J. Microbiol. Biotechnol. (2003), **13**(3), 366–372

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Anaerobic Degradation of *cis*-1,2-Dichloroethylene by Cultures Enriched from a Landfill Leachate Sediment

CHANG, YOUNG-CHEOL[1], KWEON JUNG[2]*, AND YOUNG-SIK YOO[3]

[1]*Civil and Environmental Engineering Department, University of Central Florida, P.O. Box 162450, Orlando, Florida 32816-2450, U.S.A.*
[2]*Division of Waste, Seoul Metropolitan Government Institute of Public Health and Environment, 202-3, Yangjae-Dong, Seocho-Gu, Seoul 137-130, Korea*
[3]*Department of Environmental Engineering, Seoul Health College, 212 Yangii-Dong, Soojung-Gu, Sungnam City, Kyunggi-Do 461-250, Korea*

Received: September 2, 2002
Accepted: February 28, 2003

Abstract The production of microbiologically enriched cultures that degrade *cis*-1,2-dichloroethylene (DCE) under anaerobic conditions was investigated. Among 80 environmental samples, 19 displayed significant degradation of 10 μM *cis*-DCE during 1 month of anaerobic incubation, and one sediment sample collected at a landfill area (Nanji-do, Seoul, Korea) showed the greatest degradation (94%). When this sediment culture was subcultured repeatedly, the ability to degrade *cis*-DCE gradually decreased. However, under Fe(III)-reducing conditions, *cis*-DCE degradation by the subculture was found to be maintained effectively. In the Fe(III)-reducing subculture, vinyl chloride (VC) was also degraded at the same extent as *cis*-DCE. No accumulation of VC during the *cis*-DCE degradation was observed. Thus, Fe(III)-reducing microbes might be involved in the anaerobic degradation of the chlorinated ethenes. However, the subcultures established with Fe(III) could function even in the absence of Fe(III), showing that the degradation of *cis*-DCE and VC was not directly coupled with the Fe(III) reduction. Consequently, the two series of enrichment cultures could not be obtained that degrade both *cis*-DCE and VC in the presence or absence of Fe(III). Considering the lack of VC accumulation, both cultures reported herein may involve interesting mechanism(s) for the microbial remediation of environments contaminated with chlorinated ethenes. A number of fermentative reducers (microbes) which are known to reduce Fe(III) during their anaerobic growth are potential candidates involved in *cis*-DCE degradation in the presence and absence of Fe(III).

Key words: *cis*-1,2-Dichloroethylene, vinyl chloride, anaerobic degradation, electron accepting condition, dechlorination

*Corresponding author
Phone: 82-2-570-3390; Fax: 82-2-570-3311;
E-mail: jungk719@hanmail.net

Chlorinated aliphatic hydrocarbons, such as tetrachloroethylene (PCE) and trichloroethylene (TCE), are widely used as degreasing and dry-cleaning solvents [10, 11]. These volatile organic compounds are the prevalent groundwater contaminants in many industrialized countries [26]. Since PCE and TCE are suspected carcinogens, their presence in groundwater poses a threat to public health [29]. PCE has been shown to be reductively dechlorinated to ethene by sequential dechlorination through the intermediates; TCE, *cis*-dichloroethylene (*cis*-DCE), and vinyl chloride (VC) [2, 8, 9, 14, 15, 23, 30, 32]. However, large accumulation of *cis*-DCE is often found at contaminated sites undergoing reductive dechlorination, and at some sites there is little or no dechlorination except through *cis*-DCE [10- 13]. Both DCE and VC are U.S. Environmental Protection Agency priority pollutants. The lack of understanding of the factors controlling *in situ* biodegradation of DCE and VC (in many cases, involving anoxic conditions) prevents the efficient bioremediation of many chlorinated solvent/ contaminated sites.

Recent investigations indicate that terminal electron accepting conditions (*e.g.*, Fe(III)-reducing, sulfate-reducing, and methanogenic conditions) under which biodegradation occurs are a primary determinant of the rate and extent of DCE and VC biodegradation. Bradley and Chapelle [3] proposed that oxidation of *cis*-DCE and VC occurs under Fe(III)-reducing conditions where Fe(III) is the final electron acceptor. Bradley *et al.* [6] also reported that degradation of these compounds could be linked to the reduction of humic acids.

In this study, an anaerobic culture displaying a high ability to degrade *cis*-DCE was obtained from a sediment sample collected at a landfill area (Nanji-do, Seoul, Korea). However, its subcultures under the same conditions were not able to degrade the *cis*-DCE. To obtain stable

enrichment cultures capable of degrading *cis*-DCE and VC, we focused our study on the influence of terminal electron accepting conditions [Fe(III)-reducing, sulfate-reducing, and methanogenic conditions] upon anaerobic dechlorination of the chlorinated ethenes. Since the ability to degrade DCE and VC was effectively maintained in the presence of Fe(III), its possible mechanism is discussed.

MATERIALS AND METHODS

Environmental Samples and an Assay to Determine Their Ability to Degrade *cis*-DCE

In the initial experiments to examine the occurrence of anaerobic degradation of *cis*-DCE by indigenous microorganisms, 80 environmental samples were collected from 40 different localities in total, consisting of ten sediments from lakes and rivers, three sediments from ponds receiving landfill leachate, nine sediments from drain ditches, ten subsurface soils, three anaerobic sewage sludges, two industrial wastewaters, and three river waters. These were stored at 4°C until use. The medium described by Komatsu *et al.* [20] 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). An autoclaved medium (10 ml) in a 20-ml serum vial was inoculated with 1 ml of the environmental sample and sealed with a Teflon-lined butyl rubber stopper. The samples heated at 121°C for 30 min were used as 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 at Nanji-do, Seoul, Korea was used for constructing the enrichment culture of *cis*-DCE-degrading anaerobes. The fresh sediment was suspended in MHY medium and incubated for 2 weeks, as described above. After confirming the degradation of *cis*-DCE, 1 ml of the culture suspension was repeatedly transferred to fresh MHY medium at 2-week intervals.

To investigate appropriate electron accepting conditions for the anaerobic *cis*-DCE degradation, the first 2-weeks-old sediment cultures were transferred to other media selected for their Fe(III)-reducing, sulfate-reducing, and methanogenic organisms. Fe(III) reducer-selective medium [25] 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). In the experiment to show effects of Fe(III) and glucose on *cis*-DCE degradation, concentrations of glucose and ferric citrate were varied. Sulfate reducer-selective medium [27] contained (per liter) 0.8 g of K_2HPO_4, 0.8 g of K_2HPO_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). Methanogen-selective medium [34] contained (per liter) 0.75 g of K_2HPO_4, 0.75 g of K_2HPO_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). The trace metal salts consisted of 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)- and sulfate-reducing conditions, or with H_2-CO_2 (80:20) for a methanogenic condition.

To the three culture media, 10 μM *cis*-DCE were added (as initial concentration in liquid form), and the inoculated vials were incubated at 30°C. Besides *cis*-DCE, in some experiments, VC or ethene at concentrations described were added to the anaerobic 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 of an analytical grade and used without further purification.

Microbial Enumeration

The total number of microorganisms in the environmental sample was directly counted by fluorescence microscopy, according to the method of Hobbie *et al.* [18]. Methanogens [34], sulfate reducers [27], Fe(III) reducers [7, 25], and denitrifiers (nitrate reducers) [1] were counted by the most probable number (MPN) method, as described elsewhere.

Analytical Methods

cis-DCE, VC, and ethene 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, U.S.A.), 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}, and the injector and

detector temperatures were kept at 220°C and 270°C, respectively.

Formation of methane in the gas phase 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 in the gas phase 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°C, 140°C, and 140°C, respectively.

Release of chloride anions during degradation of *cis*-DCE was measured by ion chromatography with a DX-500 system (Dionex Co., California, U.S.A.). Chloride anions in the culture supernatants were 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 [7].

Results and Discussion

cis-DCE Degradation by Environmental Samples

One sediment sample, which was collected at a landfill area located in Nanji-do, Seoul, showed the highest degree of *cis*-DCE degradation (94%) during 1 month of anaerobic culture. Also, incomplete but apparent degradation was observed in the other 18 samples: five freshwater bed sediments (23 to 59%), three drain ditch bed sediments (44 to 57%), six soils (24 to 52%), three anaerobic sewage sludges (33 to 39%), and one industrial wastewater (45%). Therefore, natural habitats of *cis*-DCE-degrading anaerobes seemed to be widespread, especially in sediment, sludge, and soil environments.

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 it showed the greatest capability of degradation. Anaerobic culture of the fresh sediment in MHY medium displayed 70% degradation of *cis*-DCE in 2 weeks. In two further cultures that were transferred into subculture, however, during the first and second subcultures the degradation degrees at 2 weeks gradually decreased to 40% and 5%, respectively. In the third culture, degradation of *cis*-DCE was no longer detectable, indicating loss of the ability as the subculture steps were repeated.

Since there was no success in maintaining this ability under conditions employing MHY, the study was re-focused on terminal electron accepting conditions, where degradation of *cis*-DCE could occur. Bradley and Chapelle [3, 5] demonstrated that terminal electron accepting conditions greatly affected the anaerobic degradation of *cis*-DCE and VC by natural sediment microcosms. In aquifer sediment microcosms, *cis*-DCE mineralization was greater under Fe(III)-reducing condition than under sulfate-reducing and methanogenic conditions. In creek bed sediment microcosms, it was greater under Fe(III)- and sulfate-reducing conditions than under methanogenic conditions [5].

The landfill leachate sediment contained (per milliliter) 6.8×10^5 of microbial cells, which comprised Fe(III) reducers (in MPN, 1.3×10^4), methanogens (9.3×10^3), and sulfate

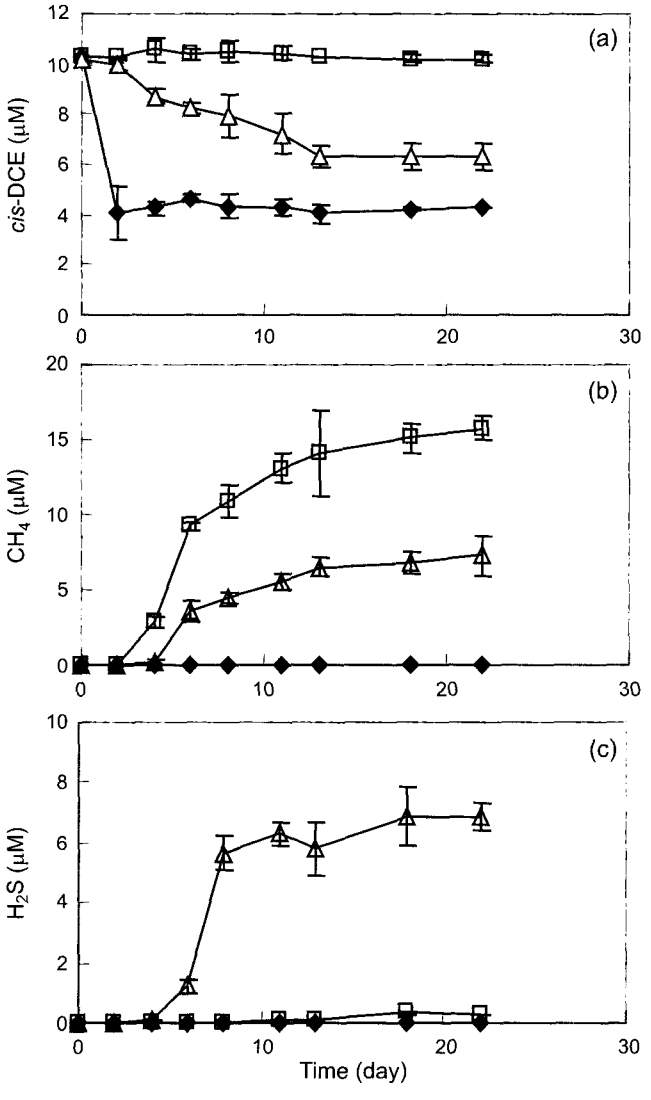

Fig. 1. Anaerobic *cis*-DCE degradation by the cultures of a sediment sample collected at a landfill area in Nanji-do, Korea. Duplicate observations for *cis*-DCE degradation (a), methane production (b), and H_2S production (c) were conducted under Fe(III)-reducing (◆), sulfate-reducing (△), and methanogenic (□) conditions. Error bars represent 1 standard deviation.

reducers (2.3×10^3). No denitrifiers were detected in this study. Therefore, degradation of *cis*-DCE under Fe(III)-reducing, methanogenic, and sulfate-reducing conditions were first examined to find the electron accepting conditions effective for *cis*-DCE-degrading organisms. The 2-week-old MHY cultures of the sediment, which displayed 70% degradation, were subcultured under the three different electron accepting conditions (Fig. 1). Development of these anaerobic conditions in cultures was demonstrated by formation of Fe(II) within the first 2 days (data not shown), and methane and H_2S (Figs. 1b and 1c). Neither methane nor H_2S was detected under Fe(III)-reducing conditions. Such preferential reduction of Fe(III) in anaerobic cultures has been previously reported [17, 19].

The Fe(III)-reducing culture degraded 60% of *cis*-DCE within 5 days (Fig. 1a). Degradation of *cis*-DCE was also observed under sulfate-reducing conditions, but the rate of degradation was slower; degradation was only 40% at 2 weeks. This degree was equivalent to that in the first subculture in MHY as described above. No *cis*-DCE was degraded within 3 weeks under methanogenic conditions (Fig. 1a), suggesting that *cis*-DCE degradation by the cultures of landfill leachate sediment did not require methanogenic conditions, nor possibly methanogens. However, this would not rule out the possible existence of latent ability in the indigenous microbes that function under methanogenic conditions, because in this study the original structure of the sediment microbial community might have been changed during the subcultures. Consequently, Fe(III)-reducing conditions appeared to be effective in releasing the latent potency of the sediment anaerobes.

Effects of Glucose and Fe(III) on *cis*-DCE Degradation Under Fe(III)-Reducing Condition

The Fe(III)-reducing conditions had the ability to degrade 60% of *cis*-DCE during the subsequent 11-times subcultures at 2-week intervals. This could be due to the involvement of Fe(III)-reducing anaerobes, which possibly utilize glucose and/or its metabolites in the medium as electron donors. The effects of glucose and Fe(III) on the *cis*-DCE degradation were further investigated. Decreasing glucose concentration from 10 to 0 g l^{-1} resulted in a gradual decrease in the degradation degree: 57% for 10 g l^{-1}, 20% for 1.0 g l^{-1}, and 7% for no addition (at the end of incubation) (Fig. 2a). Contrary to prediction, however, no significant effect of Fe(III) was observed in the concentration ranges of 10 g of glucose l^{-1} and 1.0 to 0 g of Fe(III) citrate l^{-1} (Fig. 2b). In the absence of Fe(III) citrate, moreover, the ability to degrade about 60% of *cis*-DCE was maintained during the subsequent 11-times transfers into subculture at 2-week intervals. These results suggest that the *cis*-DCE degradation observed in the presence of Fe(III) is not coupled to the Fe(III) reduction. Consequently, the two series of anaerobic mixed cultures that continuously displayed *cis*-DCE degradation in the presence and absence of Fe(III) were obtained.

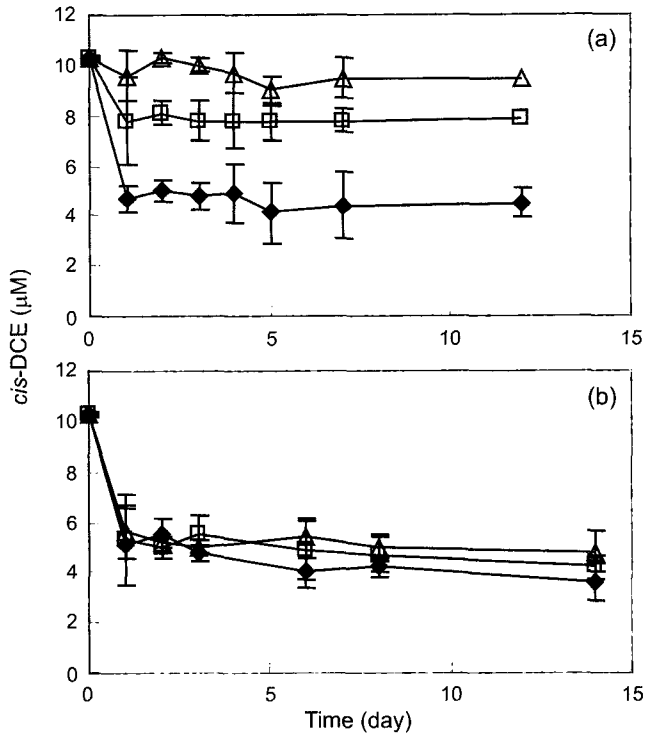

Fig. 2. Effects of glucose (a) and Fe(III) citrate (b) on *cis*-DCE degradation by the subculture developed under Fe(III)-reducing condition.
(a) Glucose concentrations are 10 (◆), 1 (□), and 0 g l^{-1} (△), respectively. (b) Fe(III) citrate concentrations are 1 (◆), 0.1 (□), and 0 g l^{-1} (△), respectively. Data points are means of duplicate observations, and error bars represent 1 standard deviation.

VC Degradation in Subcultures With and Without Fe(III)

During *cis*-DCE degradation by the subcultures maintained with Fe(III), no accumulation of VC was observed, suggesting that the subcultures had an ability to degrade VC in addition to *cis*-DCE. This was demonstrated by injection of VC in place of *cis*-DCE into the subcultures (Fig. 3a). The degradation degree was 62% within 5 days, equivalent to that of *cis*-DCE. When VC was co-injected with *cis*-DCE (Fig. 3b), simultaneous degradation of VC and *cis*-DCE occurred. The fact that VC was degraded in the subcultures implied that there was a complete dechlorination of *cis*-DCE to generate ethene. Figure 3c shows a time-dependent disappearance of ethene co-injected with VC. During a 5-day period, degradation of VC was observed and 65% of the ethene had decreased. It is, therefore, likely that ethene is an available substrate in anaerobic cultures. Degradation of VC by the subcultures without Fe(III) was equivalent to that with Fe(III) (data not shown). These cultures would also degrade ethene, because

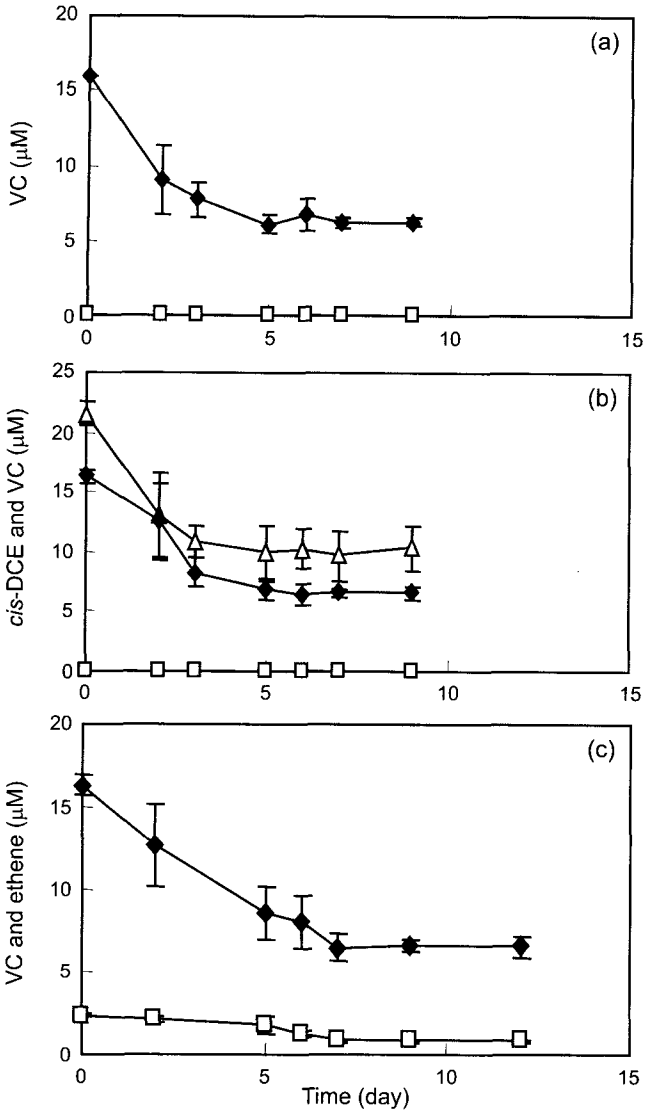

Fig. 3. Degradation of VC alone (a) and VC co-injected with *cis*-DCE (b) and with ethene (c) by the subcultures developed under Fe(III)-reducing condition.
Symbols: ◆, VC; △, *cis*-DCE; □, ethene. Data points are means of duplicate observations, and error bars represent 1 standard deviation.

no accumulation of ethene was observed during the degradation of *cis*-DCE and VC.

It was noted that *cis*-DCE degradation in the subcultures both with and without Fe(III) approached the 60% level within 5 days, but no further degradation occurred during the following incubations. A similar trend was also obtained for VC degradation. Nevertheless, it is quite unlikely that excess amounts of *cis*-DCE or VC in the cultures yielded an inhibitory effect and lowered the microbial ability after day 5. Thus, the extent of degradation of *cis*-DCE and VC was the same as those when injected separately, as shown in Fig. 3b.

Release of Chloride Ion During *cis*-DCE Degradation

To confirm that *cis*-DCE was completely dechlorinated, release of chloride ion during degradation of 1.0 mM *cis*-DCE was monitored during the subcultures without Fe(III). Initial concentrations of chloride ions in the culture was 0.03 mM. The concentrations of degraded *cis*-DCE and released chloride were 0.24 and 0.47 mM at day 1, and 0.63 and 1.0 mM at day 7, respectively (mean of duplicate experiments). Thus, two chloride ions were released from one molecule of *cis*-DCE, which is consistent with the dechlorination of *cis*-DCE to ethene.

Bradley and Chapelle [4] first demonstrated that in aquifer sediment microcosms, Fe(III) is an important factor for the anaerobic mineralization of VC. They suggested that the VC degradation is coupled to the Fe(III) reduction. In this study, Fe(III)-reducing conditions were effective in maintaining *cis*-DCE degradation in subcultures from the landfill leachate sediment. However, the role of Fe(III) in *cis*-DCE degradation was rather equivocal. The microcosms repeatedly subcultured under Fe(III)-reducing conditions expressed their ability even in the absence of Fe(III) (Fig. 2b), suggesting that the degradation of *cis*-DCE was not directly coupled to the reduction of Fe(III).

One possible explanation for these observations is that *cis*-DCE-degrading anaerobes other than Fe(III) reducers were enriched under Fe(III)-reducing conditions, and they remained in the subsequent cultures without Fe(III). Besides the oxidation with the possible electron acceptors, reductive *cis*-DCE and VC dechlorination with electron donors such as hydrogen has been demonstrated in a culture of *Dehalococcoides ethenogens* 195 [24] and a variety of mixed cultures [20, 28, 30, 31, 33]. However, the involvement of Fe(III) reducers in the degradation under the Fe(III)-free condition could not be precluded, because the subcultures maintained without Fe(III) could always produce Fe(II) without delay, when supplemented with Fe(III) citrate (data not shown). A diverse group of fermentative microbes are known to reduce Fe(III) during their anaerobic growth; they do not require Fe(III) as an electron acceptor, unlike dissimilatory Fe(III)-reducing microbes (*e.g.*, *Geobacter* spp.) [21, 22]. Such fermentative reducers may be potent candidates that are involved in *cis*-DCE degradation in the presence and absence of Fe(III).

Importantly, the subcultures either with and without Fe(III) did not accumulate detectable amounts of VC, which is more toxic than *cis*-DCE, during *cis*-DCE degradation. The lack of accumulation of VC appears to be an unusual characteristic of anaerobic *cis*-DCE degradation. Many anaerobic cultures and microcosms amended with *cis*-DCE have been shown to accumulate VC and dechlorinate it at a limited rate with a few exceptions [5, 16]. *cis*-DCE and VC are often found in the sites contaminated with PCE and TCE, and are rather difficult to remove under anaerobic

conditions. The two series of mixed cultures obtained in this study have been found to involve interesting microbes and mechanisms for bioremediating the environments which are contaminated with chlorinated ethenes. For the two series of cultures, molecular analysis of the microbial community structures is in progress.

Acknowledgments

A part of this work was supported by Kurita Water and Environment Foundation, Japan, and a grant-in-aid for JSPS fellows (No. 98124) from the Ministry of Education, Science, Sports and Culture, Japan. We thank Dr. Jae-Young Shin, Seoul Metropolitan Government Institute of Health and Environment for providing the Nanji-do sediment sample used in this study; Dr. Yukinori Tani, University of Shizuoka for advice and help with culture studies; and Dr. Hata Junko, University of Shizuoka, for technical support with chloride ion analysis.

REFERENCES

1. Alexander, M. 1965. Denitrifying bacteria, pp. 1484-1486. *In* Black C. A. (ed.). *Methods of Soil Analysis, Part 2.* American Society of Agronomy Inc., WI, U.S.A.
2. Bagley, D. M. and J. M. Gossett. 1990. Tetrachloroethene transformation to trichloroethene and *cis*-1,2-dichloroethene by sulfate-reducing enrichment cultures. *Appl. Environ. Microbiol.* **56:** 2511-2516.
3. Bradley, P. M. and F. H. Chapelle. 1997. Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions. *Environ. Sci. Technol.* **31:** 2692-2696.
4. Bradley, P. M., F. H. Chapelle, D. R. Lovley, and D. A. Vroblesky. 1996. Measuring rates of biodegradation in a contaminated aquifer using field and laboratory methods. *Ground Water* **34:** 691-698.
5. Bradley, P. M. and F. H. Chapelle. 1998. Microbial mineralization of VC and DCE under different terminal electron accepting conditions. *Anaerobe* **4:** 81-87.
6. Bradley, P. M., F. H. Chapelle, and D. R. Lovley. 1998. Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene. *Appl. Environ. Microbiol.* **64:** 3102-3105.
7. Bromfield, S. M. 1954. The reduction of iron oxide by bacteria. *J. Sci.* **5:** 129-139.
8. Chang, Y. C., M. Hatsu, K. Jung, Y. S. Yoo, and K. Takamizawa. 1998. Degradation of a variety of halogenated aliphatic compounds by anaerobic mixed culture. *J. Ferment. Bioeng.* **86:** 410-412.
9. Chang, Y. C., M. Hatsu, K. Jung, Y. S. Yoo, and K. Takamizawa. 1999. Degradation of tetrachloroethylene by enrichment cultures and its isolate. *Int. J. Environ. Conscious Design Manufactr.* **8:** 1-7.
10. Chang, Y. C. and K. Takamizawa. 2000. The current situation on PCE degrading organisms studies: A review (vol. 1). *Environ. Conserv. Eng.* **29:** 64-71.
11. Chang, Y. C. and K. Takamizawa. 2000. The current situation on PCE degrading organisms studies: A review (vol. 2). *Environ. Conserv. Eng.* **29:** 65-70.
12. Chang, Y. C., M. Hatsu, K. Jung, Y. S. Yoo, and K. Takamizawa. 2000. Isolation and characterization of a tetrachloroethylene dechlorinating bacterium *Clostridium bifermentans* DPH-1. *J. Biosci. Bioeng.* **89:** 489-491.
13. Chang, Y. C., B. C. Okeke, M. Hatsu, and K. Takamizawa. 2001. *In vitro* dehalogenation of tetrachloroethylene (PCE) by cell-free extracts of *Clostridium bifermentans* DPH-1. *Biores. Technol.* **78:** 141-147.
14. DeBruin, W. P., M. J. J. Kotterman, M. A. Posthumus, G. Schraa, and A. J. B. Zehnder. 1992. Complete biological reductive transformation of tetrachloroethene to ethane. *Appl. Environ. Microbiol.* **58:** 1996-2000.
15. DiStefano, T. D., J. M. Gossett, and S. H. Zinder. 1991. Reductive degradation of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. *Appl. Environ. Microbiol.* **57:** 2287-2292.
16. Flynn, S. J., F. E. Löffler, and J. M. Tiedje. 2000. Microbial community changes association with a shift from reductive degradation of PCE to reductive degradation of *cis*-DCE and VC. *Environ. Sci. Technol.* **34:** 1056-1061.
17. Froelich, P. N., G. P. Klinkhammer, M. L. Bender, N. A. Luedtke, G. R. Heath, D. Cullen, P. Dauphin, D. Hammond, B. Hartman, and V. Maynard. 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: Suboxic diagenesis. *Geochim. Cosmochim. Acta* **43:** 1075-1090.
18. Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33:** 1225-1228.
19. Karlin, R. and S. Levi. 1983. Diagenesis of magnetic minerals in recent haemipelagic sediments. *Nature* **303:** 327-330.
20. Komatsu, T., K. Momonoi, T. Matsuo, and K. Hanaki. 1994. Biotransformation of *cis*-1,2-dichloroethylene to ethylene and ethane under anaerobic conditions. *Water Sci. Technol.* **7:** 75-84.
21. Lovley, D. R. 1987. Organic matter mineralization with the reduction of ferric ion: A review. *Geomicrobiol. J.* **5:** 375-399.
22. Lovley, D. R. 2000. Fe(III) and Mn(IV) reduction, pp. 3-30. *In* Lovley, D. R. (ed.). *Environmental Microbe-Metal Interactions*, ASM Press, Washington, DC, U.S.A.
23. Maymó-Gatell, X., V. Tandoi, J. M. Gossett, and S. H. Zinder. 1995. Characterization of an H_2-utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis. *Appl. Environ. Microbiol.* **61:** 3928-3933.
24. Maymó-Gatell, X., I. Nijenhuis, and S. H. Zinder. 2001. Reductive degradation of *cis*-1,2-dichloroethene and vinyl chloride by "*Dehalococcoides ethenogenes*." *Environ. Sci. Technol.* **35:** 516-521.

25. Ottow, J. C. 1968. Evaluation of iron-reducing bacteria in soil and the physiological mechanism of iron-reduction in *Aerobacter aerogenes*. *Z. Allg. Mikrobiol.* **8:** 441-443.
26. Park, G. T., H. J. Hong, J. G. Kim, and S. J. Lee. 1998. Biodegradation of phenol by a trichloroethylene-cometabolizing bacterium. *J. Microbiol. Biotechnol.* **8:** 61-66.
27. Pfenning, N., F. Widdel, and H. G. Trüper. 1992. The dissimilatory sulfate-reducing bacteria, pp. 926-940. *In* Balows A., H.G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (eds.). *The Prokaryotes*, 2nd ed. vol. **1**. Springer Verlag, New York, U.S.A.
28. Rosner, B. M., P. L. McCarty, and A. M. Spormann. 1997. *In vitro* studies on reductive vinyl chloride dehalogenation by an anaerobic mixed culture. *Appl. Environ. Microbiol.* **63:** 4139-4144.
29. Shin, H. J., M. Y. Lee, and J. W. Yang. 1998. Biodegradation of trichloroethylene by phenol-degrading *Pseudomonas putida*. *J. Microbiol. Biotechnol.* **8:** 185-187.
30. Tandoi, V., T. D. DiStefano, P. A. Bowser, J. M. Gossett, and S. H. Zinder. 1994. Reductive dehalogenation of chlorinated ethenes and halogenated ethanes by a high-rate anaerobic enrichment culture. *Environ. Sci. Technol.* **28:** 973-979.
31. Villarante, N. R., P. M. Armenante, T. A. O. Quibuyen, F. Fava, and D. Kafkewitz. 2001. Dehalogenation of dichloroethene in a contaminated soil: Fatty acid and alcohols as electron donors and an apparent requirement for tetrachloroethene. *Appl. Microbiol. Biotechnol.* **55:** 239-247.
32. Vogel, T. M. and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* **49:** 1080-1083.
33. Yang, Y. and P. L. McCarty. 1998. Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture. *Environ. Sci. Technol.* **32:** 3591-3597.
34. Zeikus, J. G. 1977. The biology of methanogenic bacteria. *Bacteriol. Rev.* **41:** 514-541.