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Tetrachloroethene and 3-chlorobenzoate dechlorination activities are co-induced in *Desulfomonile tiedjei* DCB-1

James R. Cole[1], Babu Z. Fathepure[2,3] & James M. Tiedje[1,*]

[1]*Departments of Crop and Soil Sciences and of Microbiology;* [2]*Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI 48824, USA;* [3]*present address: Operations Technology & Support Environmental Services Division; Conoco, Inc. Ponca City, OK 74602, USA (*author for correspondence)*

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Abstract

Desulfomonile tiedjei, a strict anaerobe capable of reductively dechlorinating 3-chlorobenzoate, also dechlorinates tetrachloroethene and trichloroethene. It is not known, however, if the aryl and aliphatic dechlorination activities are catalyzed by the same enzymatic system. Cultures induced for 3-chlorobenzoate activity dechlorinated tetrachloroethene and trichloroethene to lower chlorinated products while uninduced parallel cultures did not dechlorinate either substrate. The observed rate of PCE dechlorination in induced cultures was 22 μmol h^{-1} g protein^{-1}, which is considerably faster than previous rates obtained with defined cultures of this organism. These results show that both dechlorination activities are co-induced and therefore, that the dechlorination mechanisms may share at least some components.

Abbreviations: PCE – tetrachloroethene, TCE – trichloroethene, *cis*-DCE – *cis*-dichloroethene, *trans*-DCE – *trans*-dichloroethene, 3FBz – 3-fluorobenzoate, 3ClBz – 3-chlorobenzoate

Contamination of groundwater by chlorinated aliphatic hydrocarbons has led to concern over their environmental fate. Many of these compounds, including tetrachloroethene (PCE), are anaerobically dechlorinated in the natural environment and by both methanogenic and sulfate reducing enrichment cultures (for reviews see McCarty 1988; Vogel et al. 1987). The anaerobic dechlorination of PCE is of special interest since, unlike trichloroethene (TCE) and lower substituted ethenes, PCE is apparently not attacked by aerobic microorganisms. Little is known, however, about either the bacteria responsible for the anaerobic dechlorination of PCE or the molecular mechanism involved in the reaction.

Desulfomonile tiedjei strain DCB-1 was originally isolated from an anaerobic enrichment growing on 3-chlorobenzoate (Shelton & Tiedje 1984). *D. tiedjei*, a sulfate reducing bacterium (DeWeerd et al. 1990), can obtaining energy by using 3-chlorobenzoate as an alternate electron acceptor, producing benzoate and HCl as products (Dolfing 1990; Dolfing & Tiedje 1987; Mohn & Tiedje 1990). Interestingly, *D. tiedjei* also dechlorinates PCE. In one study, *D. tiedjei* gave the highest rate of PCE dechlorination among several pure cultures of anaerobes tested (Fathepure et al. 1987). The slow growth rates and yields of *D. tiedjei* under the medium conditions used previously made studies of the relationship between these two dechlorination activities difficult. Medium improvements leading to increased growth rates and our own studies on induction of 3-chlorobenzoate dechlorination activity have led us to examine whether these two activities may be related. Specifically, does PCE dechlorination activity co-induce with 3-chlorobenzoate dechlorination activity. For these experiments we induced 3-chlorobenzoate dechlorination activity with 3-fluorobenzoate, an effi-

cient gratuitous inducer of halobenzoate dehalogenation in *D. tiedjei* (J. R. Cole, R. Sanford, and J. M. Tiedje, manuscript in preparation). Since 3-fluorobenzoate is not transformed by *D. tiedjei*, we were able to test for co-induction without any metabolic changes (e.g. electron transport mediated respiration) that would occur during dechlorination of 3-chlorobenzoate and that might have some indirect effect on the rate of PCE dechlorination.

Materials and methods

Cell growth

Cultures were grown using strict anaerobic technique in a synthetic minimal medium based on the medium of Widdel and Pfennig (Widdel & Pfennig 1984) and consisted of (g/liter) NaCl, 1; $MgCl_2$, 0.5; KH_2PO_4, 0.2, NH_4Cl; 0.3, KCl, 0.3; $CaCl_2$, 0.015, 1 ml/l of a trace element solution (Widdel & Pfennig 1984), 1 ml/l of a Na_2SeO_3, Na_2WO_4 solution (Brysch et al. 1987), resazurin, 10 mg/l; HEPES (hemisodium salt), 10 mM; $NaHCO_3$, 30 mM; and cysteine, 1 mM. The medium was prepared under N_2:CO_2 (95:5), the pH adjusted to 7.5, dispensed into N_2:CO_2 flushed vessels, and sterilized by autoclaving. Immediately prior to inoculation, the medium was amended with vitamins (DeWeerd et al. 1990), pyruvate at 20 mM, and 0.1 mM $Na_2S_2O_4$. 3-Fluorobenzoate (100 μM) was added to induce 3-chlorobenzoate dechlorination activity. PCE (50 μM) was added to test the ability of PCE to induce dechlorination activity. (This was the highest PCE concentration that allowed growth.) The complete medium was inoculated with a 1% transfer from homologous medium (without fluorobenzoate). Cultures were grown at 37 °C in the dark for about 14 days for fresh cells or 28 days for starved stationary phase cells. The medium of Fathepure et al. (1987) was prepared as described and assayed in parallel with cells grown with the present medium after each had grown for 19 days.

Assay conditions

Cultures were harvested by centrifugation and resuspended in 20 to 40% of the initial volume in a cold anaerobic assay buffer normally consisting of 50 mM NaCl, 10 mM sodium pyruvate, 10 mM HEPES (N-2[hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], hemisodium salt Sigma), 0.1 mg/l resazurin and reduced with 1 mM cysteine-HCl and 1 mM titanium (III) citrate. Na_2S (1 mM) replaced titanium (III) citrate where indicated. For formaldehyde addition experiments, the HEPES concentration was increased to 50 mM and formaldehyde (37%) was added to obtain a final concentration of 0.2% immediately before sealing one pair of vials. Resuspended cells were used to completely fill N_2 sparged 10 ml serum bottles after which chlorinated ethenes were added from a 0.5 or 1 mg/ml stock in methanol for PCE and TCE respectively to a final concentration of 10 μM except for experiments described in Table 1, where the concentrations were 12 μM and 15 μM for PCE and TCE respectively. The vials were immediately sealed with Teflon lined rubber septa and aluminum crimp seals, leaving no headspace. The assays were initiated by transferring the serum bottles to a 37 °C water bath. The assays were terminated after 2 h (unless otherwise noted) by transferring the serum bottles to an ice water bath, where they were stored until analyzed. Protein concentrations were determined by a modification of the Lowry method (Hanson & Phillips 1981). Data are presented as the average of duplicate vials with the sample standard deviation in parenthesis. Total Cl removed was calculated from the input concentration of chlorinated ethenes and the Mole % of recovered chlorinated products. Total recovery of chlorinated ethenes averaged greater than 90% of that of time 0 control vials. 3-Chlorobenzoate dechlorination activity was measured in parallel vials amended with 1 mM 3-chlorobenzoate.

Analytical methods

Chlorinated ethenes were monitored with a Tekmar purge and trap unit (model 4000) connected to a Hewlett-Packard GC equipped with an HP-5 capillary column (30 m × 0.53 mm × 2.6 μm film thickness) and flame ionization detector. Samples (5 ml) were purged onto Tenax TA adsorbent with helium (30 ml/min for 16 min), desorbed (180 °C for 4 min), and separated using helium as a carrier at 6 ml/min. The temperature program was 30 °C for 8 min, increasing to 200 °C at 4 °C/min and followed by an isothermal period of 5 min. The injection and detector temperatures were 220 °C and 300 °C, respectively. PCE and its dechlorinated products were identified by comparison with authentic standards.

Benzoate and 3-chlorobenzoate were analyzed by reverse phase HPLC with a Hibar RT C18 column (E. Merck), with a flow rate of 1.5 ml/min of 66:33:0.1 H_2O:CH_3CN:H_3PO_4, and a UV detector set to 230 nm.

Table 1. Rates of PCE and TCE dechlorination in induced and non-induced cells.[a]

Substrate	Induced	Mole % of recovered material			Rate (μmol h^{-1} g $prot^{-1}$
		PCE	TCE	*cis*-DCE	
PCE	+	45.7 (1.1)	54.3 (1.1)	0.0 (0.0)	22.0 (0.4)
	–	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
TCE	+	–	89.2 (0.3)	10.8 (0.3)	5.5 (0.2)
	–	–	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a]Induced and non-induced cell suspensions contained 149 and 153 μg protein/ml respectively. Incubations were for 2 h. Parenthesis indicates standard deviation in all tables.

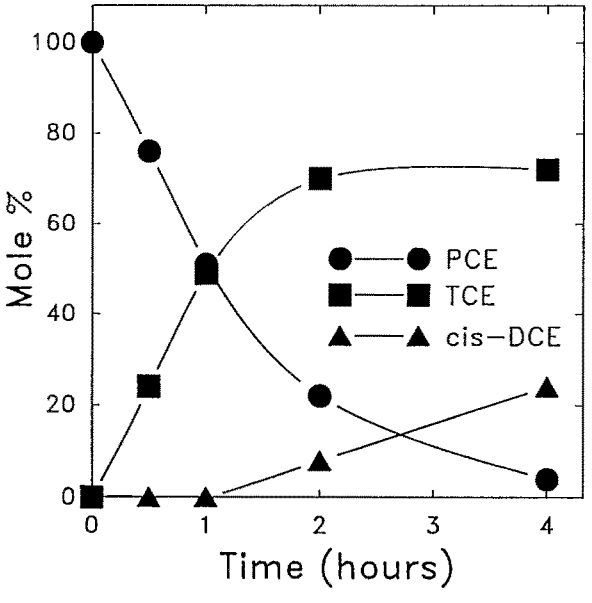

Fig. 1. Time course of PCE dechlorination. The averages of duplicate vials harvested at the indicated times are presented as the Mole % of material recovered. Suspensions contained 193 μg protein/ml.

Results and discussion

Rapid transformation of PCE to TCE and cis-DCE (cis-dichloroethene) occurred in cultures with resuspended cells grown with 0.1 mM 3-fluorobenzoate (Fig. 1), while no dechlorination was detected with uninduced cultures. Other experiments with a longer time course showed formation of small amounts of trans-DCE (trans-dichloroethene) in addition to cis-DCE (data not shown).

To further examine the transformation of both PCE and TCE and to determine if dechlorination of TCE also requires induction, we conducted a similar experiment to measure the initial transformation rates of PCE and TCE (Table 1). Only cultures pre-induced with 3-fluorobenzoate showed measurable product formation from either PCE or TCE, indicating that both activities require induction. The rate of transformation of PCE to TCE (22 μmol g $protein^{-1}$ h^{-1}) was considerably faster than the rate of transformation of TCE to DCE (5.5 μmol g $protein^{-1}$ h^{-1}), consistent with results from mixed culture systems (Bouwer & McCarty 1983).

Table 2. Dechlorination of PCE and 3ClBz by cell suspensions grown with PCE or 3-fluorobenzoate as potential inducers.[a]

Inducer	μmol Cl/g protein removed in 2 h from	
	PCE	3ClBz
None	0.1 (0.0)	7 (1)
PCE	0.2 (0.0)	10 (0)
3FBz	18.4 (0.4)	319 (19)

[a]Suspensions of cells grown with no inducer, PCE and 3-fluorobenzoate contained 717, 677, and 877 μg protein/ml respectively.

We also tested the ability of PCE to induce the dechlorination activities. Cultures of cells grown with PCE as a potential inducer instead of 3-fluorobenzoate transformed only small amounts of PCE and 3-chlorobenzoate, about the same as cultures grown with no inducer (Table 2).

There are several reports of reductive dechlorination of chlorinated methanes, ethanes and ethenes catalyzed by purified enzyme cofactors, often using a strong synthetic reductant (see eg. Klecka & Gonsior 1984; Krone et al. 1989a; Krone et al. 1989b; Wood et al. 1968). Although in our system dechlorination occurred only in pre-induced cultures, we were concerned that the dechlorination we observed might not be mediated by living cells but instead by some fac-

Table 3. Effect of different medium reductants and sterilant on PCE dechlorination.

Treatment		μmol Cl removed/g prot. in 2 h
Reductant[a]	Ti Cit	50.0 (0.4)
	Na_2S	45.4 (1.4)
Formaldehyde[b]	+	1.0 (0.1)
	–	34.1 (0.0)

[a] Suspensions contained 381 and 372 μg protein/ml for titanium (III) citrate and Na_2S reduced buffers, respectively.
[b] Suspensions contained 509 μg protein/ml, and the standard titanium (III) citrate-cysteine reductants.

Table 4. Stimulation of dechlorination by pyruvate addition to starved stationary phase cells.[a]

Pyruvate	μmol Cl removed/g protein from	
	PCE[b]	3ClBz[b]
+	29.1 (0.8)	255 (33)
–	4.1 (0.3)	30 (4)

[a] Suspensions contained 521 μg protein/ml and the standard assay reductants, titanium (III) citrate and cysteine.
[b] PCE incubation was for 2 h and 3ClBz was for 1 h.

tor(s) released only from damaged induced cells and perhaps involving the titanium (III) citrate reductant added to our buffer system.

Three experiments were conducted to test whether the PCE reduction required biological activity (Tables 3 and 4). The titanium (III) citrate in our buffer system was not required for PCE dechlorination since similar activity was obtained with sodium sulfide as the medium reductant (Table 3). Addition of formaldehyde to a final concentration of 0.2% almost totally blocked dechlorination, indicating that killed cells are not active in the dechlorination reaction (Table 3).

We had found that dechlorination of 3-chlorobenzoate was strongly dependent on the addition of pyruvate if the cells were in starved stationary phase and less dependent if they were resuspended growing cells (data not shown). *D. tiedjei* accumulates glycogen (Mohn et al. 1990) and it is probable that this acts as an endogenous electron donor until depleted. Using similarly starved cells we found that both PCE and 3-chlorobenzoate dechlorination were reduced by about the same factor in the absence of pyruvate compared to the presence of pyruvate (to about 12% for 3-chlorobenzoate dechlorination and to about 14% for PCE dechlorination) (Table 4). Taken together, these results indicate that metabolically active cells are required for PCE dechlorination.

Table 5. Dechlorination after growth in two media.[a]

Media	μmol Cl removed/g protein in 2 h from	
	PCE	3ClBz
Fathepure et al.	14.4 (0.7)	137 (4)
Present medium	63.3 (2.5)	903 (34)

[a] Suspensions of cells grown in the medium of Fathepure et al. (1987) and in the present medium contained 278 and 237 μg protein/ml respectively, and the standard titanium (III) citrate-cysteine reductants.

Two previous studies examining dechlorination of PCE by *D. tiedjei* reported much lower rates of PCE dechlorination than the present study (2.34 μmol g protein^{-1} day^{-1} reported in Fathepure et al. 1987, and 6.4 nmol/l in 5 months reported in Suflita et al. 1988). To determine if the apparent increase in rate from previous work was due to differences in growth conditions, we directly compared the dechlorination rates of cultures grown using our present method and cultures grown by the method of Fathepure et al. Cultures of cells grown by the method of Fathepure et al. dechlorinated 3-chlorobenzoate at only about 18% the rate of suspensions of cells grown by the present method (Table 5). Similarly, only about 27% as many chlorine atoms were removed from PCE by cultures grown by the older method. This rate increase is probably due to better growth conditions and better induction of the dechlorination system.

The induction of 3-chlorobenzoate dechlorination activity involves a lag consistent with the synthesis of new macromolecules (enzymes) necessary for dechlorination (J. R. Cole, R. Sanford and J. M. Tiedje, manuscript in preparation). Although 3-chlorobenzoate and PCE dechlorination might possibly involve separate enzyme systems that happen to co-induce, it seems more likely that both activities share at least one factor induced by 3-fluorobenzoate. Very little is known about the mechanism of PCE or chlorobenzoate dechlorination in *D. tiedjei*. If both PCE and 3-chlorobenzoate dechlorination share the same 'dechlorinase' enzyme then this information helps limit the plausible mechanisms for dechlorination. For example, partial ring hydrogenation by hydride ion addition para to the chlorine might make a reasonable intermediate for 3-chlorobenzoate dechlorination but is not a feasible mechanism for PCE dechlorination. If the same mechanism is responsible for the two dechlorination

reactions then a mechanism such as direct nucleophilic attack on the Cl position or electrophilic addition would appear more likely.

D. tiedjei can obtain energy for growth through the transfer of electrons to 3-chlorobenzoate (Dolfing & Tiedje 1987; Dolfing 1990, Mohn & Tiedje 1990). We do not yet know, however, if *D. tiedjei* also obtains energy from PCE dechlorination. If it can obtain energy in this manner then *D. tiedjei* and similar organisms should have a selective advantage in PCE contaminated environments. Several cultures have recently been described that are able to grow by obtaining energy from PCE dechlorination (Holliger et al. 1993; Tandoi et al. 1994). These cultures are capable of transforming PCE at higher concentrations and faster rates than found for *D. tiedjei* in the present study. The dehalogenating organism in at least one of these cultures is apparently quite different both morphologically and physiologically from *D. tiedjei*. This organism has a restricted substrate range and appears to require PCE or TCE for growth. It is not known whether the dechlorination mechanisms are similar for *D. tiedjei* and these PCE grown cultures.

Since the less chlorinated ethenes can be rapidly oxidized by aerobic microorganisms (see eg. Ewers et al. 1990; Nelson et al. 1987; Tsien et al. 1989; Vogel et al. 1987), the obligatory anaerobic first dechlorination step is often rate limiting for PCE bioremediation efforts. Therefore, the rate increases reported here may be important for PCE bioremediation. The ability of *D. tiedjei* to grow on a variety of substrates beyond chlorinated compounds (DeWeerd et a. 1990) may offer an advantage for PCE remediation in mixed contaminant environments and in environments with PCE concentrations too low to directly support the growth of organisms through PCE dechlorination.

Our knowledge that 3-chlorobenzoate both selects for an organism(s) capable of PCE dechlorination was used to develop a biofilm reactor that dechlorinates PCE as well as some chloroform and 1,1,2-trichloroethane (Fathepure & Tiedje 1994). A 1-day pulse feeding with 3-chlorobenzoate after 4 to 5 day feeding of a cheaper substrate, e.g. acetate, was required to sustain a high level of PCE dechlorination. The evidence that 3-chlorobenzoate induces this activity helps explain the pulse-feeding requirement.

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References

Bouwer EJ & McCarty PL (1983) Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45: 1286–1294

Brysch K, Schneider C, Fuchs G & Widdel F (1987) Lithoautrophic growth of sulfate-reducing bacteria, and a description of *Desulfobacterium autotrophicum* gen. nov., sp. nov. Arch. Microbiol. 148: 262–274

DeWeerd KA, Mandelco L, Tanner RS, Woese CR & Suflita JM (1990) *Desulfomonile tiedjei* gen. nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. Arch. Microbiol. 154: 23–30

Dolfing J (1990) Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. Arch. Microbiol. 153: 264–266

Dolfing J & Tiedje JM (1987) Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. Arch. Microbiol. 149: 102–105

Ewers J, Freier-Schröder D & Knackmuss HJ (1990) Selection of trichloroethene (TCE) degrading bacteria that resist inactivation by TCE. Arch. Microbiol. 154: 410–413

Fathepure BZ, Nengu JP & Boyd SA (1987) Anaerobic bacteria that dechlorinate perchloroethene. Appl. Environ. Microbiol. 53: 2671–2674

Fathepure BZ & Tiedje JM (1994) Reductive dechlorination of tetrachloroethylene by a chlorobenzoate-enriched biofilm reactor. Environ. Sci. Technol. 28: 746–752

Hanson RS & Phillips JA (1981) Chemical composition. In: Gerhardt P (Ed) Manual of Methods for General Bacteriology (pp 328–364). American Society for Microbiology, Washington, DC

Holliger C, Schraa G, Stams AJM & Zehnder AJB (1993) A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Appl. Environ. Microbiol. 59: 2991–2997

Klecka GM & Gonsior SJ (1984) Reductive dechlorination of chlorinated methanes and ethanes by reduced iron (II) porphyrins. Chemosphere 13: 391–402

Krone, UE, Laufer K, Thaure RK & Hogenkamp HPC (1989) Coenzyme F-430 as a possible catalyst for the reductive dehalogenation of chlorinated C-1 hydrocarbons in methanogenic bacteria. Biochemistry 28: 10061–10065

Krone, UE, Thauer RK & Hogenkamp HPC (1989) Reductive dehalogenation of chlorinated C-1 hydrocarbons mediated by corrinoids. Biochemistry 28: 4908–4914

McCarty PL (1988) Bioengineering issues related to in-situ remediation of contaminated soils and groundwater. In: Omen GS (Ed) Environmental Biotechnology (pp 143–162). Plenum Publishing, New York

Mohn WW & Tiedje JM (1990) Strain DCB-1 conserves energy for growth from reductive dechlorination coupled to formate oxidation. Arch. Microbiol. 153: 267–271

Mohn WW, Linkfield TG, Pankratz HS & Tiedje JM (1990) Involvement of a collar structure in polar growth and cell division of strain DCB-1. Appl. Environ. Microbiol. 56: 1206–1211

Nelson MJK, Montgomery SO, Mahaffey WR & Pritchard PH (1987) Biodegradation of trichloroethene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53: 949–954

Shelton DR & Tiedje JM (1984) Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. 48: 840–848

Suflita JM, Gibson SA & Beeman RE (1988) Anaerobic biotransformations of pollutant chemicals in aquifers. J. Ind. Microbiol. 3: 179–194

Tandoi V, DeStefano TD, Bowser PA, Gossett JM & Zinder SH (1994) Reductive dehalogenation of chlorinated ethenes and halogenated ethanes by a high-rate anaerobic enrichment culture. Environ. Sci. Technol. 28: 973–979

Tsien HC, Brusseau GA, Hanson RS & Wackett LP (1989) Biodegradation of trichloroethene by *Methylosinus trichosporium* OB3b. Appl. Environ. Microbiol. 55: 3155–3161

Vogel TM, Criddle CS & McCarty PL (1987) Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. 21: 722–736

Widdel F & Pfennig N (1984) Dissimilatroy sulfate- or sulfur-reducing bacteria. In: Krieg NR & Holt JG (Eds) Bergey's Manual of Systematic Bacteriology, Vol 1 (pp 663–679). Williams & Wilkins, Baltimore

Wood JM, Kennedy FS & Wolfe RS (1968) The reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B12. Biochemistry 7: 1707–1713