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Abiotic and Biological Transformation of Tetraalkoxysilanes and Trichloroethene/ *cis*-1,2-Dichloroethene Cometabolism Driven by Tetrabutoxysilane-Degrading Microorganisms

SANJAY VANCHEESWARAN,[†,‡] ROLF U. HALDEN,[§] KENNETH J. WILLIAMSON,[†] JAMES D. INGLE, JR.,[‡] AND LEWIS SEMPRINI*,[†]

Department of Civil, Construction and Environmental Engineering and Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, and Lawrence Livermore National Laboratory, Livermore, California

Attenuation of silicon-based organic compounds (tetraalkoxysilanes) by abiotic hydrolysis and biological mineralization was investigated. At Lawrence Livermore National Laboratory site 300, tetraalkoxysilanes are present along with trichloroethene (TCE) as subsurface contaminants. Under abiotic conditions, the alkoxysilanes such as tetrabutoxysilane (TBOS) and tetrakis(2-ethylbutoxy)silane (TKEBS) hydrolyze to 1-butanol and 2-ethylbutanol, respectively, and silicic acid. The rates of hydrolysis of TBOS and TKEBS were determined to evaluate the significance of the hydrolysis reaction in the attenuation process, and typical rates at pH 7, 30 °C, and 28 μmol/L initial concentration were 0.32 and 0.048 μmol/L/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 *cis*-1,2-dichloroethene (c-DCE). TCE and c-DCE degradation was inhibited by acetylene, indicating that a monooxygenase was involved in the cometabolism process. Acetylene did not inhibit the hydrolysis of TBOS or the utilization of 1-butanol, indicating that the above monooxygenase enzyme was not involved in the degradation of TBOS.

* Corresponding author phone: (541) 737-6895; fax: (541) 737-3099; e-mail: semprinl@ccmail.orst.edu.

[†] Department of Civil, Construction and Environmental Engineering, Oregon State University.

[‡] Department of Chemistry, Oregon State University.

[§] Lawrence Livermore National Laboratory.

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. These compounds are widely used as heat-exchange fluids, sealants, 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) $(CH_3CH_2CH_2CH_2O)_4$-Si and tetrakis(2-ethylbutoxy)silane (TKEBS) $(CH_3CH_2CH$-$(CH_2CH_3)CH_2O)_4Si$. At Lawrence Livermore National Laboratory site 300, these compounds along with trichloroethene (TCE) were used in heat-exchanger pipes at their materials testing facility. Subsurface contamination by these compounds resulted from leaking heat-exchanger pipes. TBOS and TKEBS are present as light nonaqueous phase liquids whereas TCE is present as a dense nonaqueous phase liquid in the subsurface and is also dissolved in both pure phase alkoxysilanes and groundwater.

Available literature on TBOS and TKEBS mainly focuses 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 hydrolyze abiotically to give the corresponding alcohols (*2*−*5*, *7*) and silicic acid. Thus, the hydrolysis of TBOS and TKEBS was expected to yield 1-butanol and 2-ethylbutanol, respectively. The general equation for the complete hydrolysis of tetralkoxysilanes is as follows

$$Si(OR)_4 + 4H_2O \rightarrow 4ROH + Si(OH)_4 \qquad (1)$$

where R is the substituent organic group such as $-CH_3$, $-C_4H_7$, $-C_6H_{13}$, or an aromatic ring.

Trichloroethene is the other major 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, fortuitous oxidation of TCE is catalyzed by the enzymes induced and expressed for the initial oxidation of the growth substrates (*10*−*14*). Several growth substrates such as methane, propane, butane, phenol, and toluene have been shown to induce oxygenase enzymes that cometabolize TCE (*18*).

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 microorganisms enriched from activated sludge of a wastewater treatment plant. Also tested was the ability of the aerobic microorganisms grown on TBOS to cometabolize TCE and *cis*-1,2-dichloroethene (c-DCE).

Materials and Methods

Chemicals and Stock Solutions. TCE (99.9% purity), c-DCE (99.9%), HPLC-grade dichloromethane, 1-butanol (99.9%),

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and 2-ethylbutanol (96% purity) were obtained from Aldrich Chemical Co. (Milwaukee, WI). TBOS (98% purity) and tetrapropoxysilane (TPOS) (98% purity) were 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 both as culture substrates and in the preparation of analytical standards.

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 GC connected to a HP-5971 mass-selective detector. The chromatographic separation was carried out with a Rtx-20 column (30 m × 0.25 mm, 1.0 μm film) from Restek, Inc. (Bellefonte, PA). The mass spectrometer was operated in the selective ion monitoring 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 that was added to dichloromethane prior to extraction. Because TBOS and TKEBS have low aqueous solubility (less than 0.5 mg/L) (*19, 20*), samples that contain higher than soluble amounts of TBOS and TKEBS form an emulsion with water. Prior to sampling, the sample containers were placed laterally on a high-speed reciprocating shaker table and agitated vigorously for at least 20 min to maintain homogeneity of the compounds in solution. Shaking of the containers was continued until just before the samples were collected, to obtain consistent results. The relative standard deviations of the TBOS and TKEBS measurements were approximately 10% and 15%, respectively.

Gas phase TCE and c-DCE concentrations were measured by injecting 100 μL of the headspace sample into a HP-5890 GC connected to a photoionization detector (PID) followed by a flame ionization detector (FID). Chromatographic separation was carried out with a 30 m megabore GSQ-PLOT column from J&W Scientific (Folsom, CA). The gases O_2 and CO_2 in the batch bottle headspace were measured with a HP-5890 GC 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 1000 packed column (15 ft × 0.125 in., S.S Support).

The dichloromethane extract prepared for the TBOS measurements was also used for the determination of c-DCE epoxide with TPOS as the internal standard. The GC/MS was operated in the selective ion mode, and c-DCE epoxide was measured by monitoring the ions m/z 48 and 112. Normalized area ratios were used to quantify c-DCE epoxide, as analytical standards for this compound were not available.

Experimental. Batch bottles were constructed with 310 mL serum bottles (Wheaton Industries, Millville, NJ) fitted with rubber-lined caps and butyl rubber septa. Abiotic hydrolysis studies were conducted in batch bottles with 250 mL of phosphate buffer and 60 mL of headspace. The phosphate buffer was prepared by mixing varying quantities of Na_2HPO_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 handled under a laminar flow hood to avoid microbial contamination. Autoclaved synthetic buffered medium (198 mL) and nutrient mixture (2 mL) were added to the batch bottles, and the remaining 110 mL of headspace was purged with helium (99.99% purity) 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 deionized 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 deionized water, and the minor nutrient stock was comprised of the following in 100 mL of deionized water: $ZnSO_4 \cdot H_2O$, 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.

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 total mass of TCE or c-DCE in the microcosms was determined by measuring gas phase concentrations and calculating aqueous phase concentrations assuming equilibrium Henry's law partitioning (*21*). From the volumes of liquid and headspace in the microcosms and the concentrations of TCE and c-DCE in these two compartments, the total mass balances in the microcosms were verified.

Bacterial Culture. 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 regrowth on TBOS. The enriched culture was subsequently used as an inoculum for a 2 L (1 L liquid volume) batch reactor that was continuously fed TBOS. The reactor utilized the same nutrient media as described for the batch bottles. The reactor was equipped with a fixed magnetic stir rod and stir plate for continuously mixing the contents of the reactor. 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 an inhibitor of monooxygenase enzymes (*13, 14*). Pure acetylene gas (20% of headspace volume) was added to the batch bottles to ensure maximum inactivation.

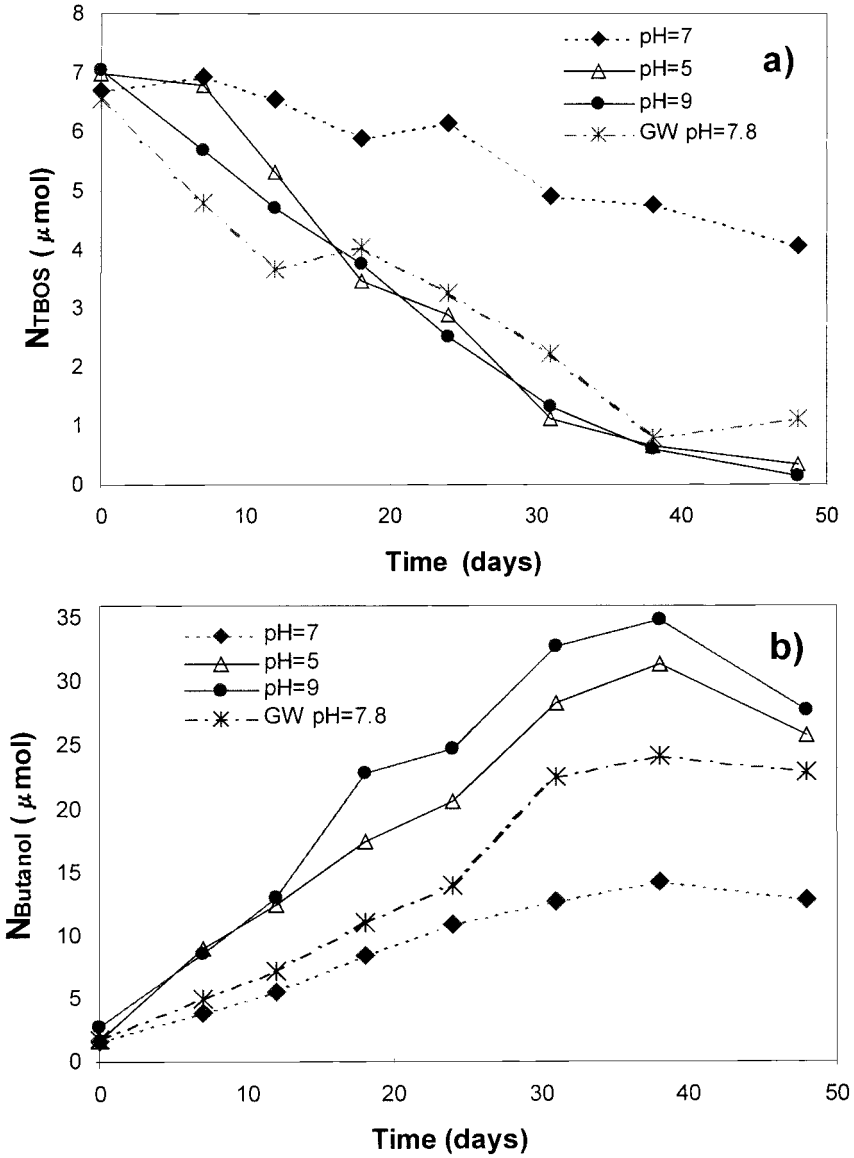

FIGURE 1. Effect of pH on the rate of hydrolysis of TBOS (above solubility limit) at 32 μmol/L initial concentration and 30 °C. The rates of hydrolysis at pH 5, 7, 7.8, and 9 were monitored (a) by the degradation of TBOS and (b) by the formation of the hydrolysis product, 1-butanol. The mixture volume was 0.25 L.

Results

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 μmol/L) "apparent" aqueous concentration is shown in Figure 1.

At the beginning of the experiment, an almost immediate loss of a small fraction (about 3−5%) of the added TBOS 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. Subsequent 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 pH 7.8 (pH of groundwater used for this experiment) relative to rates at neutral pH (Table 1). An acceptable linear regression fit was obtained with the data for the disappearance of TBOS and formation of 1-butanol.

TABLE 1. Rates of Hydrolysis of TBOS and TKEBS Based on a Linear Regression Fit[a]

			rate of hydrolysis (μmol/L/day)		
pH	temp (°C)	initial concn (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

[a] Note: Concentrations denoted in μmol/L or mg/L represent both the dissolved and the insoluble portion of TBOS or TKEBS in water. Precision of TBOS and TKEBS measurements ranged from 10 to 15% RSD.

Similar experiments were conducted with TKEBS which also hydrolyzes to stoichiometric quantities of 2-ethylbutanol. However, TKEBS hydrolyzed at a rate about 1 order of magnitude slower than TBOS.

Rates of hydrolysis under the different conditions studied are summarized in Table 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 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.

The hydrolysis of TBOS and TKEBS at pH 7 and 10 mg/L aqueous concentration was studied at 15 and 30 °C (Table 1). Hydrolysis rates were not significantly different over this temperature range. Due to the precision of TBOS and TKEBS measurements (10 to 15% RSD), a difference of less than 15% is considered insignificant.

The hydrolysis of TBOS and TKEBS was also monitored at two concentrations above the solubility limit of the compounds (Table 1). At pH 7 and 30 °C, a 10-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 10-fold increase in concentration resulted in a factor of 8 increase in the hydrolysis rates.

Aerobic Biological Degradation of TBOS and TKEBS. Initial studies focused on enriching a microbial population that could aerobically degrade TBOS and TKEBS. After several unsuccessful attempts to obtain an active culture, a microbial culture grown on TBOS was obtained from a sample of activated sludge that was incubated for about 1 year.

The record 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 2. The rate of transformation of TBOS in the presence of the microbial culture was much faster than that of the mercury poisoned control. Within 30 days, 180 μmol of TBOS (from 3 separate additions of 60 μmol) was utilized in the biologically active batch bottle, while in the poisoned control, only a small amount (<15%) 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 of a microbial culture on TBOS. Cell growth was also indicated by an increase in optical density (data not shown). The 1-butanol that was formed was subsequently utilized in the biologically active culture, while 1-butanol accumulated in the poisoned control. 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 are consistent with the TBOS utilization and the formation and disappearance of 1-butanol.

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 mol of CO_2/mol 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 9 mg (dry cell weight) of the microbial culture are shown in Figure 3. The microbial cultures used for these tests were grown on TBOS.

Figure 3a shows that TBOS at aqueous concentrations of 20 mg/L (12 μmol total) was rapidly degraded within 12 h. 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 biotic hydrolysis of TBOS or 1-butanol utilization. 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 cells and mercuric chloride, about 60% of TBOS was hydrolyzed to 1-butanol. A stoichiometric amount of 1-butanol, equivalent to the amount of TBOS degraded, was formed.

The rapid degradation of TKEBS within a matter of 50 h with the same enriched culture that was initially grown on TBOS is clear from the data in Figure 3b. Also, hydrolysis of TKEBS was complete, and the formation of 2-ethylbutanol as an intermediate was observed. No inhibition of the biotic hydrolysis of TKEBS or 2-ethylbutanol utilization 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 cells and 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 mercuric chloride poisoned controls provides some evidence that enzymes released by possible lysis of the killed cells maintain some activity for hydrolysis. The accumulation of stoichiometric amounts of the alcohol supports the conclusion that the cells were effectively poisoned and that this observed activity was entirely abiotic.

TCE Transformation by Microorganisms Grown on TBOS. The enriched microbial culture (from the activated sludge) grown 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 used: 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 4.

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 h. TBOS (20 mg/L or 12 μmol total) was also completely degraded but its percentage degradation rate was slower than that of TCE. 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 from the resting cell results was about 0.04 g of TCE/g of cells. No TCE transformation was observed in the poisoned control. The results indicate that the degradation of TBOS helped drive TCE cometabolism.

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%

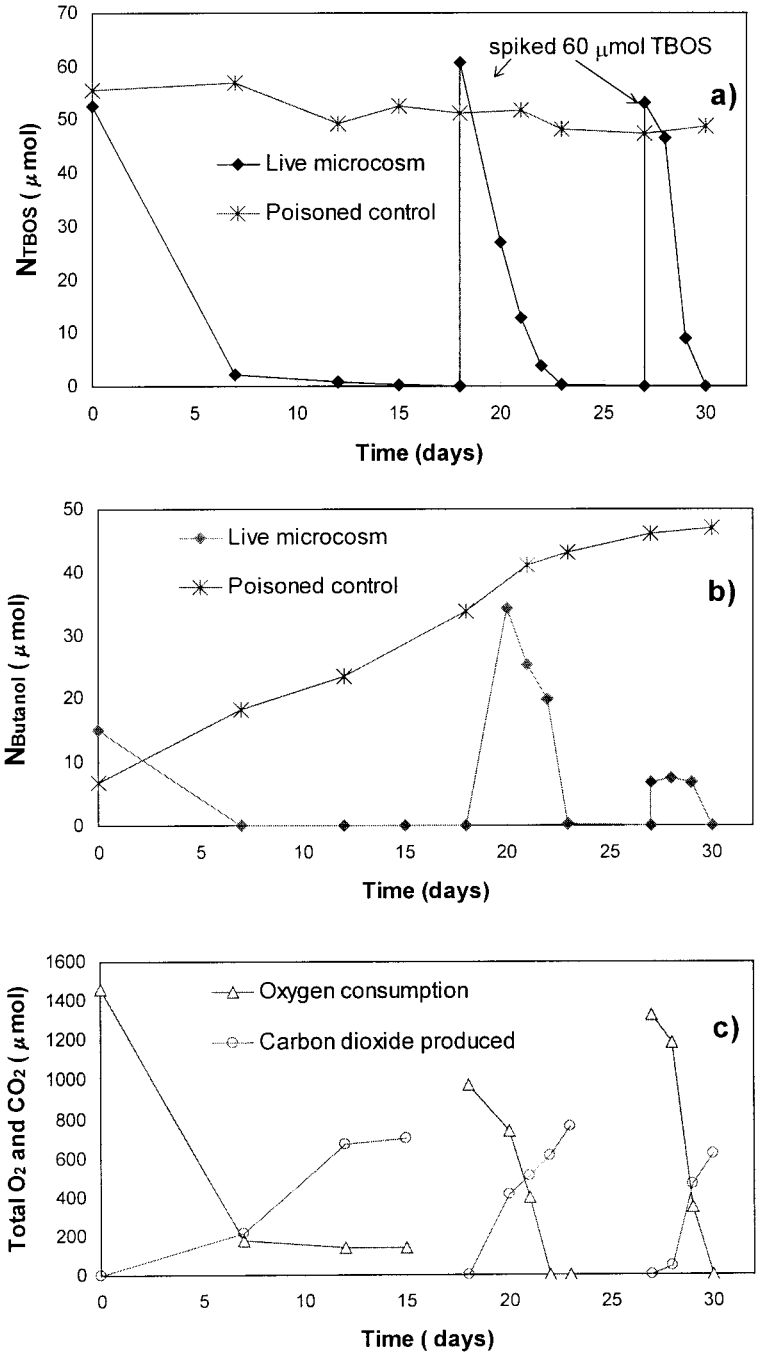

FIGURE 2. (a) TBOS degradation data providing evidence of enrichment of the microorganisms obtained from the wastewater treatment plant at Corvallis, (b) the formation of 1-butanol as an intermediary product during the aerobic degradation reaction, and (c) the utilization of O_2 and production of CO_2. The liquid mixture volume was 0.20 L.

degraded in 140 h) 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.

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 used: 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 5. 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 h 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 h), which was slightly faster than the abiotic transformation rates (half-life of 70 h) (*13*). This behavior is consistent with c-DCE cometabolism slowing after about 12 h. 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

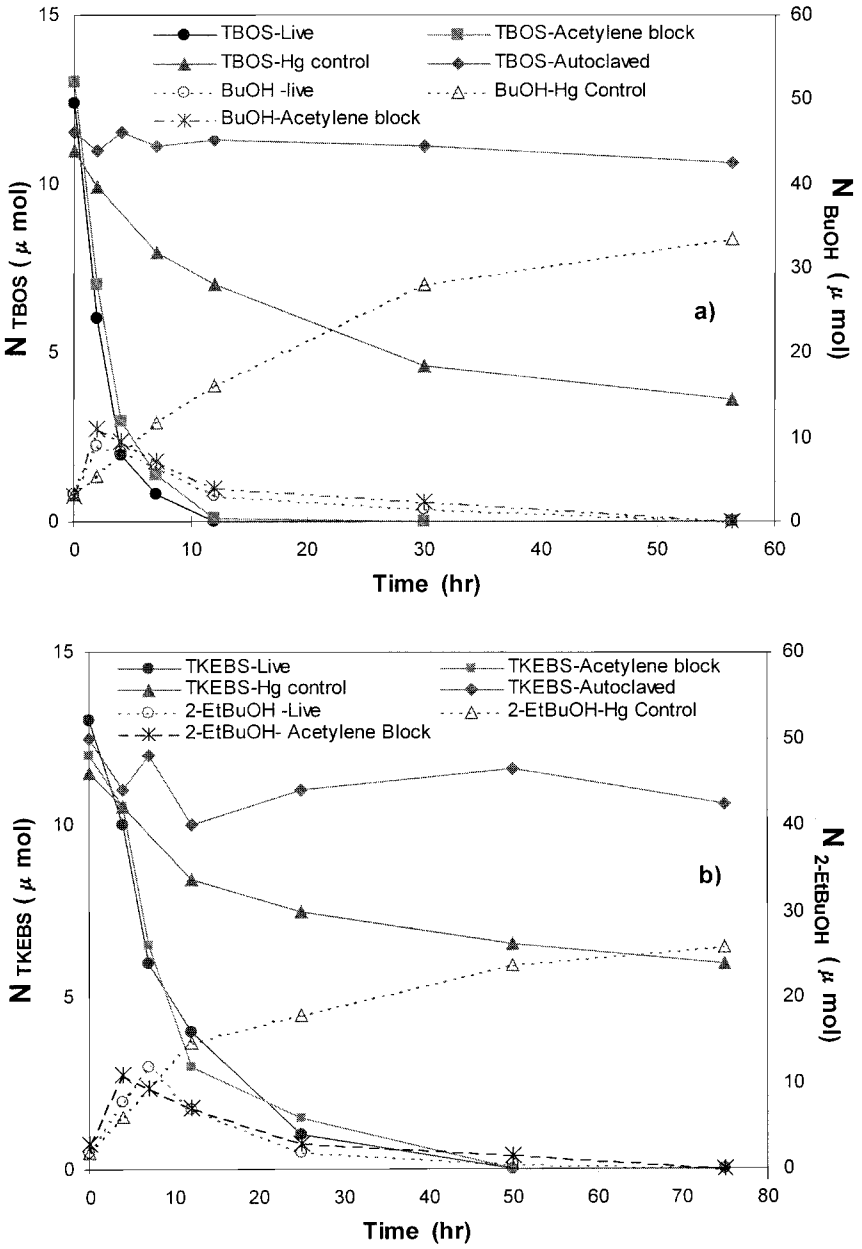

FIGURE 3. Aerobic degradation of 60 μmol/L of (a) TBOS or (b) TKEBS by the enriched microbial culture. Cells (9.0 mg, dry weight) were added to each batch bottle for the degradation study. Two controls were prepared, one by autoclaving and the other by addition of $HgCl_2$. The liquid mixture volume was 0.20 L.

reaction rate, while acetylene significantly reduced the rate of c-DCE transformation and the corresponding epoxide production. 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 of c-DCE/g of 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.

Experiments were continued 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, indicating that the cometabolism potential was exhausted from the initial exposure to c-DCE. 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 the 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, indicating that TBOS was required to induce cometabolism of c-DCE and TCE.

To evaluate the effect of O_2 and cometabolism at high concentrations of TCE or c-DCE (about 25 mg/L aqueous concentration), experiments were performed so that O_2 was completely utilized during the experiments. Upon depletion of O_2 in the batch bottles, biologically mediated hydrolysis of TBOS remained unhindered, but the degradation of 1-butanol slowed, and stoichiometric amounts of 1-butanol accumulated. These results suggest that O_2 was not required

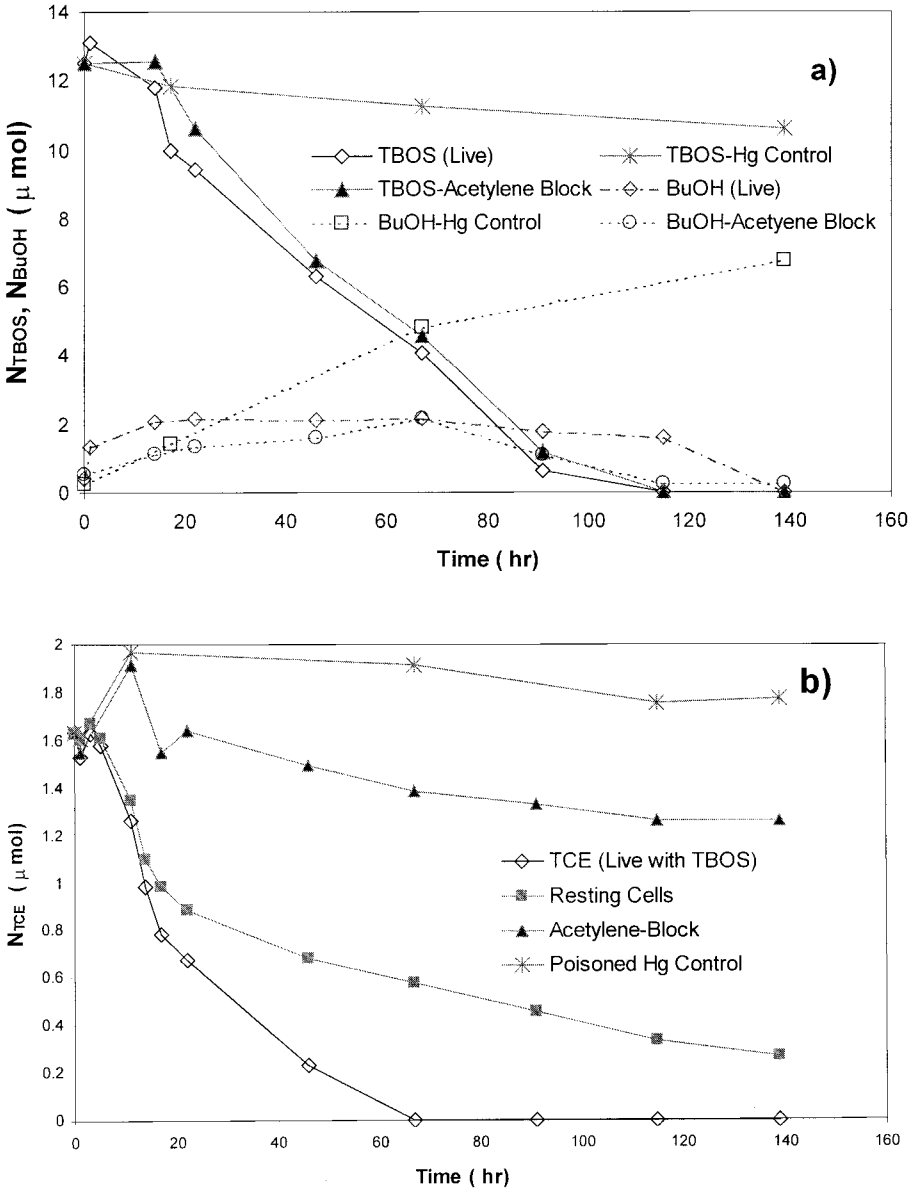

FIGURE 4. Aerobic cometabolism of TCE by microorganisms grown on TBOS. (a) Disappearance of TBOS and (b) the degradation of TCE by live microbes in the presence of TBOS and absence of TBOS (resting cells) as compared to the poison control and the acetylene block batch bottles. The liquid mixture volume was 0.20 L.

for the hydrolysis reaction and that the monooxygenase enzyme was not responsible for TBOS transformation. In the absence of O_2, c-DCE epoxide produced from c-DCE cometabolism was transformed at rates (half-life of 65 h) comparable to the abiotic transformation rates for the epoxide. Similar results were obtained with TCE, with TCE transformation stopping completely when the O_2 was depleted. The oxygen data support the acetylene block data, which indicates that different enzyme systems are involved in the hydrolysis of TBOS and the cometabolism of TCE and c-DCE. The cometabolism of TCE and c-DCE is initiated by a monooxygenase enzyme which requires O_2 and is inhibited by acetylene while the hydrolysis reaction does not require O_2 and is not inhibited by acetylene.

Discussion

The transformation of alkoxysilanes occurred under both biotic and abiotic conditions. 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 of about a factor of 2 higher than that at neutral pH. The rate of TKEBS transformation is about 1 order of magnitude lower than that of TBOS and was not substantially affected by change in pH (between 5 and 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 reaction also showed very little temperature dependence.

At concentrations of TBOS and TKEBS above the solubility limit, the rate of hydrolysis increased with concentration. 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 (or TKEBS) droplets form, the hydrolysis may be controlled by the dissolution rate of TBOS (or TKEBS) or by direct hydrolysis that 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.

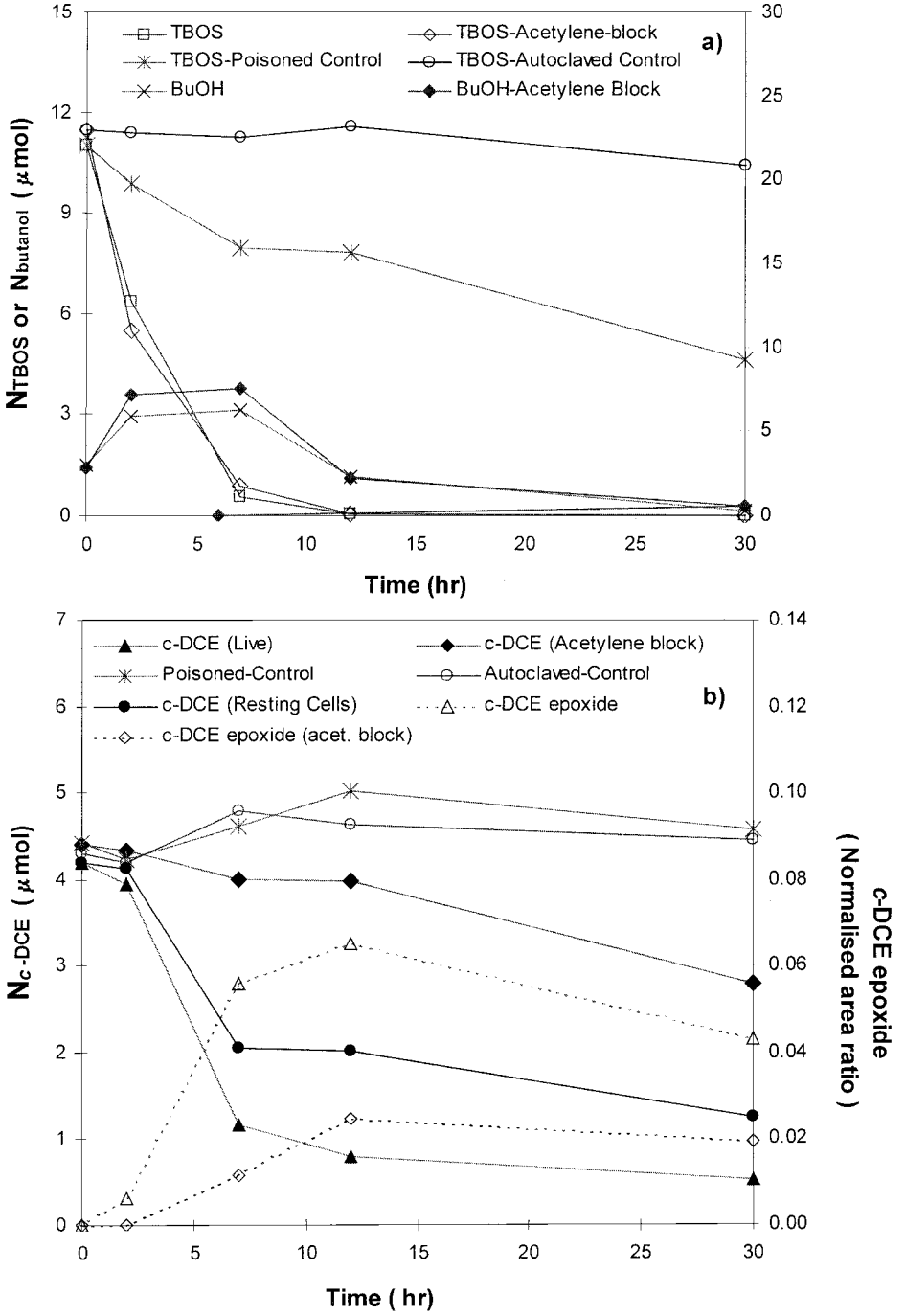

FIGURE 5. Aerobic cometabolism of c-DCE by microorganisms grown on TBOS: (a) TBOS degradation profile in the batch bottles, (b) the degradation of c-DCE by live microbes in the presence of TBOS and absence of TBOS (resting cells) as compared to the poison control, autoclaved control, and the acetylene block batch bottles. The liquid mixture volume was 0.20 L. The normalized area ratio is the peak area of c-DCE epoxide with respect to the peak area of the internal standard (TPOS).

Mineralization of TBOS and TKEBS to CO_2 was observed. $HgCl_2$ greatly reduced the rate of hydrolysis and prevented the utilization of the alcohol products. These observations indicate the biologically mediated hydrolysis of TBOS and TKEBS. The production of a monooxygenase enzyme during TBOS biodegradation is supported by the observed cometabolism of TCE and c-DCE. However, TBOS hydrolysis was not inhibited by the presence of acetylene or the temporary lack of O_2. The utilization of 1-butanol was also not inhibited by acetylene; however, 1-butanol utilization required O_2. These results indicate that the monooxygenase enzyme was not involved in TBOS transformation or 1-butanol utilization. Why an oxygenase enzyme was expressed is not known and requires future research.

The aerobic cometabolism studies demonstrated that TBOS can be used as a potential primary substrate to promote cometabolic degradation of c-DCE and TCE. To our knowledge, these are the first observations of an organosilane serving as a cometabolic substrate for chlorinated ethene transformations. 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 a monooxygenase enzyme was induced by TBOS but the monooxygenase enzyme was not required

for the degradation of TBOS. It is possible that the metabolism of the products of TBOS degradation could also stimulate monooxygenase production. Initial experiments indicate 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) (*19*). Work is underway to isolate a pure culture from the TBOS grown enrichment which will help answer some of these questions.

The transformation capacities (0.032 g of c-DCE/g of cells and 0.04 g of TCE/g of cells) were comparable to other known cometabolic substrates such as methane, propane, and butane (*10*−*14*). The ability of TBOS degraders to cometabolize c-DCE and TCE provide an opportunity to exploit the contamination of TBOS and TKEBS at Lawrence Livermore National Laboratory site 300 and take advantage of it as a preexisting electron donor. It may also be possible to add TBOS as a primary substrate to promote cometabolism of chlorinated ethenes at other contaminated sites.

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