

Microbial Reductive Debromination of Polybrominated Diphenyl Ethers (PBDEs)

JIANZHONG HE,[†]
KRISTIN R. ROBRACK,[†] AND
LISA ALVAREZ-COHEN^{*,†,‡}

Department of Civil and Environmental Engineering,
University of California at Berkeley, Berkeley,
California 94720-1710, and Earth Sciences Division,
Lawrence Berkeley National Laboratory, 1 Cyclotron Rd.,
Berkeley, California 94720

Polybrominated diphenyl ethers (PBDEs) are a class of widely used flame retardants that have recently been detected in environmental samples, diverse biota, human blood serum, and breast milk at exponentially increasing concentrations. Currently, little is known about the fate of these compounds, and in particular, about the microbial potential to degrade them. In this study, debromination of deca-BDE and an octa-BDE mixture is demonstrated with anaerobic bacteria including *Sulfurospirillum multivorans* and *Dehalococcoides* species. Hepta- and octa-BDEs were produced by the *S. multivorans* culture when it was exposed to deca-BDE, although no debromination was observed with the octa-BDE mixture. In contrast, a variety of hepta- through di-BDEs were produced by *Dehalococcoides*-containing cultures exposed to an octa-BDE mixture, despite the fact that none of these cultures could debrominate deca-BDE. The more toxic hexa-154, penta-99, tetra-49, and tetra-47 were identified among the debromination products. Because the penta-BDE congeners are among the most toxic PBDEs, debromination of the higher congeners to more toxic products in the environment could have profound implications for public health and for the regulation of these compounds.

Introduction

Polybrominated diphenyl ethers (PBDEs) have been used for more than three decades as flame retardants in a wide variety of manufactured materials such as furniture, vehicles, carpets, building materials, and electronic circuit boards because of their demonstrated fire retarding abilities (1). Usually, PBDEs are added to the materials as mixtures of penta-BDEs, octa-BDEs, or deca-BDE. Currently, deca-BDE accounts for over 80% of the total PBDE production (2). Although the penta- and octa-BDEs are being phased out in both the state of California and the European Union due to their toxicity, bioaccumulation, and persistence, deca-BDE continues to be heavily used (3).

PBDEs have very low solubility in water (ng/L to $\mu\text{g/L}$) and are lipophilic; thus, they readily accumulate in fatty tissues of humans and other animals. Although PBDEs have

low acute toxicity, they are proven endocrine disruptors that destroy thyroid hormone balance and cause developmental problems (4). Among the PBDE congeners, penta-BDEs are reportedly the most toxic, causing developmental toxicity at concentrations starting at 0.8 mg/kg body weight (5), while octa-BDEs are teratogens (6) and deca-BDE is classified by the U.S. EPA as a possible human carcinogen (7). Because of their ubiquitous use, lipophilicity and inert characteristics, PBDEs have been detected in environmental air, soil, and water samples, as well as in fish and animal tissues (2, 8). Recently, PBDEs have been detected in human blood serum and breast milk at concentrations that are doubling every five years (2).

Despite their heavy use, there have been few studies on the transformation of these compounds in the environment. PBDEs have been shown to be photolytically debrominated to lower brominated congeners by natural sunlight in a solution of water/methanol (9), and by enzymatic debromination in fish tissues (10–12). Two of the least brominated congeners—4-BDE and 4,4'-BDE—were reported to be debrominate to diphenyl ether in a sediment column containing an undefined anaerobic microbial community (13). Reductive debromination of deca-BDE to nona- and octa-BDEs was also observed in anaerobic sewage sludge after incubation for 238 days (14). Recent studies at a wastewater treatment plant found that PBDEs were removed from wastewater predominantly through adsorption to biomass solids, raising concerns about subsequent application of PBDE-laden biomass solids as fertilizers and soil amendments (15, 16).

To our knowledge, microbial degradation of octa-BDEs has not yet been demonstrated and the degradation of PBDEs by defined isolates or enrichments has not been previously described. PBDEs are relatively oxidized and are structurally similar to polychlorinated biphenyls and dioxins which can be dechlorinated via reductive dehalogenation (17–20). In this study, we investigated anaerobic dechlorinating cultures with the ability to reduce a variety of compounds for their capability to sequentially debrominate PBDEs. In particular, *Dehalococcoides ethenogenes* 195 has the ability to dechlorinate chloroethenes, chlorobenzenes and polychlorinated dibenzodioxins (19, 21) while *Dehalococcoides* sp. strain BAV1 is capable of dehalogenating dichloroethenes (DCEs), vinyl chloride (VC) and vinyl bromide (22), and *Sulfurospirillum multivorans* dechlorinates tetrachloroethene (PCE) and trichloroethene (TCE) (23, 24). Additionally, dechlorinating enrichments containing *Dehalococcoides* spp. were also examined for their debromination capabilities. Degradation of deca-BDE and an octa-BDE mixture containing 8 congeners ranging from hexa- to nona- was tested with the various microbial cultures.

Materials and Methods

Chemicals. Deca-BDE and the octa-BDE mixture were obtained from Sigma-Aldrich (Sigma-Aldrich, Inc., St. Louis, MO), with a purity >98%. The octa-BDE mixture contains two nona congeners (207 and 208), three octa congeners (196, 203, and one unidentified), two hepta congeners (183 and one unidentified), and one hexa congener (153). Decabromobiphenyl (DBB) and analysis quality isooctane were purchased from Fisher Scientific (Fairlawn, NJ).

Cultures. The following cultures were investigated in this study: *Dehalococcoides ethenogenes* 195, supplied by Stephen Zinder at Cornell University; *Dehalococcoides* sp. strain BAV1, supplied by Frank Löffler at the Georgia Institute of Technology; an enriched autotrophic culture containing *D. ethenogenes* 195 designated EC195; and an enrichment

* Corresponding author phone: 510-643-5969; fax: 510-642-7483; e-mail: alvarez@ce.berkeley.edu.

[†] Department of Civil and Environmental Engineering, University of California at Berkeley.

[‡] Earth Sciences Division, Lawrence Berkeley National Laboratory.

containing a number of *Dehalococcoides* spp. designated ANAS (25). A tetrachloroethene to dichloroethene dechlorinating bacterium, *Sulfurospirillum multivorans* (DSM no. 12446) obtained from DSMZ (the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH—German Collection of Microorganisms and Cell Cultures), was also included in this study.

Culture Media and Cultivation. All microorganisms were grown in 160-mL serum bottles filled with 50-mL bicarbonate-buffered defined mineral salts medium reduced by L -cysteine and $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (0.2 mM each) (26, He, et al., unpublished data), which were sealed with blue butyl rubber septa to ensure robust anaerobic protection (Bellco Glass Inc., Vineland, NJ) and aluminum crimp caps (Wheaton Science Products, Millville, NJ). The headspace of the bottles was filled with H_2/CO_2 (80:20 v/v) for all cultures except ANAS, whose headspace was filled with N_2/CO_2 (90:10 v/v). Acetate (5 mM) was added as the carbon source for all pure cultures, whereas lactate (10 mM) was supplied to the ANAS culture and no organic acid was fed to EC195 which grows autotrophically. After autoclaving the medium for 25 min at 121 °C and letting cool to room temperature, 5- μL of TCE carrying dissolved PBDEs (~1 mM of deca-BDE and ~13 mM of octa-BDE mixture) were added to both the sample and control bottles using a Hamilton glass syringe (Hamilton, Reno, Nev.) for final concentrations of 1 mM TCE, 0.1 μM deca- and 1.3 μM octa-BDEs. Active cultures growing on TCE or in the case of strain BAV1 on VC were inoculated (5 or 10% v/v) to the sample bottles. Each experiment was conducted with duplicate biological samples and was repeated at least once to confirm the results. Experiments also included abiotic controls with no added inoculum and autoclaved controls in order to detect possible nonbiological debromination and to account for abiotic losses. All samples and controls were incubated at 30 °C in the dark without shaking.

Sample Preparation. On a weekly basis, 1 mL of culture was removed from the bottles by disposable plastic syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) and subjected to a liquid–liquid extraction with an equal volume of isooctane in a 4-mL amber glass vial. 2- μL of methanol saturated with decabromobiphenyl (DBB), structurally similar to PBDEs, was added to the samples as an internal standard to determine extraction efficiency. The mixture was vortexed for 5 min and then shaken for 16 h in order to extract the PBDEs into the isooctane phase. The samples were then centrifuged at 12 500 rpm for 5 min to remove any particulate matter and bacterial debris. The solvent phase isooctane (~950 μL) was transferred to a 2-mL amber glass vial and concentrated to 100 μL under a stream of N_2 .

Analytical Methods. PBDE congeners were detected using a gas chromatograph (Varian 3800, Palo Alto, CA) equipped with an electron capture detector (GC–ECD). The analytical column was a 15 m \times 0.25 mm i.d. DB-5 capillary column with a 0.25 μm film thickness (J & W Scientific, Folsom, CA) and the temperature program was as follows: oven temperature held at 110 °C for 2 min, increased at 15 °C/min to 310 °C, and held for 60 min. Injector and detector temperatures were 250 °C and 320 °C, respectively. The carrier gas was nitrogen at a flow rate of 3.0 mL/min. A 5- μL amount of the extracted isooctane was injected to the GC column. The identification of specific PBDEs was performed by comparing peak retention times with a standard solution containing 27 identified mono- through deca-BDE congeners (Cambridge Isotope Laboratories Inc. Andover, MA). Six-point calibration curves for the 27 PBDE congeners were established. Extraction efficiency was determined by comparing the DBB peak areas in the samples with a four point DBB calibration curve. The instrument detection limits for PBDE congeners were 0.1–1.0 pmol, corresponding to low nM concentrations in the samples analyzed. Specific iden-

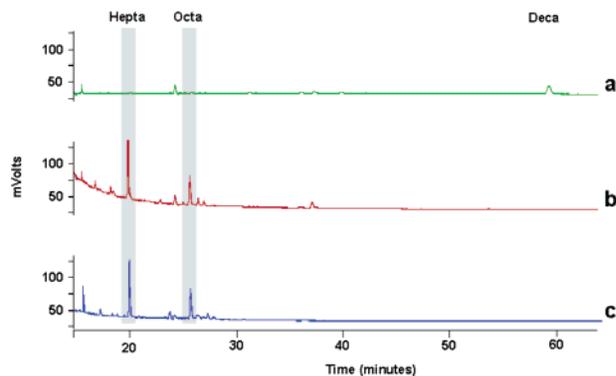


FIGURE 1. GC chromatograms of deca-BDE debromination by *S. multivorans*. (a) autoclaved control; (b) *S. multivorans* replicate 1; (c) *S. multivorans* replicate 2. Shaded areas highlight degradation products.

tification of a subset of the PBDEs was confirmed based on mass/ion ratio with a Finnigan Mat-95 GC–MS equipped with an ECNI detector (8). A standard solution containing twelve di- through hepta-BDEs was utilized for the GC–MS analysis. For enumeration of microbial cells, 16S rRNA gene based real-time polymerase chain reaction (RTm–PCR) was performed as described previously (27). The following primer pair and probe specific to *Dehalococcoides* spp. were selected, forward primer 5'-GGTAATACGTAGGAAGCAAGCG-3', probe 5'-VIC-ACATCCAACCTTGAAAGACCACCTACGCTCACT-TAM-RA-3', reverse primer 5'-CCGGTTAAGCCGGGAAATT-3'.

Results

Degradation of Deca-BDE. *S. multivorans* cells that were initially grown on 500 μM TCE and H_2/CO_2 headspace (80:20) were transferred into defined medium amended with 0.1 μM deca- or 1.3 μM octa- BDEs dissolved in TCE. The TCE was completely dechlorinated to *cis*-DCE in the samples amended with H_2 and acetate within one week, whereas no PBDE debromination was detected during that period. However, after two months incubation of *S. multivorans* without additional TCE, deca-BDE was no longer detectable while octa- and hepta-BDEs appeared in both replicates (Figure 1). In contrast, the deca-BDE peak was unchanged and there were no debromination peaks detected in the autoclaved controls. Incubation of *S. multivorans* with the octa-BDE mixture in duplicates for over one year resulted in no new debromination congeners, indicating that this organism is incapable of debrominating lower PBDE congeners.

Debromination of an Octa-BDE Mixture. *D. ethenogenes* 195, that was initially grown on defined mineral salts medium containing 500 μM TCE and H_2/CO_2 (80:20) headspace, was able to debrominate the octa-BDE mixture containing hexa- through nona-BDE congeners to hepta-, hexa-, and penta-BDEs within 6 months of incubation when 1.3 μM octa-BDEs were added to the culture dissolved in TCE (Figure 2a). Debromination was not observed in the autoclaved controls throughout the experiment (Figure 2f). Peak areas of the debromination products continued to increase throughout the one-year incubation, with tetra-BDEs appearing after six months. Analysis by GC–MS confirmed the presence of two hepta-, two hexa-, and three penta-BDEs in the 6-month samples, and was used along with available standards to positively identify hexa 154 and penta 99 in the live samples. No debromination was observed in *D. ethenogenes* 195 cultures exposed only to deca-BDE even after one full year of incubation. Complete dechlorination of TCE to VC and ethene was observed in all strain 195 incubations in the first 8 weeks. The presence of PBDEs slowed TCE dechlorination significantly, suggesting that PBDEs are toxic or inhibitory

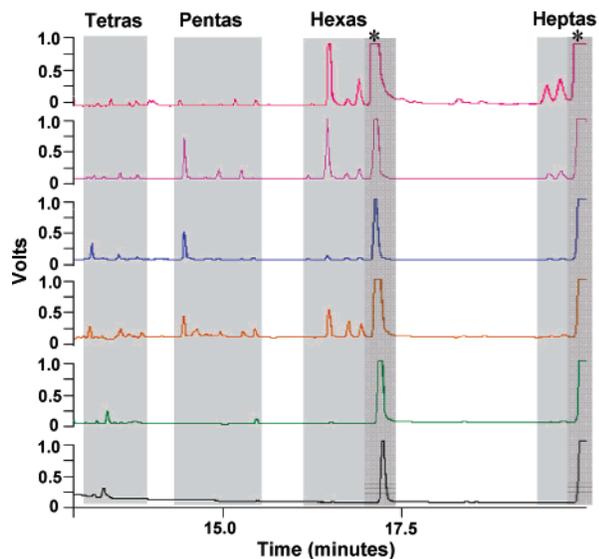


FIGURE 2. GC chromatograms of debromination of an octa-BDE mixture by cultures containing *Dehalococcoides* spp. (a) *D. ethenogenes* 195; (b) EC195; (c) EC195 plus strain BAV1; (d) ANAS195; (e) *Dehalococcoides* sp. strain BAV1; (f) autoclaved control. Samples were taken when cultures were incubated for 2 to 3 months, except the *D. ethenogenes* 195 culture which was incubated for 6 months. Shaded areas highlight different groups of PBDE isomers. Asterisks (*) denote PBDE substrate peaks.

to dechlorinating bacteria as has been observed for some other halogenated compounds (28, 29).

Enrichment cultures containing *Dehalococcoides* spp. were also investigated for their ability to debrominate deca- and octa- BDEs. A highly enriched autotrophic culture containing *D. ethenogenes* 195 (EC195) exhibited detectable debromination activity of octa-BDEs after 10 weeks of incubation in the presence of 1.3 μM octa-BDEs dissolved in TCE (Figure 2b). Complete dechlorination of TCE to VC and ethene was observed in EC195 incubations in the first 8 weeks. More congeners were detected in the EC195 culture than in the 195 isolate within a 3-month incubation, and the debromination occurred more rapidly, reflecting the approximately 3-fold higher *Dehalococcoides* cell density ($[3.90 \pm 0.11] \times 10^7$ cells/mL for the EC195 culture versus $[1.19 \pm 0.18] \times 10^7$ for pure 195 culture) of the enrichment culture, as quantified by 16S rRNA gene analysis. In addition to the hepta-, hexa-, and penta-BDE byproducts detected in the samples from the 195 isolate, a variety of tetra- and di- BDE congeners were detected in the enrichment by GC-ECD and GC-MS, while tri-BDEs were not detected in either culture. Furthermore, EC195 generated one more hexa and two more penta congeners than the isolate within 3 months incubation.

Debromination activity was not detected for *Dehalococcoides* sp. strain BAV1 after exposure to deca-BDE or the octa-BDE mixture for over one year (Figure 2e). However, when strain BAV1 was added to the EC195 enrichment, debromination beyond that observed for EC195 occurred within three months, with the appearance of additional lower congeners (Figure 2c). This augmented enrichment also demonstrated complete dechlorination of TCE to ethene with no residual vinyl chloride within 9 weeks, indicating that both strain 195 and BAV1 were active in the presence of PBDEs. The previously detected hexa and penta congeners could no longer be detected by GC-MS in the augmented enrichment and only three detectable tetra- and five detectable di-BDEs remained after 3 months incubation. Tri-BDEs were also not detected in these cultures.

An enrichment culture (ANAS) that dechlorinates 1.1 mM TCE completely to ethene in the presence of 10 mM lactate

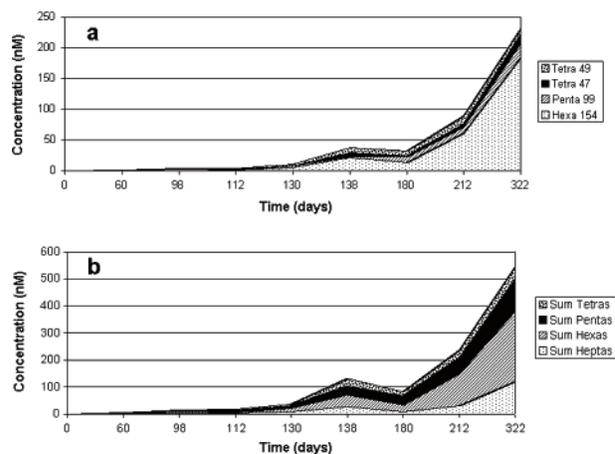


FIGURE 3. Average cumulative concentrations of octa-BDE mixture debromination products generated by ANAS195 over 12 months. (a) only positively identified products; (b) summed product concentrations for each homologue group. Values are averaged from duplicate cultures.

and that contains multiple *Dehalococcoides* spp., (25, 27) but does not contain either strain 195 or strain BAV1 was incapable of degrading the octa-BDE mixture after one year, even after a second amendment of energy-generating TCE was added resulting in final PBDE concentrations of 0.2 μM for deca- and 2.6 μM for octa-BDEs. However, when strain 195 was added to the ANAS culture (ANAS195), debromination was observed after three months and products included hepta through di-BDE congeners, similar to those observed in the EC195 cultures amended with BAV1 (Figure 2d).

In the above experiments, deca- and octa-BDEs were added to the cultures dissolved in TCE. To evaluate whether the TCE might either promote or inhibit debromination activity and to determine if the PBDEs could serve as energy-generating electron acceptors, deca- and octa-BDEs were dissolved in nonane and fed to the EC195 and the pure *D. ethenogenes* 195 cultures amended with electron donor (H_2) and carbon source, but without other electron acceptors. After one year of incubation, no lower PBDE congeners were observed in either of these cultures, indicating that PBDE debromination by these cultures required the presence of energy-generating electron acceptors. In contrast, cultures showed uninhibited TCE dechlorination activity in the presence of nonane, demonstrating a lack of inhibitory effect caused by this compound.

Quantification of Debromination Products. The concentrations of PBDE congeners produced by all cultures were in the nM range. Figure 3a shows the concentrations of identified intermediates generated over time by ANAS195. Complete dechlorination of TCE to ethene was observed in ANAS195 within the first 10 weeks. After 12 months, approximately 230 nM of positively identified products were generated, yielding approximately 11.5 nmoles of identified products from the 130 nmols octa-BDE mixture added to the culture. Most product peaks could not be positively identified, but could be classified within a certain homologue group based on mass-ion ratios from the GC-MS analysis. Using average response factors for homologue groups, the remaining peaks were quantified, yielding over 500 nM of total PBDE products (Figure 3b). In addition, losses of PBDEs during incubation, sampling and analysis were expected due to their high sorptive properties. EC195 plus strain BAV1 generated debromination products at rates similar to ANAS195. On a cell-normalized basis *D. ethenogenes* 195 exhibited about half the debromination rate of the ANAS195 culture. E.g., after 11 months of incubation, ANAS195 produced 8.8×10^{-10}

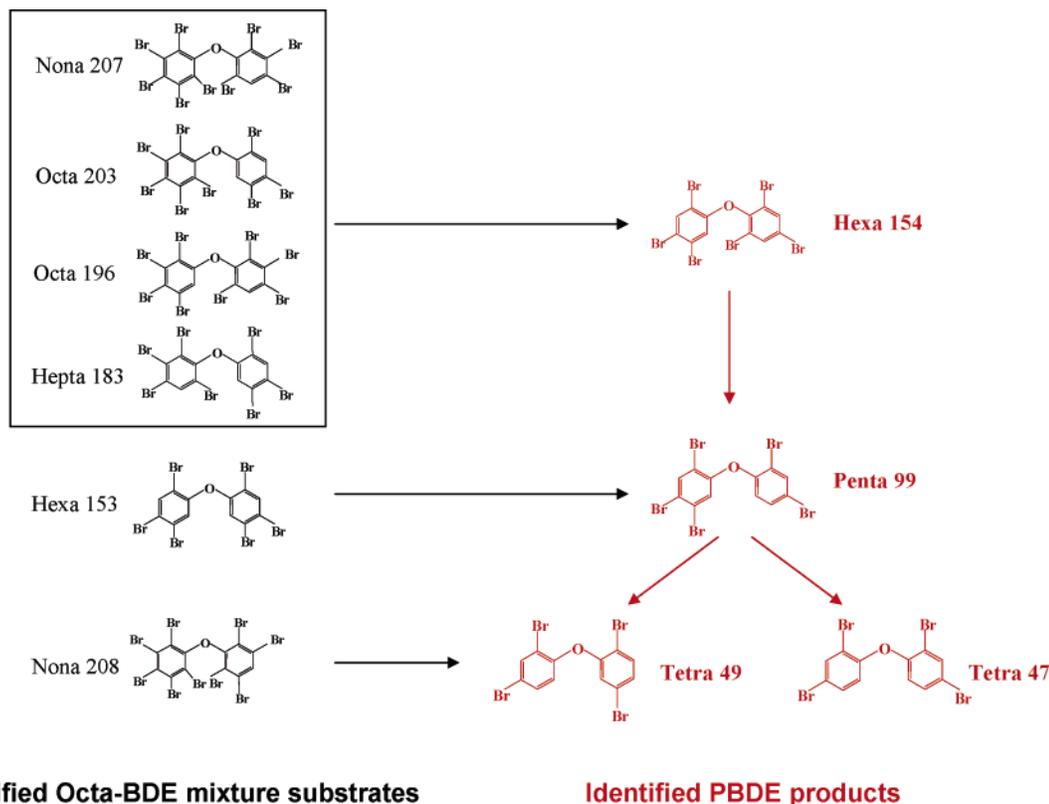


FIGURE 4. Specifically identified PBDE substrates (black) and debromination products (red) detected for ANAS195 amended with the octa-BDE mixture. Arrows connect substrates with potential debromination products assuming no isomerization.

nmol/cell of hexa 154 and 17.5×10^{-11} nmol/cell of penta 99, whereas pure *D. ethenogenes* 195 generated 4.3×10^{-10} nmol/cell of hexa 154 and 8.5×10^{-11} nmol/cell of penta 99. Of course, the rates for individual congener generation are rough estimates only, as it is likely that the debromination products were being generated and debrominated simultaneously.

Debromination Pathway. Determination of the specific PBDE degradation pathway is hindered by the difficulties associated with positively identifying the 209 different congeners, many of which coelute on a GC column (30). Furthermore, since the commercially available octa-BDE mixture contains two nona-BDEs, along with a variety of octa, hepta, and hexa congeners in significant quantities, it is not yet possible to determine which specific congeners are being debrominated. Figure 4 illustrates the identified substrates contained in the octa-BDE mixture along with identified debromination products.

Discussion

The levels of PBDEs in environmental samples are overtaking those of polychlorinated biphenyls (PCBs) which are now distributed worldwide (31). Consequently, the fate of PBDEs in the environment is attracting increased attention and concern. This study is the first report of microbial PBDE debromination beyond the octa congeners and of PBDE debromination by pure microbial isolates. The dechlorinating bacterium, *S. multivorans*, was found to debrominate deca-BDE to octa- and hepta-BDE congeners, while this culture could not debrominate an octa-BDE mixture to lower congeners. Given that *S. multivorans* can only dechlorinate the more highly chlorinated ethenes PCE and TCE, it may not be surprising that it cannot debrominate less brominated congeners.

In this study, *D. ethenogenes* 195 was found to be capable of debrominating an octa-BDE mixture producing hepta-

through penta-BDE congeners within six months. More extensive debromination was observed in the enriched culture EC195 that exhibited the generation of tetra- and di-BDE congeners within three months of incubation with PBDEs. The more rapid and extensive debromination observed with EC195 may have been due to the increased cell density of this culture or the nutritional benefits that *Dehalococcoides* gains by growing in the presence of other species. When *Dehalococcoides* sp. BAV1 was added to the enriched culture EC195, additional degradation peaks ranging from tetra-BDEs to di-BDEs were detected, while the hexa and penta intermediates disappeared. These results suggest that strain 195 was responsible for debrominating the higher congeners while BAV1 was responsible for debrominating the hexa-, penta-, and tetra-BDE intermediates. It seems that strain BAV1 debrominates certain PBDE congeners more quickly than strain 195 given the lack of accumulation of the hexa and penta congeners. This ability to debrominate the lower PBDE congeners echoes the dechlorination pattern of BAV1, a strain that grows on chloroethenes with only one or two chlorine substituents.

The TCE-dechlorinating ANAS enrichment culture was found to debrominate the octa-BDE mixture only when amended with *D. ethenogenes* 195. Though ANAS contains other yet unidentified *Dehalococcoides* strains, it is clear that strain 195 is responsible for the debromination of octa-BDE. Given the abilities of ANAS to dechlorinate TCE to ethene without residual vinyl chloride, it is possible that the debromination products generated when strain 195 was added to ANAS were further debrominated by other indigenous *Dehalococcoides* spp. within ANAS. This hypothesis was supported by the production of a new tetra-BDE congener by ANAS195 that was also produced when the EC195 culture was amended with strain BAV1 yet was absent from the EC195 culture. This observation suggests that *Dehalococcoides* strains capable of metabolizing mono-

chlorinated ethenes likely participate in the debromination of the lower PBDE congeners. In all cases, PBDE transformation by *Dehalococcoides* spp. proceeded slowly, with approximately 500 nM (25 nmoles) of debromination products detected over a twelve month period in the most active culture. Previous studies conducted with sewage sludge generated 0.5 nmol of products within 8 months from the debromination of deca-BDE, that however, did not debrominate beyond octa-BDEs (14). Kinetics may be limited by PBDE toxicity effects to the microorganisms or by mass transfer of the more highly brominated congeners.

Recent work suggests that each strain of *Dehalococcoides* spp. has multiple reductive dehalogenases that have diverged to fill unique functional niches (32). Strain 195 and BAV1 possess dissimilar reductive dehalogenases (33, 34) that may target different PBDE congeners. Although strain 195 and ANAS both contain the *tceA* gene that codes for a TCE reductive dehalogenase (33, 35), the former culture debrominates octa-BDEs, whereas the latter does not. Therefore, it is likely that a reductase other than *tceA* is responsible for the debromination of the higher congeners. Moreover, strain 195 has been reported to share with *Dehalococcoides* sp. strain CBDB1 the ability to dechlorinate chlorobenzenes and polychlorinated dibenzodioxins, although strain CBDB1 does not contain a *tceA*-like gene and cannot dechlorinate TCE (17, 19), indicating the potential role of uncharacterized reductive dehalogenases in a variety of reductive dehalogenations. Attempts to test the capability of strain CBDB1 to debrominate PBDEs were unsuccessful because the titanium citrate present in the CBDB1 medium debrominated PBDEs abiotically at rates sufficiently high to make it impossible to detect biological debromination.

The appearance of identified hexa, penta, and tetra congeners along with unidentified hepta, and di congeners (Figure 4) indicates that debromination beyond octa-BDEs is occurring in the *Dehalococcoides* spp.-containing cultures. It is possible that multiple substrates are being debrominated given the variety of debromination byproducts. Moreover, individual substrates could be debrominated to multiple products. It also appears that bromines in the 2 (ortho) and 4 (para) positions (e.g., penta 99 and tetra 47) are the most recalcitrant since these positions are more commonly brominated in the identifiable byproducts. This raises public health concerns since a recent study found that when PBDEs are hydroxylated with bromines in the ortho and para position they can compete with the natural hormone thyroxine to bind with transthyretin, the thyroid hormone-binding transport protein, thereby disrupting normal endocrine function and causing significant toxicity (36). *D. ethenogenes* 195 can remove the meta chlorines from PCBs and dioxins, which would appear to match the debromination pattern exhibited in this study (19). In all, debromination products generated by cultures containing *Dehalococcoides* spp., including penta 99 and tetra 47, compounds known to be more potent endocrine disruptors and more bioavailable than the higher brominated congeners, raise a potential health concern since the higher congeners are considered to be relatively safe and continue to be used heavily as fire retardants.

Acknowledgments

Funding was provided by the Superfund Basic Research Project NIEHS ES04705, National Science Foundation grant BES-05-04244, and the University of California Toxics Substances Research and Teaching Program. Thanks go to Arthur Holden and Jianwen She at the California Department of Toxic Substances Control for assistance with the GC-MS. We also thank Stephen Zinder at Cornell University, Frank Löffler at the Georgia Institute of Technology, and Lorenz Adrian at the Berlin Technical University for providing *D.*

ethenogenes strain 195, *Dehalococcoides* sp. strain BAV1, and *Dehalococcoides* sp. strain CBDB1 respectively, and Patrick Lee for providing the ANAS culture.

Literature Cited

- Alcock, R. E.; Sweetman, A.; Prevedouros, K.; Jones, K. C. Understanding levels and trends of BDE-47 in the UK and North America: an assessment of principal reservoirs and source inputs. *Environ. Int.* **2003**, *29*, 691–698.
- de Wit, C. A. An overview of brominated flame retardants in the environment. *Chemosphere* **2002**, *46*, 583–674.
- California Assembly Bill 302, 2004, European Parliament Directive 2002/95/EC.
- Eriksson, P.; Jakobsson, E.; Fredriksson, A. Developmental neurotoxicity of brominated flame-retardants, polybrominated diphenyl ethers and tetrabromo-bis-phenol A. *Organohalogen Compd.* **1998**, *35*, 375–377.
- Eriksson, P.; Jakobsson, E.; Fredriksson, A. Brominated flame retardants: a novel class of developmental neurotoxins in our environment? *Environ. Health Perspect.* **2001**, *109*, 903–908.
- Darnerud, P. O.; Eriksen, G. S.; Jöhanncsson, T.; Larsen, P. B.; Viluksela, M. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* **2001**, *109* suppl. 1, 49–68.
- CDC. Public Health Statement for Polybrominated Diphenyl Ethers (PBDEs). September **2004**.
- She, J.; Holden, A.; Tanner, M.; Sharp, M.; Adelsbach, T.; Hooper, K. Highest PBDE levels (max 63 ppm) yet found in biota measured in seabird eggs from San Francisco Bay. *Organohalogen Compd.* **2004**, *66*, 3939–3944.
- Eriksson, J.; Green, N.; Marsh, G.; Bergman, Å. Photochemical decomposition of 15 polybrominated diphenyl ether congeners in methanol/water. *Environ. Sci. Technol.* **2004**, *38*, 3119–3125.
- Stapleton, H. M.; Alae, M.; Letcher, R. J.; Baker, J. E. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (*Cyprinus carpio*) following dietary exposure. *Environ. Sci. Technol.* **2004**, *38*, 112–119.
- Stapleton, H. M.; Letcher, R. J.; Baker, J. E. Debromination of polybrominated diphenyl ether congeners BDE 99 and BDE 183 in the intestinal tract of the common carp (*Cyprinus carpio*). *Environ. Sci. Technol.* **2004**, *38*, 1054–1061.
- Kierkegaard, A.; Balk, L.; Tjarnlund, U.; de Wit, C. A.; Jansson, B. Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **1999**, *33*, 1612–1617.
- Rayne, S.; Ikononou, M. G.; Whale, M. D. Anaerobic microbial and photochemical degradation of 4,4'-dibromodiphenyl ether. *Water Res.* **2003**, *37*, 551–560.
- Gereke, A.; Hartmann, P.; Heeb, N.; Kohler, H.-P.; Giger, W.; Schmid, P.; Zennegg, M.; Kohler, M. Anaerobic degradation of decabromodiphenyl ether. *Environ. Sci. Technol.* **2005**, *39*, 1078–1083.
- Rayne, S.; Ikononou, M. G. Polybrominated diphenyl ethers in an advanced wastewater treatment plant. Part 1: Concentrations, patterns, and influence of treatment processes. *J. Environ. Eng. Sci.* **2005**, *4*, 353–367.
- de Boer, J.; Wester, P. G.; van der Horst, A.; Leonard, P. E. G. Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from The Netherlands. *Environ. Pollut.* **2003**, *122*, 63–74.
- Bunge, M. L.; Adrian, A. K.; Opel, M.; Lorenz, W. G.; Andreesen, J. R.; Görlich, H.; Lechner, U. Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. *Nature* **2003**, *421*, 357–360.
- Cutter, L. A.; Watts, J. E. M.; Sowers, K. R.; May, H. D. Identification of a microorganism that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl. *Environ. Microbiol.* **2001**, *3*, 699–709.
- Fennell, D. E.; Nijenhuis, I.; Wilson, S. F.; Zinder, S. H.; Häggblom, M. M. *Dehalococcoides ethenogenes* strain 195 reductively dechlorinates diverse chlorinated aromatic pollutants. *Environ. Sci. Technol.* **2004**, *38*, 2075–2081.
- Wu, Q.; Watts, J. E. M.; Sowers, K. R.; May, H. D. Identification of a bacterium that specifically catalyzes the reductive dechlorination of polychlorinated biphenyls with doubly flanked chlorines. *Appl. Environ. Microbiol.* **2002**, *68*, 807–812.
- Maymón-Gatell, X.; Chien, Y.-T.; Gossett, J. M.; Zinder, S. H. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* **1997**, *276*, 1568–1571.

- (22) He, J.; Ritalahti, K. M.; Yang, K.-L.; Koenigsberg, S. S.; Löffler, F. E. Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. *Nature* **2003**, *424*, 62–65.
- (23) Scholz-Muramatsu, H.; Neumann, A.; Messmer, M.; Moore, E.; Diekert, G. Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. *Arch. Microbiol.* **1995**, *163*, 48–56.
- (24) Luijten, M. L. G. C.; deWeert, J.; Smidt, H.; Boschker, H. T. S.; deVos, W. M.; Schraa, G.; Stams, A. J. M. Description of *Sulfurospirillum halorespirans* sp. nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 787–793.
- (25) Richardson, R. E.; Bhupathiraju, V. K.; Song, D. L.; Goulet, T. A.; Alvarez-Cohen, L. Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques. *Environ. Sci. Technol.* **2002**, *36*, 2652–2662.
- (26) Löffler, F. E.; Sanford, R. A.; Tiedje, J. M. Initial characterization of a reductive dehalogenase from *Desulfitobacterium chlororespirans* Co23. *Appl. Environ. Microbiol.* **1996**, *62*, 3809–3813.
- (27) Freeborn, R. A.; West, K. A.; Bhupathiraju, V. K.; Chauhan, S.; Rahm, B. G.; Richardson, R. E.; Alvarez-Cohen, L. Phylogenetic analysis of TCE-dechlorinating consortia enriched on a variety of electron donors. *Environ. Sci. Technol.* **2005**, *39*, 8358–8368.
- (28) He, J.; Sung, Y.; Krajmalnik-Brown, R.; Ritalahti, K. M.; Löffler, F. E. Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. *Environ. Microbiol.* **2005**, *7*, 1442–1450.
- (29) Gerritse, J.; Drzyzga, O.; Kloetstra, G.; Keijmel, M.; Wiersum, L. P.; Hutson, R.; Collins, M. D.; Gottschal, J. C. Influence of different electron donors and acceptors on dehalorespiration of tetrachloroethene by *Desulfitobacterium frappieri* TCE1. *Appl. Environ. Microbiol.* **1999**, *65*, 5212–5221.
- (30) Korytár, P.; Covaci, A.; de Boer, J.; Gelbin, A.; Brinkman, U. A. T. Retention-time database of 126 polybrominated diphenyl ether congeners and two Bromkal technical mixtures on seven capillary gas chromatographic columns. *J. Chromatogr. A* **2005**, *1065*, 239–249.
- (31) PBDEs overtaking PCBs. http://pubs.acs.org/subscribe/journals/esthag-w/2005/sep/science/kb_pbde.html, accessed **2005**.
- (32) Hölscher, T.; Krajmalnik-Brown, R.; Ritalahti, K. M.; Wintzingerode, F. v.; Görisch, H.; Löffler, F. E.; Adrian, L. Multiple nonidentical reductive-dehalogenase-homologous genes are common in *Dehalococcoides*. *Appl. Environ. Microbiol.* **2004**, *70*, 5290–5297.
- (33) Seshadri, R.; Adrian, L.; Fouts, D. E.; Eisen, J. A.; Phillippy, A. M.; Methe, B. A.; Ward, N. L.; Nelson, W. C.; Deboy, R. T.; Khouri, H. M.; Kolonay, J. F.; Dodson, R. J.; Daugherty, S. C.; Brinkac, L. M.; Sullivan, S. A.; Madupu, R.; Nelson, K. E.; Kang, K. H.; Impraim, M.; Tran, K.; Robinson, J. M.; Forberger, H. A.; Fraser, C. M.; Zinder, S. H.; Heidelberg, J. F. Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethenogenes*. *Science* **2005**, *307*, 105–108.
- (34) Krajmalnik-Brown, R.; Hölscher, T.; Thomson, I. N.; Saunders, F. M.; Ritalahti, K. M.; Löffler, F. E. Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp. strain BAV1. *Appl. Environ. Microbiol.* **2004**, *70*, 6347–6351.
- (35) Johnson, D. R.; Lee, P. K. H.; Holmes, V. F.; Alvarez-Cohen, L. An internal reference technique for accurately quantifying specific mRNAs by real-time PCR with application to the *tceA* reductive dehalogenase gene. *Appl. Environ. Microbiol.* **2005**, *71*, 3866–3871.
- (36) Meerts, I. A. T. M.; Zanden, J. J. v.; Luijks, E. A. C.; Leeuwen-Bol, I. v.; Marsh, G.; Jakobsson, E.; Bergman, Å.; Brouwer, A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* **2000**, *56*, 95–104.

Received for review December 14, 2005. Revised manuscript received March 23, 2006. Accepted April 28, 2006.

ES052508D