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Biodegradation of *cis*-1,2-dichloroethylene and vinyl chloride in anaerobic cultures enriched from landfill leachate sediment under Fe(III)-reducing conditions

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Abstract

An anaerobic, Fe(III)-reducing enrichment culture, which originated from a sediment sample collected at a landfill in Nanji-do, Seoul, Korea, was capable of degrading *cis*-1,2-dichloroethylene (*cis*-DCE) and vinyl chloride (VC). Although it exhibited the ability under Fe(III)-reducing conditions, the chlorinated ethenes degradation was not linked to the Fe(III) reduction. During *cis*-DCE degradation, no VC, ethene, or ethane was detected through the experimental period. Also, this culture did not accumulate ethene and ethane during the VC degradation. It was unlikely that *cis*-DCE was reductively dechlorinated to VC and then the VC formed was dechlorinated fast enough. Because the kinetic data showed that the rate of *cis*-DCE degradation was 3.5 times higher than that of VC. Whereas glucose supported the culture growth and the degradation, formate, acetate, butyrate, propionate, lactate, pyruvate, and yeast extract did not. The results appeared consistent with the involvement of oxidative degradation mechanism rather than reductive dechlorination mechanism. The traits of the culture described here are unusual in the anaerobic degradation of chlorinated ethenes and may be useful for searching an effective organism and mechanism regarding anaerobic *cis*-DCE and VC degradation.

Introduction

Chlorinated aliphatic hydrocarbons, such as tetrachloroethylene (PCE) and trichloroethylene (TCE), are widely used as degreasing and dry-cleaning solvents. These volatile organic compounds are prevalent groundwater contaminants in many industrialized countries. Since PCE and TCE are suspected carcinogens, their presence in groundwater poses a threat to public health. PCE has been reductively dechlorinated to ethene by sequential dechlorination through the intermediates TCE, *cis*-dichloroethylene (*cis*-DCE), and vinyl chloride (VC) (Vogel & McCarty 1985; Bagley & Gossett 1990; DiStefano et al. 1991; Debruin et al. 1992; Tandoi et al. 1994; Maymó-Gatell et al. 1995).

However, large accumulations of *cis*-DCE and VC are often found at contaminated sites undergoing reductive dechlorination. At some sites, there is little or no dechlorination past *cis*-DCE (Parsons et al. 1984; Milde et al. 1988; Hirata et al. 1992). Both *cis*-DCE and VC are priority pollutants for the U.S. Environmental Protection Agency. Much attention has been focused on the achievement of effective dechlorination of PCE and TCE to ethene under anaerobic conditions. Some anaerobic enrichment cultures have been reported to reductively dechlorinate *cis*-DCE to ethene (Komatsu et al. 1994; Tandoi et al. 1994; Rosner et al. 1997; Yang & McCarty 1998; Bloom et al. 2000; Flynn et al. 2000; Duhamel et al. 2002). In most of the enrichment cultures, however, the VC dechlorination is a rate-limiting step for the complete dechlorination

of *cis*-DCE and VC is often detected as a dead-end product along with ethene. Furthermore, only one bacterium, *Dehalococcoides ethenogenes* strain 195, is known to completely dechlorinate *cis*-DCE and VC in addition to PCE and TCE (Maymó-Gatell et al. 1997, 2001). Given that many contaminated sites accumulate *cis*-DCE and VC, establishing the enrichment cultures capable of degrading these chlorinated ethenes efficiently and conducting a search for new effective organisms are needed.

While *cis*-DCE and VC are preferentially mineralized by aerobic bacteria having cometabolic activities, a few studies have demonstrated that such oxidative degradation of the lower chlorinated ethenes can also occur under anaerobic conditions. *cis*-DCE and VC were shown to be mineralized by a variety of anaerobic sediment microcosms under Fe(III)-reducing, sulfate-reducing, and methanogenic conditions (Bradley & Chapelle 1996, 1997, 1998) and humic acids-reducing conditions (Bradley et al. 1998). Among these conditions, occurrence of the oxidative degradation was confirmed at least under Fe(III)-reducing (for VC) and humic acids-reducing (for *cis*-DCE and VC) conditions. Under the conditions, no dechlorinated ethenes were detected through the experimental periods (Bradley & Chapelle 1997, 1998; Bradley et al. 1998). Although the organisms and their mechanisms involved remain uncertain, these studies suggest that the oxidative degradation under different electron-accepting conditions functions as an alternative tool for removal of lower chlorinated ethenes from the contaminated sites.

In this study, we describe the production and characterization of an anaerobic enrichment culture capable of degrading *cis*-DCE and VC without accumulation of dechlorinated ethene. This culture, which originated from a sediment sample collected at a landfill in Nanji-do, Seoul, Korea and was developed under Fe(III)-reducing conditions, has attractive traits for searching the new organism and mechanism regarding anaerobic degradation of *cis*-DCE and VC.

Materials and methods

Environmental samples and assay for their ability to degrade cis-*DCE*

In the initial experiments, 80 environmental samples including sediments, soils, and sludges were collected from 40 different locations to examine anaerobic degradation of *cis*-DCE by indigenous microorganisms. For sediments, ten samples were taken from lakes and rivers, three were from ponds receiving landfill leachate, and nine were from drain ditches. Ten samples were subsurface soils, three were anaerobic sewage sludges, two were industrial wastewaters, and three were river waters. These were stored at 4 °C until use. The medium described by Komatsu et al. (1994) was modified and used as MHY medium for the examination of *cis*-DCE degradation. It contained (per liter) 200 mg of $NaHCO_3$, 280 mg of K_2HPO_4, 29 mg of $(NH_4)_2HPO_4$, 30 mg of KCl, 35 mg of NH_4Cl, 33 mg of $FeCl_3 \cdot 6H_2O$, 17 mg of $MgCl_2 \cdot 6H_2O$, 11 mg of $MgSO_4 \cdot 7H_2O$, 0.8 mg of $CoCl_2 \cdot 6H_2O$, 6 mg of $CaCl_2 \cdot 6H_2O$, 1 mg of resazurin, and 2 g of yeast extract (pH 7.0). Autoclaved medium (10 ml) in a 20-ml serum vial was inoculated with 1 ml of an environmental sample and sealed with a Teflon-lined butyl rubber stopper. The samples autoclaved at 121 °C for 30 min were used for abiotic control. After replacing headspace air in the vials with N_2, *cis*-DCE dissolved in ethanol was injected to yield an initial liquid concentration of 10 μM. Duplicate cultures for each sample were incubated at 30 °C for 1 month in an orbital shaker at 100 rpm. Biodegradation of *cis*-DCE in the cultures was estimated by subtracting the abiotic loss from the final concentration of *cis*-DCE. During the incubation period, loss of *cis*-DCE in the abiotic cultures was below 2%.

Enrichment of anaerobic cis-*DCE degrader from a sediment sample*

A sediment sample from a pond receiving landfill leachate in Nanji-do, Seoul, Korea was used for constructing an enrichment culture. The fresh sediment was suspended in MHY medium and incubated for 2 weeks, as described above. After confirming degradation of *cis*-DCE, 1 ml of the culture suspension was repeatedly transferred to the same medium (9 ml) at 2-week intervals.

To investigate appropriate electron-accepting conditions for anaerobic *cis*-DCE degradation, the first sediment cultures of 2 weeks old were transferred to the media for selecting Fe(III)-reducing, sulfate-reducing, and methanogenic organisms. The Fe(III) reducer medium (Ottow 1968) contained (per liter) 3.0 g of K_2HPO_4, 0.8 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 5.0 g of L-asparagine, 10 g of D-glucose, and 1.0 g of ferric citrate (pH 7.2). To study the effects of Fe(III) and glucose on *cis*-DCE de-

gradation, concentrations of ferric citrate and glucose were altered. The sulfate reducer medium (Pfenning et al. 1992) contained (per liter) 0.8 g of K_2HPO_4, 0.8 g of KH_2PO_4, 2.0 g of $MgSO_4{\cdot}7H_2O$, 0.2 g of $FeSO_4{\cdot}7H_2O$, 1.5 g of Na_2SO_4, 1.6 g of Na lactate, 1.4 g of Na acetate, 0.7 g of Na formate, 1.0 g of yeast extract, and 2.0 g of polypeptone (pH 7.0). The methanogen medium (Zeikus 1977) contained (per liter) 0.75 g of K_2HPO_4, 0.75 g of KH_2PO_4, 0.36 g of $MgCl_2{\cdot}6H_2O$, 0.90 g of NH_4Cl, 41 mg of nitrilotriacetic acid, 5.0 g of $NaHCO_3$, 0.5 g of $Na_2S{\cdot}9H_2O$, 0.5 g of cysteine·HCl, 5.0 g of Na formate, 5.0 g of Na acetate, 2.0 g of yeast extract, 2.0 g of polypeptone, 20 g of NaCl, 10 ml of methanol, and trace metal salts (pH 7.0). Trace metal salts consisted of (per liter) 36 mg of $FeCl_2{\cdot}4H_2O$, 0.9 mg of $MnCl_2{\cdot}6H_2O$, 1.5 mg of $CoCl_2{\cdot}6H_2O$, 0.9 mg of $ZnCl_2$, 0.18 mg of $CaCl_2$, 0.17 mg of H_3BO_4, 0.09 mg of Na_2MoO_4, and 0.02 mg of $NiCl_2$. Serum vials containing 10 ml of each medium were sealed with septum and purged with N_2 (purity, >99.9%) for Fe(III)-reducing conditions and sulfate-reducing conditions and with H_2—CO_2 (80 : 20) for methanogenic conditions.

To the three culture media, *cis*-DCE was added at 10 μM (as an initial concentration in liquid), and the inoculated vials were incubated at 30 °C. Besides *cis*-DCE, in some experiments VC at prescribed concentrations were added to the cultures. *cis*-DCE (99% in liquid form; Tokyo Kasei Kogyo Co., Japan), VC (50 mg per liter of ethanol; GL Sciences Inc., Japan), and ethene (99.5% in gas form; GL Sciences Inc.) were used without further purification.

Analytical methods

cis-DCE, VC, ethene, and ethane in the anaerobic cultures were determined by injecting 200 μl of headspace gas into a gas chromatograph (GC-17A; Shimadzu Co., Japan), equipped with a capillary column, VOCOL (0.25 mm × 30 m; Supelco Inc., Pennsylvania, USA) and a flame ionization detector (FID). N_2 was used for the carrier gas. The column temperature was kept at 35 °C for 2 min and then raised to 180 °C at a rate of 4 °C min^{-1}. The injector and detector temperatures were kept at 220 °C and 270 °C, respectively. Release of chloride ion during degradation of *cis*-DCE was measured by ion chromatography with a DX-500 system (Dionex Co., California, USA). Chloride ion was separated by a column, IonPac AS 12A (Dionex Co.), with 2.0 mM $NaHCO_3$ at a flow rate of 1.0 ml min^{-1} and was monitored with an electric conductivity detector.

In the anaerobic cultures under Fe(III)-reducing conditions, formation of Fe(II) was checked by the α, α'-dipyridyl method (Bromfield 1954). Formation of methane gas was analyzed by gas chromatography using a Shimadzu GC-4C equipped with a packed column, Unibeads C 60/80 (Shimadzu Co.) and a thermal conductivity detector (TCD). Hydrogen sulfide gas was analyzed by gas chromatography (GC-9A, Shimadzu Co.) equipped with a packed 1,2,3-Tris(2-cyanoethoxy)propane column (Shimadzu Co.) and a flame photometric detector (FPD). For these analyses, temperatures of column ovens, injectors, and detectors were kept at 70, 140, and 140°C, respectively.

The culture growth was monitored as optical density at 600 nm (OD_{600}) with a UV-1600 spectrophotometer (Shimadzu Co.).

Results

cis-*DCE degradation by landfill leachate sediment under different electron-accepting conditions*

The sediment collected at Nanji-do landfill was used for enriching organism(s) capable of degrading *cis*-DCE because of its greatest degradation capability among 80 environmental samples. Anaerobic culture of the fresh sediment using MHY medium degraded 70% of initial *cis*-DCE at 2 weeks. When it was subcultured repeatedly, the degradation yield decreased to 40% then 5%. In the third subculture, *cis*-DCE degradation was no longer detectable, indicating a complete loss of the ability during subculture.

The first culture conditions (using MHY medium) were considered inadequate to maintain and enrich the *cis*-DCE degrader(s). Fe(III)-reducing, methanogenic, and sulfate-reducing conditions were applied to find the electron-accepting conditions effective for *cis*-DCE degradation. The 2-week-old MHY cultures, which displayed 70% degradation, were subcultured under the three electron-accepting conditions (Figure 1). The development of each anaerobic condition was demonstrated by formation of Fe(II) (within 2 days; data not shown), methane (Figure 1b), and H_2S (1c). Neither methane nor H_2S was detected under Fe(III)-reducing conditions. The Fe(III)-reducing culture degraded 60% of initial *cis*-DCE within 5 days (Figure 1a). Degradation of *cis*-DCE was also observed under sulfate-reducing conditions; however,

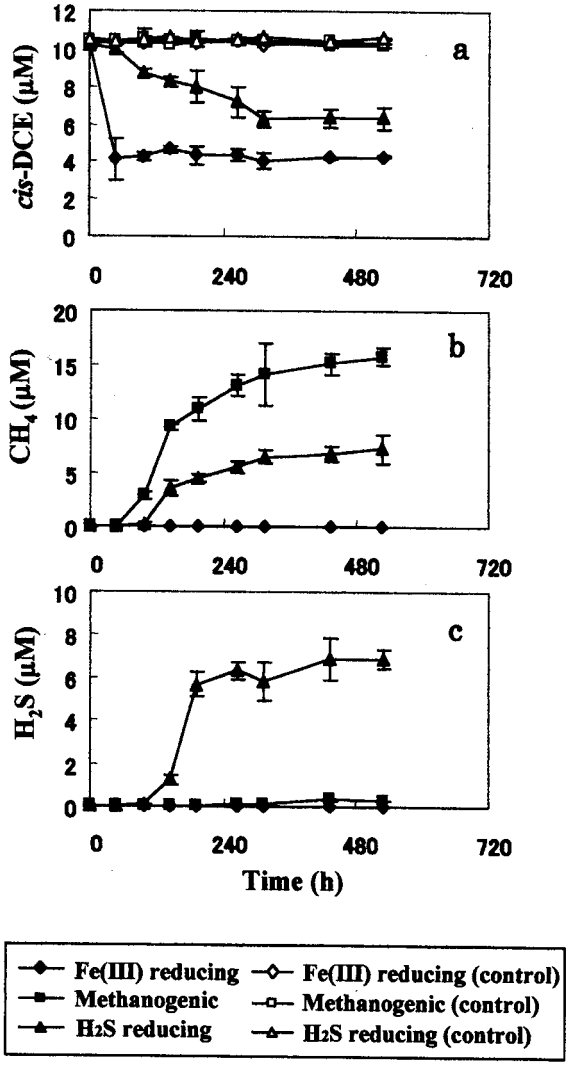

Figure 1. Anaerobic *cis*-DCE degradation in cultures of sediment sample collected at a landfill in Nanji-do, Korea. *cis*-DCE degradation (a), methane production (b), and H_2S production (c) were examined under Fe(III)-reducing, sulfate-reducing, and methanogenic conditions. Data points are means of duplicate observations and error bars represent 1 standard deviation.

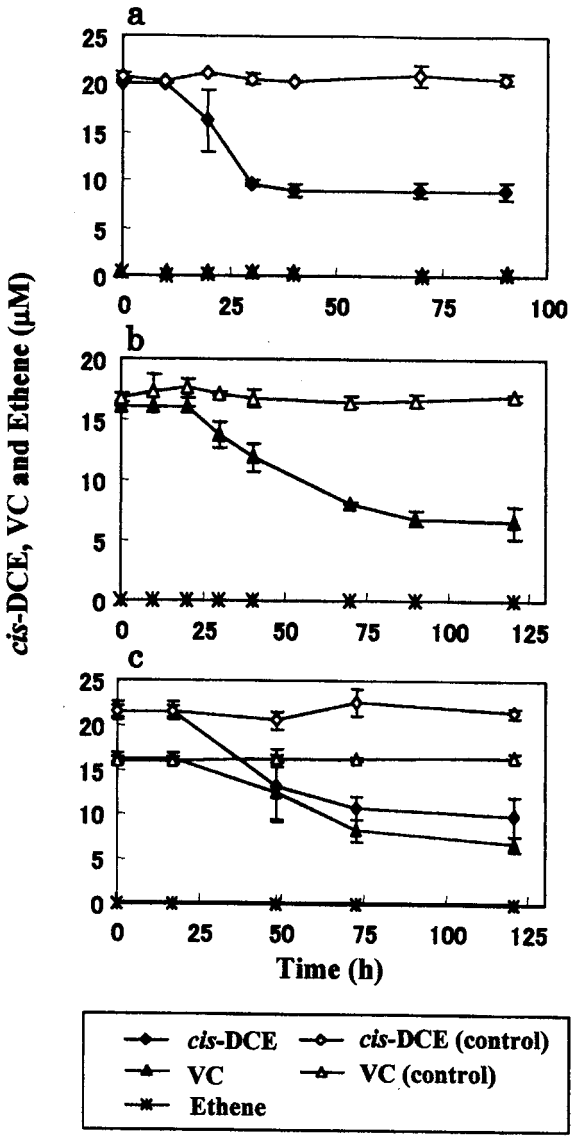

Figure 2. Degradation of *cis*-DCE (a), VC (b), and VC plus *cis*-DCE (c) under Fe(III)-reducing conditions. Data points are means of duplicate observations and error bars represent 1 standard deviation.

the rate of degradation was slower (40% at 2 weeks). No *cis*-DCE was degraded over 3 weeks under methanogenic conditions (Figure 1a). No significant decrease in *cis*-DCE added was observed for all the abiotic controls. Consequently, Fe(III)-reducing conditions appeared to be effective for eliciting the potent ability of the sediment anaerobes. Under Fe(III)-reducing conditions, the ability of cultures to degrade 60% of *cis*-DCE was maintained over 20 subsequent transfers at 2-week interval.

cis-*DCE and VC degradation under Fe(III)-reducing conditions*

Figure 2 shows the time courses of *cis*-DCE and VC in the cultures established under Fe(III)-reducing conditions. No VC, ethene, or ethane (not shown in Figure 2) was detected during the decrease in *cis*-DCE at 20 μM, indicating that the degradation of *cis*-DCE was not accompanied by accumulation of the dechlorinated ethenes (Figure 2a). In addition, VC added to

the culture was also degraded without accumulation of ethene and ethane (Figure 2b). Even when VC and *cis*-DCE were coinjected, each of them appeared to decrease independently (Figure 2c). These results demonstrated that the anaerobic culture degrades both *cis*-DCE and VC to the equivalent extent and suggested that during the degradation, no dechlorinated ethene or ethane was produced as the intermediate. A release of Cl^- accompanied by *cis*-DCE and VC degradation was not investigated. Because it was worried that a large quantity of soluble Fe(II) contained in the culture supernatants was re-oxidized to insoluble Fe(III) and deteriorated the ion chromatography column under the analytical conditions used [eluent: 2.0 mM $NaHCO_3$ (pH 7.9)]. Instead of that, the release of Cl^- in the anaerobic cultures omitting Fe(III) citrate was investigated (see below).

Kinetics of cis*-DCE and VC degradation*

To show the kinetics of *cis*-DCE and VC degradation, first, growth of the cultures in the presence of 20 and 100 μM *cis*-DCE and 16 and 48 μM VC was monitored (Figure 3). Since the population densities of *cis*-DCE- and VC-degrading cultures increased until they approached the maximum population densities, the growth kinetics were analyzed using the logistic equation represented as follows (Schmidt et al. 1985):

$$B = B_{\max}/(1 + [(B_{\max} - B_0/B_0] \cdot e^{-\mu \cdot t}), \qquad (1)$$

where μ is the maximum specific growth rate (h^{-1}), $B_{\max}$ is the maximum population density, and B_0 is the population density at time zero. The maximum specific growth rates (Table 1) were estimated by non linear regression analysis of the growth curves shown in Figure 3. The cultures with *cis*-DCE were indicated to grow somewhat faster than the cultures with VC. A higher concentration of VC caused a slight inhibition of growth, whereas that of *cis*-DCE did not.

In Figure 3, the fractions of remaining substrate (S/S_0) are also plotted as a function of culture time. Degradation of *cis*-DCE and VC appeared to be accompanied by the increase in the population densities. Assuming that the chlorinated ethenes do not support growth of the cultures and the degradation rates are not saturated at the range of substrate concentrations tested, the degradation kinetics can be represented by the following equation (Schmidt et al. 1985):

$$-\frac{dS}{dt} = \frac{k \cdot S \cdot B_{\max}}{1 + (B_{\max} - B_0) \cdot \exp(-\mu \cdot t)/B_0}. \qquad (2)$$

Its integral form is as follows:

$$S/S_0 = [\phi(e^{\mu \cdot t} - 1) + 1]^{-k/r}, \qquad (3)$$

where k is the degradation rate constant (h^{-1}), S_0 is the substrate concentration at time zero, and ϕ is the $B_0/B_{\max}$ ratio. Non linear regression analysis revealed that the time courses of *cis*-DCE and VC degradation followed the Equation (3) with high correlation coefficients (Table 1). The estimated k values for *cis*-DCE at 20 and 100 μM were comparable (0.047 and 0.045 h^{-1}, respectively) and those for VC at 16 and 48 μM were also comparable (0.013 and 0.012 h^{-1}, respectively). The fact that comparable k values were obtained for one substrate supports the initial assumption that the degradation rate is not saturated at the given concentrations. As the k values for *cis*-DCE were 3.5 times those for VC, in the Fe(III)-reducing cultures *cis*-DCE was shown to be degraded faster than VC.

Effect of Fe(III) on cis*-DCE and VC degradation*

To study the role of Fe(III) in the *cis*-DCE and VC degradation, the Fe(III)-reducing culture was grown on a fresh medium omitting Fe(III) citrate and the degradation in the absence of Fe(III) was examined. The lack of Fe(III) did not significantly affect the fate of *cis*-DCE observed in the culture with Fe(III) (Figure 4). The cultures without Fe(III) did not accumulate VC, ethene, and ethane (not shown in Figure 4) as well as Fe(III)-reducing cultures. These were also the case for VC: no influence on VC degradation and no accumulation of ethene or ethane were observed for the cultures without Fe(III) (data not shown). The equivalent results were obtained after the subculture was transferred 20 times at 2-week interval in the absence of Fe(III) citrate. These results indicated that the anaerobic degradation of the chlorinated ethenes is not linked to the reduction of Fe(III) to Fe(II).

In the absence of Fe(III), we investigated whether Cl^- was stoichiometrically released as *cis*-DCE was degraded. As shown in Figure 5, ratios of Cl^- formed to the *cis*-DCE decreased were 2.0, 2.0, and 1.6 at days 1, 2, and 7, respectively, consistent with the view that 2 moles of chlorides were released from one mole of *cis*-DCE.

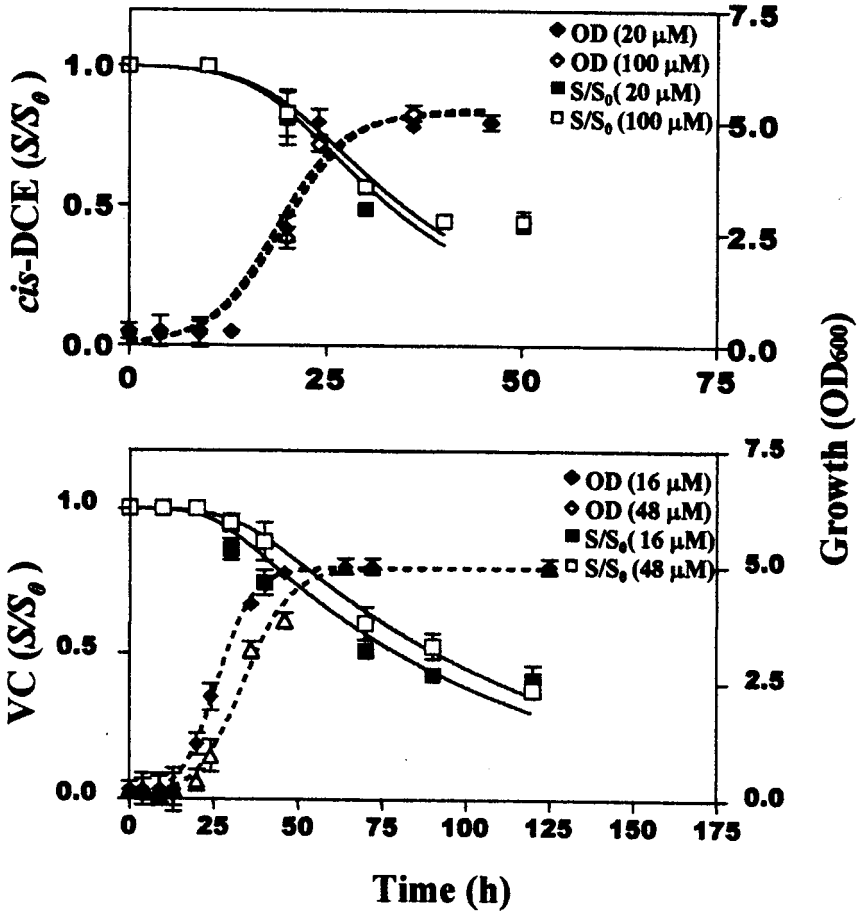

Figure 3. Culture growth and *cis*-DCE and VC degradation under Fe(III)-reducing conditions. *cis*-DCE was added at 20 and 100 μM and VC was added at 16 and 48 μM. Data points are means of duplicate observations and error bars represent 1 standard deviation. The growth (dotted) and degradation (solid) curves were fit by non linear regression; calculations for *cis*-DCE and VC degradation were conducted until the S/S_0 values approached steady states.

Table 1. Kinetics of culture growth and *cis*-DCE and VC degradation under Fe(III)-reducing conditions

Substrate (μM)		Growth[a]		Degradation[a]	
		μ (h^{-1})	r^2	k (h^{-1})	r^2
DCE	(20)	0.238 ± 0.017	>0.99	0.047 ± 0.005	0.89
	(100)	0.228 ± 0.013	>0.99	0.045 ± 0.003	0.92
VC	(16)	0.185 ± 0.003	>0.99	0.013 ± 0.001	0.92
	(48)	0.142 ± 0.003	0.99	0.012 ± 0.001	0.95

[a] Maximum specific growth rate (μ) and degradation rate constant (k) were estimated by non linear regression analysis (see Figure 3 and text). The values are represented by mean $\pm$1 standard deviation.

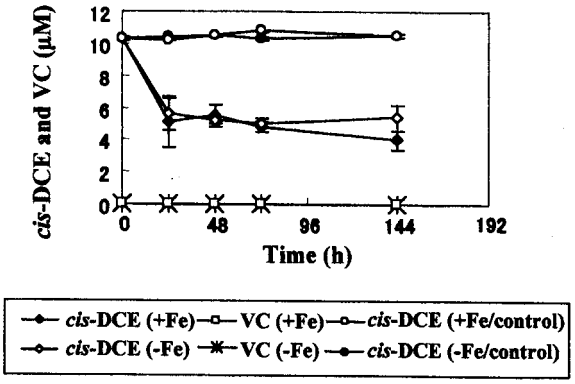

Figure 4. Degradation of *cis*-DCE by the enrichment culture in the absence and presence of 1 g of Fe(III) citrate per liter. Data points are means of duplicate observations and error bars represent 1 standard deviation.

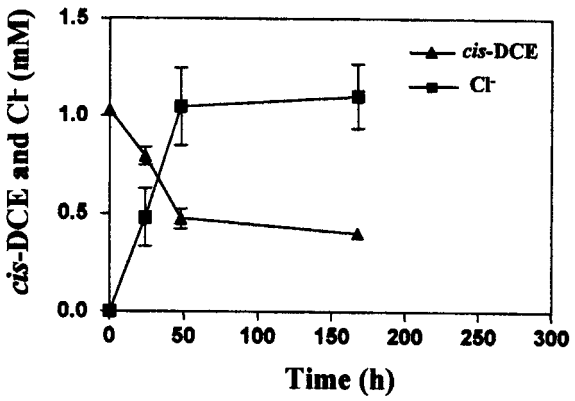

Figure 5. Release of chloride ion during degradation of *cis*-DCE in the absence of Fe(III). Data points are means of triplicate observations and error bars represent 1 standard deviation.

Effect of glucose on cis*-DCE degradation under Fe(III)-reducing conditions*

The effect of glucose, which was included in the Fe(III)-reducing culture conditions, on *cis*-DCE degradation was investigated (Figure 6). When the glucose concentration of 10 g l^{-1} was reduced to 1 g l^{-1}, the degradation yield at the end of culture decreased to 24%. When was omitted, the degradation was 17%. No VC, ethene, or ethane was detected at all the glucose concentrations. Although the culture medium contained glucose, asparagine, and citrate as carbon source, glucose was shown to mostly contribute to the expression of *cis*-DCE-degrading ability. When glucose was not added, no significant growth was observed ($OD_{600} < 0.3$). The culture growth and the *cis*-DCE degradation did not recover, even when it was supplemented with formate, acetate, butyrate, propionate,

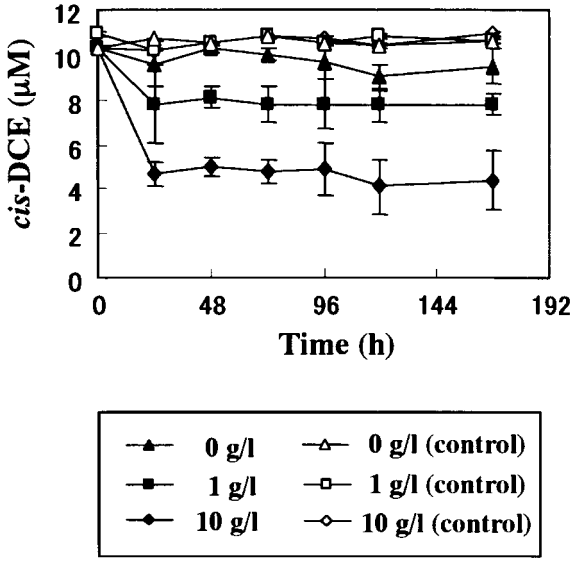

Figure 6. Effect of glucose concentration on *cis*-DCE degradation under Fe(III)-reducing conditions. Glucose was added at 1 and 10 g l^{-1}. Data points are means of duplicate observations and error bars represent 1 standard deviation.

lactate, pyruvate (each 55 mM), or 10 g l^{-1} yeast extract (data not shown).

Discussion

To obtain anaerobic enrichment culture capable of degrading *cis*-DCE effectively, the subcultures originating from a landfill leachate sediment were examined for their ability under Fe(III)-reducing, sulfate-reducing, and methanogenic conditions. The result demonstrates that the Fe(III)-reducing condition used is effective to give rise to the stable ability to degrade *cis*-DCE and VC. More importantly, the time courses of the *cis*-DCE and VC added to the culture show that their degradation processes are not accompanied by accumulation of dechlorinated ethenes, VC and ethene, or ethane. On the other hand, the cultures under sulfate-reducing and methanogenic conditions showed slower degradation and no significant degradation, respectively.

The fact that H_2S and methane production is strongly suppressed under Fe(III)-reducing conditions (Figure 1) indicates that the chlorinated ethenes degradation in the Fe(III)-reducing culture does not link to sulfate-reducing and methanogenic activities. Such preferential reduction of Fe(III) in anaerobic cultures has been previously reported (Froelich et al. 1979;

Karlin & Levi 1983). Also, the *cis*-DCE and VC degradation did not link to the reduction of Fe(III) to Fe(II) (Figure 4), despite the effectiveness of Fe(III) for the initial enrichment steps. Instead of that, the degradation of *cis*-DCE depended upon the presence of glucose as shown in Figure 6. Since other carbon source tested did not support the culture growth and the degradation, the culture may respond to a relatively narrow range of substrates.

The reductive dechlorination of initially added *cis*-DCE to ethene has been shown in *D. ethenogenes* strain 195 (Maymó-Gatell et al. 2001) and some anaerobic enrichment cultures (Komatsu et al. 1994; Tandoi et al. 1994; Rosner et al. 1997; Yang & McCarty 1998; Bloom et al. 2000; Flynn et al. 2000). In the process, chlorinated ethenes function as the electron acceptors and H_2 functions as the electron donor. However, most of the researchers reported the transient accumulation of VC during the *cis*-DCE degradation: dechlorination of accumulated VC proceeds after residual *cis*-DCE is fully consumed. Based on the release of Cl^- from *cis*-DCE in the anaerobic culture omitting Fe(III) (Figure 5), we confirmed that *cis*-DCE is fully dechlorinated through the culture time. In this regard, the results are inconsistent with the involvement of the reductive dechlorination mechanism. It was unlikely that the VC dechlorination rate is fast enough to maintain the formed VC at the non-detectable concentration. Because the kinetic parameters for *cis*-DCE and VC degradation (Table 1) imply that *cis*-DCE is degraded 3.5 times faster than VC. If *cis*-DCE is first converted to VC, amount of VC formed would surpass the amount consumed and then this would cause an accumulation of VC. Furthermore, neither of formate, acetate, butyrate, propionate, lactate, pyruvate, and yeast extract, which have been generally used as H_2 source for the reductive dechlorination, supported the culture growth and its *cis*-DCE degradation ability.

The Fe(III)-reducing culture obtained in this study appears to include a oxidative degradation mechanism rather than the reductive dechlorination mechanism. Bradley & Chapelle (1996, 1997, 1998) demonstrated that under Fe(III)-reducing conditions, aquifer and creek bed sediments microcosms can convert VC to CO_2 without accumulation of ethene or ethane and proposed that VC is directly oxidized to CO_2 under the conditions. Since our Fe(III)-reducing culture showed the Fe-independent degradation of *cis*-DCE and VC, the Fe(III)-coupled oxidation mechanism would be inapplicable. However, the Fe(III)-reducing conditions are not only a case for the oxidative degradation. The mineralization of *cis*-DCE and VC without accumulation of dechlorinated ethenes has been found in the streambed sediment microcosms placed under humic acid-reducing conditions (Bradley et al. 1998). Thus, the microcosms utilized humic acids as the electron acceptors for *cis*-DCE and VC. Although it has not been clarified if sulfate-reducing and methanogenic conditions roles in the oxidative degradation (Bradley & Chapelle 1997, 1998), these studies suggest that *cis*-DCE and VC can be oxidized by anaerobic cultures under a diverse range of electron-accepting conditions. The possibility that in our Fe(III)-reducing culture glucose or its metabolites function as the electron acceptors would not be ruled out.

The aim of this study was to develop and characterize the enrichment culture capable of degrading *cis*-DCE and VC under anaerobic conditions. Because of this focus, firm conclusions regarding of the degradation mechanism were not drawn in this study. However, the results could show that the Fe(III)-reducing culture has unusual traits, i.e., no accumulation of dechlorinated ethenes during anaerobic *cis*-DCE and VC degradation and no requirement of electron acceptors [Fe(III) and humic acids] as reported elsewhere. Given that many ground water systems contaminated with highly chlorinated ethenes have yielded *cis*-DCE and more toxic VC as dead-end products (Parsons et al. 1984; Milde et al. 1988; Hirata et al. 1992), our anaerobic culture may be involve useful organism(s) and mechanism(s) for the development of efficient bioremediation strategies. Studies to elucidate community structures of the enrichment culture are under way.

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