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Enhanced reductive dechlorination of PCE DNAPL with TBOS as a slow-release electron donor

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ABSTRACT

Tetrabutoxysilane (TBOS), which yields 1-butanol upon abiotic hydrolysis, was evaluated as a slow-release substrate for the reductive dechlorination of tetrachloroethylene (PCE) as a dense non-aqueous-phase liquid (DNAPL). Dechlorination was achieved using an anaerobic binary mixed (BM) culture, which consisted of the Pt. Mugu (PM) and the Evanite (EV) mixed cultures. In batch reactor experiments, TBOS was mixed with PCE DNAPL to achieve different PCE mol fractions (PCE mol/(PCE mol + TBOS mol)), and different PCE aqueous concentrations based on Raoult's Law. The reductive dechlorination activity was determined based on the amount of chloride ions released and the mass balances of the transformation products formed. The mass balances of the total chlorinated aliphatic hydrocarbons (CAHs) between water, NAPL and gas phases were performed using independently measured NAPL/water partition coefficients. The amounts of chloride released (directly measured in aqueous samples) agreed with the total chloride produced based on the mass balances. The abiotic rates of TBOS hydrolysis were first-order with respect to TBOS NAPL concentration. A higher electron transfer efficiency to dechlorination was correlated with lower rates of TBOS hydrolysis. The total amounts of PCE DNAPL and TBOS were important factors for the reductive dechlorination of PCE. The dechlorination activity was suppressed at high NAPL concentrations. Direct contact of the PCE/TBOS NAPL mixture may have caused toxicity to the dechlorinating bacteria. Decreases in pH likely lowered the microbial activity for reductive dechlorination due to the accumulation of acetate and/or butyrate. These studies showed the potential of TBOS as a slow-release substrate for enhancing bioremediation of DNAPL contaminated sites.

1. Introduction

Tetrachloroethylene (PCE) and trichloroethylene (TCE) are common and recalcitrant contaminants in soil and groundwater. Sites contaminated with DNAPLs are among the most difficult to remediate; PCE and TCE DNAPLs can be long-term sources of soil and groundwater contamination [1]. For the clean-up of DNAPL contaminants, bioremediation technologies are being considered [2–7].

Anaerobic reductive dechlorination is a promising technology for the remediation of high concentrations of PCE and TCE associated with dissolution of the DNAPL source zone [2,8,9]. Enhanced DNAPL PCE dissolution due to biotransformation processes has been observed [2–4,10–12], suggesting their potential for the biological dechlorination of PCE or TCE DNAPL.

The use of slow-release substrates for the enhanced anaerobic biodegradation of a DNAPL source zone has been investigated [5,13–19], and can potentially reduce the operational costs resulting from the repeated or continuous injection of commonly used soluble substrates, such as lactate [17]. Another possible advantage of slow-release substrates, such as vegetable oil and TBOS (silicon-based organic compound), is the potential partitioning of chlorinated solvents into insoluble or semi-soluble substrates injected near a DNAPL zone. The partitioning may reduce the toxicity or inhibition of high concentrations of PCE, TCE and *cis*-dichloroethylene (*c*-DCE) [17]. The treatment of DNAPL contaminants using slow-release substrates may also enhance the rates of biological reductive dechlorination.

A number of *Dehalococcoides* strains have shown different abilities to use chlorinated ethylenes as electron acceptors for growth. *Dehalococcoides ethenogenes* strain 195 can grow on PCE, TCE and *c*-DCE, and cometabolically dechlorinates vinyl chloride (VC) to ethylene (ETH) [20]. *Dehalococcoides* sp. strain FL2 reductively dechlorinates TCE and *c*-DCE, with cometabolic PCE and VC transformations [21], while *Dehalococcoides* sp. strain BAV1 grows on *c*-DCE and VC and can cometabolize PCE and TCE in the presence of a growth-supporting chlorinated ethylene [22,23]. A single culture has not been shown to grow on all of the chlorinated ethylenes. A mixed culture containing two or more dechlorinating microorgan-

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isms that can grow on all dechlorination steps of PCE to ETH would likely be more competitive and robust than a pure culture under field conditions.

A mixed culture (BM) consisting of two enriched dechlorinating cultures was used in this study, with one enriched from Pt. Mugu, CA (PM), the other from the Evanite site, Corvallis, OR (EV) described by Yu et al. [24]. Yu and Semprini [25] showed when these two cultures were mixed, the resulting culture had transformation abilities, which represented both the PM and EV cultures. Yu and Semprini [17] demonstrated that TBOS was an effective slow-release substrate for transforming low aqueous concentrations of TCE (15–381 μM) to ETH. TBOS slowly and abiotically hydrolyzes to 1-butanol, which ferments to butyrate and/or acetate, with the production of hydrogen. The objectives of this research were to: (1) evaluate the enhancement of reductive dechlorination of NAPL by mixing PCE with TBOS as a slow-release substrate, (2) compare mass balances of the parent and daughter products that include partitioning between NAPL, aqueous, and the batch reactor headspace with chloride release measurements, (3) determine PCE/TBOS NAPL mixtures that result in effective CAH transformation, and (4) investigate how the rates of TBOS hydrolysis affect the rates of reductive dechlorination and electron transfer efficiencies.

2. Materials and methods

2.1. Chemicals

PCE (99%, spectrophotometric grade), TCE (99.9%) and *c*-1,2-DCE (97%) were purchased from Acros Organics (Pittsburgh, PA), and VC and ETH (both 99.5%) from Aldrich Chemical Co. (Milwaukee, WI). 1-butanol (99.8%, HPLC grade), sodium butyrate (98%) and sodium acetate (99+%) were purchased from Aldrich Chemical (Milwaukee, WI), and used for preparing the analytical standards. TBOS (97%) used as a slow-release substrate was purchased from Aldrich Chemical (Milwaukee, WI). Dichloromethane (DCM) (99.9%, HPLC Grade, Fisher Scientific Co., Pittsburgh, PA) was used for the solvent extraction of 1-butanol prior to analysis via gas chromatography (GC).

2.2. Analytical methods

PCE, TCE, *c*-DCE, VC, ETH and methane were measured using a Hewlett-Packard gas chromatograph (model 6890) equipped with a 30 m × 0.53 mm GS-Q column (J&W Scientific, Folsom, CA). The batch reactor headspace sample (20–100 μL) was injected into the GC equipped with photoionization (PID) and flame ionization detectors (FID) connected in series. The GC oven was initially set at 80 °C for 1.5 min, heated to 170 °C at 65 °C/min and to 220 °C at 40 °C/min, and then maintained at 220 °C for 2.7 min. The hydrogen concentrations in headspace gas samples were determined using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220 °C employing argon as the carrier gas.

The chloride ion concentrations were measured using a Dionex DX 500 ion chromatography system, consisting of a C25 chromatography oven, CD20 conductivity detector and Dionex IonPac AS14 4 mm column, with 0.371 g/L Na_2CO_3 and 0.084 g/L of $NaHCO_3$ as eluent.

1-Butanol extracted with DCM was determined using GC, equipped with an FID and Rtx-5 column (30 m × 0.32 mm, 0.25 μm film) (Restek, Inc.) as described by Yu and Semprini [17]. Fatty acids were measured using high performance liquid chromatography (HPLC) with UV absorbance detector at 210 nm (Dionex, Sunnyvale, CA) [17].

2.3. Culture enrichment and growth condition

Two different mixed cultures, from Point Mugu, CA, and the Evanite site in Corvallis, OR, were enriched and maintained in separate batch reactors (total 1.2 L with 1 L liquid) at 20 °C, with continuous shaking at 200 rpm for about 1 year. The characterization of the kinetics of the cultures is provided by Yu and Semprini [25]. The PM culture transformed PCE, TCE, and *c*-DCE rapidly, but showed very slow dechlorination of VC to ETH, with the last step being cometabolic in nature. The EV culture dechlorinated PCE to ETH with the accumulation of *c*-DCE, indicating *c*-DCE strongly inhibits VC dechlorination to ETH. These two cultures showed no competition or synergistic effects for dechlorination of CAHs, confirmed by comparison of the experimental and modeling results [25]. Yu et al. [24] characterized the cultures using molecular methods and found *Dehalococcoides* sp. microorganisms present in both the EV and PM cultures. Details of the batch growth conditions were provided by Yu and Semprini [24]. Both cultures were grown in sterile basal medium containing trace nutrients, modified from Yang and McCarty [26] by replacing the Cl^- with salts containing Br^- to make a low chloride content medium, to allow measurements of the amount of chloride released. Each mother reactor was observed to reach a steady-state biomass concentration of about 40 mg/L, based on protein.

2.4. Batch experiments

All batch reactor experiments were performed in serum bottles (total volume 156 mL) fitted with rubber-lined caps and butyl rubber septa (Wheaton Industries, Millville, NJ). 100 mL of the liquid culture harvested from the mother reactor and 25 mL fresh medium

Table 1
Experimental conditions for PCE/TBOS mixtures in batch reactors with the BM culture. All reactor studies were conducted in duplicate and poisoned controls were constructed for each set.

Batch reactors	PCE (μmol)	TBOS (μmol)	PCE mol fraction (mol-PCE/mol-NAPL)	Resulting aqueous concentration (μM)[a]	Total volume of NAPL (mL)	Hydrolysis rate of TBOS (μM/day)[b]
BP-1	182	8660	0.02	18	3.20	29.9
BP-2	1060	8660	0.11	99	3.30	29.9
BP-3	8660	8660	0.50	450	4.10	29.9
BP-4	182	1490	0.11	99	0.57	8.6
BP-5	424	3460	0.11	99	1.32	13.9
BP-6	741	6060	0.11	99	2.30	24.1
BP-7	200	200	0.50	450	0.09	0.8
BP-8	200	467	0.30	270	0.19	1.7
BP-9	200	667	0.23	207	0.27	2.1
BP-10	200	1333	0.13	117	0.51	3.2
BP-11	200	2000	0.09	81	0.76	6.3

[a] Calculated based on Raoult's Law. All batch reactor sets were duplicates and had killed control bottles.
[b] Determined by measuring butanol production from abiotic hydrolysis of TBOS in each control bottle.

were added to the serum bottles in an anaerobic glove box filled with 10% H_2 and 90% N_2. The fresh media contained 0.03 M Na_2CO_3 to buffer acid production from the dechlorination reactions and potential fermentation reactions. After construction in the glove box, the headspace of each reactor bottle was purged with the mixed gas (10% CO_2 and 90% N_2), treated in a tube furnace to remove trace oxygen. At the onset of the experiment, PCE/TBOS NAPL mixtures were added to create different NAPL conditions in the bottles, as shown in Table 1. The reactors were incubated at 20 °C on a shaker table, operated at 200 rpm, to enhance mass transfer between the headspace, aqueous and NAPL phases.

All the batch bottles in the study used the BM culture, consisting of equal volumes of the PM and the EV cultures harvested from the mother reactors. For all experimental sets, control bottles were prepared in the same manner as for the live batch reactors, but were poisoned with 50 mg/L formaldehyde. The poisoned controls showed no methane and hydrogen production during the course of the experiments. These control reactor sets were used to determine the abiotic rates of TBOS hydrolysis. All the reactor tests were conducted in duplicate.

2.5. Total mass balance for partitioning into the NAPL phase

The partitioning of the chlorinated ethylenes and ETH between the gas, aqueous and NAPL phases was determined in order to complete the reaction mass balances. Headspace samples of all the chlorinated ethylenes and ETH were analyzed by gas chromatography, and the total mass was calculated using the partition coefficients assuming equilibrium between the three different phases. The total mass balances in the batch reactors were calculated using Eq. (1),

$$M_T^i = C_W^i V_W + C_G^i V_G + C_N^i V_N = \frac{C_G^i}{H_{CC}^i}(V_W + H_{CC}^i V_G + K_{N-W}^i V_N) \quad (1)$$

where M_T^i (μmol) represents total mass of a chlorinated ethylene; C_W^i, C_G^i and C_N^i (μmol/L) the concentrations of the chlorinated ethylene (i) in the aqueous, headspace and NAPL phases, respectively; V_W, V_G, and V_N (L) the volumes of the aqueous, headspace and NAPL phases, respectively; $H_{CC}^i (= C_G^i/C_W^i)$ the dimensionless Henry's constant for partitioning between the aqueous and gas phases, and $K_{N-W}^i (= C_N^i/C_W^i)$ the dimensionless partition coefficient between the aqueous and NAPL phases. The Henry's constants used for partitioning between gas and aqueous phases were those reported by Gossett [27] and Perry et al. [28], with values of 0.545, 0296, 0.122, 0.905 and 7.636 for PCE, TCE, c-DCE, VC and ETH, respectively.

Since TBOS was the dominant phase in most of the tests, the partition coefficients between the aqueous and TBOS NAPL phases were independently determined for PCE, TCE, *c*-DCE, VC and ETH. TBOS and PCE, TCE or *c*-DCE NAPL mixtures were generated by adding TBOS with a known amount of the neat chlorinated ethylene, and the mixture was added to batch bottles completely filled with anaerobic media (no headspace) under the same conditions as the batch reactor experiments. TBOS and VC or ETH NAPL mixtures were generated by adding TBOS with a known volume of gaseous VC or ETH. The bottles with TBOS NAPL and the chlorinated ethylene or ETH were agitated on a wrist-action shaker at 20 °C for 24 h and allowed to sit quiescently for another 24 h to allow complete phase separation. Aqueous-phase samples were taken and analyzed using GC (Hewlett-Packard model 6890), equipped with a purge-and-trap (HP purge-and-trap concentrator with autosampler), employing the GC method previously described. Dimensionless partition coefficients were determined based on the mass balances between the NAPL and aqueous phase from triplicate batch experiments.

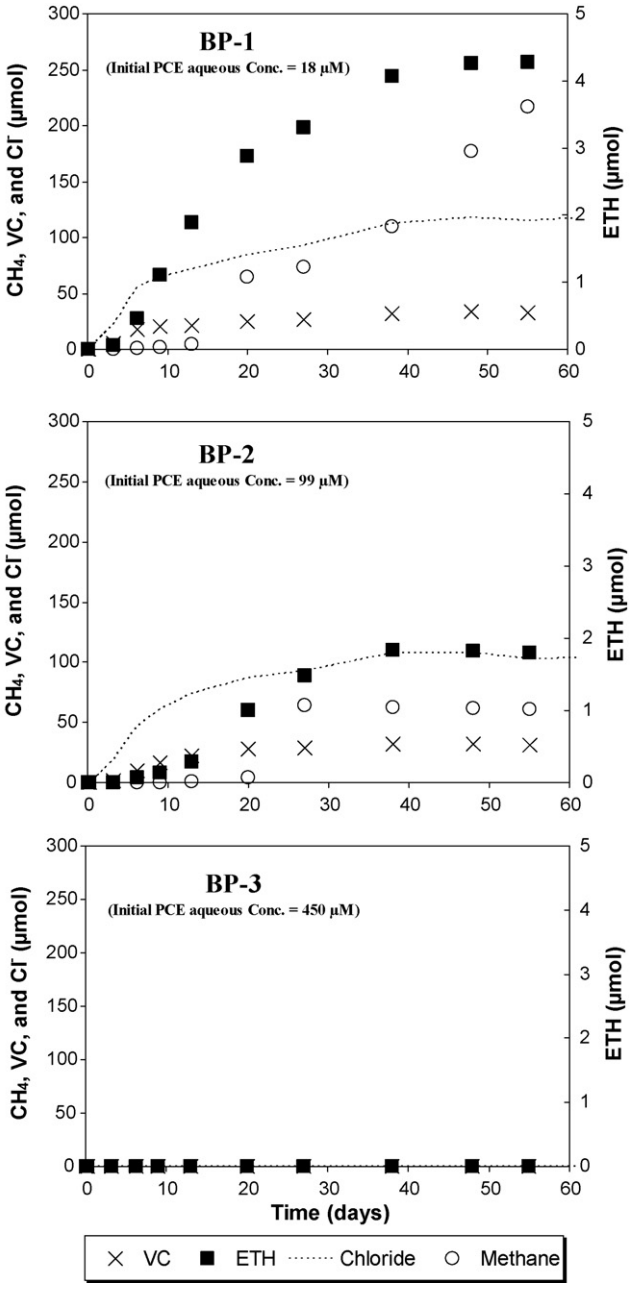

Fig. 1. Effect of PCE aqueous concentrations on reductive dechlorination for the BM culture. Chloride ions were calculated from the concentrations of the dechlorination products. The results show average values in duplicate. The ranges of duplicate values are generally smaller than the size of the symbols.

3. Results

3.1. Partitioning of chlorinated ethylenes between three different phases

Dimensionless aqueous/TBOS NAPL partition coefficients for PCE, TCE, *c*-DCE, VC and ETH, based on triplicate measurements, were 2860, 450, 72, 27 and 1.8, respectively. These experimental values (K_{N-W}^i) showed a similar trend as the octanol–water partition coefficients (K_{OW}^i) of 2510, 263, 72, 42 and 13 reported by Yaws [29]. Since the TBOS was generally greater than 80% of the NAPL in the batch experiments on a mole fraction basis, the partition coefficient should be representative of the experimental conditions for performing mass balances.

Table 2
Electron mass balances of PCE dechlorination mixed with TBOS during incubation of 56 days.

e⁻ equiv.		BP-1 (e⁻ μequiv.)		BP-2 (e⁻ μequiv.)		BP-3 (e⁻ μequiv.)		BP-4 (e⁻ μequiv.)		BP-5 (e⁻ μequiv.)		BP-6 (e⁻ μequiv.)	
		Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56
TBOS[a]	96	20,064	0	20,064	0	20,064	0	5760	0	9312	0	16,224	0
Butanol	24	11,496	4284	13,428	9504	6876	25,956	3492	0	6948	276	11,232	4512
Butyrate	20	10,280	34,680	10,060	31,990	9820	10,820	10,870	15,100	9890	23,630	9820	29,580
Acetate	8	2812	5948	2864	6260	2884	3044	2580	5576	2732	4284	2724	5728
TCE	2	0	0	0	0	0	0	0	0	0	0	0	27
c-DCE	4	0	0	0	0	0	0	0	0	0	0	0	22
VC	6	0	195	0	186	0	0	0	663	0	858	0	384
ETH	8	0	34	0	14	0	0	0	492	0	152	0	26
CH_4	8	0	1736	0	492	0	0	0	440	0	420	0	524
H_2	2	0	0.2	0	2.5	0	4.7	0	2.5	0	1.3	0	0.6
Total		44,652	46,877	46,416	48,449	39,644	39825	22,702	22,274	28,882	29,621	40,000	40,804
e⁻ recovery (%)			105		104		100		98		103		102
e⁻ distribution to dechlorination at day 56 (%)[b]			0.5		0.5		0		5.2		3.4		1.3

e⁻ equiv.		BP-7 (e⁻ μequiv.)		BP-8 (e⁻ μequiv.)		BP-9 (e⁻ μequiv.)		BP-10 (e⁻ μequiv.)		BP-11 (e⁻ μequiv.)	
		Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56
TBOS[a]	96	518	0	1152	0	1411	0	2131	0	4205	0
Butanol	24	143	0	106	0	143	0	367	0	433	0
Butyrate	20	190	0	212	47	137	82	171	206	141	1683
Acetate	8	2819	3278	2751	3510	3027	4100	2817	4118	2694	4582
TCE	2	0	5	0	25	0	13	0	0	0	0
c-DCE	4	0	461	0	292	0	394	0	470	0	617
VC	6	0	398	0	398	0	461	0	696	0	313
ETH	8	0	6	0	3	0	4	0	10	0	5
CH_4	8	0	0	0	0	0	0	0	0	0	0
H_2	2	0	0.2	0	0	0	0.1	0	0.3	0	3
Total		3670	4148	4221	4275	4718	5054	5486	5500	7473	7203
e⁻ recovery (%)			113		101		107		100		96
e⁻ distribution to dechlorination at day 56 (%)[b]			20.9		16.8		17.3		21.4		13.0

The values are represented as average in duplicates.
[a] Based on TBOS hydrolyzed to BuOH, calculated from control bottle for the same period of the experiment.
[b] Calculated based on the ratio of the electrons used for dechlorination to the total electrons present except butanol at day 56.

3.2. Effect of PCE aqueous concentrations on reductive dechlorination

The first set of experiments in the batch reactors; BP-1, BP-2 and BP-3 (Table 1), contained different PCE mol fractions in TBOS (0.02, 0.11 and 0.5) in order to investigate the effect of the PCE aqueous concentration on the reductive dechlorination. The resulting PCE aqueous concentrations were 18, 99 and 450 μM, respectively, consistent with Raoult's Law.

A greater mass of ETH was produced at the lower aqueous PCE concentration (BP-1) compared to those for BP-2 and BP-3 (Fig. 1). The total chloride released, based on the mass balance of transformation products of BP-1, was slightly higher than for BP-2. Essentially, no TCE or *c*-DCE was detected in the BP-1 and BP-2 reactors during the experiments, indicating that the dechlorination of VC to ETH was the slowest dechlorination step, consistent with the kinetic tests reported for the PM and EV cultures [24] and in previous studies [30,31]. No reductive dechlorination of PCE occurred in BP-3 with the highest PCE concentration, and the continuous accumulation of butanol due to TBOS hydrolysis was observed (data not shown). These results indicated that high PCE aqueous concentrations are likely to inhibit the fermentation process of butanol to butyrate and/or acetate producing H_2, as well as the reductive dechlorination of PCE [2,34]. The fermentation of butanol to butyrate and/or acetate with the production and consumption of H_2 was observed in BP-1 and BP-2 (data not shown). After 30 days, the rate of methane production in BP-1 was much higher than in BP-2, while BP-3 showed no methane production. Yang and McCarty [2] and Distefano et al. [32] found high concentrations of PCE were inhibitory to methanogens, which is consistent with the observations in BP-3. The reductive dechlorination activities in BP-1 and BP-2 were observed to slow down and eventually cease after 40 days. The reason for this is not known, but toxicity or inhibition due to the direct exposure to the PCE and TBOS NAPL mixture may have caused the loss of dechlorination activity. The fermentation of butanol to butyrate and/or acetate was extensive in BP-1 and BP-2 (see Table 2), which likely lowered the pH of the reactors, and potentially inhibited dehalogenation activity.

3.3. Effect of PCE mass in NAPL on reductive dechlorination

The inhibition or toxicity resulting from the amount of PCE/TBOS NAPL mixture was also evaluated. This was accomplished using equal PCE mol fractions to yield the same aqueous concentration, but with various masses of the PCE/TBOS mixture. The PCE mol fraction in the PCE/TBOS mixtures was set to 0.11, resulting in a PCE aqueous concentration of 99 μM, but the total volume of the NAPL mixture was increased from 0.57 to 3.30 mL (BP-4, BP-5, BP-6, and BP-2 (Table 1)). The tests were performed in duplicate.

The duplicates showed nearly identical results, therefore average values are plotted in Fig. 2. Good mass balances achieved by applying Eq. (1) and using the measured headspace concentrations, as represented by the total CAH data. PCE (182 μmol) was completely transformed in BP-4 after 40 days, which had the lowest amount of PCE/TBOS NAPL. The amounts of PCE transformed within 40 days in reactors BP-5, BP-6 and BP-2 were 121, 80 and 35 μmol, respectively, i.e. decreasing with increasing amount of PCE/TBOS NAPL. This indicates that the rate of PCE dechlorination was affected

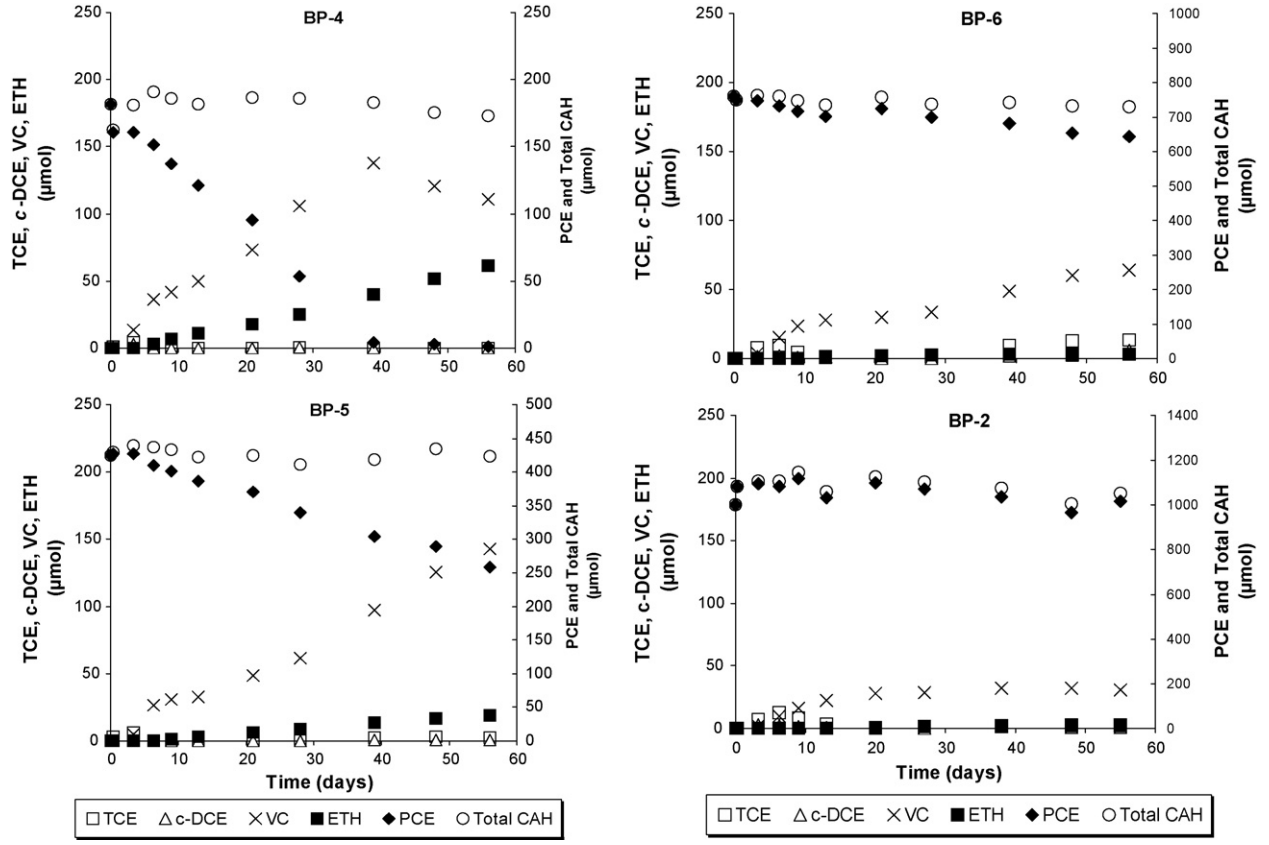

Fig. 2. Effect of constant PCE mol fraction and increased NAPL concentration on reductive dechlorination with the BM culture. The results show average values of the duplicates. The ranges of duplicate values are generally smaller than the size of the symbols. The total CAH and ethylene (open circle) is the mass balance using Eq. (1).

by the mass of PCE or PCE/TBOS NAPL present. Very little accumulation of TCE and *c*-DCE was observed during the incubations (Fig. 2). Higher ETH mass production was observed in the reactors with lower volumes of the PCE/TBOS NAPL mixtures (BP-4 and BP-5); with BP-4 producing three times as much ETH as BP-5. The production of VC in BP-6 was about twice that in BP-2, which had the greatest amount NAPL present.

The chloride ion concentrations calculated from the reductive dechlorination products (TCE, *c*-DCE, VC, and ETH) concentrations and mass balance partitioning between the aqueous, gas and NAPL phases were compared with measured chloride ion concentrations in the aqueous samples (Fig. 3). Very good agreement was achieved between the calculated and measured amounts of Cl^-. The results presented in Figs. 2 and 3 demonstrate that the partitioning model given in Eq. (1) yields good mass balances using the determined NAPL partition coefficients. The results also showed that the amounts and rates of chloride released decreased with increasing amount of PCE/TBOS NAPL.

The dechlorination process was inhibited by the amount of PCE/TBOS NAPL present, which has several possible causes. One possibility is that the direct contact of dechlorinating microorganisms with the NAPL resulted in toxicity, as proposed by Yang and McCarty [2]. Another possibility is the reduction in pH in the batch reactors. At the end of the incubations (55 days), the pH was less than 5 in all four reactors, below the optimal pH range of 6.8–7.8 for dechlorinating microorganisms [35]. The acetate and butyrate, as fermentation products along with H^+ liberated from the dechlorination reactions exceeded the buffering capacity of the 700 µmol of Na_2CO_3 added to the reactors [33]. The highest concentration of PCE/TBOS NAPL released the most butanol via abiotic hydrolysis, followed by the fermentation of butanol to butyrate and/or acetate, with the production of H_2. It was interesting to note that, after 40 days, once the PCE NAPL had been completely utilized in BP-4, the rates of dechlorination decreased. This was likely to be due to the slow rate of VC transformation, consistent with the low k_{max} and high K_S values for VC [24]. It was also interesting to note the greatest accumulation of butyrate and H_2 occurred in the reactors with the poorest dechlorination, i.e. BP-6 and BP-2 (data not shown). Electron balances presented in Table 2 show a greater production of butyrate and acetate resulting from the fermentation of the butanol

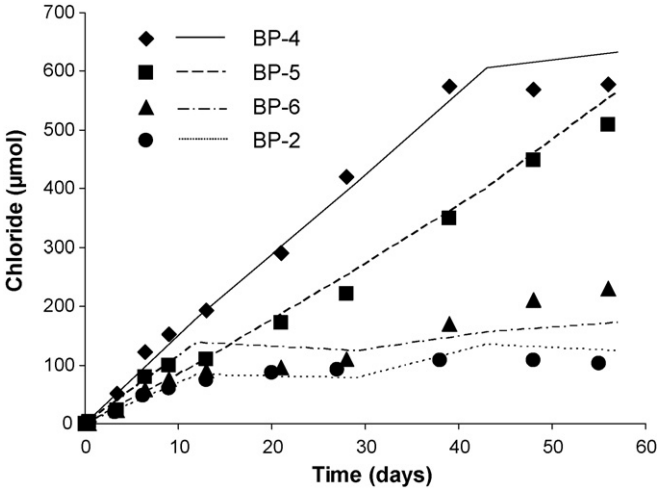

Fig. 3. Total reductive dechlorination activity based on chloride ion released as calculated from dechlorination products, and chloride ion measured from aqueous samples in the batch reactors. Calculated from dechlorination products (♦, ■, ▲, and ●) and ion chromatography measured from aqueous samples (solid and dashed lines). The results show average values of duplicates.

released by hydrolysis. Thus, there was greater potential for developing a lower pH in BP-6 and BP-2, which may have affected the rates of transformation.

3.4. Effect of TBOS amounts on reductive dechlorination

Controlling the rate of TBOS hydrolysis to yield butanol as a fermenting substrate may be a means of achieving effective substrate utilization for dehalogenation [17]. A series of tests were performed where PCE (200 μmol) was mixed with different amounts of TBOS (200, 467, 667, 1333 and 2000 μmol in BP-7, BP-8, BP-9, BP-10 and BP-11, respectively) in order to correlate the rates of hydrolysis with the efficiency of the PCE dechlorination.

Fig. 4 shows that BP-10, with the lowest NAPL volume, gave the best overall dechlorination, as based on the release of chloride ions. No significant differences were observed between BP-7, BP-8, BP-9 and BP-11. No limitations in the production of hydrogen occurred in any of the batch reactors, as the concentrations remained higher than 5–10 nM at all times in each reactor (data not shown). These hydrogen concentrations were higher than the hydrogen threshold of the dechlorinators (0.3–2 nM) reported by Löffler et al. [36] and Yang and McCarty [26]. However, no methane was produced during the course of the experiments, which was likely due to the inhibition of PCE to methanogenesis [2,31]. BP-11, which had the greatest amount of TBOS in this experimental set, showed a decreased dechlorination rate at around 30 days (data not shown), presumably due to the lower pH resulting from the higher concentrations of the fermentation products butyrate and acetate. These results show that the best dechlorination activity was achieved with PCE mol fractions from 0.09 to 0.13 in reactors with lower amounts of TBOS.

3.5. Comparison of electron mass balances

Electron mass balances were conducted for the electron donors and acceptors. Table 2 gives the overall electron equivalence mass balances for the biologically active batch reactors (BP-1 to BP-11) on days 0 and 56. Good electron equivalence mass balances were obtained, with electron recoveries varying from 96 to 113%. The electron mass balances were calculated based on the hydrolyzed butanol, fermentation products, CH_4, H_2 and amount of dechlorination. Most of the electron flow in BP-1 to BP-6 was channeled into the formation of butyrate from hydrolyzed butanol, and to a lesser extent into the creation of acetate. These results indicate that the fermentation of butanol to butyrate dominated, compared to the fermentation of butyrate to acetate, which might be explained by the standard free energy change (ΔG° = 16.3 kJ/mol for the fermentation of butanol to butyrate with H_2 production, compared to 48.1 kJ/mol for butyrate to acetate) [37]. However, in BP-7 to BP-11, butyrate was almost not detected, indicating that the butyrate produced from the hydrolyzed butanol was rapidly fermented to acetate. These reactors also had the highest electron distribution to dechlorination, which was associated with several effects, such as the comparably lower PCE and/or TBOS masses. The dechlorination reactions, which utilized hydrogen, were likely to help drive the fermentation reactions of butyrate to acetate.

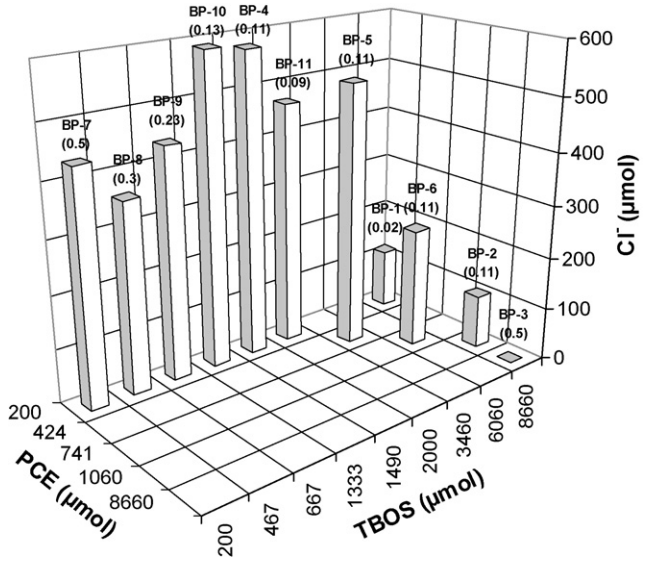

Fig. 4. Total dechlorination as chloride release calculated from dechlorination daughter products and partitioning mass balances for different PCE/TBOS NAPL conditions. PCE mol fractions in NAPL mixtures are represented in parentheses.

The electron distribution to dechlorination was calculated based on the ratio of the electrons used for dechlorination to the total electrons present at day 56. The distributions ranged from 0 (BP-3) to 21.4% (BP-10). For BP-1 to BP-6, the distributions ranged from 0 to 5.2%. With these high NAPL concentration conditions, BP-4 had the highest distribution to dechlorination of 5.2%, which was associated with the lower PCE/TBOS NAPL volume present. For BP-7 to BP-11, the distributions ranged from 13 to 21.4%. These reactors contained lower amounts of TBOS NAPL. BP-10 showed the highest electron distribution of dechlorination (21.4%), with the highest total dechlorination to chloride, as calculated from the dechlorination products.

3.6. Electron distribution to dechlorination and hydrolysis rates of TBOS

The effect of the rates of TBOS hydrolysis on the electron distribution to dechlorination was evaluated in more detail. The abiotic hydrolysis rate of TBOS was determined based on the production of butanol in the control bottles (Table 1). Fig. 5(a) shows the rates of TBOS hydrolysis as a function of the TBOS NAPL concentration. The rates of TBOS hydrolysis were found to follow first-order kinetics with respect to the TBOS concentration, with a rate coefficient (k) of 4.6×10^{-4} day^{-1}. The hydrolysis of NAPL TBOS was likely governed by direct hydrolysis on the surface of TBOS droplets [38]. Accordingly, the increase in the rates of hydrolysis with the higher TBOS concentrations likely resulted from the greater TBOS NAPL surface area [17].

Fig. 5(b) shows the electron distribution to dechlorination and the total amount of dechlorination as a function of the rate of TBOS hydrolysis. Lower rates of TBOS hydrolysis resulted in lower rates of butanol production and slower rates of fermentation. Higher electron distributions to dechlorination (electron transfer to dechlorination reactions) were obtained with lower rates of TBOS hydrolysis. However, the greatest amount of dechlorination was observed with rates of TBOS hydrolysis between 3 and 14 μM/day. The lowest rates of TBOS hydrolysis may have limited the electron donors available for reductive dechlorination, despite the high electron distribution to dechlorination. The rates of TBOS hydrolysis are important in achieving effective electron transfer efficiencies to dechlorination, with the highest dechlorination efficiencies associated with the lowest rates of TBOS hydrolysis.

4. Discussion

TBOS was shown to be an effective slow-release substrate for the promotion of the dechlorination of PCE DNAPL. TBOS might be applied as a substrate for the remediation of CAH DNAPL contamination and to enhance the rates of reductive dechlorination. TBOS injected into the DNAPL zone and co-mixing with the NAPL would

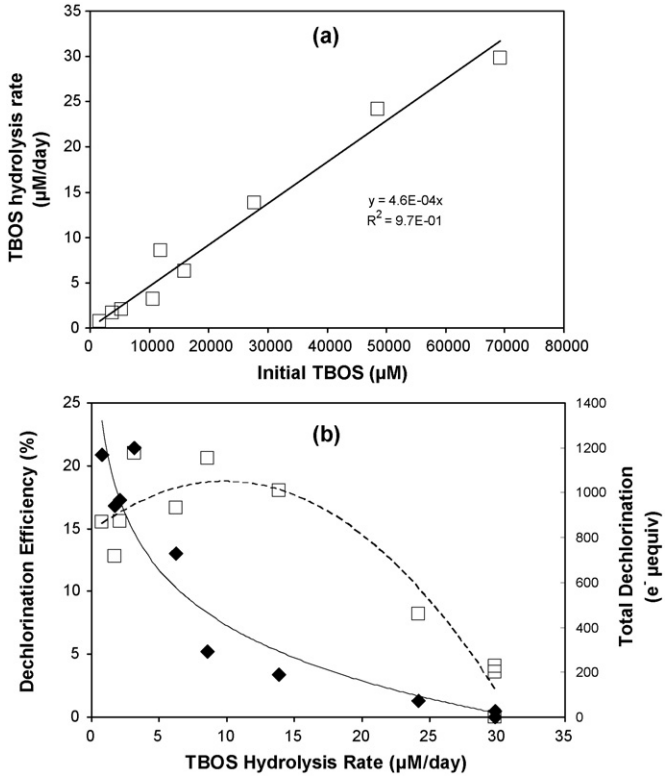

Fig. 5. (a) Hydrolysis rates of TBOS over a wide range of TBOS NAPL concentrations, (b) electron distribution to dechlorination as dechlorination efficiency (♦) and total dechlorination (□) at different hydrolysis rates of TBOS. The average of duplicates is shown.

reduce the aqueous concentrations of PCE and TCE, and potentially decrease the toxicity or inhibition, which could enhance the overall rates of reductive dechlorination [17].

Fig. 4 summarizes the total chloride released from the dechlorination product mass balances as a function of the amounts of PCE and TBOS. Generally, the volumes or amounts of PCE and TBOS NAPL are key factors affecting the reductive dechlorination of PCE DNAPL. Lower degrees of reductive dechlorination were associated with higher amounts of PCE and/or TBOS. Additionally, at given PCE mol fractions of 0.11 (BP-4, BP-5, BP-6 and BP-2) and 0.5 (BP-3 and BP-7), the dechlorination of PCE decreased with increasing amounts of PCE and TBOS. Greater amounts of PCE/TBOS NAPL are potentially toxic to dechlorinating microorganisms. This toxicity can result from the direct contact of the NAPL with the microorganisms in these well shaken batch reactors. The lack of fermentation and dehalogenation reactions in BP-3, which had the highest NAPL concentration, indicates the potential toxicity to fermenting microorganisms as well. PCE mol fractions are also an important factor in promoting better reductive dechlorination of PCE DNAPL. The greatest release of chloride ions was obtained with PCE mol fractions of around 0.1–0.13 (BP-4 and BP-10), and dechlorination ability decreased on decreasing the PCE mol fraction to 0.02. As shown in Fig. 5(b), high dechlorination efficiencies were associated with the lowest rates of hydrolysis, which were associated with the lowest TBOS NAPL concentration. The lower rates of hydrolysis were also associated with lower production of butyrate and/or acetate (Table 2), which would result in less reduction in pH.

NAPL toxicity and pH decreases encountered in batch systems might be overcome in a groundwater aquifer setting with a continuous flow. First, the possibility of continuous direct contact of the PCE NAPL with dechlorinating bacteria would be lessened in subsurface systems. Second, the dilution effects in flow-through systems would likely prevent the accumulation of fatty acids, which might limit the decrease in the pH. Overall, the results obtained herein indicate that the application of TBOS as a slow-release substrate has potential for the remediation of CAH DNAPL contamination as well as aqueous CAHs. TBOS could be added to a DNAPL zone to create a reactive barrier system [13]. The butanol released due to the hydrolysis of TBOS was found to be mainly fermented to butyrate. Butanol, which has a high aqueous solubility (60–80 g/L), may be beneficial in supporting the reductive dechlorination of high CAH concentrations, since the fermentation of butanol could possibly maintain high hydrogen tensions. In addition, the butyrate produced is a slow fermenting fatty acid [39], and would be effective for promoting reductive dechlorination in the down-gradient plume containing chlorinated products.

The binary mixed culture, composed of two mixed cultures, was capable of transforming NAPL PCE to ETH. However, microbial growth will vary spatially. To better understand these interactions in a groundwater flow system, continuous flow column studies are required, with molecular techniques applied to characterize the microbial communities that develop spatially. The development of flow and transport models, including hydrolysis, fermentation, dechlorination reactions and the partitioning processes, is also required for the better engineer of slow-release substrate remediation systems.

5. Conclusions

- TBOS was an effective slow-release substrate for remediating PCE DNAPL contamination and enhancing the rates of transformation.
- Direct measurements of the chloride released and CAH mass balances, including partitioning between the three phases, were in good agreement.
- The reductive dechlorination of PCE was affected by the aqueous PCE concentrations, the amount of PCE/TBOS NAPL and the PCE/TBOS mol fractions.
- The highest dechlorination efficiencies were associated with the lowest rates of hydrolysis, which were associated with the lowest TBOS concentration.
- High rates of hydrolysis to butanol, which is easily fermented to acid products, results in the lowering of pH which would inhibit microbial processes.

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