Pathways for the Anaerobic Microbial Debromination of Polybrominated Diphenyl Ethers

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Received August 21, 2007. Revised manuscript received January 14, 2008. Accepted January 23, 2008.

The debromination pathways of seven polybrominated diphenyl ethers (PBDEs) by three different cultures of anaerobic dehalogenating bacteria were investigated using comprehensive two-dimensional gas chromatography (GC × GC). The congeners analyzed were the five major components of the industrially used octa-BDE mixture (octa-BDEs 196, 203, and 197, hepta-BDE 183, and hexa-BDE 153) as well as the two most commonly detected PBDEs in the environment, penta-BDE 99 and tetra-BDE 47. Among the dehalogenating cultures evaluated in this study was a trichloroethene-enriched consortium containing multiple Dehalococcoides species, and two pure cultures, Dehalobacter restrictus PER-K23 and Desulfitobacterium hafniense PER-K23. The congener mixtures were analyzed by GC × GC coupled to an electron capture detector to maximize separation and identification of the product congeners. All studied congeners were debrominated to some extent by the three cultures and all exhibited similar debromination pathways with preferential removal of para and meta bromines. Debromination of the highly brominated congeners was extremely slow, with usually less than 10% of nM concentrations of PBDEs transformed after three months. In contrast, debromination of the less brominated congeners, such as penta 99 and tetra 47, was faster, with some cultures completely debrominating nM levels of tetra 47 within weeks.

Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants that have been integrated into plastics and textiles for about three decades (1). Recent studies found that the penta- and tetra-substituted BDEs are endocrine disruptors, thus raising concern over their elevated levels of detection in the environment and particularly in human breast milk (2, 3). Concentrations in environmental samples and human tissues have reportedly been increasing exponentially over time, doubling approximately every five years (4). A recent study of sediments from the San Francisco Bay Area reported PBDE concentrations up to 200 ppb (5). The penta- and octa-substituted PBDEs have been banned in the European Union and in several states in the United States and their production has ceased. However, the fully brominated deca-BDE continues to be used (6).

PBDEs can be debrominated under anaerobic conditions by dehalogenating bacteria (7) (8). Less brominated congeners, such as penta- and tetra-BDEs, are more toxic and bioavailable than more highly brominated congeners, such as octa-BDEs (9). It is therefore important to understand the dominant debromination pathways in order to predict the likely congeners produced from the transformation of environmentally prevalent PBDEs. Although specific rates of debromination determined in the laboratory can not be directly extrapolated to the environment, nonetheless, improved understanding of preferential transformation pathways can provide important information about the relative biodegradability or recalcitrance of individual PBDE congeners.

We previously found that a commercial octa-BDE mixture could be reductively debrominated to a variety of less brominated congeners including penta-BDE 99 and tetra-BDE 47 (8). Because of the complexity of the numerous starting substrates present in the octa mixture and the diversity of products that were formed, it was not possible to delineate specific PBDE debromination pathways in that study. To clearly identify specific debromination pathways, products generated by bacteria exposed to individual PBDE congeners must be identified. There are analytical challenges associated with PBDE congener identification since many of them (up to 50%) coelute when analyzed by conventional gas chromatography. Comprehensive two-dimensional chromatography employs two different and sequential chromatography columns that dramatically reduces the number of coelutions, significantly improving the separation and identification of PBDE congeners (10).

Our goal was to determine the debromination pathway of seven environmentally relevant PBDE congeners by a variety of dehalogenating bacteria. The congeners studied were those that dominate the industrial octa-BDE mixture DE-79, specifically octa 196, octa 203, octa 197, hepta 183, and hexa 153. In addition, two of the most frequently detected congeners in the environment, penta 99 and tetra 47, were included in this study. Bacteria representing three different dehalogenating genera, Dehalococcoides, Dehalobacter, and Desulfitobacterium, were evaluated to provide a broad understanding of anaerobic PBDE debromination. Our results describe the debromination pathways and the PBDE products that might be expected to occur in the environment under anaerobic conditions. In addition, this work represents the first use of comprehensive two-dimensional gas chromatography to understand PBDE debromination processes.

Materials and Methods

Chemicals. Individual congeners purchased from Accustandard (New Haven, CT) or Wellington Laboratories (Guelph, ON, Canada) were dissolved in nonane at a concentration of 50 µg/L. The octa-BDE mixture was obtained from Sigma-Aldrich (Sigma-Aldrich, Inc., St. Louis, MO). The major congeners in the octa-BDE mixture are deca (209), two nona congeners (207 and 206), three octa congeners (196, 203, and 197), three hepta congeners (183, 180, and 171), one hexa congener (153), and additional trace congeners (hexes 154 and 144; heptas 140, 138, and 191; octa 201 and 194; nona 208) (11). Decabromobiphenyl (DBB) and isooctane were purchased from Fisher Scientific (Fairlawn, NJ).
Cultures. Three cultures were investigated in this study: (1) a trichloroethene-dechlorinating enrichment culture containing multiple *Dehalococcioides* strains (12, 13) mixed in a 1:10 ratio with the tetrachloroethene (PCE) dechlorinating strain *D. ethenogenes* 195 to form a mixed culture called ANAS195 (strain 195 was kindly donated by Professor Zinder at Cornell University (14)); (2) a PCE dechlorinating bacterium *Dehalobacter restrictus* PER-K23 (DSM 9455) (15); and (3) a pentachlorophenol degrading bacterium *Desulfitobacterium hafniense* PCP-1 (DSM 12420) (16), both obtained from the German Collection of Microorganisms and Cell Cultures. Additionally, we tested *Desulfitobacterium chlororespirans* Co23 (DSM 11544) (17), *Desulfitobacterium dehalogenans* JW/IU-DC1 (DSM 9161) (18), and *Desulfitomonile tiedjei* DCB-1 (DSM 6799) (19) for their ability to debrominate the octa-BDE mixture.

Culture Media and Cultivation. Cultures were grown in 160 mL serum bottles with 100 mL of medium and sealed with butyl rubber septa (Bellco Glass Inc., Vineland, NJ) and aluminum crimp caps (Wheaton Science Products, Millville, NJ) to ensure a robust anaerobic environment over the course of the experiment. ANAS195 was grown in mineral salts medium (20) reduced with 0.2 mM cysteine sulfide with 20 mM lactate and 685 µM PCE. *D. restrictus* was grown in the same medium as ANAS195 with 5 mM acetate, 685 µM PCE, and 100 mg/L of peptone. The headspace of the bottles was H2/CO2 (80:20 v/v). *D. hafniense*, *D. dehalogenans*, and *D. chlororespirans* were grown in DSMZ medium 720 reduced with 1.25 mM Na2S with 5 µM pentachlorophenol, 10 mM 3-chloro-4-hydroxyphenylacetate, and 10 mM 3-chlorobenzoic acid. Octa-BDE was added to these cultures dissolved in nonane for final concentrations of 1 µM. Individual PBDE congeners were detected using comprehensive two-dimensional gas chromatography with electron capture detection at the Wageningen IMARES laboratories in IJmuiden, Netherlands (10). The primary analytical column was a 30 m × 0.25 mm × 0.25 µm DB-1 column (J & W Scientific, Folsom, CA) and the secondary column was a 1 m × 0.10 mm × 0.1 µm uncoated fused-silica deactivated column (BGB Analytik, Aalst, Switzerland), which served as the modulator loop. The temperature program for both columns was as follows: oven temperature held at 110 °C for 2 min, increased...
The complexity of multiple starting substrates and products generated from transformation of the octa-BDE mixture precluded the identification of specific PBDE debromination pathways. Three diverse and representative debrominating cultures, ANAS195, \textit{D. restrictus}, and \textit{D. hafniense}, were therefore exposed individually to the five major components of the octa-BDE mixture (octas 196, 203, and 197; hepta 183, and hexa 153). Additionally, two environmentally important congeners that had been detected as products of the octa-BDE mixture transformation (penta 99 and tetra 47) were also tested. As a representation, debhalogenation products of hepta-BDE 183 by the ANAS195 culture are shown in Figure 2. Product peaks are clearly visible from hepta to tribrominated congeners. With the improved separation enabled by GC × GC, a number of peaks could be matched to PBDE standards and identified, including penta-BDE 99 and tetra-BDE 47—two of the most toxic PBDE congeners (23, 24).

Since the objective of this study was identification of debromination pathways rather than the calculation of biotransformation kinetics, and because the likely variability in extraction efficiencies for the wide variety of individual PBDE congeners detected in this study could not be adequately quantified using the single internal standard method employed herein, mass balances on debromination products were not calculated. Therefore, debromination products in Table 1 are represented as percent of the total molar concentration of recovered PBDEs. Some products were present only in trace quantities and are listed as detectable but not quantifiable (DNQ).

All tested congeners were found to be biotransformable by all cultures within three months of incubation, although...
TABLE 1. Products Generated by the Degradation of Individual PBDE Congeners by Microbial Culture after Three Months

<table>
<thead>
<tr>
<th>culture</th>
<th>octa 197</th>
<th>octa 203</th>
<th>octa 196</th>
<th>hepta 183</th>
<th>hepta 153</th>
<th>penta 99</th>
<th>tetra 47</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANAS195</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hepta 184 0.8%</td>
<td>(0.5–1.1%)</td>
<td>hepta 187* 2.6%</td>
<td>(2.3–3.0%)</td>
<td>hepta 183 0.8%</td>
<td>(0.4–1.5%)</td>
<td>hepta 182 0.5%</td>
<td>(0.2–1.5%)</td>
</tr>
<tr>
<td>hepta 184 0.8%</td>
<td>(0.4–0.9%)</td>
<td>hepta 187* 2.6%</td>
<td>(2.5–2.8%)</td>
<td>hepta 183 0.8%</td>
<td>(0.2–1.5%)</td>
<td>hepta 182 0.5%</td>
<td>(0.3–0.5%)</td>
</tr>
<tr>
<td>hepta 176 0.4%</td>
<td>(0.3–0.7%)</td>
<td>hepta 180 0.3%</td>
<td>(0.25–0.3%)</td>
<td>hepta 182 0.5%</td>
<td>(0.1–0.6%)</td>
<td>hepta 175* 1.2%</td>
<td>(0.4–2.6%)</td>
</tr>
</tbody>
</table>

| D. hafniense |          |          |          |           |           |          |          |
| hepta 184 0.3% | (0.1%–0.4%) | hepta 187* 2.3% | (1.4–3.3%) | hepta 183 3.4% | (1.7–5.1%) | hepta 182 3.1% | (0.9–5.3%) | hepta 180 1.2% | (0.4–2%) | hepta 180 1.2% | (0.4–2%) |
| hepta 184 0.3% | (1.9–2.9%) | hepta 187* 2.3% | (4.0–7.9%) | hepta 183 3.4% | (1.7–5.1%) | hepta 182 3.1% | (0.9–5.3%) | hepta 180 1.2% | (0.4–2%) | hepta 175* 2.1% | (1.1–3.1%) |
| hepta 176 0.9% | (0.7–1.2%) | hepta 176 0.9% | (0.7–1.2%) | hepta 183 3.4% | (1.7–5.1%) | hepta 182 3.1% | (0.9–5.3%) | hepta 182 3.1% | (0.9–5.3%) | hepta 175* 2.1% | (1.1–3.1%) |

| D. restrictus |          |          |          |           |           |          |          |
| hepta 184 4.6% | (2.4–6.7%) | hepta 187* 7.6% | (7.1–8.1%) | hepta 191 4.1% | (1.6–6.9%) | hepta 183 7.5% | (2.2–11.8%) | hepta 183 2.3% | (0.8–3.2%) | hepta 175* 14.2% | (4.2–22%) |
| hepta 184 4.6% | (2.2–2.6%) | hepta 187* 7.6% | (6.0–6.5%) | hepta 183 7.5% | (2.2–11.8%) | hepta 183 2.3% | (0.8–3.2%) | hepta 175* 14.2% | (4.2–22%) | hepta 175* 14.2% | (4.2–22%) |
| hepta 176 2.6% | (1.1–3.4%) | hepta 180 0.2% | (0.24–0.25%) | hepta 182 2.4% | (6.0–6.5%) | hepta 183 7.5% | (2.2–11.8%) | hepta 182 2.3% | (0.8–3.2%) | hepta 175* 14.2% | (4.2–22%) |

The products from each substrate are listed in separate columns beneath the substrate name. The products are divided by culture, listed on the left. The average mole percent of each product at the end of the sampling period is listed next to the name. The range of mole percents for the replicate samples where available is listed in parentheses beneath the average value. The D. restrictus 47 data were collected after two weeks and the ANAS195 197 data were collected after 6 months. DNQ indicates that the congener was detected but was not quantifiable. * Indicates congener that is presumptively identified due to lack of available standards.

The extent of debromination varied greatly between cultures and between congeners (Table 1). However, the debromination pathway for each congener was surprisingly similar for all tested cultures. For simplification in describing the pathways, we assumed that there was no isomeric rearrangement of bromines around the PBDE ring during debromination. Except for two unavailable congeners, all substrate and product PBDE peaks were matched with standards for specific identification and quantification. Figure 3 summarizes the observed debromination pathway for all tested congeners. Based on the geometries of the substrate molecules, possible debromination products ranged from two to eight possible congeners, although in most cases many of these congeners were never produced. All cultures exhibited preferences for removing bromines at certain positions, typically the meta and para bromines.

In some cases, due to the synthesis process, the substrates contained impurities that were also possible debromination products. For example, octa 203 contained trace (around 0.1 mol percent) quantities of heptas 183 and 187 and hepta 183 contained hexa 153 and 154. However, in each case we were able to confirm that these PBDEs were being biologically produced because the concentrations of the products increased significantly (at least 2-fold) in the active bottles while remaining constant in control bottles during the three month incubation.

Biotransformation of the octa congeners was rather slow and limited for all tested cultures, in most cases with less than 10 mol% of the original substrate being degraded within three months. Octa 197 was debrominated by ortho substitution to hepta 183, by meta substitution to hepta 184, and by para substitution to 176 by all three cultures. All congeners were generated in approximately equal quantities, cumulatively amounting to 9 mol% or less of the total recovered molar concentration of PBDEs at the end of three months. Octa 203 debromination produced two major congeners for all three cultures: hepta 183 and presumptively hepta 187—the meta and para bromine removal products respectively. Both peaks represented between 2 and 7 mol% after three months. An ortho debromination product, hexa 180, was produced in trace quantities, accounting for only 0.3 mol%. Congener 187 is considered presumptively identified because that congener was not available for purchase. However, given the geometry of the bromines positioned on the octa 203 molecule, there are only six possible debromination products (heptas 180, 183, 187, 190, 185, and 181) and the availability of standards for all of these except for 187 facilitated identification by elimination. That is, of the three product peaks detected in all samples, two have matching standards (180 and 183), while the other three standards (191, 185, and 181) did not match the third product peak, leaving 187 as the lone possible candidate for the unidentified peak.

Four congeners were identified as debromination products from octa 196, including heptas 183, 182, and presumptively identified 175 for all species. D. restrictus also
produced the ortho debromination product 191 whereas *D. hafniense* and ANAS195 produced 180—a different ortho product. One of the possible debromination products of octa 196, hepta 174 was not available for purchase and therefore the production of this congener could not be verified. Although one of the product peaks perfectly matches the 175 standard, it could not be excluded that this peak might be 174. According to relative retention time estimates by Wang et al. (25) and Rayne and Ikonomou (26), 175 and 174 have very similar retention times and might coelute even on a two-dimensional GC. Furthermore, both 175 and 174 are para bromine removal products and thus equally likely possibilities. However, 175 is a doubly flanked para removal whereas 174 is a singly flanked para removal. Since doubly flanked bromines are preferred over singly flanked ones in the other PBDE pathways, we have presumptively designated hepta 175 as the product.

Hepta 183 was debrominated relatively rapidly by all three cultures, producing many products including hexas 154 (meta removal), 153 (ortho removal), 149, and 144 (para removal products). Additionally, 183 was debrominated to the meta substitution product hexa 139 by only *D. restrictus*. Oddly, the two para removal products varied significantly in concentrations, with 149 being a major product with concentrations up to 4 mol% and 144 produced only in minor quantities, representing on average 0.5 mol%. Hexa 153 was debrominated to pentas 99 and 101 by all three cultures. Penta 118 required an ortho bromine removal and was produced only by the ANAS195 culture in trace quantities at around 0.6 mol%.

In all cultures, penta 99 was debrominated to tetras 47 and 49 by meta and para substitution respectively. ANAS195 and *D. hafniense* also produced tetra 66, an ortho bromine removal product, whereas *D. restrictus* and *D. hafniense* substituted a para bromine, producing tetra 48. Tetras 49 and 48 were the predominant congeners, representing up to 22 mol%, whereas 47 and 66 were detected at concentrations less than 1 mol% after three months. In particular, tetra 47 appeared only at trace concentrations probably because it is so biodegradable. When tetra 47 was exposed to bacteria as a substrate, it was biotransformed relatively rapidly particularly in the *D. hafniense* and *D. restrictus* cultures where the original congener was almost completely debrominated by the conclusion of the experiment. *D. hafniense* produced the ortho debromination product 191 whereas *D. hafniense* and ANAS195 produced 180—a different ortho product. One of the possible debromination products of octa 196, hepta 174 was not available for purchase and therefore the production of this congener could not be verified. Although one of the product peaks perfectly matches the 175 standard, it could not be excluded that this peak might be 174. According to relative retention time estimates by Wang et al. (25) and Rayne and Ikonomou (26), 175 and 174 have very similar retention times and might coelute even on a two-dimensional GC. Furthermore, both 175 and 174 are para bromine removal products and thus equally likely possibilities. However, 175 is a doubly flanked para removal whereas 174 is a singly flanked para removal. Since doubly flanked bromines are preferred over singly flanked ones in the other PBDE pathways, we have presumptively designated hepta 175 as the product.

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and *D. restrictus* produced only tri-BDE 17, which was quickly debrominated further to di-BDE 4. This di-BDE congener represented a major product at 28 and 83 mol% in the *D. hafniense* and *D. restrictus* cultures, respectively. It is possible that further debromination products such as mono-BDEs were produced in the *D. hafniense* and *D. restrictus* cultures, but these congeners were never detected, perhaps due to the low sensitivity of electron capture detection to mono-BDEs, interference with compounds in the bacterial medium that elute at the same time as mono-BDEs, and low mono-BDE concentrations. The ANAS195 culture, in addition to the para substitution product tri-BDE 17, generated the ortho bromine removal product tri-BDE 28, although in trace concentrations (0.2 mol%). Di-BDE 4 was never observed in the ANAS195 samples.

Although Figure 3 depicts only the products of one bromine removal for each tested congener, further debromination products were frequently detected. These additional products are not represented in the pathway schematic as it is not always evident which congener they were derived from, since often multiple debromination pathways (para, meta, ortho) are possible. These subsequent debromination products are listed in Table 1 along with all of the single bromine removal products for each congener and bacterial culture.

Discussion

All of the PBDE congeners evaluated in this study were found to be debrominated to some degree by anaerobic dehalogenating microorganisms. The debromination pathways were found to be generally consistent across the different cultures, with exceptions typically represented by a single congener produced in trace quantities. In general, debromination was slower for the more brominated congeners, such as the octas, than for the less brominated penta and tetras—a trend that has also been observed with PCBs (27) and that can be explained by the increasing hydrophobicity of PBDEs as the number of bromines increases. The *D. hafniense* and *D. restrictus* cultures were able to dehalogenate almost all of the tetra 47 they were exposed to (the least hydrophobic PBDE tested), while transforming typically less than 10 mol% of the octa congeners in the same time period.

Para and meta bromines were preferentially removed from the rings by the cultures, often with multiple para or meta bromine removal products being formed. The most commonly substituted bromines were those that are double flanked. In the case of octa-BDE 203, the doubly flanked meta and para bromines were removed whereas the two other singly flanked para and meta bromines were never removed. Given that repulsion between adjacent bromines results in increased enthalpies of formation, it is not surprising that doubly flanked bromines are removed more frequently than singly flanked bromines (28).

Ortho bromines were also frequently removed. Typically, however, the ortho substitution products were minor ones compared to the para and meta bromine removal products in terms of concentration and were sometimes not even produced (as is evident with the lack of production of tri 28 from tetra 47 and penta 118 from hexa 153 for both the *D. hafniense* and *D. restrictus* cultures). Furthermore, as is evident with hepta 183, only one of the ortho-bromine removal products (hexa 153) was formed, while all the possible meta and para bromines were removed. This emphasizes the difficulty in removing ortho halogenes, as had been observed with PCBs and polybrominated biphenyls (PBBs) (29–31). The most notable pathway differences between species occurred with ortho bromine removals. For example, *D. hafniense* and ANAS195 preferred to remove the ortho bromine on the fully brominated ring of octa 196, creating hepta 180 whereas *D. restrictus* preferred the ortho removal on the other ring, creating hepta 191. The ANAS195 culture was better than *D. hafniense* and *D. restrictus* at ortho bromine removals, which might be explained by the fact that *D. ethenogenes* 195 is known to dechlorinate chlorophenols by ortho removal in the presence of an adjacent meta chlorine (32). Oddly, the PBDE dehalogenation patterns for *D. hafniense* differed from its chlorophenol dehalogenation patterns in that it prefers to remove the ortho chlorines from chlorophenols, whereas it preferentially removes meta and para bromines from PBDEs (16).

Our study is the first to show that bacteria in the *Dehalococcoides* and *Dehalobacter* genera can transform PBDEs. In particular *Dehalobacter* had not been previously shown to dehalogenate aromatic compounds, unlike *Dehalococcoides* and *Desulfotobacterium* (33–35). Both *D. restrictus* and *D. hafniense* exhibited more extensive PBDE debromination producing higher concentrations of products compared to the ANAS195 culture, which degraded all congeners much more slowly. This difference is perhaps due to the faster growth exhibited in the *D. restrictus* and *D. hafniense* cultures. *D. restrictus* and *D. hafniense* were both capable of rapid removal of multiple bromines as is particularly evident with tetra 47, which both cultures transformed to mostly di-BDE 4 within two weeks in the case of *D. restrictus* and within three months in the case of *D. hafniense*.

The debromination results using individual congeners described here correlate well with previously reported debromination of an octa mixture in that many of the same products were generated (8). For example, the dominant hexas generated from hepta 183 in this study (hexas 154, 153, 149, and 144) were the same as those generated from debromination of the octa-BDE mixture. The observed debromination products of hexa 153 (pentas 99 and 101) were also detected in that study, as were two of the debromination products of penta 99 (tetras 47 and 49) and the tetra 47 debromination product tri 17. There were also other products in the octa mixture samples that did not match our individual congener studies. This was expected given that there were additional minor components in the octa mixture which were not evaluated in this study. Furthermore, since the octa mixture samples were incubated for almost a year, many of the generated products likely resulted from multiple bromine removal steps.

An interesting aspect of these results is their striking similarity to PCB and PBB dehalogenation studies. The prevalent removal strategies observed in this study were meta and para debrominations, as is common with PCBs and PBBs.
Specifically, Kuipers et al. found that octa-CB 203 produced hepta-CB 183 similar to what was shown here for PBDEs (36). Hepta-CB 181 was also generated, whereas this congener was never detected in PBDE samples. Octa-CB 196 was dechlorinated to hepta-CB 183, as was observed with the PBDE analog. Morris et al. found that with PBBS anaerobic sediment microorganisms dechlorinated hexa-BB 153 to tetra-BBs 49 and 47 and tetra-BB 47 to tri-BB 17, exactly as has been observed in this study (30).

Dehalococcoides is known to dechlorinate PCBs (27, 34, 37). Fagervold et al. found that microbial microcosms dechlorinated hepta-CB 183 to hexa-CB 154, hexa-CB 153 to penta-CB 99, and penta-CB 99 to tetra-CB 47, as analogous to the observation in our study (27). One notable difference between PCB and PBDE degradation, however, is that ortho dechlorination appears to be quite rare with PCBs, whereas ortho dechlorination products were detected in all of our experiments.

The extent of PBDE dehalogenation observed in this study, in which nM quantities were transformed over the course of months, is orders of magnitude less than reported PCB dechlorination, in which µM quantities were transformed over the same time period (27, 33, 34). This may in part be due to higher cell densities of dechlorinating organisms, which ranged from 106 to 109 cells/mL in pure and mixed cultures. However, it may also be due to the approximately 10-fold higher hydrophobicity of PBDEs compared to PCBs (9, 36).

As evidenced by the lack of de bromination observed with D. tiedjei DCB-1, not all dehalogenating bacteria are capable of catalyzing PBDE de bromination. Although we have not identified the specific enzymes responsible for these reactions, it is possible that the de bromogeneses responsible for the congener sequences of these isolates is cometabolic, requiring the concomitant absence of PCE, although the mechanism behind this reaction is unclear.

In conclusion, the similarity of de bromination pathways exhibited by these three different bacterial cultures analyzed in this study suggests that pathways for PBDE de bromination are conserved across some species and genera. An additional notable observation is that although tetra 47 and penta 99, two of the most toxic PBDE congeners, are products of higher congeners, they are also relatively readily de brominated by anaerobic bacteria to at least di-BPs. This suggests that it is possible that their accumulation could be transient, leading to the generation of lesser toxic final products.

Acknowledgments
We thank Patrick Lee for providing the ANAS culture and Professor Steve Zinder for providing D. ethenoxidans 195. Funding was provided by the University of California Center for Water Resources and the NIEHS Superfund Basic Research Program under grant ES04705. K.R. also thanks Dr. Gregory Cost for excellent scientific discussions and advice.

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ES0720917