Influence of Hydraulic Retention Time on Extent of PCE Dechlorination and Preliminary Characterization of the Enrichment Culture

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Abstract: The extent of tetrachloroethene (PCE) dechlorination in two chemostats was evaluated as a function of hydraulic retention time (HRT). The inoculum of these chemostats was from an upflow anaerobic sludge blanket (UASB) reactor that rapidly converts PCE to vinyl chloride (VC) and ethene. When the HRT was 2.9 days, PCE was converted only to *cis*-dichloroethene (cDCE). When the HRT was 11 days, the end products were VC and ethene. Further studies showed that the dechlorinating microbial community in the UASB reactor contained two distinct populations, one of which converted PCE to cDCE and the other cDCE to VC and ethene. Methanogenic activity was very low in these cultures. The cDCE dechlorinating culture apparently has a lower growth rate than the PCE dechlorinating culture, and as a result, at a shorter HRT, the cDCE dechlorinating culture was washed out from the system leading to incomplete dechlorination of PCE. Both enrichment cultures used pyruvate or hydrogen as electron donors for dechlorination. Acetate was the carbon source (but not energy source) when hydrogen was used. Both cultures had undefined nutrient requirements and needed supplements of cell extract obtained from the mixed culture in the UASB reactor. However, the two cultures were different in their response to the addition of an inhibitor of methanogenesis (2-bromoethanesulfonate [BES]). BES inhibited the dechlorinating activity of the enriched cDCE dechlorinating culture, but had no influence on the PCE dechlorinating culture. Preliminary studies on BES inhibition are presented.

Introduction

The prevalence of tetrachloroethene (PCE) and trichloroethene (TCE) in the environment has driven the need to develop remediation strategies capable of effecting the complete removal of these compounds. One such strategy, anaerobic in situ bioremediation, has shown promise as a remediation process based on cost, the potential for complete transformation of contaminants to a nontoxic species (i.e., ethene) (Bradley and Chapelle, 1996; de Bruin et al., 1992; Freedman and Gossett, 1989; Komatsu et al., 1994), and recent evidence has demonstrated microbially enhanced removal of chlorinated ethenes-containing nonaqueous phase liquids (Carr et al., 2000).

Anaerobic microbial transformation of chlorinated ethenes occurs through dechlorination, a process that is either cometabolic (Damborsky, 1999; El Fantroussi et al. 1998; Fathepure and Boyd, 1988a; 1988b; Fathepure et al. 1987; Middeldorp et al. 1999) or respiratory (Gerritse et al., 1996; Holliger et al., 1998; Holliger et al., 1993; Krumholz, 1997; Krumholz et al., 1996; Maymo-Gatell et al., 1997; Neumann and Scholz-Muramatsu, 1994; Scholz-Muramatsu et al., 1995; Wild et al., 1996). Because PCE- and TCE-halorespiring organisms exhibit dechlorination rates orders of magnitude greater than that of cometabolism (Eisenbeis et al., 1997), much interest has developed in isolating, characterizing, and exploiting the metabolic capabilities of these organisms. To date, six PCE-

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halorespiring bacteria have been isolated. Only one organism, *Dehalococcoides ethenogenes*, can dechlorinate PCE completely to ethene (Maymo-Gatell et al., 1997). The other isolates dechlorinate PCE to TCE (Gerritse et al., 1996) or cDCE (Holliger et al., 1998; Krumholz, 1997; Krumholz et al., 1996; Neumann and Scholz-Muramatsu, 1994; Scholz-Muramatsu et al., 1995; Wild et al., 1996). In many contaminated sites, *cis*-dichloroethene (cDCE) has been observed to be the principle end product of PCE degradation (Lee et al., 1997; Wiedemeier et al., 1999). Incomplete dechlorination could jeopardize the application of natural attenuation and anaerobic in situ bioremediation of PCE due to the accumulation of suspected carcinogen cDCE or the known carcinogen VC. To further the use of dechlorination in the field, more information on factors that control the rate and extent of chlorinated ethenes halorespiration is needed.

The objective of this research was to study the influence of hydraulic retention time (HRT) on the extent of dechlorination by a microbial community in a upflow anaerobic sludge blanket (UASB) reactor maintained in our laboratory for over 5 years (Carr and Hughes, 1998). High rates of PCE dechlorination indicated that dechlorination by this microbial community was occurring via halorespiration. Efforts were made to isolate PCE and cDCE dechlorinators through serial dilutions in liquid medium. Two highly purified enrichment cultures were developed. Information gained on the nutritional requirements of this culture is presented. Also, information is presented that demonstrates the negative impact that inhibiting the methanogenic activity of the cultures (using 2-bromoethanesulfonate [BES]) on dechlorination activities of the highly purified enrichment cultures. This negative impact of BES on the activity of dechlorination served to significantly complicate the ability to isolate the dechlorinating organisms from the methanogenic mixed culture

Materials and Methods

Chemicals

Liquid compounds used in this study include tetrachloroethene (99+%; Acros), trichloroethene (99.5%; Aldrich); *cis*-dichloroethene (97%; Acros); 2-bromoethanol (97%; Acros), and *n*-hexadacane (99%, Sigma). Mixed gases used in this study include vinyl chloride (8% in N_2; Matheson Gas Products), 1% CH_4, CO, CO_2, C_2H_2, C_2H_4, and C_2H_6 in N_2 (Supelco), N_2/CO_2 (90/10%, TriGas), and H_2/CO_2 (80/20%, TriGas). Sodium 2-Bromoethanesulfonate (98%) was from Acros, and sodium 2-mercaptoethanesulfonate (≥ 98%) was Fluka.

Medium. The nutrient medium contained (mg/L) KCl, 400; $MgCl_2 \cdot 6H_2O$, 400; NH_4Cl, 400; KH_2PO_4, 140; $CaCl_2 \cdot 2H_2O$, 25; $NaHCO_3$, 7500; $Na_2S \cdot 6H_2O$, 240; $FeCl_2 \cdot 4H_2O$, 15; $ZnCl_2$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.5; H_3BO_3, 0.5; $CoCl_2 \cdot 6H_2O$, 2.5; $NiCl_2 \cdot 6H_2O$, 0.5; $Na_2MoO_4 \cdot 2H_2O$, 0.5; $(NaPO)_{16}$, 10; KI, 2.5; NH_4VO_3, 0.5; and vitamins (μg/L) 4-aminobenzoic acid, 0.04; D(+)-biotin, 0.01; nicotinic acid, 0.1; Ca-D(+)-pantothenate, 0.05; pyridoxamine dihydrochloride, 0.15; thiamine hydrochloride, 0.1; and cyanocobalamin, 0.05. All the chemicals were reagent grade. The medium had an adjusted pH of 7.0. Substrates were 20 mM pyruvate or H_2/CO_2 (80/20%) and 2 mM acetate. Filter-sterilized cell extract (FSCE, see below) at 1% (w/v) or filter-sterilized spent medium (FSSE, see below) at 1% (v/v) was also added to the nutrient medium.

Analytical Method

Gas chromatography was used to quantify methane, ethene, and chlorinated compounds. Headspace samples (100 μL) were injected onto a gas chromatograph (GC) (Hewlett-Packard) equipped with a packed column (6 ft. × 1/8 in outer diameter [OD], 60/80 Carbopack B/1% SP-1000, Supelco), and a flame ionization detector (FID). GC operating parameters were as described previously (Carr et al., 2000). Standards were prepared by adding methanol-dissolved PCE, TCE, or cDCE and known volumes of VC, methane, and ethene to anaerobic culture tubes (27 mL) that contained 10 mL fresh medium and 100 μL hexadecane (resembling the cultivation conditions of the enrichment). The tubes were capped with Teflon™-lined butyl rubber septa and aluminum crimp caps.

Bromide concentration in the aqueous phase was quantified using an ion chromatograph (IC) (Dionex) equipped with an IonPac AS14 analytical column (4 × 250 mm, Dionex). The detection of ions was obtained by suppressed conductivity at 10 SFS ASRS-II‘ (Dionex). The IC operation parameters were as the manufacturer recommended (eluent, 3.5 mM Na_2CO_3/1 mM $NaHCO_3$; flow rate, 1.2 mL/min, and injection volume, 10 μL).

Inoculum for Chemostat and Enriched Cultures

The inoculum for chemostats and enrichment cultures used in this study was obtained from a methanogenic UASB reactor fed methanol and PCE for over 5 years (Carr and Hughes, 1998). The feed of the reactor contained 52 m*M* methanol, 0.52 m*M* (86 mg/L) PCE, 0.2g/L yeast extract, and the nutrient medium. PCE was converted to VC (ca. 80% in molar) and ethene (ca. 20% in molar).

Chemostats

Two continuously fed complete mixed reactors were established. The feed for the reactors was the identical to the UASB reactor, including PCE at a concentration of 0.52 mM (86 mg/L), and both reactors were operated at room temperature (22°C). The hydraulic volume of Reactor A was 1.3 L and for Reactor B, 0.3 L. Feed was added to each reactor using a syringe pump at the rate of 0.072 mL/min, resulting in an HRT of 11 days for Reactor A and 2.9 days for Reactor B. After 80 days of operation (Phase I), the HRT for both reactors was changed to 5.8 days by changing the feeding rate to 0.036 mL/min and reducing the volume of Reactor A to 0.3 L. The reactors were operated for another 92 days (Phase II).

Batch Test

Batch tests were used to verify the ability of the microbial community in each chemostat to dechlorinate PCE, TCE, cDCE, and VC. Effluent was collected from each reactor and chlorinated ethenes were removed by sparging with N_2/CO_2 (90/10%). Effluent subsamples (4 mL) were then distributed to anaerobic test tubes (27 mL) containing fresh medium (0.5 mL). Methanol (2 μL) containing PCE, TCE, or cDCE was added to individual test tubes, making the final aqueous concentration equal to 0.47 mM for the corresponding compounds. VC was added as gas (8%). The test tubes were put on a shaker table at room temperature for a total of 16 days. Dechlorination products were quantified by GC.

Preparation of FSSE and FSCE

To prepare FSSE, the effluent from the UASB reactor was collected and sparged with N_2/CO_2 (90/10%) to remove dechlorination products. The effluent was then adjusted to pH 7.0 and filtered through 0.2-μm filter. To prepare FSCE, biomass was collected from the UASB reactor. The cell pellets were washed three times with fresh medium. After washing, the cell pellets were weighed and resuspended in fresh medium to a final concentration of 10% (w/v). The mixtures were then homogenized in a tissue grinder, followed by cell disruption using ultrasonication and French Press (1000 to 1500 psig, SLM Aminco). The mixture was centrifuged (30,000 × g, 5 min, 4°C) and the supernatant was stored at –20°C. The supernatant was thawed and filtered through 0.2–mm filter before use.

Similarly, FSSE and FSCE were prepared from a sample of digested sludge obtained from an anaerobic municipal sewage sludge digester (MSSD) in Urbana, IL. The procedure used for MSSD FSCE and MSSD FSCE were identical to those presented previously, except for the origin of the initial material.

PCE/Hexadacane, PCE/Tridecane, TCE/Hexadacane, cDCE/Hexadacane Mixtures

Filter sterilized (0.2 μm) PCE, TCE, or cDCE was added to hexadecane or tridecane that had been autoclaved for one hour at 121°C (15 psi). The final concentrations for PCE and TCE were 0.46 and 0.08 M, respectively. The final concentration of cDCE was gradually increased from 0.04 M to 0.4 M, in order to inhibit methanogens in the enriched culture.

Enrichment Method

Two highly enriched cultures were obtained through serial liquid dilutions using PCE or cDCE as the electron acceptor. Effluent from the UASB was serially diluted in sterile anaerobic nutrient medium containing 2 mM acetate and 100 μL PCE/tridecane or cDCE/hexadecane. Dilutions were conducted using either pyruvate (20 mM final concentration) or hydrogen as electron donor and were prepared in anaerobic culture tubes (Bellco) closed with Viton stoppers (Wheaton) and aluminum crimp caps. Before capping, the culture tube headspace was replaced with sterile H_2/CO_2 or N_2/CO_2 (pyruvate). Culture tubes were stored on a shaker table (150 rpm) at room temperature (25°C). Headspace samples were periodically analyzed by GC for dechlorination end products, and once active dechlorination was observed, serial dilutions were repeated using the highest dilution demonstrating dechlorination activity.

Dechlorination in the Presence and Absence of FCSE or FSSE

The dependency of dechlorination activity on the presence of FSCE or FSSE was evaluated in anaerobic culture tubes containing the highly enriched cultures and pyruvate (20 mM) or H_2/CO_2 as electron donor. Cultures were incubated on a shaker table at 22°C. PCE or cDCE and dechlorination end products were quantified by GC.

Carbon Source in Hydrogen-Fed Cultures

The purified enrichment culture grown on hydrogen was tested to determine whether the addition of acetate was necessary for dechlorination. The culture was transferred (10% dilution) either into anaerobic nutrient medium containing 2 mM acetate or into medium without acetate. Dechlorination was monitored by GC.

Results

Influence of HRT on Dechlorination Extent

Table 1 compares the extent of PCE dechlorination in the two chemostats at different HRTs. During Phase I, Reactor A (HRT = 11 days) converted PCE to mainly VC (over 85%), some cDCE, and ethene. Reactor B (HRT = 2.9 days), one the other hand, converted PCE to mainly cDCE (over 85%) and some VC. During Phase 2 when the HRT was changed to 5.9 days for both reactors, the makeup of dechlorination end products also changed. For both reactors, PCE was converted to mainly cDCE (about 70%) and some VC.

Dechlorinating Abilities of the Microbial Community

Batch tests were conducted at the end of each phase of chemostat operation to evaluate the dechlorinating abilities of the microbial community in each reactor. The results are summarized in Table 2. The test demonstrated that the microbial community in Reactor A during Phase I (HRT = 11 days) had the abilities to dechlorinate PCE, TCE, and cDCE. On the contrary, in Reactor B with a shorter HRT, the microbial community could only dechlorinate PCE and TCE. When its HRT increased to 5.9 days, this community regained its ability to dechlorinate cDCE.

Enrichment

Serial dilutions were conducted over a period of several months with hydrogen or pyruvate as electron donor in an attempt to isolate PCE-dechlorinating bacteria. Within the first four transfers, it became evident that transference of dechlorination activity was dependent upon the addition of FSCE. Filter-sterilized spent medium (10% v/v) from the packed column could not replace the FSCE, nor could autoclaved cell extract (1% w/v), yeast extract (0.2% v/v), or bacto-peptone (0.2% w/v). Loss of cDCE dechlorination was also observed in the first dilution series (10,000-fold dilution). After this point, the culture consistently dechlorinated PCE or TCE to cDCE. No differences were observed between the hydrogen- and pyruvate-fed series with regard to dechlorination activity or morphologies of organisms present (based on microscopic analysis). Two dominant morphologies were present: curved, motile rods (3 to 4 μm long, 0.5 to 1 μm wide), and cocci (1 to 1.5 μm in diameter) that were often observed singularly, in pairs, or in clusters.

Because the original inoculum had the ability to convert PCE to VC (about 80% molar based) and ethene (20%), it is likely that, in the microbial community, there was another group of bacteria that can convert cDCE to VC and ethene. A second culture was enriched on cDCE using hydrogen or pyruvate as electron donor. The enriched cDCE-dechlorinating culture converted cDCE to about 80% VC and 20% ethene. The culture could also dechlorinate TCE, but not PCE. Under the microscope, slightly curved and motile rods (1 to 2 μm long, about 0.5 μm wide) were found to be dominant.

Carbon Source in Hydrogen-Fed Cultures

In the absence of 2 mM acetate, dechlorination in the hydrogen-fed PCE dechlorinating culture was found to lag over a 16-day period (when compared with tubes containing acetate). The addition of acetate to the former tubes resulted in a marked increase in dechlorination activity (mass of TCE and cDCE doubled within a period of 48 hs). Subsequent transfers demonstrated continued increases in dechlorinating activity in the presence of acetate. Acetate was considered a carbon source rather than electron donor because control tubes grown in the absence of hydrogen with acetate and

Table 1. Dechlorination extent observed in chemostats in Phase I and Phase II

	PCE (molar %)	TCE (molar %)	cDCE (molar %)	VC (molar %)	Ethene (molar %)
Phase I					
Reactor A	N.D.[a]	N.D.	4.8 ± 4.9[b]	86 .± 5.6	8.9 ± 3.6
Reactor B	N.D	0.8 ± 1.00	87 ± 2.7	11 ± 2.3	N.D.
Phase II					
Reactor A	N.D.	1.1 ± 1.1	69 ± 4.6	30. ± 4.9	0.1 ± 0.2
Reactor B	N.D.	0.7 ± 1.5	68 ± 4.2	31 .± 4.9	0.2 ± 0.4

[a] N.D. not detected.
[b] Average and standard deviation of measurements for over 40 days.
[c] The mass of PCE in the feed (0.5 mM) was used to calculate the reported mass balance.

Table 2. Dechlorinating abilities of microbial communities in Reactor A and B at the end of Phases I and II

	Electron acceptor added	Electron acceptor and dechlorination products (molar %)				
		PCE	TCE	cDCE	VC	Ethene
Phase I						
Reactor A	PCE	0.0	0.0	5	95	0.0
	TCE		0.0	67	34	0.0
	cDCE			50	50.	0.0
	VC				100.	0.0
Reactor B	PCE	0.0	47	53	0.0	0.0
	TCE		12	88	0.0	0.0
	cDCE			100	0.0	0.0
	VC				100	0.0
Phase II						
Reactor A	PCE	0.0	11	75	14	0.0
	TCE		24	60	14	0.0
	cDCE			85	15	0.0
	VC				100	0.0
Reactor B	PCE	0.0	0.0	77	23	0.0
	TCE		3.6	80	17	0.0
	cDCE			78	20	1.8
	VC				100	0.0

FSCE present (see following section) would not dechlorinate.

Similarly, the cDCE-dechlorinating culture showed very low dechlorinating activity when hydrogen was eliminated from feed that contained acetate (Figure 1a). In the presence of hydrogen, the deletion of acetate did, however, delay cDCE dechlorination (Figure 1b).

Dechlorination in the Presence and Absence of FSCE

Results from an experiment in which the enriched PCE-dechlorinating culture was grown in the presence and absence of FSCE using pyruvate as the electron donor are shown in Figure 2. In the absence of FSCE, dechlorination was not observed for a period of 16 days. No trace TCE or cDCE was formed during this time. Cultures containing FSCE started dechlorinating after a 1-day lag phase. TCE production in these cultures was minimal, and the majority of the reduced PCE was present as cDCE.

Similarly, the cDCE-dechlorinating culture also required cell extracts from the UASB reactor (Figure 3). Cell extract prepared from anaerobic municipal digested sewage (MSSD FSCE) sludge was found to be able to support dechlorination of this culture, but to a lesser extent (Figure 3). FSSE from the UASB reactor could not support the growth.

Inhibition of cDCE Dechlorination by BES

The two enriched cultures were different in their response to BES inhibition. BES (10 mM) was used to inhibit methanogens during the enrichment processes for PCE and cDCE dechlorinating cultures. It was found that BES delayed dechlorination of the cDCE-dechlorinating culture, but not the PCE-dechlorinating culture. This is presented in Figure 4 where hydrogen was the electron donor. Methanogenesis was low in the cDCE-dechlorinating culture after several months of transfer. In the absence of BES, methane production was at least two orders of magnitude lower than those of dechlorination products. Therefore, it is believed that methanogens were not responsible for cDCE dechlorination in this culture. BES (10 mM) also inhibited TCE dechlorination by this culture.

Compounds (CoM and BEOH) with structures similar to BES were tested for their influence on cDCE dechlorination. BEOH inhibited the dechlorination of cDCE in both H2/acetate- and pyruvate-fed cultures (Figure 5). On the other hand, CoM did not exhibit any inhibition (Figure 5). Unlike methanogenesis of which BES inhibition could be relieved by CoM, no dechlorination was observed when CoM was added to cultures that were inhibited by BES (Figure 5). No production of bromide was detected after one month of incubation in the BEOH- or BES-inhibited cultures.

Discussion

Complete anaerobic dechlorination of PCE involves four reductive steps, each of which replaces one chloride with hydrogen. A study on dechlorinating enzymes in *Dehalococcoides ethenogenes* 195 (Maymo-

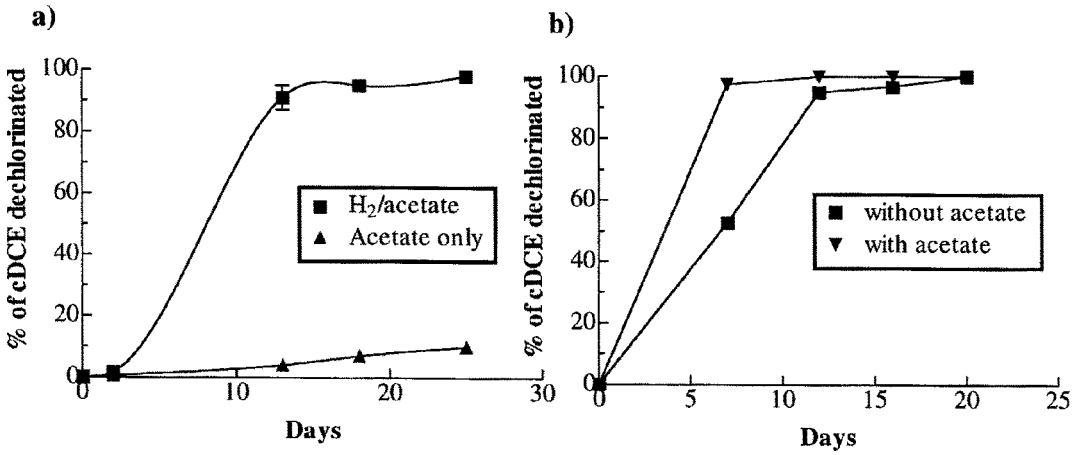

Figure 1. Influence of hydrogen (a) and acetate (b) on cDCE dechlorination. Data in (a) are averages of duplicates and error bars show the standard deviations.

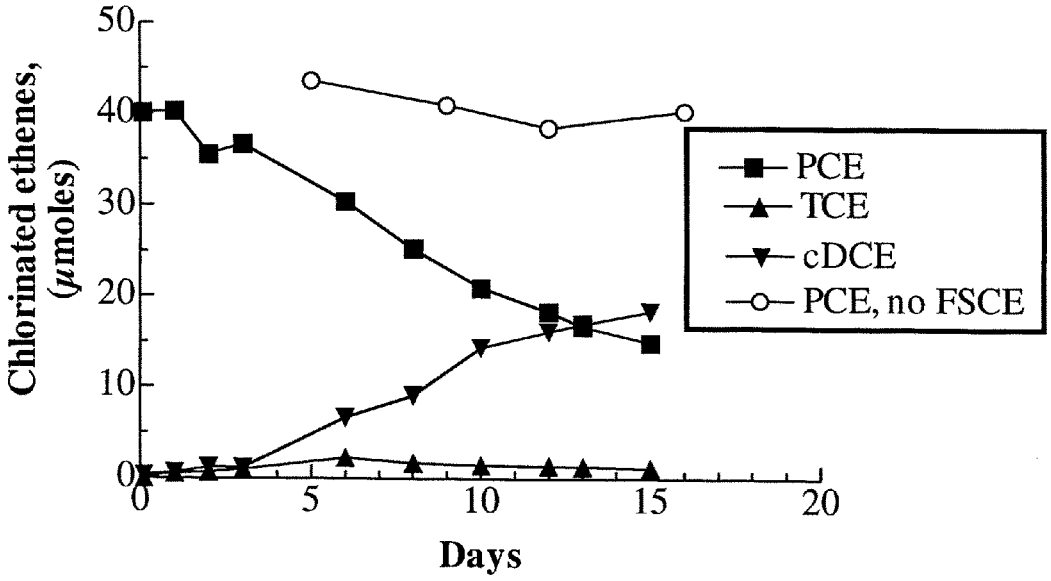

Figure 2. PCE dechlorination in the absence and presence of FSCE with pyruvate as the electron donor. Solid symbols represent data taken from cultures in which FSCE was added, and hollow symbols represent data taken from cultures with no FSCE. In both cases, data shown are averages from duplicate sets of tubes.

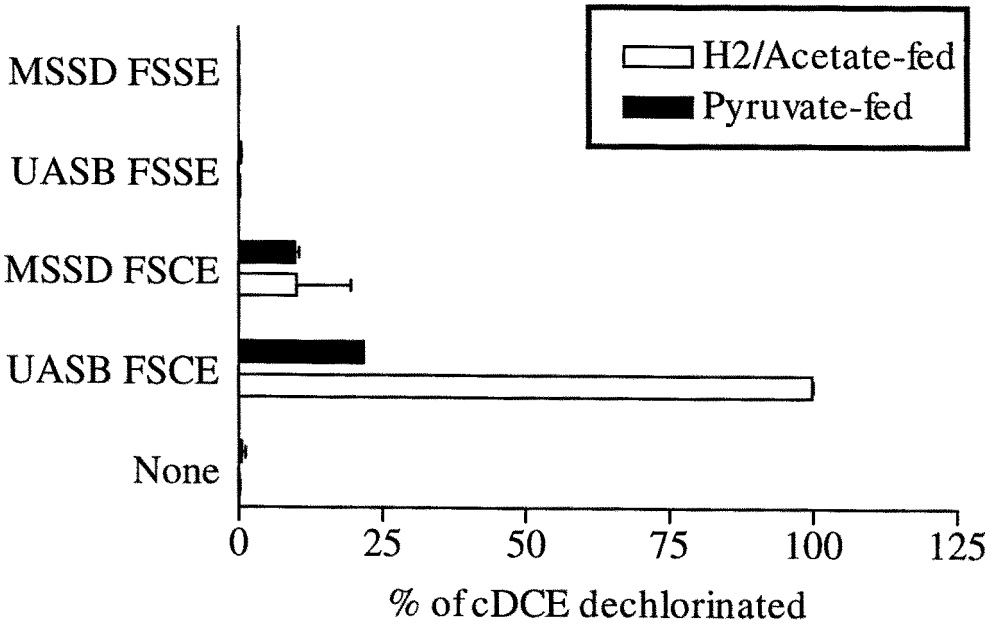

Figure 3. cDCE dechlorination in the absence and presence of FSCE and FSSE. Data are averages of duplicate and error bars show the standard deviations.

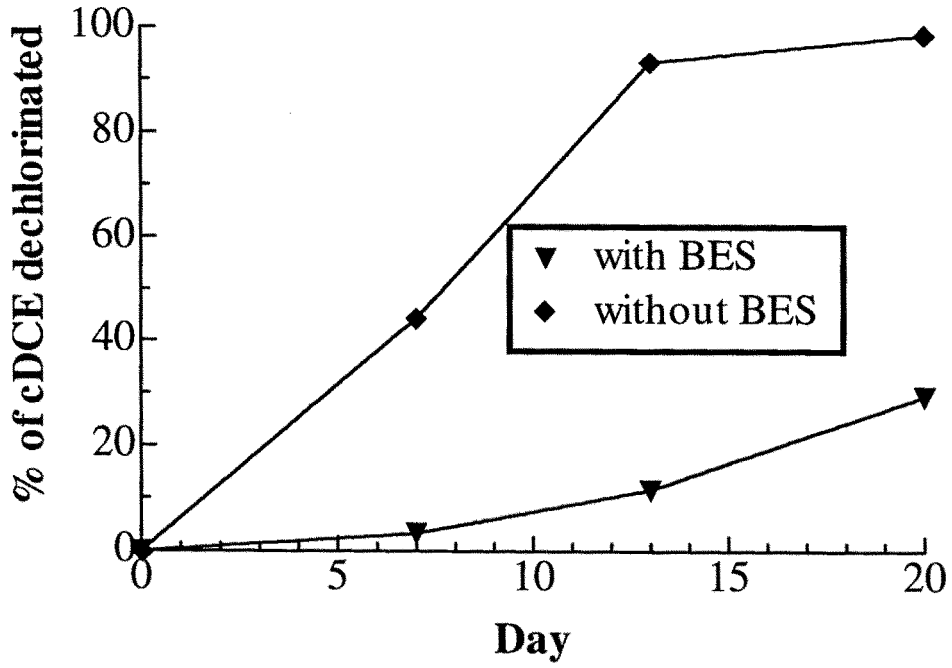

Figure 4. Influence of 10 mM BES on cDCE dechlorination with hydrogen as the electron donor.

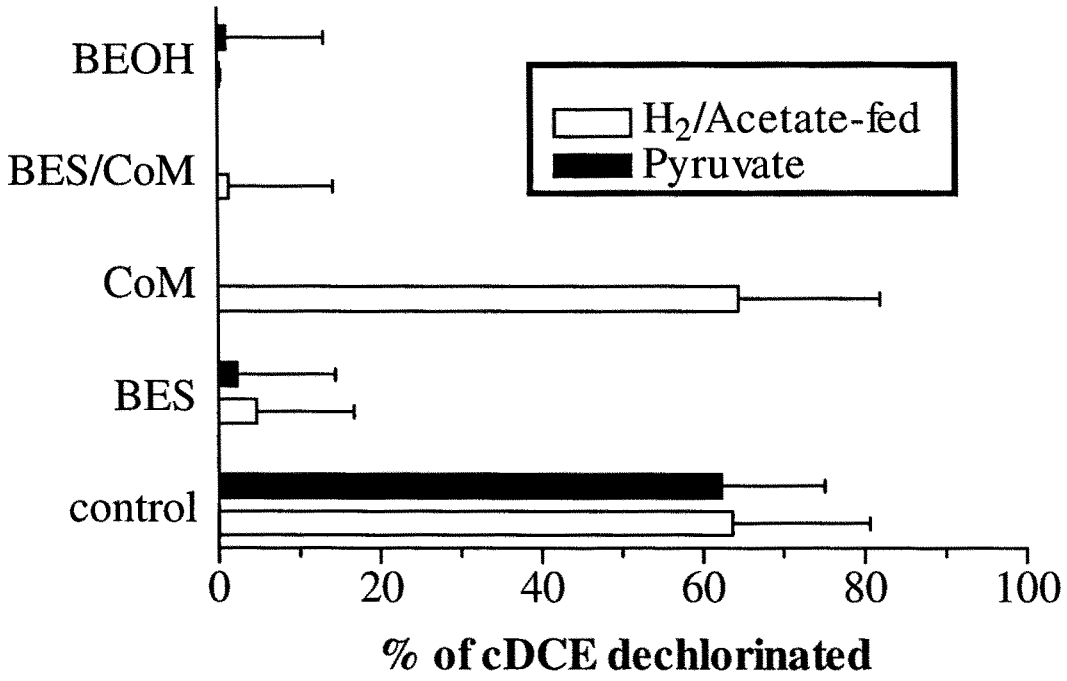

Figure 5. Influence of BES and compounds of similar structure on cDCE dechlorination. Data are averages of duplicate and error bars showed the standard deviations.

Gatell et al., 1997) showed that the reaction rates are lower for dechlorination of cDCE and VC than PCE and TCE. Our findings that a shorter HRT prevented the full extent of PCE dechlorination could be explained by the slower reaction of cDCE and VC dechlorination. However, beyond reaction rate, the growth rate of the dechlorinating organism is believed to play a role in the results observed.

Two enrichment cultures were developed from the UASB reactor. One of the cultures converted PCE and TCE to cDCE, and the other one converted TCE and cDCE to VC and ethene. It is likely that these two cultures worked consecutively to transfer PCE to VC and ethene in the UASB reactor. It is not uncommon to find that complete dechlorination of PCE is achieved by two or more dechlorinating organisms. A few dechlorinating isolates that converted PCE to only cDCE were obtained from mixed cultures in which PCE was converted to ethene (e.g., Holliger et al., 1993; Sharma and McCarty, 1996; Wild et al., 1996). In addition, cultures that could only dechlorinate cDCE to ethene were developed from the initial cultures in which ethene was the end product of dechlorination (e.g., Flynn et al., 2000). So far, in only one instance has an organism been found to dechlorinate PCE completely to ethene (Maymo-Gatell et al., 1997). The batch test demonstrated that the microbial community lost its ability to dechlorinate cDCE when HRT was 2.9 days. This indicated that the cDCE-dechlorinating bacteria had a relatively low growth rate and were washed out from the chemostat. Nevertheless, it was observed that about 10% of initial PCE was converted to VC when the HRT was 2.9 days and that the community regained its ability to produce VC after the HRT was increased to 5.9 days (based on batch test results). This indicates that a very low population of cDCE dechlorinating bacteria was retained through the short HRT period.

The two enrichment cultures developed were similar in some aspects. First, they both could use pyruvate and H_2 as electron donor. When H_2 served as electron donor, acetate had to be added as carbon source. Second, both cultures required FSCE for growth. The necessary addition of FSCE indicated that the dechlorinating bacteria in both cultures possessed an undefined nutritional dependency on one or more organisms in the microbial community in the UASB reactor. Because the medium was consistent with that used in the UASB reactor, the inability to sustain dechlorination in the enriched cultures could only be explained by the loss of an essential organism(s) supplying an undefined growth factor. This study also showed that the constituent in the FSCE required for dechlorination is either absent or present in unsatisfactory concentrations in FSSE. The requisite addition of an undefined growth factor from a parent culture, such as filter-sterilized spent medium and cell extracts, has been

observed in the isolation of other PCE-halorespiring bacteria (Maymo-Gatell et al., 1997; Maymo-Gatell et al., 1995). The ability to sustain cDCE dechlorination by FSSE from MSSD indicated that these essential organisms also are present in MSSD but may be at relatively lower levels.

In addition to their different morphologies, the two enrichment cultures are different in the response to BES inhibition. With 10 mM of BES, the activity of dechlorination by PCE-enriched culture was not influenced, while the activity by cDCE-enriched culture was severely inhibited. Other researchers reported similar observations that BES inhibited TCE and cDCE dechlorination (Loffler et al., 1997; Ye et al., 1999). Interestingly, although both the cDCE- and PCE-dechlorinating cultures could dechlorinate TCE to cDCE, BES inhibited the conversion in the cDCE-dechlorinating culture, but not in the PCE-dechlorinating culture. Other researchers have also reported that the inhibition of BES on dechlorination was culture dependent (Loffler et al., 1997). This is consistent with the phylogenetic and physiologic diversity of dechlorinating bacteria.

BES is similar in structure to CoM, a coenzyme involved in methanogenesis. It is generally accepted as a selective inhibitor for methanogens. It has become a general practice to use BES in determining whether methanogens are involved in dechlorination processes. However, both the current and previous studies (Loffler et al., 1997) have demonstrated that methanogens are not responsible for dechlorination in these TCE- and cDCE-dechlorinating cultures that were inhibited by BES. Based on these findings, caution should be taken when interpreting the roles of methanogens in dechlorination merely relying on BES inhibition.

To date, the mechanism of BES inhibition on TCE and cDCE dechlorination is still unknown. For compounds with similar structure to BES tested in this study, BEOH exhibited inhibition while CoM did not. Therefore, it can be assumed that inhibition was associated with the bromo group in BES and BEOH. Further studies are needed to explore the mechanisms of BES inhibition on dechlorination.

While in situ bioremediation holds a promising future for cleaning up chlorinated solvents contaminated sites, our current knowledge on dechlorinating organisms is still very limited. An improved understanding on these organisms will allow for the appropriate application of dechlorination-based bioremediation technology in the field. Our research on a PCE-dechlorinating microbial community in a laboratory-scale UASB reactor indicated that at least two dechlorinating populations work consecutively to convert PCE to ethene. The cDCE-dechlorinating bacteria have a lower growth rate than PCE-dechlorinating bacteria. In a continuously mixed system, a long HRT is needed to sustain the cDCE-dechlorinating bacteria in order to obtain the full dechlorination extent. In addition, both populations have undefined nutrient requirements that could be supplied by cell extracts. This indicates the syntrophism between the dechlorination bacteria and other organisms presented in mixed cultures. Pure cultures or defined mixed cultures are needed for further research to gain comprehensive understanding of the PCE- and cDCE-dechlorinating bacteria enriched for in this study.

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