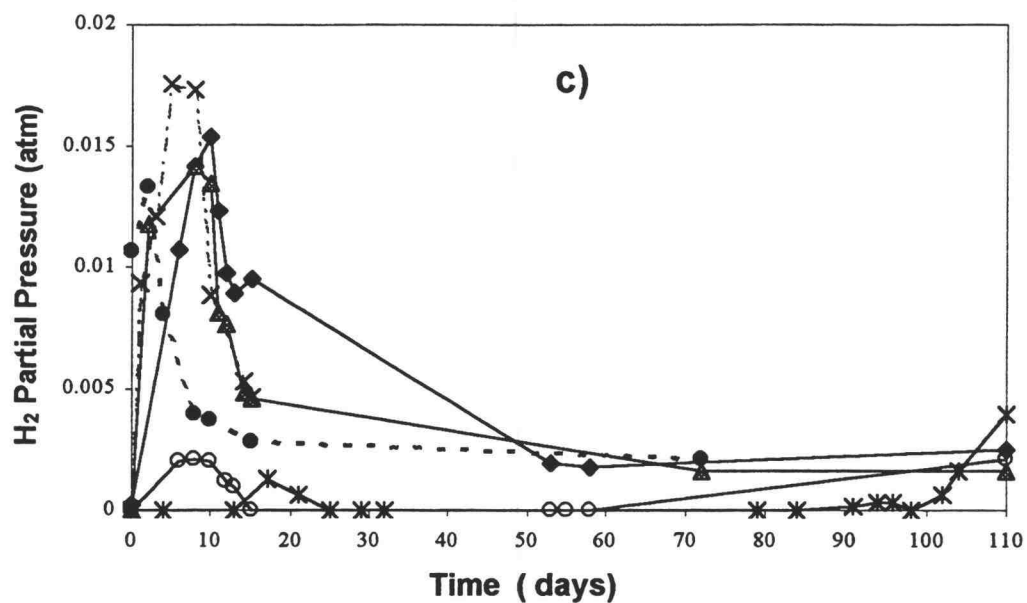
Figure 4.6. Continued.

At the end of the incubation period in all the microcosms, the concentration of 2-ethylbutanol was lower than 5 mg/L and its formation was limited to hydrolysis of TKEBS present (about 60-80 mg/L). The H_2 production after the incubation period is attributed mainly to the acid fermentation. After 100 days of incubation, the H_2 levels were found to remain stable between the range of 0.001-0.004 atm in all the microcosms.

In Figure 4.6d the final CO_2 concentrations in the headspace of the microcosms with varying TCE concentrations are compared with data from control microcosm with no TCE added. The CO_2 concentration in the headspace increased with higher TCE concentration and the final pH in the microcosms decreased from 7.6 to about 5.5. This pH shift increased the relative ratio of CO_2 in the headspace to the solution. For the control microcosm containing no TCE, the volume of 1 M HCl added was determined based on the equivalent amounts of H^+ liberated by the dechlorination reaction of TCE to c-DCE. The increase in CO_2 levels between the microcosms with added TCE and the controls amended with HCl were comparable which suggests that the pH was the controlling factor rather than increased fermentation. These results indicate that the dechlorination of TCE to c-DCE causes a significant drop in pH of the groundwater and hence influences the aqueous chemistry of the groundwater.

In this and previous experiments, the concentration of TKEBS was monitored. The TKEBS concentration decreased by less than 15% relative to its initial concentration during the course of the experiments (10-100 days). The uncertainty in the rate of TKEBS degradation is high because TKEBS could only be estimated with about 15 % precision. However, these degradation rates are similar to the abiotic hydrolysis rates studied previously (20) for TKEBS (0.32 µM/day) at an initial concentration of 185 µM

(80 mg/L). Thus little biotic transformation of TKEBS occurred under anaerobic conditions.

4.3.3 Evidence of TCE Transformation In-Situ from Field Sampling

The results of the analysis of the chlorinated ethenes, TKEBS, the alcohols and the fermentation products at Site-300 in wells D3 and D8 (Figure 4.7) provide convincing evidence of the role played by TKEBS in supporting TCE transformation to c-DCE. Several other wells were also monitored and the contaminant profile of well D4 was similar to that of D3 and those of wells D7 or D14 were similar to that of well D8. For the sake of comparison, only two of the wells studied, D3 and D8, are discussed.

Monitoring wells D3 and D8 are located about 100 feet apart and their profiles of TCE transformation are quite different. The TCE transformation activity at well D3 (Figure 4.7a) is primarily attributed to the presence of TKEBS and its hydrolysis product, 2-ethylbutanol and the subsequent fermentation products, 2-ethylbutyric acid and acetic acid. The observed persistence of c-DCE at high aqueous concentrations (about 200 mg/L) at the site is also consistent with the microcosm data. Methanogenesis was not observed in any of the selected monitoring wells consistent with microcosm results. There was evidence of sulfides and traces of sulfates at locations around well D3 indicating sulfate reduction. No TCE transformation was observed in well D8 (Figure 4.7b) and this could likely be due to the absence of TBOS, TKEBS or their transformation products. Recent field results also indicate a significant change in the pH of the groundwater at the D3 and D4 monitoring wells from a pH of 7.6 (February 1997) to a pH of 6.6 (September 1997). Surrounding monitoring wells had a pH of 7.5. These

results are consistent with our microcosm observation of lower pH resulting from the

dechlorination reaction.
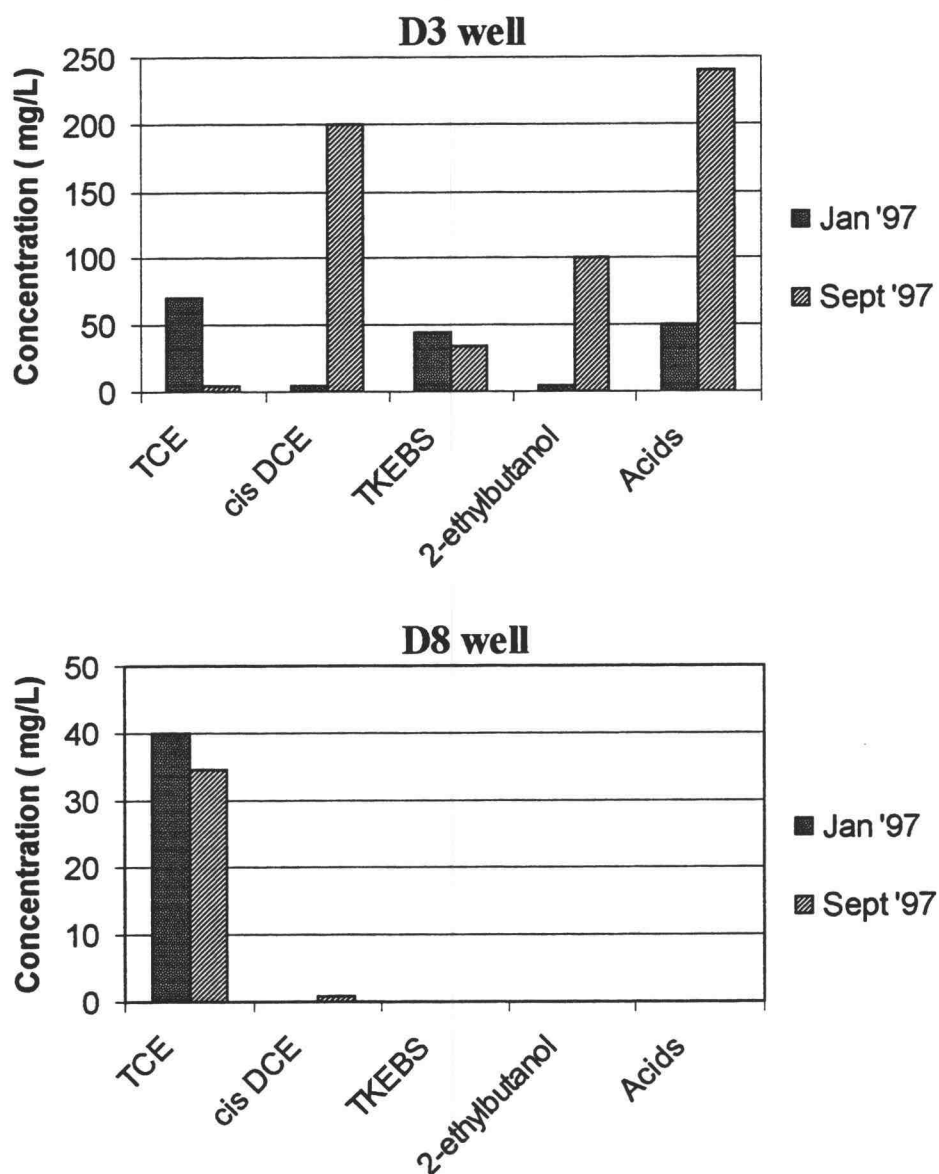
Figure 4.7. Evidence of TKEBS driven TCE transformation at Site-300, LLNL,CA.
a) Concentration profiles of D3 monitoring well, b) Concentration profiles
of D8 monitoring well

4.4 Discussion

The intrinsic transformation of TCE to c-DCE at Site-300 at selected well sites was demonstrated in anaerobic microcosms and through field sampling. The transformation of TCE was driven by TKEBS, which acted as the "apparent" electron donor. The hydrolysis of TKEBS to 2-ethylbutanol and the subsequent fermentation of the alcohol was observed in the microcosms. H_2 produced during the fermentation reaction appears to be serving as the ultimate electron donor for the TCE dechlorination to c-DCE. Similar observations have been made about microorganisms such as PER-K23 (25) and *Dehalospirillum multivorans* (26) that use H_2 as the electron donor and dechlorinates TCE to c-DCE.

Field sampling provides further evidence that in-situ TCE transformation occurs only at locations contaminated with TKEBS. In locations around the monitoring wells D3 and D4 that were substantially contaminated by TKEBS, transformation of TCE to c-DCE was almost complete. Aqueous concentrations of c-DCE (about 200 - 300 mg/L) were high. These results are consistent with our laboratory microcosm studies. 2-ethylbutanol and its fermentation products, 2-ethylbutyric acid and acetic acid, are also present in the groundwater from these wells. Consistent with proposals of McInerney et al.(23) and Stieb and Schink (24), 2-ethylbutyric acid (a fatty acid with even number of carbon atoms) should ferment directly to acetic acid and H_2. For locations around wells D7, D8 and D14 which have no TBOS and TKEBS contamination, no TCE transformation was observed and the concentrations of TCE remain high at around 60 to 80 mg/L. In microcosms, the pH decreased significantly during the dechlorination of

TCE to c-DCE. Similar results were also observed at wells D3 and D4. The pH dropped about 1 pH unit in wells where high concentrations of TCE were dechlorinated.

Based on the microcosm results, the descriptive pathway for the transformation of TKEBS to CO_2 and H_2 is hypothesized and is shown in Figure 4.8. The 2-ethylbutanol formed from the hydrolysis of TKEBS ferments to 2-ethylbutyric acid, which likely further ferments to acetic acid and H_2.
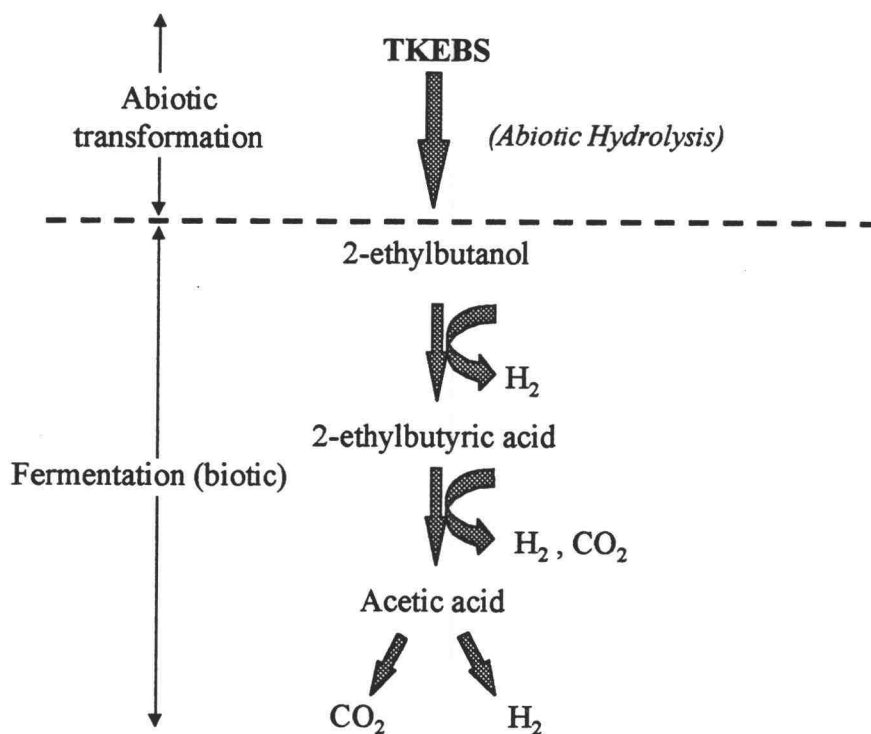
Figure 4.8. Anaerobic transformation pathway for TKEBS

Although the products in all the proposed steps are observed, we do not have direct evidence for the pathway for acetic acid formation. The rates of TKEBS hydrolysis to 2-ethylbutanol observed with anaerobic microcosms correlated well with abiotic hydrolysis rates (20) suggesting that the initial hydrolysis step was abiotic. However, the rates of hydrolysis could possibly be enhanced by the continuous removal of the hydrolysis product, 2-ethybutanol. In this study, the presence of high concentrations of 2-ethylbutanol in the background limited our ability to monitor the significance of this possibility. Though biologically-mediated hydrolysis is also a possibility, it was not observed in our studies. The abiotic hydrolysis rates could however be used to predict the lowest rate of transformation that is obtainable.

TCE dechlorination was correlated to the level of hydrogen in the microcosms. In microcosms with 2-ethylbutyric acid, H_2 levels are low and slow rates for TCE dechlorination were observed. Addition of external H_2 into the microcosm increased the rate of TCE transformation. The microcosms with 2-ethylbutanol exhibited high H_2 partial pressures, hence smaller lag periods, and higher dechlorination rates. Similar correlation of rates of TCE dechlorination to the partial pressure of H_2 was observed by Fennel et al, 1997. Their studies demonstrated that dechlorination was slow with butyric and propionic acid which fermented at a lower partial pressure. Faster dechlorination was observed with ethanol which ferments at a low or high partial pressure. This result is expected from thermodynamic free-energy calculations. Our studies also indicated that the presence of TCE as an electron acceptor in the microcosms enhanced the rate of fermentation of 2-ethylbutanol. The persistence of 2-ethylbutanol in the groundwater at

some sites may be a direct result of the absence of electron acceptors that drive the fermentation reaction.

TCE at high concentrations, about 1/4 of the solubility limit was dechlorinated which suggests that the dechlorinators may not be inhibited by high TCE concentrations. After a long lag period, the dechlorination occurred at high rates of about 110 μM/day. The dechlorination might be limited by the inhibitory effect of high TCE concentrations on the fermenting microorganisms and in turn on the production of H_2. The long lag was probably a direct result of the inhibitory effect. Overall the dechlorinating mechanism appears to be very efficient and about 25-30 % efficiency based on electron donor utilization was observed

Methanogenesis was absent both at the field site and in the microcosms. Plausible explanations include 1) TCE at high concentrations inhibit methanogens as observed by Semprini et al, 1995 (29) at the St.Joseph's field site; 2) the fluctuating water table at the site introduced O_2 that inhibits methanogenesis; 3) initial slow release of 2-ethylbutanol and subsequent fermentation might have resulted in low levels of H_2 where dechlorinators out compete the methanogens; d) methanogens are not present in the subsurface under investigation.

Ballapragada et al., 1997, and Smatlak et al., 1996, observed that the half-velocity constants for H_2 utilization for the dechlorinators are lower than the methanogens by an order of magnitude. The slow release of 2-ethylbutanol from the hydrolysis of TKEBS may have resulted in low H_2 concentrations where the dechlorinators hold a significant advantage and could have survived over the methanogens. It could also be hypothesized that the H_2 partial pressures can be

effectively regulated by the application of different homologues of alkoxysilanes that hydrolyze at required rates and hence effectively directing it towards the dechlorination reaction.

Based on the understanding of the system that emerged from the microcosm studies and field analysis, a conceptual model of the various chemical and transport processes governing the intrinsic remediation in the region around the D3 well was developed and is shown in Figure 4.9. In the winter months, the groundwater is recharged, the water table is high, and anaerobic transformations dominate. During this period, the TBOS and TKEBS hydrolysis is slow and limited by the abiotic rates of hydrolysis. With the lower rate of release of alcohols, the rate of production of H_2 is lower and a slower rate of TCE transformation to c-DCE results. However, during the summer months, the water table drops low introducing O_2 into the aquifer and the vertical spreading of the TBOS and TKEBS LNAPL. During this period, accelerated hydrolysis of TBOS and TKEBS by indigenous aerobic microorganisms may occur (20). Aerobic cometabolism of c-DCE and TCE, as a consequence of TBOS and TKEBS degradation is also plausible.
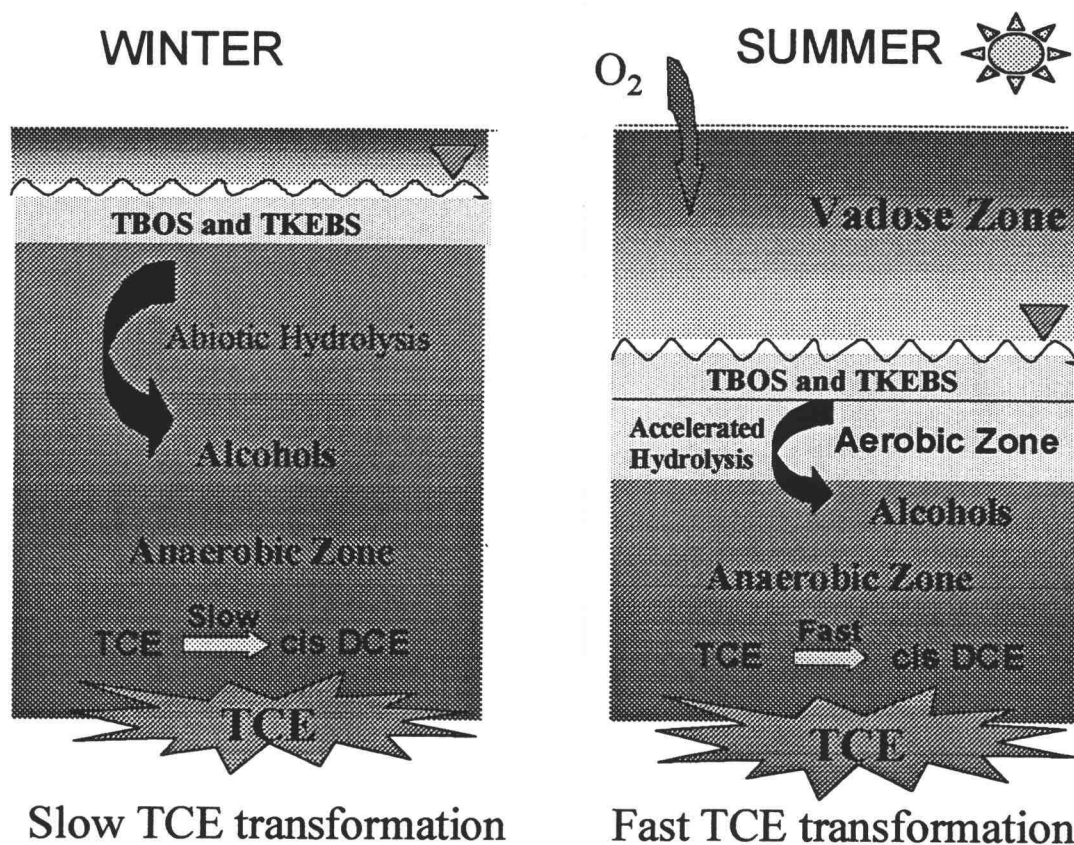
Figure 4.9. Conceptual model of transformations occurring at locations in the aquifer where TBOS, TKEBS and TCE are present.

In separate studies we have demonstrated that microorganisms grown on TBOS can cometabolize TCE and c-DCE with high transformation yields (20). TBOS and TKEBS were hydrolyzed under aerobic conditions by these enrichment cultures. If a similar aerobic process occurs at the site, significant amounts of highly soluble alcohols would be released and then easily transported down to the anaerobic sections of the aquifer as the groundwater level recedes. This phenomenon was observed in the subsurface around the D3 well where the concentrations of 2-ethylbutanol and 2-

ethylbutyric acid were substantially higher in September 1997 as compared to January 1997. Active fermentation of high concentrations of these alcohols and acids result in high levels of H_2 which may serve as the electron donor for dechlorinating microorganisms resulting in rapid dechlorination of TCE to c-DCE. Aerobic cometabolism may also occur when the water table rises through the vadose zone that has been exposed to O_2. The natural attenuation of TCE and c-DCE via a sequential aerobic and anaerobic pathway due to the seasonal variations in the water table is hypothesized to have occurred over the years at the site. This hypothesis requires confirmation through more detailed site and microcosm studies.

This study has provided insight into the natural processes that occur at LLNL Site-300. Natural attenuation of TCE driven by TKEBS could be explored as a remedial alternative at the site. This natural process could be exploited to enhance TCE transformation by a) injecting a substrate (maybe TBOS or TKEBS) for supporting the anaerobic transformation of TCE, b) exploring the possibility of using TBOS and TKEBS present at site and distributing them in the wells which could then serve as substrates for TCE transformation, c) addition of an anaerobic culture to the site that effectively dehalogenates TCE to ethene using TBOS and TKEBS, 1-butanol and 2-ethylbutanol as substrates.

The other interesting finding is the potential for TBOS and TKEBS to act as effective electron donors. These compounds transform to an alcohol and a non-toxic silicic acid and hence could potentially be applied at other sites to serve as electron donors for enhancing intrinsic transformation of TCE.

4.5 References

(1) Westrick, J. J.; Mello, J. W.; Thomas, R. F., *J. Am. Water Works Assoc.*, **1984**, *76* (*5*), 52-59.

(2) Federal Register, **1989**, *National Primary and Secondary Drinking Water Regulation*, *54*, 22062-22160.

(3) Hopkins, G. D.; Semprini, L.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1993**, *59* (*7*), 2277-2285.

(4) Mars, A. E.; Houwing, J.; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 886-891.

(5) Johan, E. T.; Vlieg, H. V.; Koning, W. D.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 3304-3312.

(6) Gibson, S. A.; Sewell, G. W., *Appl. Environ. Microbiol.*, **1992**, *58*, 1392-1393.

(7) Maymo-Gatell, X.; Chien, Y.; Gossett, J. M.; Zinder, S. H., *Science*, **1997**, *276*, 1568-1571.

(8) Distefano, T. D.; Gossett, J. M.; Zinder, S. H., *Appl. Environ. Microbiol.*, **1992**, *58*, 3622-3629.

(9) Fennell, D. E.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1997**, *31*, 918-926.

(10) Smatlak, C.R.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1996**, *30*, 2850-2858.

(11) Ballapragada, B. S.; Stensel, D. H.; Puhakka, J. A.; Ferguson, J. F., *Environ. Sci. Technol.*, **1997**, *31*, 1728-1734.

(12) Hasegawa, H.; Sakka, S., *J. Non-crystalline solids*, **1988**, *100*, 201-205.

(13) Aelion, R.; Loebel, A.; Eirich, F., *J. Am. Chem. Soc.*, **1950**, *72*, 5705-5712.

(14) McNeil, K. J.; DiCaprio, J. A.; Walsh, D. A., *J. Am. Chem. Soc.*, **1980**, *102:6*, 1859-1864.

(15) Eaborn, C., *Organo-silicon Compounds*, Buttersworth publications limited.: Edinburgh, **1960**.

(16) Alagar, A.; Krishnasamy, V., *Chem. Tech. Biotechnol.*, **1988**, *36*, 577-582.

(17) Von Ebelman, J., *Ann. Chem.*, **1845**, *57*, 319.

(18) Arthur, C. L.; Killam, L. M.; Buchholz, K. D.; Pawliszyn, J., *Anal. Chem.*, **1992**, *64*, 1960-1966.

(19) Zhang, Z.; Yang, M. J.; Pawliszyn, J., *Anal. Chem.*, **1994**, *66*, 844A-853A.

(20) Vancheeswaran, S.; M. S. Thesis, *Abiotic and Biological Transformation of TBOS and TKEBS, and their Role in the Biological Transformation of TCE and c-DCE*, **1997**.

(21) Vancheeswaran, S.; Semprini, L.; Williamson, K. J.; Ingle, J. D.; Daley, P., Proceedings of the First International Conference on " Remediation of Chlorinated and Recalcitrant compounds", Monterey, CA., May 8th, **1998.**

(22) Thauer, R. K.; Jungermann, K.; Decker, K., *Bacteriol. Rev.*, **1977**, *41*, 100-180.

(23) McInerney, M. J.; Bryant, M. P.; Hespell, R. B.; Costerton, J. W., *Appl. Environ. Microbiol.*, **1981**, *41*, 1029-1039.

(24) Stieb, M.; Schink, B., *Arch. Microbiol.*, **1993**, *140*, 2991-2997.

(25) Holliger, C.; Schraa, G.; Stams, A. J. M.; Zehnder, A.J. B., *Appl. Environ. Microbiol.*, **1993**, *59*, 2991-2997.

(26) Neumann, A.; Scholtz-Muramatzu, H.; Diekert, G.; *Arch. Microbiol.*, **1994**, *162*, 295-246.

(27) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M., *Environmental Organic Chemistry*, John-Wiley & Sons, Inc., NY, **1993**.

(28) Sharma, P. K.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1996**, *62*, 761-765.

(29) Semprini, L.; Kitanidis, P. K.; Kampbell, D. H.; Wilson, J. T., *Water Resources Research*, **1995**, *31(4)*, 1051-1062.

CHAPTER 5
CONCLUSIONS

A thorough investigation and evaluation of the biodegradation and abiotic

hydrolysis of TBOS and TKEBS and the intrinsic anaerobic transformation of TCE at

Site-300, LLNL, CA, was conducted. The results obtained from the lab scale studies

were consistent with the transformations observed at the site. These studies provided

insight about the transformation processes that occur at the site and unique strategies that

could be applied for the clean-up of the contamination at the site.

5.1. Summary

The significant results that were obtained from this research are summarized

below.

1) <u>Abiotic hydrolysis of TBOS and TKEBS</u>: Hydrolysis rates of TBOS and TKEBS

were determined for a range of pH (5-9), temperature (15 and 30 °C) and

concentrations (10 and 100 mg/L) that are typical of natural environments. The

abiotic hydrolysis rates of TBOS and TKEBS are slow with half-lives on the order of

several months to years. Above the solubility limits of TBOS and TKEBS, these

rates are approximately proportional to the concentration of these compounds.

Laboratory and field evidence indicates that these compounds are naturally

transformed at the site at rates faster than abiotic hydrolysis rates. This observation

supports the presence of other processes such as biologically-mediated hydrolysis that

may accelerate the transformation of these compounds. The rapid utilization of 1-butanol and 2-ethylbutanol by biotic processes could also accelerate the hydrolysis.

2) <u>Aerobic biodegradation of TBOS and TKEBS</u>:

    a) An aerobic culture capable of growing on TBOS and TKEBS was enriched. This culture was stimulated from an aerobic activated sludge obtained from the wastewater treatment plant at Corvallis. The aerobic culture rapidly hydrolyzes TBOS and TKEBS to 1-butanol and 2-ethylbutanol, respectively. The alcohols are subsequently degraded to CO_2.

    b) Several attempts to stimulate indigenous aerobic microorganisms from the site that could grow on TBOS and TKEBS were unsuccessful. However, aerobic microorganisms from the site that grow on 2-ethylbutanol and hydrolyze TKEBS were observed. Hence, hydrolysis products formed from abiotic hydrolysis could support an aerobic microbial population which would in-turn hydrolyze TBOS and TKEBS.

3) <u>Intrinsic transformation of TCE</u>: Reductive dechlorination of TCE to c-DCE under anaerobic conditions occured naturally around wells D3 and D4 contaminated with TBOS and TKEBS. Around these wells, more than 95% of TCE (initially around 100 mg/L) transformed to cis-DCE and high concentrations of c-DCE (around 200 mg/L) were observed. No transformation of TCE was observed in the wells D7, D8 and D14 where TBOS and TKEBS were absent.

4) <u>Role of TBOS and TKEBS in driving reductive dechlorination of TCE</u>: Laboratory studies were conducted with groundwater from wells contaminated with TCE and TKEBS to understand the role of TKEBS as an "apparent" electron donor for driving

TCE transformation. TKEBS hydrolyzes to 2-ethylbutanol that ferments to 2-ethylbutyric acid and subsequently to acetic acid. H_2 produced during each of these fermentation reactions likely serves as the ultimate electron donor for reductive dechlorination. The efficiency of H_2 utilization was estimated to be 20-30% and TCE at concentrations as high as 300 mg/L was dechlorinated to c-DCE. c-DCE persisted in all the microcosms and no further transformation was observed. No VC or ethene was detected in the microcosms.

5) <u>Aerobic cometabolism of TCE and c-DCE by microorganisms grown on TBOS</u>: The aerobic microbial culture grown on TBOS was capable of cometabolizing TCE and cis-DCE. It appears that the compound TBOS induced a monooxygenase enzyme in the aerobic microorganism. This enzyme was not directly involved in the hydrolysis of TBOS, but this extraneous enzyme catalyzed the oxidation of TCE and c-DCE. The microbial culture displayed a high potential for cometabolizing TCE and cis-DCE with TBOS being used as the substrate. High concentrations of TCE and c-DCE (about 25 mg/L) were rapidly transformed to the epoxide.

5.2 Engineering Significance

Based on a thorough analysis of the results obtained from the lab scale study and field observations, several remediation strategies are possible and are discussed below. The following remedial alternatives for TBOS and TKEBS could be explored:

- Natural Attenuation: The abiotic hydrolysis of TBOS and TKEBS occurs naturally at a slow but significant rate. Unless an immediate remediation of these compounds is necessary, these compounds should be left to naturally attenuate at the site.

- Bioventing: Biological degradation of TBOS and TKEBS could be enhanced by aeration of the subsurface to stimulate aerobic microorganisms that grow on the hydrolysis products of TBOS and TKEBS.

- Bioaugmentation: If immediate remediation is necessary, biological treatment employing the aerobic microorganisms that grow on TBOS and TKEBS could be explored. The enriched culture could be injected into the subsurface to promote rapid degradation of TBOS and TKEBS.

The potential avenues for bioremediation of TCE and enhancing the intrinsic TCE transformation that could be explored include:

- Enhance anaerobic reductive dechlorination by injecting a substrate (electron donor), such as TBOS or TKEBS, that drives TCE transformation.

- Explore the possibility of distributing TBOS and TKEBS present in some locations at the site using surfactants. They could then serve as substrates for TCE transformation.

- Sequential anaerobic and aerobic treatment - Reductive dechlorination of TCE to c-DCE under anaerobic conditions followed by aerobic cometabolism of cis-DCE by microorganisms grown TBOS could be exploited.

- Aerobic cometabolism of TCE and cis-DCE at the site with the enriched aerobic culture grown on TBOS.

5.3 Future Work

The results of this study have demonstrated the potential for intrinsic transformation of TCE under subsurface conditions at Site 300, especially in areas where TBOS and TKEBS are co-contaminated with TCE. The objectives of future research should be to determine how remediation activities might be optimized to take advantage of this intrinsic process and to accelerate in-situ remediation of TCE to non-toxic products.

Pumping operations and natural fluctuations in the water table at the site from summer to winter are likely causing parts of the aquifer to transition from anaerobic to aerobic conditions. Laboratory observations are consistent with a hypothesis that aerobic conditions accelerate the hydrolysis of TBOS and TKEBS and hence, enhance release of 1-butanol and 2-ethylbutanol into solution. These substrates then drive the anaerobic transformation of TCE to cis-DCE. If aerobic conditions can be maintained, there is also the potential for TBOS and TKEBS driving the aerobic cometabolism of cis-DCE and TCE.

Future research should focus on laboratory investigations that support future field remediation efforts to enhance intrinsic remediation processes that are already occurring at the site.

1) Anaerobic degradation of TCE driven by TBOS and TKEBS should be compared to anaerobic degradation with 1-butanol and 2-ethylbutanol added. This would determine whether more effective transformation is achieved by TBOS and TKEBS as they slowly hydrolyze, or by equivalent amounts of 1-butanol and 2-ethylbutanol.

2) The potential for complete anaerobic dechlorination to ethene at the site should be investigated. It may be that the microorganisms required for the complete dehalogenation are missing from the subsurface at Site-300. Microcosms, containing Site-300 groundwater, should be augmented with microbes that dechlorinate TCE completely to ethene and use TBOS and TKEBS to drive the dechlorination process.

3) The ability of organisms grown on TBOS to cometabolize TCE and cis-DCE is a new discovery from this work. This process needs to be evaluated more extensively for application under high concentrations of TCE and c-DCE, from the point of view of Site-300, and for potential application to other TCE contaminated sites.

4) The sequential anaerobic-aerobic treatment of TCE with TBOS and TKEBS should be evaluated in microcosms to confirm that this proposed process could be occurring under field conditions.

5) Continuous-flow column studies should be used to demonstrate the processes that have been studied in the laboratory microcosm tests. These tests should closely mimic subsurface conditions and evaluate the following:

- the feasibility of introducing and maintaining anaerobes in site groundwater and aquifer solids

- the potential for augmentation of TBOS-degrading aerobes for TBOS and TKEBS transformation and the aerobic cometabolism of cis-DCE.

- the sequential combination of anaerobic and aerobic processes driven by TBOS and TKEBS.

6) The promising processes identified through the lab scale studies should be evaluated under field conditions through push-pull experiments. Potential experiments include:

- The addition of oxygen to demonstrate the potential for aerobic cometabolism of TCE and cis-DCE.

- The addition of TBOS and TKEBS aerobes to demonstrate accelerated TBOS and TKEBS transformation and aerobic cometabolism of TCE and cis-DCE.

- The augmentation of anaerobic microorganisms to promote the complete dehalogenation to ethene.

- The engineering process to promote the sequential anaerobic/aerobic conditions at the field site.

- The addition of substrates (TBOS and TKEBS) to enhance anaerobic processes that are already occurring.

- The addition of biodegradable surfactants to enhance the anaerobic transformations.

g) More fundamental studies should focus on the following

- Model the hydrolysis of alkoxysilanes at concentrations above the solubility limits. Specifically, the hydrolysis rates of TBOS and TKEBS at concentrations below their aqueous solubility should be determined and the factors such as rate of dissolution and surface hydrolysis should be evaluated.

- Radio-labeled TBOS and TKEBS biodegradation experiments under both aerobic and anaerobic conditions should be conducted to identify the pathways.

- Evaluation of the effect of TCE and c-DCE concentrations on the growth of microorganisms that degrade TBOS.

- Study the reasons for inhibition of methanogens at the site.

- Determine the half-velocity constants of the dechlorinating microorganisms at the site.

BIBLIOGRAPHY

Aelion, R.; Loebel, A.; Eirich, F., *J. Am. Chem. Soc.*, **1950**, *72*, 5705-5712.

Alagar, A.; Krishnasamy, V., *Chem. Eng. Comm.*, **1989**, *80*, 1-9.

Alagar, A.; Krishnasamy, V., *Chem. Tech. Biotechnol.*, **1988**, *36*, 577-582.

Alvarez-Cohen, L. M.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1993**, *57*, 228-235.

Arthur, C. L.; Killam, L. M.; Buchholz, K. D.; Pawliszyn, J., *Anal Chem.*, **1992**, *Vol.64*, 1960-1966.

Ballapragada, B. S.; Stensel, D. H.; Puhakka, J. A.; Ferguson, J. F., *Environ. Sci. Technol.*, **1997**, *31*, 1728-1734.

Barry Arkles, *A Survey of Properties and Chemistry*, Gelest, **1995**.

Chapelle, F.H; Haack, S.K; Adriaens, P; Henry, M.A; Bradley, P.M, *Environ. Sci. Technol*, **1996**, *30*, 3565-3569.

Distefano, T. D.; Gossett, J. M.; Zinder, S. H., *Appl. Environ. Microbiol.*, **1992**, *58*, 3622-3629.

Eaborn, C., *Organo-silicon Compounds*, Buttersworth publications limited: Edinburgh, **1960**.

Fathepure, B. Z.; Boyd, S. A., *Appl. Environ. Microbiol*, **1988**, *54*, 2976-2980.

Federal Register, **1989**, *National Primary and Secondary Drinking Water Regulation*, *54*, 22062-22160.

Fennell, D. E.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1997**, *31*, 918-926.

Gibson, S. A.; Sewell, G. W., *Appl. Environ. Microbiol.*, **1992**, *58*, 1392-1393.

Glod, G.; Angst, W.; Holliger, C.; Schwarzenbach, R. P., *Environ. Sci. Technol*, **1997**, *31*, 253-260.

Hamamura, N.; Page, C.; Long, T.; Semprini, L.; Arp, D. J., *Appl. Environ. Microbiol.*, **1997**, *63* (*9*), 3607-3613.

Hasegawa, H.; Sakka, S., *J. Non-crystalline solids*, **1988**, *100*, 201-205.

Holliger, C.; Schraa, G.; Stams, A. J. M.; Zehnder, A.J. B., *Appl. Environ. Microbiol.*, **1993**, *59*, 2991-2997.

Hopkins, G. D.; Semprini, L.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1993**, *59* (*7*), 2277-2285.

Johan, E. T.; Vlieg, H. V.; Koning, W. D.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 3304-3312.

Leahy, J. G.; Byrne, A. M.; Olsen, R. H., *Appl. Environ. Microbiol.*, **1996**, *62*, 825-833.

Lovley, D.R; Chapelle, F.H; Woodward, J.C, *ES&T Research*, 1994, *28*, 1205-1209.

Lovley, D.R; Ferry, J. G., *Appl. Environ. Microbiol.*, **1985**, *49*, 247-249.

Madigan, M.T; Martinko, J.M; Parker, J., *Brock Biology of Microorganisms*, **1997**, 8th ed., Prentice hall, NJ.

Mars, A. E.; Houwing, J.; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1996**, *62*, 886-891.

Matheson, L.J; Tratnyek, P. G., *Environ. Sci. Technol*, **1994**, *28*, 2045-2053.

Maymo-Gatell, X.; Chien, Y.; Gossett, J. M.; Zinder, S. H., *Science*, **1997**, *276*, 1568-1571.

McInerney, M. J.; Bryant, M. P.; Hespell, R. B.; Costerton, J. W., *Appl. Environ. Microbiol.*, **1981**, *41*, 1029-1039.

McNeil, K. J.; DiCaprio, J. A.; Walsh, D. A., *J. Am. Chem. Soc.*, **1980**, *102:6*, 1859-1864.

Neumann, A.; Scholtz-Muramatzu, H.; Diekert, G.; *Arch. Microbiol.*, **1994**, *162*, 295-246.

Landa, A. S.; Sipkema, E. M.; Weijma, J.; Beenackers, A. A. C. M,; Dolfing, J.; Janssen, D. B., *Appl. Environ. Microbiol.*, **1994**, *60*, 3368-3374.

Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M., *Environmental Organic Chemistry*, John-Wiley & Sons, Inc., NY, **1993**.

Shurtliff, M. M.; Parkin, G. F.; Weathers, L. J.; Gibson, D. T., *J. Envir. Eng.*, **1996**, *122*, 581-589.

Smatlak, C.R.; Gossett, J. M.; Zinder, S. H., *Environ. Sci. Technol.*, **1996**, *30*, 2850-2858.

Stieb, M.; Schink, B., *Arch. Microbiol.*, **1993**, *140*, 2991-2997.

Thauer, R. K.; Jungermann, K.; Decker, K., *Bacteriol. Rev.*, **1977**, *41*, 100-180.

Vogel, T. M.; McCarty, P. L., *Appl. Environ. Microbiol.*, **1985**, *49*, 1080-1083.

Von Ebelman, J., *Ann.Chem.*, *57*, **1845**, 319.

Westrick, J. J.; Mello, J. W.; Thomas, R. F., *J. Am. Water Works Assoc.*, **1984**, *76 (5)*, 52-59.

Wilson, J. T.; Noonan, M.J.; McNabb, J. F., *Groundwater. Qual.*, **1985**, Wiley & Sons, New York.

Zhang, Z.; Yang, M. J.; Pawliszyn, J., *Anal. Chem.*, **1994**, *66*, 844A-853A.

APPENDICES

APPENDIX A

A.1 Batch-Bottle and Microcosm Construction

The following sections provide a detailed step-by-step procedure for the preparation of batch bottles/microcosms for experiments involving abiotic hydrolysis, aerobic biodegradation and the anaerobic dechlorination.

A.1.1 Aerobic Batch-Bottle Experiments

Batch bottles were constructed with the sequence of steps described below:

a) Autoclave the apparatus consisting the following:

- media bottles (125/250/500 mL)
- phenolic screw caps (for 250 and 500-mL media bottles)
- measuring cylinders
- beakers
- spatula
- 2-L conical flasks containing buffer-media (pH 7) with nutrients

Most experiments were conducted in 250-mL media bottles and the quantities described in the steps below refer to the preparation of these batch bottles.

b) Cool the apparatus to room temperature under a laminar-flow hood.

c) Meantime, remove 30-50 mL suspension of aerobic microorganisms from the continuous-feed batch reactor and dispense into a 50-mL centrifuge tube.

d) Centrifuge at 3000 rpm for about 15 min. Remove supernatant (centrate) from centrifuge tube and add 25 mL of autoclaved buffer-media solution. Centrifuge and repeat this procedure 3-4 times.

e) Several such centrifuge tubes with washed suspension of cells are prepared. One centrifuge tube is set aside for weighing the amount of cells in each tube.

f) Add about 200 mL of buffer-media into the media bottles using a measuring cylinder.

g) Add centrifuged cells from a single centrifuge tube into each media bottle.

h) Cap the media bottles with the phenolic-screw cap and butyl-rubber septa.

i) For some experiments, pure helium gas is used at this stage to purge the headspace (to remove air) of the batch bottles. Next 25-mL of pure O_2 gas is added.

j) Spike required amounts of pure TBOS/TKEBS/1-butanol/2-ethylbutanol using microliter syringes by piercing the septum

k) For acetylene-block experiments, about 25-mL of pure acetylene gas is added at this stage to the headspace of the batch bottles. Appropriate amounts of pure O_2 gas are added to maintain a partial pressure of 0.21 atm in the headspace.

l) Spike required amounts of TCE/c-DCE saturated aqueous solutions to achieve low concentrations of TCE or c-DCE. A 100-μL syringe was used.

m) Incubate the batch bottles in an environmental chamber at 30 °C with continuous shaking at 150 rpm using a gyratory-shaker.

Autoclaved Controls: Steps a) to g) are performed as explained above. The media bottle with the cells is autoclaved for 15 min and cooled to room temperature. Steps h) to k) are subsequently carried out.

Poisoned Controls: Steps a) to g) are performed as explained above. 2 mL of 2.5 g/L $HgCl_2$ solution are added to the media bottles. Steps h) to k) are subsequently carried out.

A.1.2 Abiotic Hydrolysis Experiments

The abiotic experiments were carried out by poisoning with $HgCl_2$, to completely exclude any biological activity. The preparation of apparatus for these studies is similar to that described in the preparation of aerobic batch bottles excluding the steps involving the preparation of the microbial inoculum and its addition.

A.1.3 Anaerobic Microcosm Experiments

Anaerobic microcosms were constructed in the sequence of steps described below:

a) Autoclave the apparatus consisting the following:

- media bottles (125/250/1000 mL)
- phenolic screw caps (for 250 and 1000-mL media bottles)
- measuring cylinders
- beakers
- spatula

Most experiments were conducted in 250-mL media bottles and the quantities described in the steps below refer to the preparation of these microcosms.

b) Cool the apparatus to room temperature under a laminar-flow hood.

c) Transfer the apparatus into the anaerobic glove box.

d) Groundwater from the site (stored in the refrigerator at 4 °C) is purged with N_2 treated in a tube furnace (to remove traces of O_2) to remove the background TCE and c-DCE in the groundwater.

e) The groundwater is then placed in the anaerobic glove box and allowed to equilibrate to room temperature.

f) Add about 250 mL of groundwater into the media bottles using a measuring cylinder.

g) Cap the media bottles with the phenolic-screw cap and butyl-rubber septa.

h) Remove the bottles from the anaerobic glove box.

i) The microcosms with the groundwater is again purged with N_2 treated in a tube furnace (to remove traces of O_2) to remove the H_2 introduced from the glove box atmosphere.

j) Spike required amounts of pure TBOS/TKEBS/1-butanol/2-ethylbutanol using microliter syringes by piercing the septum

k) Spike required amounts of pure TCE (for high concentrations) using microliter syringes by piercing the septum

l) Spike required amounts of TCE/c-DCE saturated aqueous solutions to achieve low concentrations of TCE or c-DCE.

m) Incubate the microcosms in an environmental chamber at 30 °C with periodic shaking.

Poisoned Controls: Steps a) to g) are performed as explained above. 2-mL of 2.5 g/L $HgCl_2$ solution is added to the media bottles. Steps h) to k) are subsequently carried out.

A.2 Analytical Methodology

A.2.1 Preparation of Stock and Standard Solutions

1) <u>TBOS and TKEBS stock solutions in dichloromethane (DCM)</u>: Appropriate volumes of liquid TBOS (97 % purity) and TKEBS (95 % purity) are added to HPLC-grade DCM to obtain 100 mg/L, 1 g/L and 20 g/L stock solutions of TBOS and TKEBS.

2) <u>1-butanol and 2-ethylbutanol stock solutions in methanol</u>: Appropriate volumes of 1-butanol (>99 % purity) and 2-ethylbutanol (96 % purity) are added to HPLC-grade methanol to obtain 100 mg/L, 1 g/L and 20 g/L stock solutions of 1-butanol and 2-ethylbutanol.

3) <u>Stock solution of tetrapropoxysilane (TPOS) (Internal Standard) in dichoromethane</u>: A stock solution of 10 mg/L TPOS in DCM is prepared by injecting 1.1 mL of liquid TPOS into 100 mL of DCM.

4) <u>Preparation of TBOS, TKEBS, 1-butanol and 2-ethylbutanol standards in water</u>: Appropriate volumes of TBOS and TKEBS in DCM stock and 1-butanol and 2-ethylbutanol in methanol stock solution is spiked into 20 mL of buffered water (pH 7) to obtain TBOS and TKEBS standards in water. The concentration range of the standards prepared should range from 0.1 to 1000 mg/L. The amount of DCM stock solution added to water should never exceed 100 µL because of the low solubility of DCM in water.

6) <u>Sample Preparation</u>: 1-mL samples are removed from the microcosms using a gas-tight syringe and transferred into 2-mL glass vials with propylene cap and septa. The microcosms are agitated vigorously for about 5 min prior to collecting the 1-mL

aqueous sample. This is done to homogenize the solution due to the low solubility of

TBOS and TKEBS and their tendency to form emulsions. Dirty samples (biological

samples) are centrifuged to obtain clean samples prior to extraction.

7) Extraction Procedure: Liquid-liquid extraction (LLE) is performed on 1-mL

standards or samples by addition of 0.5 mL of the 1-mg/L TPOS stock solution in

DCM. The mixture was agitated vigorously to extract the analytes of interest into

the DCM phase. The mixture is allowed to settle to allow complete separation of the

two immiscible phases. The DCM phase is selectively removed and used in analysis.

*A.2.2 Solid Phase Micro Extraction (SPME) Procedure*

SPME was performed with a variety of coated fibers such as 7, 30, 100-μm

polydimethylsiloxane (PDMS) fibers or 85-μm polyacrylate fiber and 2-mL aqueous

volumes of the standards or sample.

A brief summary of the SPME conditions is listed below:

1) Condition the fiber by placing it in the GC inlet port for 5 min and opening the split

vent to bypass the column and remove the carryover from the fiber.

2) Take 2 mL of sample in a 2-mL vial (with septa) with mini stirrer.

3) Place the vial on a magnetic stirring plate and insert the fiber into the vial by

piercing the septa. Be sure as to pierce the septa using the needle prior to extending

the fiber from inside the hollow needle.

4) Allow 20 min for exhaustive extraction of the analytes in the sample. Keep the

stirring rate constant for all the runs.

5) Remove fiber and immediately introduce it into the GC inlet port and perform GC/MS analysis.

6) Leave the fiber in the injection port for 3 min to allow complete desorption and avoid carry over into the subsequent runs.

7) Repeat the procedure with different samples and attempt to maintain all the parameters constant.
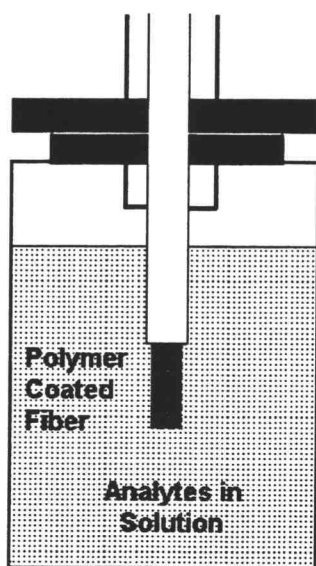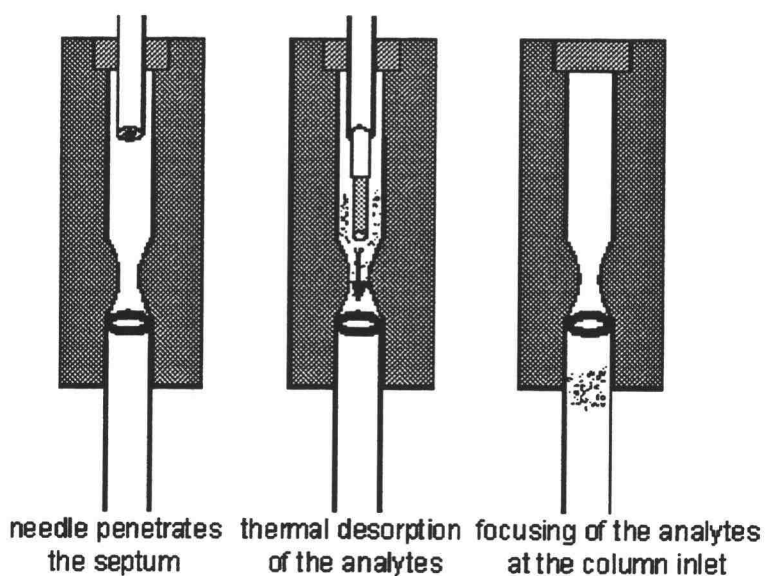

Figure A.1.   Illustration of SPME operation involving the various stages of sampling, extraction, concentration and desorption step.

The following different parameters are to be optimized for the SPME technique:

i. Equilibration time for complete partitioning of the analyte into the fiber.

ii. Desorption time for complete desorption of sorbed analytes in the fiber.

iii. Stirring rate to ensure proper mixing.

iv. Placement of the fiber in the injection port with respect to its height.

v. Desorption temperature to ensure removal of all adsorbed analytes.

vi. Temperature of the sample being analyzed.

A.2.3 GC/MS Operating Conditions

The qualitative analysis was performed in the scanning mode. The GC/MS was typically operated to scan in the range of m/z 30 - 450. The scan mode was also used to determine the mass spectrum of the target analytes and to choose the appropriate ions for use in the quantitative determination. The quantitative analysis was performed using the selective ion-monitoring mode (SIM).

A detailed description of the GC/MS operating conditions for both the quantitative and qualitative analysis are provided below.

For concentrations of TBOS and TKEBS in the range of 0.1 -25 mg/L and 1-butanol and 2-ethylbutanol in the range of 0.5 -100 mg/L

Splitless flow mode

Purge vent on time: 3.00 min

Purge vent off time: 17.00 min

Column gas flow: 0.8 mL/min

For concentrations of TBOS and TKEBS in the range of 25 -1000 mg/L and 1-butanol and 2-ethylbutanol in the range of 100-1000 mg/L

Split flow mode

Septum purge flow: 3 mL/min

Split vent flow: 100 mL/min

Purge vent on time: 0.00 min (Split Flow)

Column gas flow: 0.8 mL/min

Solvent delay: 6.2 min (mandatory)

Oven Program

Oven temperature (Initial): 40 °C

Initial Time: 3.00 min

Rate: 10 °C /min

Final Temperature A: 70 °C

Rate A: 40 °C /min

Final Temperature: 250 °C

Final Time: 6.50 min

Total Run time: 18.00 min

Injector Temperature: 250 °C

Detector Temperature: 280 °C

Column Head Pressure: 8 psi

A.2.4 GC/PID-FID Operating Conditions

The GC operating conditions were as follows:

Injector temperature: 250 °C; Detector temperature: 250 °C;

<u>Split flow mode</u>

Septum purge flow: 3 mL/min

Split vent flow: 80 mL/min

Purge vent off time: 0.00 min (Splitless-Flow)

Column gas flow: 15 mL/min

Column Head Pressure: 9.1 psi

<u>Oven Program</u>

Oven temperature (Initial): 40 °C

Initial Time: 2.00 min

Rate: 15 °C /min

Final Temperature: 220 °C

Total Run time: 10.80 min

A.2.5 GC/FID Operating Conditions

The GC operating conditions were as follows:

Injector temperature: 200 °C; Detector temperature: 250 °C;

<u>Split flow mode</u>

Septum purge flow: 3 mL/min

Split vent flow: 15 mL/min

Column gas flow: 1 mL/min

Column Head Pressure: 10 psi

Oven Program

Oven temperature (Initial): 80 °C

Initial Time: 4.00 min

Rate: 10 °C /min

Final Temperature: 200 °C

Total Run time: 16.00 min

A.2.6 GC/TCD Operating Conditions

The following GC operating conditions were as follows:

Injector temperature, 275 °C; Detector temperature, 275 °C;

Packed Column

Column gas flow: 30 mL/min

Regulator Pressure: 40 psi

Oven Program

Oven temperature (Initial): 50 °C

Initial Time: 4.00 min

Rate: 0.5 °C /min

Final Temperature A: 51 °C

Rate A: 32 °C /min

Final Temperature B: 115 °C

Rate B: 20 °C /min

Final Temperature : 225 °C

Final Time: 2.50 min

Total Run time: 16.00 min

A.2.7 Retention Times of Compounds Monitored.

Table A.1 lists the retention times and the concentration ranges of the target
analytes monitored with the various gas chromatographs (GC) used for analysis of the
target compounds. Some target analytes were determined in more than one GC with
different types of detectors.

Table A.1 List of retention times and concentration ranges measured with the various gas chromatographs

Target Analyte	GC/MS			GC/PID-FID		GC/TCD		GC/FID	
	m/z	RT min	CR (mg/L)	RT min	CR (mg/L)	RT min	CR (%v/v)	RT	CR (mg/L)
TBOS	277	13.4	0.1-500						
TKEBS	361	18.2	0.1-500						
TPOS (IS)	235	11.6	10						
1-Butanol	56	6.5	0.5-500						
2-EtBuOH	70	8.9	0.5-500						
2-EtBuacid								14	1-500
Acetic acid								7.9	1-500
TCE	131	5.4	N/A	9.2	0.1-50				
c-DCE	96	4.5	N/A	8.0	0.1-50				
t-DCE	96		N/A	7.4	N/A				
1,1-DCE	96		N/A	7.0	N/A				
VC				5.4	0.1-5				
Ethene				1.6	0.1-5				
CH_4				0.9	N/A	11.2	0.01-95		
H_2						1.3	0.001-20		
N_2						4.3	N/A		
O_2						4.6	0.01-40		
CO_2						14.2	0.01-40		

RT -Retention Time, CR - Concentration Range, m/z - mass to charge ratio

A.3 Properties of Chlorinated Ethenes

The physical and chemical properties of TCE and its transformation products determine the distribution of these chemicals in the environment. Some important physical and chemical properties of TCE and its transformation products are summarized in Table A.2.

Table A.2   General properties of chlorinated ethenes

	TCE	c-DCE	t-DCE	1,1-DCE	VC
Formula	$CHCl=CCl_2$	$CHCl=CHCl$	$CHCl=CHCl$	$CCl_2=CH_2$	$CH_2=CHCl$
Molecular Weight	131.5	96.95	96.95	96.95	62.5
Boiling Pt.[a] (20 °C)	87.2	60.0	47.5	32.0	-13.4
Solubility[b] @ 20 °C (mg/L)	1100	3500	6300	2250	2670
Density[a] @ 20 °C (g/mL)	1.464	1.284	1.257	1.218	0.911
Dimensionless Henry's Constant[a] 'H' @ 30 °C	0.43	0.164	0.163	0.608	2.575
Log K_{ow}[b]	2.38	0.70	0.48	1.84	1.38
U.S Drinking water MCL[c] (μg/L)	5	70	100	7	2

[a] (Schwarzenbach, 1993),   [b] (Concawe, 4/79),   [c] (McCarty and Semprini, 1994)

A.4 Mass Balance - Calculations

The mass balance of TBOS, TKEBS, 1-butanol, 2-ethylbutanol, 2-ethylbutyric acid, acetic acid, TCE, c-DCE, O_2, CO_2, H_2 or CH_4 in the microcosms was verified by measuring aqueous or headspace concentrations depending on the compound monitored. From the volumes of liquid and headspace in the microcosms and the concentrations of TCE and c-DCE in these two compartments, the mass balance in the microcosm were verified.

The following equations were used to determine the total mass of compounds in the microcosm.

$$n = C_w V_w + C_g V_g \qquad (A-1)$$

where 'n' is the total mass of the compound, C_w and C_g are aqueous and headspace concentrations, respectively. V_w is the liquid volume and V_g is headspace volume in the microcosm.

Partitioning of the compounds between the aqueous phase and headspace can be determined by assuming equilibrium partitioning and applying Henry's law.

$$H = C_w/C_g \qquad (A-2)$$

For non-volatile compounds such as TBOS, TKEBS, 2-ethylbutanol, 2-ethylbutyric acid and acetic acid, the aqueous phase concentrations (C_w) are measured directly. In this case, equation A-1 can be written as follows:

$$n = C_w V_w + (C_w/H) V_g$$

The values for V_w, V_g, H and C_w are known, and hence the total mass is easily calculated.

For volatile compounds or gases, such as TCE, c-DCE, 1-butanol, O_2, CO_2, H_2 or CH_4, the headspace concentrations (C_g) are measured directly. In this case, equation A-1 can be written as follows:

$$n = (C_g H) V_w + C_w V_g$$

The values for V_w, V_g, H and C_g are known, and hence the total mass is easily calculated. For CO_2, the speciation in the aqueous phase as H_2CO_3, HCO_3^- and CO_3^{2-} should be considered at the measured pH.

Tha values of 'H' used for all the calculations performed are summarized in Table A.3.

Table A.3   Basic properties of compounds monitored in the microcosms

Compound	Molecular Wt. (MW) (g/mole)	Dimensionless Henry's Constant (H)
TBOS	320.4	0
TKEBS	432	0
1-Butanol	76	0.00045[a]
2-Ethylbutanol	102	0
2-Ethylbutyric acid	116	0
Acetic acid	60	0
TCE	131.5	0.432[b]
c-DCE	96.5	0.164[b]
VC	62.5	2.575[b]
CO_2	44	1.22[c]
H_2	2	55[c]
CH_4	16	27.9[c]

[a] (Merck Index,),
[b] (data adapted from Schwarzenbach (1993) and Metcalf & Eddy (1990)),
[c] (data adapted from Stumm and Morgan (1989) and Smatlak et al.(1996))

A.5 Solubility Measurement

Purpose:

To determine the solubility of TBOS in water.

Rationale:

TBOS was observed to form an emulsion with water. This emulsion was stabilized by air preventing accurate determination of dissolved TBOS concentration. The formation of an emulsion is avoided by the use of a column-type apparatus shown in Figure A.2. When de-ionized water is circulated in a down-flow mode through a column containing TBOS coated beads, only the soluble portion of TBOS is expected to partition into the aqueous phase. TBOS being lighter than water floats on water and is retained in the column. Re-circulation provides for sufficient time to allow equilibrium partitioning. After sufficient equilibration is achieved, aqueous samples are withdrawn for determination of dissolved TBOS concentration.
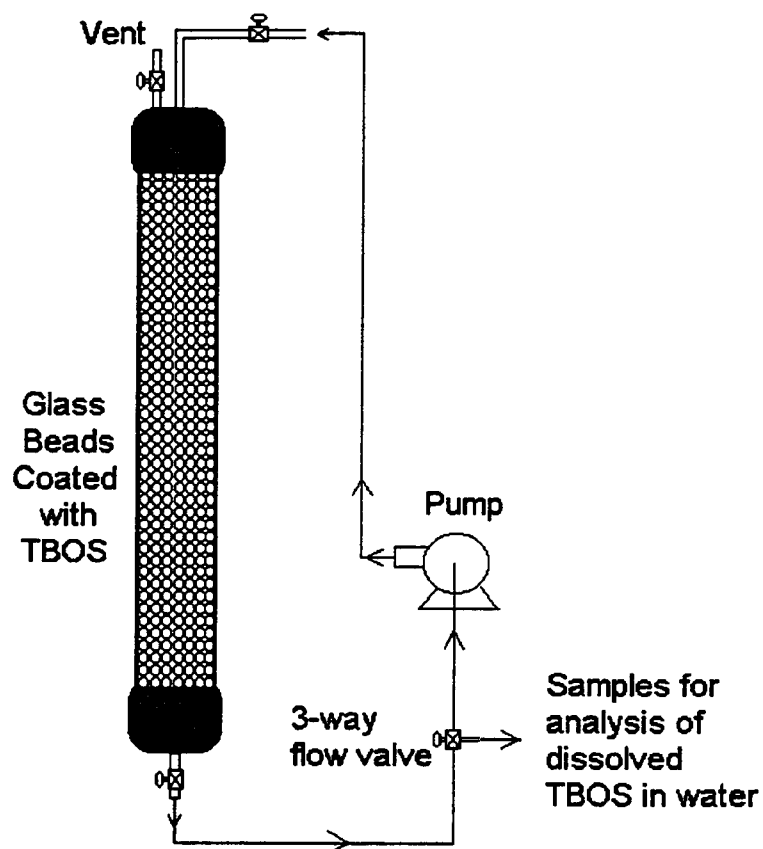
Figure A.2. Column apparatus for determination of solubility of TBOS in water.

Procedure :

The method for determining solubility involved the following steps:

1. Spherical (3 mm dia, uniformly sized) glass beads are coated with TBOS by adding

   pure liquid TBOS to a beaker containing the glass beads followed by vigorous

   agitation. Excess TBOS in the beaker is drained out.

2. The column (40 cm x 4 cm ID) is packed with these coated glass beads

3. The apparatus is then set-up as shown in Figure A.2. The apparatus comprised of a column containing the coated glass beads, a FMI piston pump, plastic tubing, three 2-way flow valves and a 3-way flow valve.

4. De-ionized water is circulated through the column in a down-flow mode at the rate of 2-5 mL/min.

5. The water is re-circulated back into the column using a peristaltic pump to allow equilibration partitioning of TBOS into water.

6. The water should be re-circulated through the column for at least 24 hr prior to removing a 1-mL sample of water from the outlet of the column (See Figure A.2)

7. The TBOS or TKEBS in the water is extracted with dichloromethane and the extract is injected into a GC/MS. The GC operating procedures are provided in the previous section of the Appendix A.

Note: It is important to not allow the formation of air bubbles in the column, tubing or pump to avoid formation of TBOS or TKEBS emulsion in water.

Results:

The solubility of TBOS in water at 25 °C was determined to be in the range of 0.4 - 0.6 mg/L from GC/MS measurements.

APPENDIX B
Supporting Data

B.1 Abiotic Hydrolysis

The abiotic transformation of TBOS to 1-butanol and TKEBS to 2-ethylbutanol

for a range of pH, temperature and concentration was studied to determine the rates of

hydrolysis and its significance as a natural attenuation pathway. The disappearance of

TBOS and TKEBS and the subsequent formation of their transformation products were

monitored. The abiotic hydrolysis data that were not included in Chapter 3 is provided

below.

B.1.1 Effect of pH

The effect of pH on the hydrolysis of TKEBS at 30 °C and 10 mg/L (23 µM)

aqueous concentration was evaluated (Figure B.1). The total moles of TKEBS

hydrolyzed and 2-ethylbutanol formed was plotted as a function of time.

TKEBS hydrolyzed to stoichiometric amounts of 2-ethylbutanol. There was no

notable effect of pH on the rates of hydrolysis. The rates of hydrolysis for TKEBS were

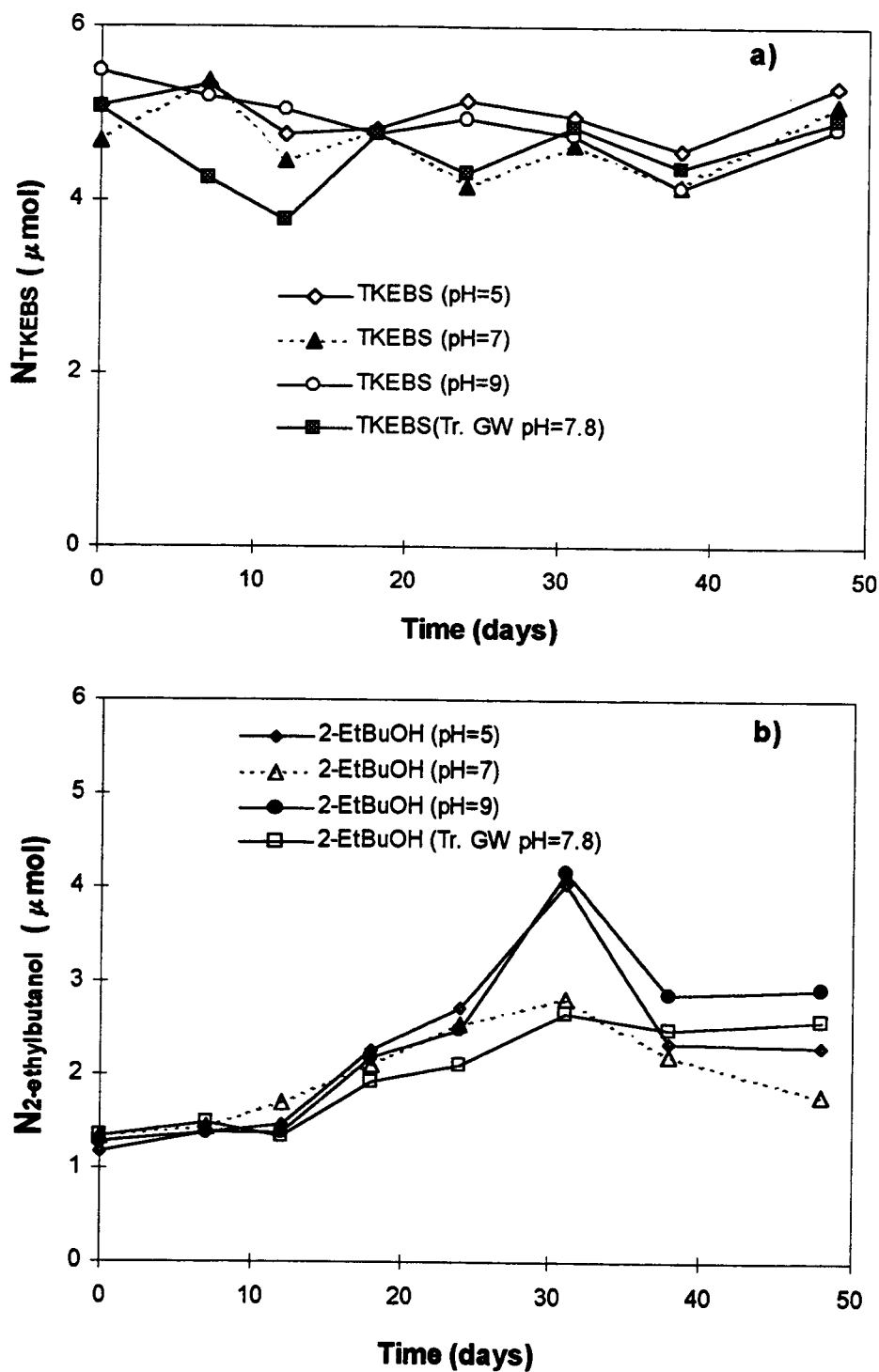about an order of magnitude lower than TBOS.

Figure B.1. Effect of pH on the hydrolysis rates of TKEBS. The disappearance of TKEBS (a) and the formation of 2-ethylbutanol (b) at a pH 5, 7, 7.8 and 9 were monitored at 30 °C and 10 mg/L initial TKEBS concentration.

B.1.2 Effect of Temperature

The hydrolysis of TBOS and TKEBS at pH 7 and 10 mg/L aqueous

concentration was studied at two different temperatures 15 and 30 °C (Figures B.2 and

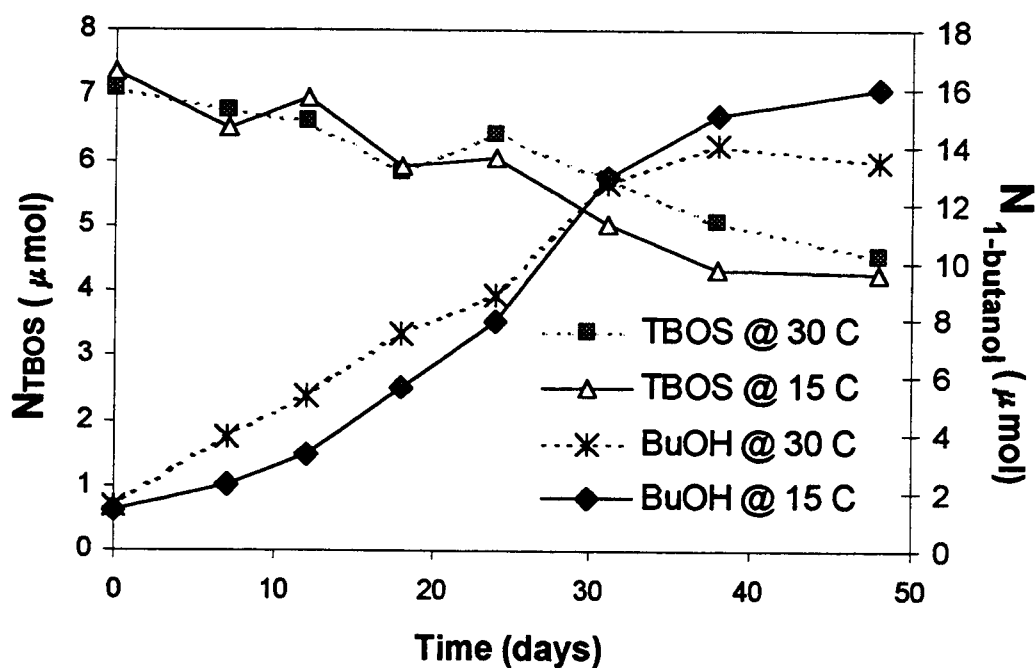B.3). No significant difference in hydrolysis was noted.

Figure B.2.   Effect of temperature on the rates of hydrolysis of TBOS. Disappearance
of TBOS and the formation of 1-butanol at pH 7 and 10 mg/L aqueous
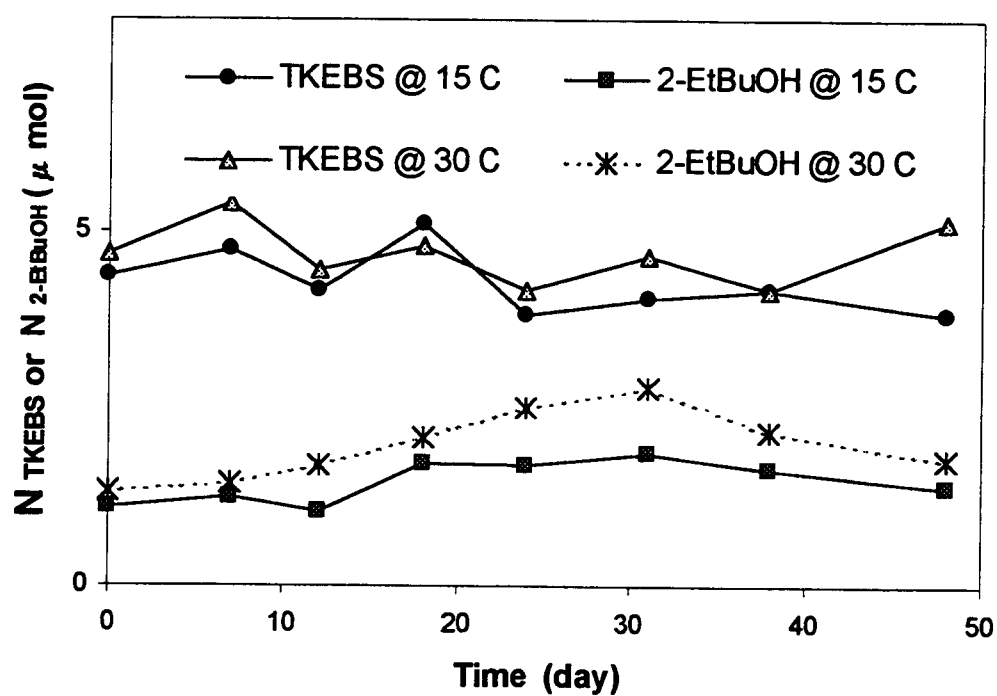concentration at 15 and 30 °C are shown.

Figure B.3. Effect of temperature on the rates of hydrolysis of TKEBS. Disappearance
of TKEBS and the formation of 2-ethylbutanol at pH 7 and 10 mg/L
aqueous concentration at 15 and 30 °C are shown.

B.2 Aerobic Cometabolism of High Concentrations of c-DCE

To evaluate the effect of O_2 and cometabolism at high concentrations (about 25

mg/L aqueous concentration) of c-DCE, several microcosm experiments were operated

to run out of O_2 during the course of the experiments. The microcosms contained 9.0

mg cells (dry weight). The results of a microcosm study with initial concentrations of 25

mg/L c-DCE (aq) and 250 mg/L of TBOS are shown in Figure B.4.
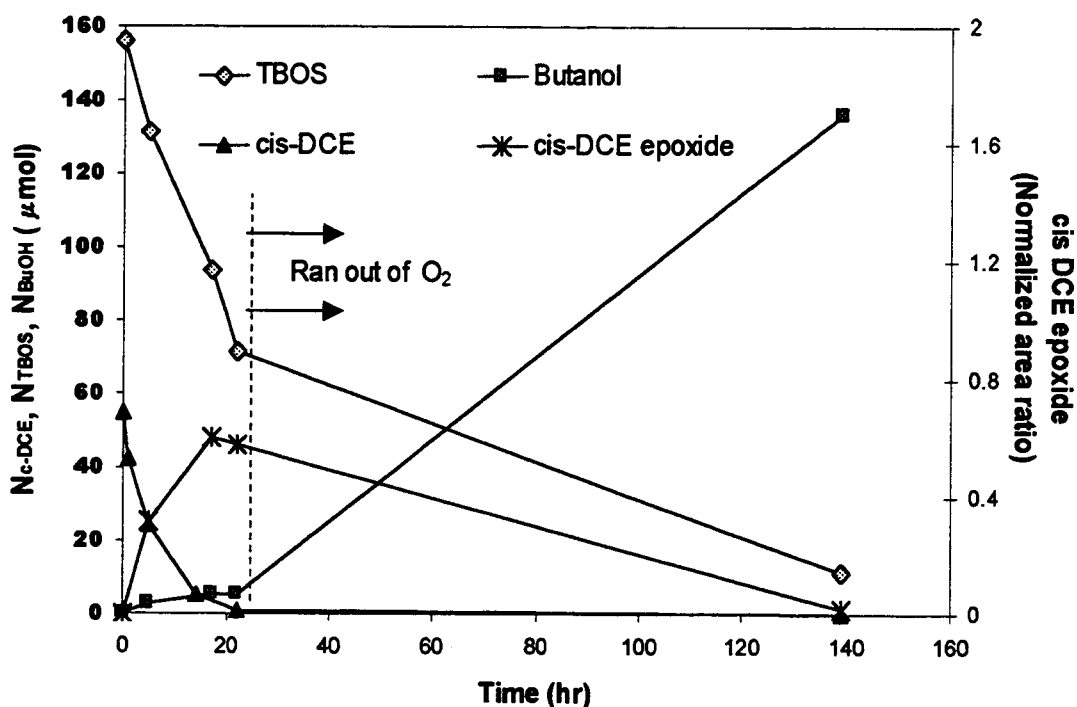

Figure B.4.   Aerobic cometabolism of high concentrations of c-DCE by
microorganisms grown on TBOS. TBOS degradation profile in the
microcosms, the degradation of c-DCE, formation and degradation of c-
DCE epoxide and the formation of 1-butanol. After 22 hr of operation,
the microcosm was depleted of oxygen and turned anoxic.

The TBOS concentration in the microcosm dropped from 158 μmol (total) to about 70 μmol in about 22 hr and simultaneously c-DCE (55 μmol total) was completely oxidized by the microorganisms to c-DCE epoxide. 1-butanol formed during the degradation of TBOS was actively degraded and its concentration never exceeded 6 μmol (total) in the microcosm. After 22 hr of operation, the O_2 in the microcosm was completely depleted and the microcosm was turned anoxic. Immediately after the depletion of O_2 in the microcosm, biologically-mediated hydrolysis of TBOS remained unhindered but the degradation of 1-butanol slowed drastically. Large amounts of 1-butanol accumulated in the microcosm. Active sulfate reduction was observed following this transition period. The c-DCE epoxide that had built-up in the microcosms was then degraded abiotically.

The aerobic cometabolism studies demonstrated the application of TBOS as a potential cometabolic substrate for c-DCE and TCE degradation. The reasons for the production of a monooxygenase enzyme are yet to be substantiated. The acetylene-block experiment indicated that the degradation of TBOS was not linked to the production of the monooxygenase enzyme. It is possible that the products of TBOS degradation could initiate the monooxygenase enzyme. However, separate experiments indicated that the cometabolism was not related to 1-butanol degradation and TBOS was required for cometabolism. It is proposed that that the monooxygenase enzyme was induced as a side-reaction and that it did not directly support the degradation of TBOS. Figure B.5 illustrates the proposed mechanism of TBOS degradation and the cometabolism of TCE and c-DCE.
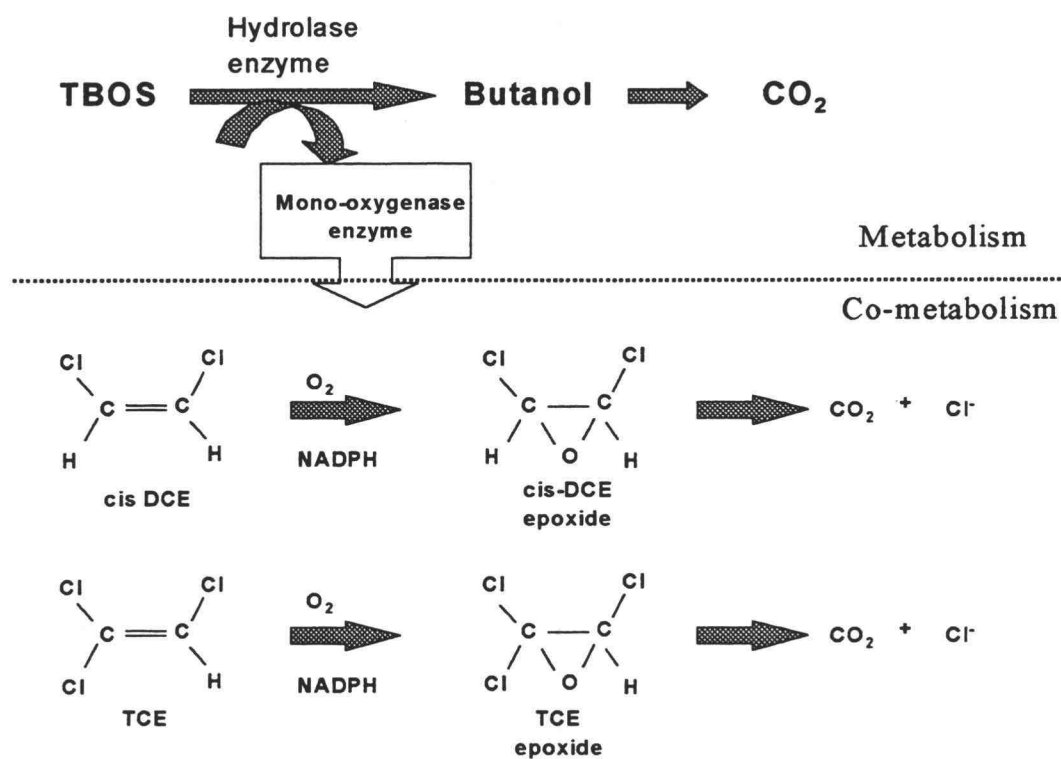
Figure B.5.   Proposed oxidation pathway for the aerobic degradation of TBOS showing the induction of the monooxygenase enzyme.