Microbial dechlorination of dioxins in estuarine enrichment cultures: effects of respiratory conditions and priming compound on community structure and dechlorination patterns

Q. Shiang Fu a,*, Andrei L. Barkovskii b, Peter Adriaens c

a Environmental Engineering and Science, Department of Civil and Environmental Engineering, Stanford University, Stanford, CA 94305-4020, USA
b Department of Biological and Environmental Sciences, Georgia College and State University, Milledgeville, GA 31061-0490, USA
c Environmental and Water Resources Engineering, Department of Civil and Environmental Engineering, The University of Michigan, Ann Arbor, MI 48109-2125, USA

Received in revised form 22 January 2004; accepted 6 April 2004

Abstract

The effect of respiratory conditions and priming compound on dechlorination patterns of heptachlorodibenzo-p-dioxins (HpCDD) was investigated using estuarine sediment-eluted cultures in the presence and absence of 20 mM sulfate, and 0.2 μM 2-bromodibenzo-p-dioxin (2-BrDD) as a priming compound. Electron balance calculations based on fatty acid turnover, hydrogen production, and electron acceptor depletion/methane formation indicated that whereas fermentative processes dominated in sulfate-free incubations, sulfate-reduction was predominant in the sulfate-amended incubations. The dechlorination of 1,2,3,4,6,7,8-HpCDD exhibited the following trends: (i) the relative yields of product formation did not exceed 30% and the presence of 2-BrDD increased the yield by up to 10%; (ii) sulfidogenic conditions resulted in a lower 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) formation, and the presence of 2-BrDD decreased the formation of 2,3,7,8-TCDD by additional 4–5-fold; (iii) the presence of 2-BrDD effected a predominance in lateral (2,3,7,8 positions) over peri (1,4,6,9 positions)-dechlorination. Denaturing gradient gel electrophoresis (DGGE) banding patterns indicated significant shifts of microbial community structure in response to terminal electron accepting
processes as well as to the presence of the priming compound. The latter resulted in a similar community structure independent of dioxin spike, indicating that subsets of populations in the sediment are capable of exploiting the new niche provided by the priming compound.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Chlorinated dioxins; Reductive dechlorination; Community structure; Degradation; Sediment pollution; Microorganisms

1. Introduction

Fate and transformation of polychlorinated dibenzo-p-dioxins (PCDD) in the environment are of particular importance due to their high toxicity, bioaccumulation, and suspected carcinogenicity. Freshwater, estuarine, and marine sediments serve as the primary sinks for this group of compounds. Chlorinated dioxins, especially 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins, have been shown to undergo reductive dechlorination reactions under reducing conditions (Adriaens, Fu, & Grbic’-Grlic’, 1995; Adriaens, Chang, & Barkovskii, 1996; Albrecht, Barkovskii, & Adriaens, 1999; Barkovskii & Adriaens, 1996, 1998; Beurskens et al., 1995; Bunge, Ballerstedt, & Lechner, 1999; Fu, Barkovskii, & Adriaens, 1999, 2001). Using cultures eluted from Passaic River sediments, microbial dechlorination has been demonstrated under freshwater and estuarine conditions (Barkovskii & Adriaens, 1996; Fu et al., 2001). Lateral dechlorination patterns (chlorine removal at 2,3,7,8 positions) were found to be dominant under freshwater conditions.

Little is known about the microbiology of PCDD dechlorination, through several lines of evidence indicate that non-methanogenic subpopulations may be responsible for reductive dechlorination under freshwater conditions (Barkovskii & Adriaens, 1996). Microbial PCDD dechlorination under estuarine conditions is less clear. Recently, we documented microbial PCDD dechlorination under high salinity and sulfate conditions by estuarine microbial consortia from Passaic River sediments (Fu et al., 2001). However, nothing is known about the impact of PCDD on microbial community structure and the relationships between microbial community composition and dechlorination patterns in estuarine and marine environments. Numerous investigations have demonstrated that the presence of organic compounds or contaminants affects the diversity and structure of microbial communities (Abed et al., 2002; Colores, Macur, Ward, & Inskeep, 2000). Recent insights in the microbial ecology of polychlorinated biphenyl (PCB) dechlorination have indicated that ortho- or meta/para-dechlorination activity can be enriched (Cutter, Sowers, & May, 1998; Pulliam-Holoman, Elberson, Cutter, May, & Sowers, 1998). Furthermore, community analysis using genetic methods shows that the community structure associated with ortho-dechlorination patterns differs markedly from that of cultures not spiked with PCBs (Prieur et al., 1987).

Polychlorinated biphenyl studies also revealed that various compounds structurally related to PCBs exhibit a ‘priming’ effect towards PCB dechlorination. The actual role of the priming compounds is not trivial. In particular, it is unknown whether such
primers cause increased production of proteins involved in reductive dehalogenation (e.g., components of electron transport chains) or the priming effect is the result of the emergence of new populations in response to selective pressure by the priming agents.

The natural production of halogenated compounds in marine environments (Gribble, 1994) has prompted investigations on the potential of these compounds to induce or stimulate microbial degradation activities of (structurally analogous) anthropogenic compounds (Allard, Hynning, Remherger, & Nielson, 1992; King, 1988). Dechlorination of historical PCB contamination in freshwater sediments has been stimulated using both chloro- and bromobiphenyls (Bedard & van Dort, 1997; Bedard, van Dort, & DeWeerd, 1998; DeWeerd & Bedard, 1993), and bromobenzoates (DeWeerd & Bedard, 1993). More recently, it was demonstrated that dioxin-contaminated sediments amended with 2-bromodibenzo-p-dioxin (2-BrDD) exhibited extensive dechlorination activity, resulting in the production of 2-chlorodibenzo-p-dioxin (2-monoCDD) (Albrecht et al., 1999).

In estuarine environments, microbial diversity and metabolic activity are mainly affected by salinity gradients, high sulfate concentrations, and competition for energy sources. Sulfate reduction has been reported to be the dominant microbial respiratory process in estuarine and marine environments (Capone & Kiene, 1988). Whereas earlier studies suggested that methanogenesis and sulfate reduction were spatially separated or that methanogenesis only became significant after sulfate was depleted, the coexistence of sulfate reduction and methanogenesis has been shown in marine sediments that exhibit sulfate reduction as the dominant respiration (Kiene & Capone, 1994).

Previously, freshwater sediments were shown to dechlorinate PCBs under methanogenic, but not under sulfate-reducing conditions (Alder, Häggblom, Openheimer, & Young, 1993). However, when estuarine sulfate-reducing sediments were incubated with PCBs, dechlorination ensued (Ofjord, Puhakka, & Ferguson, 1994). This suggests that freshwater and estuarine environments select for different populations, which exert differences in PCB dechlorination activity (Bedard & Quensen, 1995).

One of the most challenging aspects of microbial ecology remains the demonstration of cause–effect relationships between the effect of external amendments on community structure and activity endpoints. The goals of this study are to determine: (i) whether there are structural and physiological changes related to the presence of PCDDs and their structural analogues (brominated dioxins), (ii) whether the latter can prime PCDD dechlorination under estuarine and marine-mimicking conditions, and (iii) whether the diversity in structural and physiological composition affects PCDD dechlorination.

2. Materials and methods

2.1. Inoculum

The sediment core was collected from the lower Passaic River (NJ) near the outfall of 24 Lister Ave., one of the presumed sources of the dioxin contamination in
this estuary (Albrecht et al., 1999; Bopp et al., 1991). A 30-cm core section derived from approximately 1 m below the riverbed was used to obtain the microbial fraction. Estuarine bottom water was collected at the same time from the same location. The salinity of the water column 1 m above the sediment ranged from 16‰ to 22 ‰.

2.2. Experimental design

Estuarine microbial consortia were prepared under strictly anaerobic conditions in a glove box (N₂–CO₂–H₂, 85:10:5) (Coy Laboratory Products, Inc., Ann Arbor, MI). Two estuarine media for the experiment included modified basal medium (Sowers, Johnson, & Ferry, 1984) (in g/L): Na₂CO₃, 3.0; Na₂HPO₄·7H₂O, 1.12; NH₄Cl, 0.5; cysteine-HCl·H₂O, 0.25; and resazurine, 0.001. Trace element and vitamin solutions (1% each) were supplemented to the media (Wolin, Wolin, & Wolfe, 1963). Estuarine medium with sulfate (E-Sulfate medium) (Berkaw, Sowers, & May, 1996) contained (g/L): NaCl, 8.4; MgSO₄·7H₂O, 4.78; KCl, 0.27; and CaCl₂·2H₂O, 0.05. The final sulfate concentration in the E medium was 19.4 mM. Estuarine medium without sulfate (E medium) was similar to E-Sulfate medium with the exception that MgSO₄ was replaced by MgCl₂ (3.9 g/L). All of the components except for Na₂CO₃ and the vitamins were subjected to a stream of N₂–CO₂ (75:25). The vitamin solution was filter-sterilized. The pH of the media was about 6.8. The salinity in the two estuarine media was 17‰.

Wet sediment (1.2 kg) was transferred from the sediment core into glass jars in the glove box and amended with 2.4 L of the Passaic River bottom water and 100 mg/L (final concentration) of a primary growth substrate cocktail (acetic acid, 75 mg/L; butyric acid, 15 mg/L; and benzoic acid 10 mg/L). The jar was covered with aluminum foil and the sediment suspension was incubated in the anaerobic chamber for 6 months at room temperature in the dark and was shaken daily for 1 min. After 6 months, a Tween 80 solution (0.1% v/v) was added to the sediment suspension, which was then transferred into 800-ml centrifuge tubes, capped with a butyl rubber stopper, and sonicated for 5 min. The suspension was then centrifuged (500 rpm; 5 min) to separate solid particles, and the supernatant containing cells and colloidal material was harvested. The supernatant was centrifuged (3700 rpm; 15 min) to pellet the cells, which were resuspended separately in E and E-Sulfate medium (600 ml), and amended with 100 mg/L (final) of substrate cocktail. These cell suspensions were incubated for another 6 months to enrich communities in the presence and absence of sulfate and to increase biomass densities, centrifuged (3700 rpm; 20 min), and left overnight for sedimentation. The pelleted cells were resuspended separately with E and E-Sulfate media (700 ml each) and spiked with substrate cocktail (100 mg/L). These enriched microbial consortia were used as the inoculum for subsequent incubations. A 5-ml aliquot of the enrichment cultures was transferred into 20-ml sterilized serum bottles sealed with Teflon lined butyl rubber stoppers (The West Co., Lionville, PA).

The cell suspensions were set up in five replicates in addition to background (no spike of dioxin congeners) and sterile controls (autoclaved for 2 h on two consecutive days prior to the addition of dioxin congeners). One set of active cultures was spiked
separately with 0.47 \pm 0.01 \mu M of either 1,2,3,4,6,7,8- or 1,2,3,4,6,7,9-HpCDD (AccuStandard Inc., New Haven CT) from a 90 \mu M stock solution in decane. Another set of cultures was spiked with the HpCDD congeners and 2-bromodibenzo-p-dioxin (2-BrDD) (0.19 \mu M) (Dow Chemical, Midland, MI). Duplicate incubations were used for analysis of methane, hydrogen, sulfate, protein, and molecular (16S rRNA) characterizations. All cultures (a total of 240) were incubated with daily manual shaking (twice a day and 2 min each time) at 30 °C in the dark for a year. Substrate cocktail (100 mg/L, final concentration) was added every two months.

2.3. Sample preparation and PCDD analysis

Triplicate microcosms were sacrificed for congener-specific dioxin (mono-through heptaCDD) analysis. Octachlorodibenzo-furan (100 \mu l of a 5 ppm stock) was used as the internal standard to monitor extraction efficiency. A NaOH solution (2 ml, 5 M) was added to the cell suspensions to break up bacterial cell walls. Manual triplicate extractions with toluene (4 ml) were performed and the extracts were pooled and dried over a gentle stream of nitrogen gas to approximately 2 ml. Elemental sulfur was removed from E-Sulfate medium samples based on a modified procedure with tetrabutylammonium sulfate and sodium sulfite (Jensen, Renberg, & Reutergardh, 1977). The treated samples were dried again with nitrogen to reduce the amount of solvent to about 2 ml, and approximately 0.5 g of anhydrous sodium sulfate was added to remove residual water from the organic phase. Sample cleanup was performed in a micro Florisil column (~0.2 g in a disposable Pasteur pipette with glass-wool at the bottom) eluted with 10 ml of hexane. After evaporation of hexane, 100 \mu l of toluene was added to the sample to concentrate the dioxins prior to analysis.

Mono- to heptaCDD congeners were analyzed on a 5890-II Hewlett-Packard GC equipped with a 5972A Mass Selective Detector (MSD) in selective ion monitoring mode (SIM) as described previously (Albrecht et al., 1999). Recovery efficiencies for PCDDs were 85–100% based on the internal standard used. Quantitation was based on a six-level calibration curve established for a custom-made mixture of dioxin congeners. The detection limit for native dioxins was 0.1 ng on-column. Identification of congeners was based on the comparison of the retention times of known congeners and the ratios of relative abundance of key ion clusters in the mass spectrum corresponding to the values calculated with the natural isotopic abundance of chlorines (EPA, 1986).

2.4. Hydrogen analysis

A 0.4-ml aliquot of the gas sub-sample was taken from microcosms with a Hamilton (Reno, NV) gas-tight sample-lock syringe, and the Teflon valve was closed until analysis. Hydrogen gas was analyzed using a Trace Analytical (Menlo Park, CA) reduction gas analyzer equipped with Carbosorb and molecular sieve 5A columns in series and quantified against a 10-point calibration curve. The detection limit of this analysis was 20 ppb (v/v). The gas concentration was then converted to
an equilibrium liquid phase concentration using the Oswald phase partitioning
constant for hydrogen of 0.08211 L atm/K mole (Lovley, Chapelle, & Woodward,
1994).

2.5. Sulfate analysis

Sulfate in filtered culture medium was analyzed using a Dionex 4500i ion chro-
matograph (IC) (Sunnyvale, CA) equipped with an IonPac AS4A column (4 × 250
mm) (Dionex, Sunnyvale, CA), and a conductivity detector with an eluent consist-
ing of both sodium bicarbonate (1.7 mM) and sodium carbonate (1.8 mM) pumped at 2
ml/min. Ions were identified and quantified by comparing retention times and peak
areas, respectively, with those of external standards. External calibration was based
on a 10-point standard curve (0.1–10.0 mM, \( R^2 = 0.99 \)). Time zero microcosms were
diluted 2-fold prior to analysis.

2.6. Methane analysis

Headspace gases were collected from GC vials via purge with helium carrier gas.
The subsample was injected onto a 30 m DB-624 column through a split/splitless
port operating at 140 °C in the splitless mode. Methane was detected within 4 min by
Flame Ionization Detector (FID). A 4-point calibration was used for quantitation.
Standards were prepared by taking 1 ml methane from a sealed gas-tight container
and diluted into a 160-ml sealed vial: 0.25, 0.5, 0.75, and 1 ml subsamples were in-
jected into four 10-ml headspace GC vials.

2.7. Organic acid analysis

HPLC analysis was performed on a Hewlett–Packard Series II 1090 Liquid
Chromatograph equipped with an HP Chemstation and diode array detector. Pri-
mary substrate cocktail mixtures, benzoic, butyric, and acetic acids were analyzed on
a Spherisorb C8 column (250 mm × 4.6 mm × 5 μm) (Alltech, Deerfield, IL). The
mobile phase consisted of 90% 50 mM KH₂PO₄ (pH was adjusted to 2.5 with
H₃PO₄) and 10% CH₃CN. The 50 mM KH₂PO₄ solution was prepared with Milli-Q
water and filtered with 0.2-μm filter using a Masterflex pump. Identification and
quantification was performed by retention time and peak area of external standards.
External calibration was based on a 9-point standard curve for each compound
(0.10–10.0 mM, \( R^2 = 0.99 \)).

2.8. Protein analysis

Bacterial growth was determined by measuring the amount of protein produced in
the replicate experimental cultures, based on 1-ml aliquots. A 10-ml culture tube
containing the cells and 0.4 ml of 5 N NaOH (to break up cell membranes) was
heated for 10 min at 98 °C with a dry bath incubator (Fisher Scientific). The test
tubes were then centrifuged at 13,000 rpm for 15 min with a Tomy MC-140 high-
speed micro centrifuge (Tomy Tech USA, Inc., Palo Alto, CA). The protein concentration was measured by using the Bio-Rad Protein Assay Kit II (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as standard.

2.9. DNA extraction procedure

DNA was extracted from each active culture for 16S ribosomal DNA (rDNA) analysis based on a reported procedure (Oelmüller, Krügger, Steinbüchel, & Friedrich, 1990). The concentration and purity of the extracted DNA were determined by absorption spectrophotometry.

2.10. PCR amplification of rDNA fragments

The 16S rRNA was amplified by PCR with a Mastercycler personal® thermocycler (Eppendorf Scientific, Inc. Westbury, NY). The universal primers were f984GC (forward primer with a GC clamp) (5’CGCCCCGGGCGCGCCCGGCGGCGGCGGGGCGCACGGGGGAACGCGAACCTTAC3’) and r1378 (reverse primer) (5’CGGTGTGTACAGGCCCGGAACG3’). These two primers amplify the bacterial 16S rDNA fragment at positions 968–1401 (fragment 968–1401; Escherichia coli numbering; Brosius, Dull, Sleeter, & Noller, 1981). The GC clamp was attached to the forward primer 984 to prevent melting of the PCR products during separation of DNA bands in the denaturing gradient gel electrophoresis (DGGE) (Myers, Maniatis, & Lerman, 1987). The PCR mixtures were added to a 1.5-ml autoclaved Eppendorf tube as follows: 5 µl 10× PCR buffer (100 mM Tris–HCl, 15 mM MgCl₂, and 500 mM KCl, pH 8.3), 200 µmol of each deoxyribonucleoside triphosphate, 100 nmol of each primer, 2 µl of the extracted DNA sample, and 40.5 µl of autoclaved ultra-filtered water (pH 7.0). The mixture was centrifuged with a microcentrifuge (Micro12, Fisher Scientific) to pellet all components. After initial denaturation at 94 °C for 5 min, Taq DNA polymerase of 0.5 µl (1.25 U) was added to each reaction tube without removing the tubes from the thermocycler. This hot start was effective to prevent non-specific annealing of the primers to non-target DNA molecules (Chou, Russel, Birch, Raymond, & Bloch, 1992). The reaction tubes were centrifuged again before reaction cycles were initiated.

Amplification was performed with 31 cycles of reaction: 1 min denaturing at 94 °C, 1 min annealing at 54 °C for primer annealing, 1 min at 72 °C for primer extension, followed by final extension at 72 °C for 5 min, and cooling to 4 °C. Products of PCRs were first examined by running electrophoresis in 1% (w/v) agarose gels and ethidium bromide staining (Sambrook, Fritsch, & Maniatis, 1989).

2.11. DGGE analysis

A D-Code 16/16-cm gel system with a 1.5-mm gel width (Bio-Rad, Hercules, CA) was used to perform DGGE analysis. The DNA separation was maintained at a constant temperature of 60 °C in 6 L of 0.5× TAE buffer (20 mM Tris acetate and
0.5 m MEDTA [pH 8.0]). Gradients were formed with 6% (w/v) acrylamide stock solutions (acrylamide–N,N’-dimethylene-bis-acrylamide, 37:1) which contained 15% and 55% denaturant (with 100% denaturant defined as 7 M urea dissolved in 40% [v/v] formamide). Gels were run at 35 V for 16 h. Gels were stained in Milli-Q water containing ethidium bromide (0.5 mg/L) and destained twice in 0.5× TAE buffer for 15 min each. Images were obtained with the Alpha-Imager software (Alpha Inotech, San Leandro, CA). A tif-formatted image of the gel was loaded into GelCompar™ (Applied Maths, Kortrijk, Belgium) for comparative correlation analyses of the lanes. The Pearson correlation algorithm in association with either Unweighted Pair Group Method with Arithmatic Mean (UPGMA) or Ward’s methods was used to establish clustering patterns. Analysis was limited to the region of the gel containing bands (top region near wells were excluded). The dendrogram is from clustering analysis with UPGMA using the “Fine Optimization” option of GelCompar™. The observed pattern was detected with both UPGMA and Ward’s clustering methods using either the global optimization or fine optimization options in GelCompar.

2.12. Extraction of DNA from DGGE gels and sequence analysis

The central 1-mm² portions of strong DGGE bands were excised with a razor blade and soaked in 50 µl of sterile Milli-Q water overnight. A 15 µl portion of the solution was reamplified by PCR as described in the PCR procedure. The reamplified PCR products were purified by electrophoresis though a 1.2% agarose gel in 0.5× TAE running buffer followed by glass-milk extraction (Gene-Clean Kit; Bio 101). Purified DNA was sequenced with an ABI-Prism model 373 automatic sequencer. Sequence identification was performed by use of BLASTN facility of the National Center for Biotechnology Information and the Sequence Match facility of the Ribosomal Database Project (BLASTN, 1997; Maidak et al., 1999; Sequence Match, 1999). Not all bands produced high quality sequence and no further sequence analysis was done on those sequences due to lack of PCR products.

3. Results

3.1. Impact of environmental conditions and dioxin isomers on microbial respiratory activity

Respiratory activity was evaluated by means of sulfate, methane, and hydrogen analysis. The data for sulfate consumption and methane production over the period of incubation are presented in Table 1. It is clear that salinity and sulfate affect the distribution of predominant respiratory activities. Methane production in the sulfate-free incubations accounted for the consumption of over one-third of the available reducing equivalents (primary substrates). The methane concentrations were comparable in all incubations regardless of dioxin congener spiked and the presence of 2-BrDD. Methane concentration in the sulfate-amended systems indi-
cated a low level of methanogenic activity. Less than 1% of the reducing equivalents was converted via methanogenesis, most likely due to the presence of high sulfate concentrations. Under these conditions, methane formation was not likely hydrogenotrophic as sulfate reducers compete more efficiently for substrates such as hydrogen (Yang & McCarty, 1998). The sulfate consumption was nearly stoichiometric based on the loss of the reducing equivalency, indicating that the predominant respiration in the sulfate-amended incubations was sulfate reduction. Even though sulfate consumption was not monitored during the course of the incubation, residual sulfate (0.94–2.29 mM) was found in all active incubations spiked with dioxins in sulfate-amended microcosms at the end of the incubation. The presence of brominated dioxin appeared to have no effect on either methane production or sulfate consumption. Electron balance calculations based on turnover of primary substrates, dissolved hydrogen concentration, and sulfate reduction/methane formation indicated that whereas sulfate-free incubations were dominated by fermentative processes, sulfate-reduction was predominant in the sulfate-amended incubations. Dissolved hydrogen concentrations were measured in order to determine their correlation with the dominant terminal electron accepting processes (TEAP) and dechlorination patterns. Dissolved hydrogen concentrations were measured for both sulfate-amended (in the range of 2.11–3.76 nM) and sulfate-free incubations (in the range of 1.96–3.53 nM).

Table 1

<table>
<thead>
<tr>
<th>Incubations</th>
<th>Sulfate reduction</th>
<th>Methane production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual loss</td>
<td>Predicted lossa (%)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Unspiked control</td>
<td>9.96</td>
<td>16.19 (61.5)</td>
</tr>
<tr>
<td>1234678-HpCDD</td>
<td>15.67</td>
<td>16.22 (96.6)</td>
</tr>
<tr>
<td>1234678-HpCDD + 2-BrDD</td>
<td>17.38</td>
<td>16.15 (107.6)</td>
</tr>
<tr>
<td>1234679-HpCDD</td>
<td>16.29</td>
<td>16.2 (100.5)</td>
</tr>
<tr>
<td>1234679-HpCDD + 2-BrDD</td>
<td>15.96</td>
<td>16.17 (98.7)</td>
</tr>
</tbody>
</table>

a Calculated based on the amount of primary substrates added and the equation (use acetate as an example): CH₃COO⁻ + SO₂⁻ = 2HCO₃⁻ + HS⁻.

b Ratio of actual loss over predicted loss expressed in percent.

c Methane production was estimated by the subtracting of the time zero from the endpoint.

d Obtained by multiplying the number of moles of total loss of the reducing equivalent added by the theoretical stoichiometric ratio of methane production without cell biomass production from the equation (use acetate as example): CH₃COO⁻ + H₂O = CH₄ + HCO₃⁻.
3.2. Impact of environmental conditions and dioxin compounds on microbial community structure

The bacterial community structure of each incubation type was characterized and compared by PCR-DGGE analysis (Fig. 1). Several features were observed from the DGGE banding patterns: (i) the addition of the priming compound caused major shifts in microbial composition in both sulfate-amended and sulfate-free incubations; (ii) the presence of the priming compound resulted in a similar community structure independent of the dioxin spiked, but dependent on the geochemical conditions.

The sulfate-free incubation without dioxins (unspiked control) showed two highly visible bands (A-1 and A-2), indicating two specific populations. The derived sequences of the first band (A-1) indicated its close relation (98% match) to Pseudomonas stutzeri within the γ subgroup of the proteobacteria. This band remained in incubations with either heptaCDD isomers alone (lanes B and D), but almost entirely disappeared in the presence of 2-BrDD (lanes C and E). The second band (A-2) was present in incubations spiked with either heptaCDD isomer and in the presence of 2-BrDD. The sequence analysis revealed its close match (98%) in sequence to Marinobacter sp., also within the γ subgroup of the proteobacteria. Sulfate-free incubations with 2-BrDD (lanes C and E) generated a novel unidentified band (C-1). The unspiked control (lane F) in the sulfate-amended incubations showed a high diversity in microbial community structure with several distinct populations. One band (H-1) showed close resemblance (97% match) in sequence to Thiorhodovibrio winogradskyi, a sulfur oxidizer within the γ subgroup of the proteobacteria.

Cluster analysis of DGGE gel banding patterns demonstrated significant diversity between microbial communities originating from sulfate-amended and sulfate-free incubations as shown by two separate clusters in Fig. 2, with exception for the first lane (represents lane G in Fig. 1). While the impact of PCDDs on community structure under sulfate-amended conditions was not quite clear due to lane G as an outlier in Fig. 1, under sulfate-free conditions both heptaCDD-spiked communities clustered with the dioxin-free control, demonstrating the absence of selective pressure due to the presence of PCDDs. This is counter to observations with PCBs, where the addition of 2,3,5,6-tetrachlorobiphenyl effected the selection of species (e.g., Thermotogales, Dehalococcoides ethenogenes-similar organisms) that were either absent or less predominant in the absence of the spiked congener (Pulliam-Holoman et al., 1998).

3.3. Microbial dechlorination activity

The dechlorination patterns are similar between the two heptachlorodioxins investigated. The dechlorination pattern interpretation was based on the dechlorination of 1,2,3,4,6,7,8-HpCDD as it can potentially produce 2,3,7,8-tetrachlorodibenzo-p-dioxin, which is arguably the most toxic chlorinated compound. The effect of 2-BrDD on dechlorination of 1,2,3,4,6,7,8-heptaCDD in sulfate-amended and sulfate-free incubations was assessed in terms of both dechlorination yield and homologue distribution (Figs. 3 and 4). Autoclaved controls did not yield lesser
chlorinated products (data not shown). Not all the loss of the starting 1,2,3,4,6,7,8-heptaCDD can be accounted for as only dechlorination products were monitored and analyzed. In all active incubations, PCDD dechlorination has occurred. The
main effect of the presence of 2-BrDD is that the percentage of lateral reactions in PCDD dechlorination increased under all the conditions studied, even though laterally (2,3,7,8)-substituted PCDDs were formed, including 2,3,7,8-TCDD. The yields (amount of products formed as a percent of total parent compound removed) of 2,3,7,8-substituted hexaCDDs among dechlorination products in 2-BrDD incubations represented 10% in the presence and 30% in the absence of sulfate (Figs. 3 and 4), whereas in the absence of 2-BrDD these values were 21% and 40% for sulfate-amended and sulfate-free incubations, respectively (data not shown). 1,2,3,7,8-PentaCDD was also detected, but its level was below the quantitation limit. In the presence of 2-BrDD, the yield of 2,3,7,8-TCDD resulting from dechlorination of 1,2,3,4,6,7,8-heptaCDD under both tested conditions was up to one order of magnitude lower than in the absence of 2-BrDD (1% and 2% in the presence of 2-BrDD; 10% and 20% in the absence of 2-BrDD).

Under sulfate-free conditions, the presence of 2-BrDD also caused the greatest extent of the dechlorination resulting in the formation of monoCDDs (Figs. 3 and 4). A similar trend was reported earlier for PCDD dechlorination in Passaic River sediments (Albrecht et al., 1999). Moreover, the presence of 2-BrDD caused a significant increase in dechlorination yield. Dechlorination of 1,2,3,4,6,7,8-heptaCDD yielded a total of 55 and 72 nM of lesser-chlorinated dioxin congeners in sulfate-amended and sulfate-free incubations corresponding to a stoichiometric conversion of, respectively, 26% and 32% of the 1,2,3,4,6,7,8-heptacDD (data derived from the difference in 1,2,3,4,6,7,8-heptaCDD concentration before and after the incubations, 211 and 226 nM in sulfate-amended and sulfate-free conditions respectively). The absolute yield of products increased nearly 2-fold due to the presence of 2-BrDD (Fig. 5).

Hexa-through diCDD isomers were produced under both conditions, while monoCDDs were the end products under sulfate-free conditions only, and only in the presence of 2-BrDD (Fig. 4). Here, 1- and 2-monoCDD were formed in nearly equimolar concentrations. Tri- and dichlorodibenzo-p-dioxin congeners were the major congeners accounting for 28–41% of all dechlorination products except for sulfate-free incubations without 2-BrDD (Fig. 6).

4. Discussion

The presence of 2-BrDD caused the most distinctive shifts in microbial community structure under both sulfate-amended and sulfate-free conditions, relative to the control community. Moreover, DGGE profiles originating from 2-BrDD incubations exhibited highest structural similarity among themselves both in the presence or the absence of sulfate (78% and 69%, respectively, Fig. 2). The distinctive patterns of community structure in the presence of 2-BrDD indicated that subsets of populations in the estuarine enrichment cultures are capable of exploiting the new niche provided by this compound, even at the low concentrations provided (0.2 μM). Bedard et al. (1998) observed that the addition of specific brominated PCBs primed specific dechlorinating processes. It was shown that brominated congeners with a
Fig. 3. Dechlorination pathway of 1,2,3,4,6,7,8-heptaCDD in sulfate-amended incubations amended with 2-BrDD under estuarine conditions. The microcosms were incubated at room temperature for 12 months.
Fig. 4. Dechlorination pathway of 1,2,3,4,6,7,8-heptaCDD in sulfate-free incubations amended with 2-BrDD under estuarine conditions. The microcosms were incubated at room temperature for 12 months.

meta bromine primed Dechlorination Process N (flanked meta dechlorination) and congeners with an unflanked para bromine primed Dechlorination Process P (flanked para dechlorination). The authors hypothesized that brominated analogues primed specific PCB-dechlorinating populations (Bedard et al., 1998). However, no
observations on microbial community shifts have been demonstrated following amendments of PCB-dechlorinating systems with brominated analogues.

The result from our experiment clearly indicates that the changes are directly attributable to 2-BrDD as evidenced by the new bands (e.g., band #1 in lane C for sulfate-free microcosms and band #2 in lane J for sulfate-amended microcosms). It is unclear, however, whether those species associated with the new bands possess specific dechlorinating activities towards either highly or lesser chlorinated dioxins. In order to understand their actual role in PCDD dechlorination, those microorganisms should be isolated and further studied, and the temporal changes in microbial community structure as related to the degree and extent of PCDD dechlorination in the presence of 2-BrDD should also be followed.

Recently, Yang and McCarty (1998) reported on the use of different organic acids (which ferment to produce hydrogen) to stimulate reductive dechlorination in a benzoate-acclimated dehalogenating mixed culture. Dissolved hydrogen concentrations between 2 and 11 nM were found to be optimal for dechlorination reactions and did not result in competition by methanogenic bacteria. Dissolved hydrogen concentrations in the presence of 2-BrDD correlated with sulfate-reduction as the dominant TEAP (Vroblesky & Chapelle, 1994). In the absence of sulfate, and a predominance of methanogenic activity, H2 concentrations are expected to be in the 5–25 nM range, unless other coupling (e.g., halorespiring) processes are active. Since mixed cultures were used in this study and no dechlorinating organisms were identified, the measured dissolved hydrogen concentration itself does not necessarily indicate halorespiration as the respiratory process for the observed dechlorination activity.
No correlation between respiratory and dechlorination activity could be inferred from the metabolic endpoints. The onset of dechlorination activity was unknown since no kinetic analysis was performed. It has been reported that dechlorination of PCBs started at a later stage in the presence of sulfate in estuarine media (Berkaw et al., 1996) and that inhibition of sulfate-reduction negatively impacted PCB dechlorination (Cutter et al., 1998). Several observations can be made regarding PCDD dechlorination as a function of the presence of 2-BrDD and incubation conditions: (i) PCDD dechlorination occurred under sulfate-reducing conditions; (ii) the presence of 2-BrDD caused more extensive dechlorination; (iii) the homologue distribution among dechlorination products was significantly affected by the presence of 2-BrDD; (iv) concentrations of lesser chlorinated dioxin congeners were higher under sulfate-free conditions; and (v) monoCDDs were only produced in the presence of 2-BrDD under sulfate-free conditions.

We conclude that in the presence of 2-BrDD, shifts among the patterns and the extent of PCDD dechlorination correlate to changes in microbial community.
structure as revealed by 16S rRNA analysis. Therefore, the presence of 2-BrDD likely resulted in the priming of microbial populations with novel dechlorinating capabilities and may indicate that PCDD dechlorination is not as fortuitous as postulated earlier. Whether this indicates that populations with predominantly peri- and lateral-dechlorinating capabilities can be stimulated in sediments remains to be investigated. The indications of cause-and-effect relationships between PCDD dechlorination activity and community composition may lead to further development of predictive tools for natural and enhanced dechlorination of PCDD in estuarine and marine environments.

Acknowledgements

Funding for this research was provided by the Office of Naval Research (ONR). Since this paper has not been reviewed by this agency, no endorsement should be inferred. We thank Upassri Sorachart for technical assistance. We are indebted to Dr. Terence L. Marsh of the Center for Microbial Ecology and Department of Microbiology at Michigan State University for performing cluster analysis of the DGGE gel banding patterns.

References


