Degradation of Polybrominated Diphenyl Ethers by Anaerobic Dehalogenating Cultures

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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 foams, textiles and plastics. PBDEs are most commonly added to products as mixtures of penta-BDEs, octa-BDEs and deca-BDE. There are three major commercial mixtures of PBDEs produced: deca-BDEs (mostly deca-BDE with some nona- and octa-BDE congeners), octa-BDEs (mostly octa- and hepta-BDE congeners), and penta-BDEs (mostly penta- and tetra-BDE congeners). Among them, deca-BDE accounts for over 80% of the total PBDE production1.

Recent toxicology studies have shown that penta-BDEs are endocrine disruptors at low concentrations. Octa-BDEs are also endocrine disruptors and deca-BDE is a suspected carcinogen at high concentrations2,3,4,5. 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.

Currently, little is known about the fate and transport of these compounds in the environment, and in particular about their potential for microbial degradation. Recent studies have demonstrated that PBDEs can be photolyticallydebrominated to less brominated congeners by natural sunlight[6]. Transformation of higher brominated PBDEs to lower brominated congeners has also been observed in fish tissues[7,8,9]. One study focused on the debromination of deca-BDE in an anaerobic sediment column and found that after one year 5% of the deca had been degraded to nona-BDE[10].

Anaerobic reductive dehalogenation may be an effective mechanism for removing bromines from the highly brominateddiphenyl ethers. Anaerobic dechlorinating cultures have demonstrated the ability to successfully degrade recalcitrant and aromatic compounds such as PCBs and dioxins, and therefore, may play an important role in removing bromines from PDBEs[11,12]. If higher brominated congeners are microbially transformed to more toxic lower congeners, such as penta-BDE, this will have profound implications for public health and for the regulation of these compounds. Alternatively, PBDEs might also be completely debrominated to diphenyl ether, which is relatively non-toxic.

Materials and Methods

In this study we have exposed a variety of different dehalogenating bacteria to PBDEs to assess their ability to debrominate these compounds. We have tested the following microorganisms: *Dehalococcoides ethenogenes* 195, known to degrade chlorinated ethenes as well as dioxins, *Dehalococcoides sp.* BAV1, a dichloroethene and vinyl chloride respiring strain, *Dehalococcoides sp.* CBDB1, a strain capable of degrading chlorinated aromatics, and mixed consortia containing *Dehalococcoides spp*. We have tested *Desulfomonile tiejdei*, *Desulfitobacterium chlororespirans* and *Desulfitobacterium dehalogenans*—all species capable of degrading chlorinated phenols. We have also tested a tetrachloroethene to dichloroethene dechlorinating bacterium—*Sulfurospirillum multivorans*.

All microorganisms were grown in defined mineral salts media. All cultures were fed either deca-BDE or an octa-BDE mixture at approximately 1uM concentration along with their usual electron acceptors, donors and carbon sources. When possible, the PBDEs were dissolved in one of the substrates and added to the microbial sample. Otherwise, PBDEs were dissolved in nonane. Some samples were autoclaved as controls to ensure that all observed degradation was microbially mediated. PBDEs were added to the controls after autoclaving. The samples were stored in an incubator at 30°C in the dark.

On a weekly basis, 1 ml aliquots were removed from the samples and subjected to a liquid-liquid extraction with an

equal volume of isooctane. Decabromobiphenyl was added to the samples as an internal standard. Only amber colored glassware was used to reduce possible photodegradation. PBDE congeners are detected using a Gas Chromatograph equipped with an Electron Capture Detector (GC-ECD) for measuring the highly brominated congeners and a Flame Ionization Detector (FID) for measuring the lower brominated compounds and for biphenyl ether—the ultimate debromination byproduct.

The identification of PBDEs was done by comparing peak retention times with a standard solution containing 27 mono- through deca-BDE congeners (Cambridge Isotope Laboratories Inc. Andover, MA). Since not all peaks could be positively identified, some PBDE peaks were confirmed based on mass/ion ratio with a GC-MS equipped with an ECNI detector.

Results and Discussion

After three months of exposure to PBDEs, new peaks appeared in highly enriched consortia samples containing *Dehalococcoides ethenogenes* 195 that were exposed to octa-BDE. Similar peaks did not appear in the pure *D. ethenogenes* 195 samples, nor in the autoclaved controls. (Data not shown.) Since PBDEs were being degraded by cultures containing *D. ethenogenes* 195, but not by the pure strain after three months of incubation, it was unclear whether *D. ethenogenes* 195 was actually the functional bacterium. To explore this question, a study was carried out in which *D. ethenogenes* 195 was added to a dehalogenating consortium (ANAS culture) that did not originally contain *D. ethenogenes* 195. Activity by this augmented consortiumwas then compared to the original ANAS culture and to the pure strain of *D. ethenogenes* 195. The augmented consortium was capable of degrading octa-BDE whereas both the pure strain of *D. ethenogenes* 195 and the ANAS culture were not. The reason for this is unclear and may have to due with the slowness of growth of the pure strain or simply due to nutritional benefits from other bacteria in the consortium. Figure 1 shows the appearance of peaks in the ANAS culture augmented with *D. ethenogenes* 195 as compared to the ANAS culture, a pure culture of *D. ethenogenes* 195 and an autoclaved control.

The new peaks in the ANAS-*D. ethenogenes* 195 samples were identified as hepta- through tetra- BDE congeners using GC-MS and continued to increase in size over time. Some of the congeners were positively identified using the available standards as hexa 154, penta 99 and tetra 47 and 49. The concentrations of the by-products were in the low nM range (Figure 2). When *Dehalococcoides sp.* BAV1, which is capable of degrading lower chlorinated ethenes, was added to the highly enriched *D. ethenogenes* 195 culture, even further debromination of octa-BDE was observed, with the production of tri-BDEs.

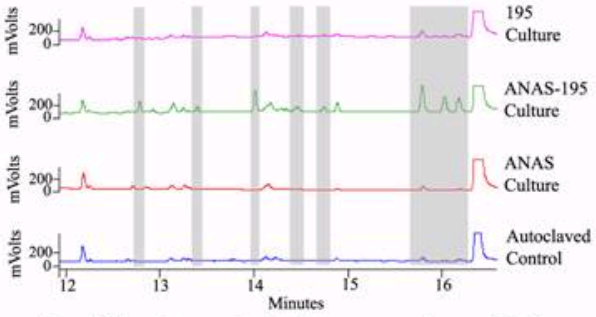

Figure 1. Chromatograms showing the appearance of new peaks in the ANAS-*D. ethenogenes* 195 culture as compared to pure *D. ethenogenes* 195, the ANAS culture and an autoclaved control. Shaded areas highlight the new peaks. The large peak at 16.5 mins is a hexa-BDE substrate peak.

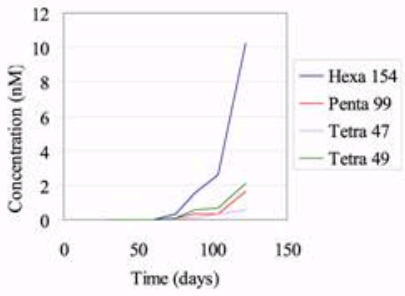

Figure 2. Concentrations over time of identifiable PBDE by-products that appeared in the ANAS-*D. ethenogenes* 195 culture fed octa-BDE.

Currently, the degradation pathway cannot be determined because not all by-products have been positively identified. Furthermore, since the octa-BDE mixture contains a nona-BDE, some octa, hepta and hexa congeners, it is not clear which congener is being degraded. It appears, however, that bromines in the 2 (ortho) and 4 (para) positions are the most recalcitrant since many of these positions are still brominated in the identifiable by-products, whereas the other positions are occupied by hydrogens. It also appears that two different substrates are being degraded given the differences in the degradation by-products. Hexa 153 (2,2',4,4',5,5'-BDE) present in the octa-BDE mixture is being degraded to hepta 49 (2,2',4,5'-BDE). Three other congeners—hexa 154 (2,2',4,4',5,6'-BDE), penta 99 (2,2',4,4',5-BDE), tetra 47 (2,2',4,4'-BDE) are being produced from some other unidentified substrate.

To date, we have not seen degradation of deca-BDE in any *D. ethenogenes* 195 samples. However, another culture, *Sulfurospirillum multivorans*, has been able to degrade deca-BDE with the production of octa and hepta-BDEs (Figure 3).

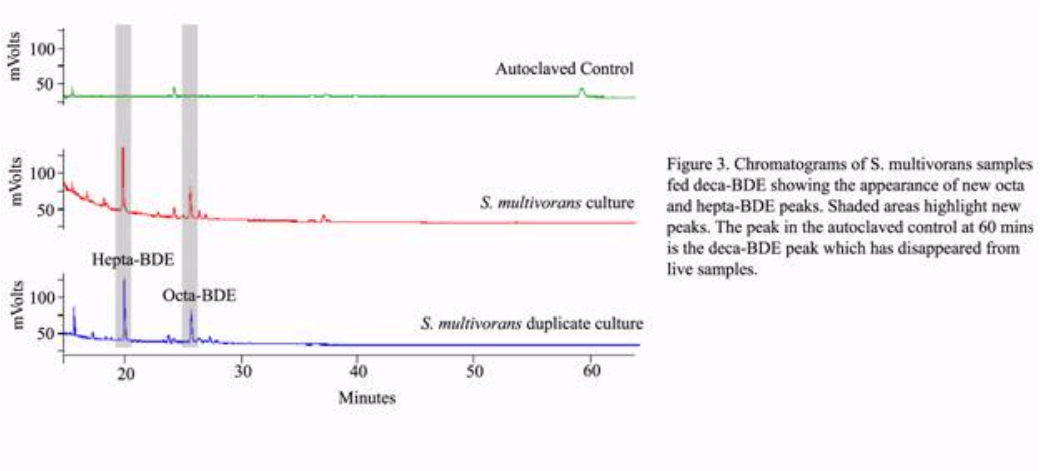

Figure 3. Chromatograms of S. multivorans samples fed deca-BDE showing the appearance of new octa and hepta-BDE peaks. Shaded areas highlight new peaks. The peak in the autoclaved control at 60 mins is the deca-BDE peak which has disappeared from live samples.

The degradation by-products produced by the debromination of octa-BDE by *D. ethenogenes* 195—penta 99 and hepta 47—are among the most toxic congeners. This raises a health concern because deca-BDE is considered safe and continues to be heavily used, whereas our studies indicate that it could be easily degraded to more toxic forms.

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