

Anaerobic Biotransformation of Tetrabromobisphenol A, Tetrachlorobisphenol A, and Bisphenol A in Estuarine Sediments

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Biotransformation of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A, and their ultimate biodehalogenation product, bisphenol A, was examined in anoxic estuarine sediments. Dehalogenation of tetrabromobisphenol A and tetrachlorobisphenol A was examined under conditions promoting either methanogenesis or sulfate reduction as the primary terminal electron-accepting process. Complete dehalogenation of tetrabromobisphenol A to bisphenol A with no further degradation of bisphenol A, was observed under both methanogenic and sulfate-reducing conditions. Dehalogenation of tetrachlorobisphenol A under both methanogenic and sulfate-reducing conditions resulted in the accumulation of a persistent dichlorinated bisphenol A isomer, while no bisphenol A was formed. Co-amendment of sediment enrichments with either 2,6-dibromo- or 2,6-dichlorophenol did not affect the extent of dehalogenation as compared to sediments that were amended only with the flame retardants. Sediment cultures pre-acclimated on 2-bromophenol dehalogenated the flame retardants in a manner similar to that of fresh sediments. No loss of bisphenol A was observed in separate incubations within 162 days under conditions promoting either methanogenesis, sulfate-reduction, iron(III)-reduction, or nitrate-reduction. Furthermore, identical enrichments that readily degraded 4-hydroxybenzoate, a structural analogue of bisphenol A, did not exhibit bisphenol A degradation. The dehalogenation of tetrabromo- and tetrachlorobisphenol A and the potential for accumulation of bisphenol A in anoxic sediments is significant given the widespread use of these chemicals.

Introduction

Tetrabromobisphenol A (4,4'-isopropylidenebis(2,6-dibromophenol), TBBPA) is a widely used brominated flame retardant that has been detected in environmental samples from around the world, including Japan, Sweden, and the U.S. (1–4). TBBPA is added to synthetic resins, polycarbonates, and plastics that are used to make computer and electronics housings, laminated electronic circuit boards,

carpets, upholstery, and many other consumer goods (5–7). Tetrachlorobisphenol A (4,4'-isopropylidenebis(2,6-dichlorophenol), TCBPA) is also used as a flame retardant, but to a lesser extent than TBBPA. TBBPA is preferred over other brominated flame retardants because it can be covalently bound to the polymer in the manufacturing process (7). Even so, one study found that a small fraction of the TBBPA remains unreacted and leachable in the finished product (2). In contrast, the brominated flame retardants, polybrominated diphenyl ethers (PBDEs), are additive components in polymers and significant amounts can volatilize directly from the finished product into the environment (8).

TBBPA has primarily been reported in sediments near manufacturing facilities (2–4, 9) and in samples from a wastewater treatment plant that received leachate from a landfill containing wastes from the plastics industry (2). It should be expected that as aging computer inventories are disposed of, the input of brominated flame retardants to the environment through recycling facility dusts and wastes, recycled plastic pellets, or from disposal of components in landfills has the potential to increase dramatically (10, 11). TBBPA and PBDE concentrations were elevated in a computer component recycling facility relative to those of a factory that assembles circuit boards, a computer repair facility, or offices equipped with computers (12). A method for detection of TCBPA and TBBPA in human plasma has been developed, and a preliminary test revealed the presence of the compounds in occupationally exposed persons (13).

Bisphenol A (4,4'-isopropylidenediphenol, BPA) is widely used in the manufacture of plastics, resins, food packaging, and coatings. In the United States alone, 1.65 billion lb of BPA is produced each year (14). Exposure to this compound has produced estrogenic effects in a variety of organisms (15). BPA has been detected in bay sediments (16), wastewater treatment plant effluents (17, 18), septage (17), and landfill leachate (19, 20). Ronen and Abeliovich also reported production of BPA through the biological reductive dehalogenation of TBBPA (9). BPA was not degraded under anoxic conditions (9), but degradation by aerobic microorganisms has been demonstrated (9, 21, 22).

Halogenated compounds with structural similarity to TBBPA (e.g., polychlorinated biphenyls and dioxins) are known to negatively affect various aspects of human physiology (15). Because of its widespread use in everyday products and presence in environmental samples, TBBPA is currently under study to determine whether it also causes negative physiological effects in mammalian and human systems (23, 24).

Similar to halogenated PCBs or dioxins, TBBPA and TCBPA would likely be ultimately transported to sediments because of their hydrophobicity. The fate of these compounds in anoxic sediments—where they may be subject to anaerobic reductive dehalogenation—is thus of great interest. Various redox zones exist in estuarine sediments, depending upon the location and pollutant input (25). Nitrate-reducing, sulfate-reducing, iron(III)-reducing, and methanogenic conditions could be encountered within sediment layers. Dehalogenation, a process through which dehalogenating bacteria utilize halogenated compounds as electron acceptors, can be enhanced or inhibited by other electron acceptors present in the sediment (for reviews, see 26 and 27). For example, studies have found that under sulfate-reducing conditions (the primary electron-accepting process in the top layers of marine and estuarine sediments), the process of dehalogenation may be inhibited. Dehalogenation may be suppressed by the following: direct inhibition of deha-

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logenases by sulfate, sulfite, or hydrogen sulfide; preferential use of sulfate (over the halogenated compound) as an electron acceptor within the same organism; or, by successful competitive exclusion of dehalogenating bacteria through competition for electron donor by sulfate-reducing bacteria (28, 29).

The purpose of this study was to examine the effect of different electron-accepting conditions on the dehalogenation of TBBPA and TCBPA and on the ultimate degradation of the expected product of dehalogenation, BPA. Additionally, we examined the effect of the addition of structural analogue cosubstrates 2,6-dibromophenol (2,6DBP) and 2,6-dichlorophenol (2,6DCP) on the dehalogenation of TBBPA and TCBPA, respectively, in unacclimated sediments and in sediment cultures that had been previously enriched on 2-bromophenol (2BP). In separate enrichments, we attempted to stimulate BPA degradation under a variety of redox conditions in sediments amended only with BPA and in sediments that were amended with 4-hydroxybenzoate (4OHB), a readily degraded aromatic compound and an intermediate in aerobic BPA degradation (9).

Experimental Methods

Chemicals. 4OHB, Phenol, 2BP, 2-chlorophenol (2CP), 2,6DBP, 2,6DCP, TBBPA, TCBPA, and BPA were obtained from Aldrich Chemical Co. (Milwaukee, WI) and were of minimum 97% purity. All other reagents for medium preparation, extractions, and high-performance liquid chromatography (HPLC) analyses were obtained from Fisher Scientific (Fair Lawn, NJ).

Sediment. Sediment grab samples were collected from the Arthur Kill tidal strait located between Staten Island and New Jersey by using a gravity corer. Sediment was transferred to sterile glass jars, which were sealed and transported back to the lab and stored at 4 °C until use.

Sediment Enrichments for TBBPA and TCBPA Dehalogenation. Anaerobic enrichments (50 mL) containing 25% v/v live Arthur Kill sediment were established in 60-mL serum bottles.

Prior to addition of live slurries, TBBPA and TCBPA were added to serum bottles in the following manner. A total of 1 g of dry, sterile (autoclaved for 30 min on three successive days) Arthur Kill sediment was added to each serum bottle. TBBPA and TCBPA were delivered to the dry sediment through the addition of hexane stock containing either 2.58 mM TBBPA (3.87 mL/serum bottle) or 4 mM TCBPA (2.5 mL/serum bottle). This volume of stock completely saturated the sediment. The hexane was allowed to evaporate from vented bottles under a fume hood over a period of several days, thereby leaving a coating of TBBPA or TCBPA on the dry sediment.

Next, sediment enrichment slurries were prepared using 25% v/v live Arthur Kill sediment. First an inorganic anaerobic medium was prepared. The following medium components were added to distilled water: (g/L) KCl, 1.3; KH₂PO₄, 0.2; NaCl, 1.17; CaCl₂·2H₂O, 0.1; and MgCl₂·6H₂O, 0.18. For sulfate-reducing medium, 20 mM Na₂SO₄ was also added. The medium was boiled for 30 min then cooled under a stream of deoxygenated N₂. After the medium was cooled, the gas purge was changed to N₂/CO₂ (70/30%). The following were added from anoxic, sterile stock solutions via deoxygenated sterile syringes: NaHCO₃ (2.5 g/L); resazurin (1 mg/L); vitamin solution (3I); FeCl₂·4H₂O, 370 (methanogenic) or 1.5 (sulfate-reducing) mg/L; and trace metals (mg/L: MnCl₂·4H₂O, 20; CoCl₂·6H₂O, 30; H₃BO₃, 5.7; CuCl₂·2H₂O, 2.7; Na₂MoO₄·2H₂O, 2.6; ZnCl₂, 2.1; and NiCl₂·6H₂O, 0.5); and Na₂S·9H₂O, 0.5 g/L. Next, live Arthur Kill sediment was added to bring the medium to volume.

Serum bottles containing the dry sediment coated with TBBPA or TCBPA were purged under a N₂/CO₂ (70/30%) gas

stream, and 50 mL of the 25% v/v sediment slurry was added using a purged, sterile pipet. The bottles were capped with Teflon-lined serum caps and were shaken vigorously to mix the dry sediment into the slurry. Although the nominal concentration of TBBPA and TCBPA in the enrichments (discounting partitioning) was 225 to 275 μM, greater than 95% of the TBBPA and TCBPA was partitioned to the sediment (data not shown).

2,6DBP and 2,6DCP were dissolved in ultrapure water (0.1 M NaOH) to create 10 mM stock solutions, and the solutions were stored under argon headspace. These solutions were added via syringe, aseptically and anaerobically, after the serum bottles had been sealed. The final concentration of 2,6DBP and 2,6DCP was between 200 μM and 260 μM.

Sediment enrichment slurries with no substrate, 2,6DBP, 2,6DCP, TBBPA, and TCBPA as the sole amendment, and those containing TBBPA and TCBPA combined with 2,6DBP and 2,6DCP, respectively, were set up in triplicate. Sterile controls were autoclaved at 121 °C for 30 min on two consecutive days after the addition of TBBPA, TCBPA, 2,6DBP, or 2,6DCP. The sediment enrichments were incubated at 28 °C in the dark without shaking.

2-Bromophenol Enrichment Cultures. Methanogenic and sulfidogenic sediment cultures previously enriched on 2BP [25% v/v Arthur Kill sediment enrichments set up in 1-L bottles using the medium described above and re-spiked repeatedly with 2BP over a period of 3 years] were also tested for their ability to dehalogenate TBBPA and TCBPA in the presence or absence of 2,6DBP or 2,6DCP, respectively. Aliquots of the cultures were set up using a procedure similar to that described above. The culture volume was 10 mL in a 30-mL serum bottle.

BPA Degradation. Sediment enrichment slurries (10%, v/v) were prepared using the anaerobic medium described above. No alternate electron acceptor, Na₂SO₄ (20 mM), freshly precipitated amorphous iron as ferric oxyhydroxide (200 mM), or KNO₃ (30 mM) was added to promote methanogenic, sulfate-, iron(III)-, or nitrate-reducing conditions, respectively. Sixty-mL serum bottles were purged under a N₂/CO₂ (70/30%) gas stream, and 50 mL of slurry was added using a purged, sterile pipet. BPA and 4OHB were added via syringe to the sealed serum bottles to a concentration of 200 μM using sterile, anoxic aqueous stock solutions. Sediment enrichment slurries with no substrate, BPA, and 4OHB were set up in triplicate for each electron acceptor condition. After the 4OHB-enriched sediments had consumed two doses of 4OHB, BPA was added to a final concentration of 200 μM along with a third dose of 4OHB. Sterile controls spiked with BPA and 4OHB were autoclaved at 121 °C for 30 min on two consecutive days.

HPLC Analyses. Serum bottles were vigorously shaken to ensure mixing while a slurry sample (0.2 mL) was withdrawn using a deoxygenated syringe fitted with an 18-gauge needle. The samples were extracted with one (BPA degradation study) or three (TBBPA and TCBPA dehalogenation study) vols of methanol per volume of sample by shaking for 1 h at 60 rpm and then centrifuging for 5 min at 10,000 rpm. The resulting supernatant was then filtered through a 0.45-μm Millipore filter and analyzed via HPLC equipped with a UV-Vis detector. A Spherclone 5-μ ODS (2) (250 × 4.60 mm; 5-micron particle size; Phenomenex Inc.) analytical column was used at ambient temperature. Concentrations were calculated by comparison to either three or four point external standard curves. The extraction efficiency was above 85%.

For the TBBPA and TCBPA study, the eluent was composed of 80% methanol, 18% ultrapure water, and 2% glacial acetic acid at a flow rate of 1 mL/min. The retention times of the amended compounds and their transformation products were as follows: 2CP, 3.2 min; 2BP, 3.3 min; 2,6DCP, 3.6 min; 2,6DBP, 3.9 min; BPA, 3.3 min; TCBPA, 5.7 min; and TBBPA,

6.9 min. A wavelength of 280 nm was used to detect the compounds 2CP, 2BP, 2,6DCP, and 2,6DBP. A wavelength of 238 nm was used to detect BPA, TCBPA, and TBBPA.

For the BPA study, the eluent was composed of 60% methanol, 38% ultrapure water, and 2% glacial acetic acid at a flow rate of 1 mL/min. The retention times of the analytes were as follows: 4OHB, 3.4 min; phenol, 4.1 min; and BPA, 7.6 min. A wavelength of 280 nm was used.

Gas Chromatography–Mass Spectrometry (GC–MS). Samples were acetylated by taking 1 vol of the HPLC sample, evaporating the methanol, and then adding 10 vol of 1 M K_2CO_3 and 0.5 vol of acetic anhydride. This solution was then vigorously mixed and allowed to sit for 10 min. After 10 min the solution was extracted with 5 vol of hexane, and the organic phase was removed for analysis. Samples were analyzed on a Hewlett-Packard 5890 gas chromatograph with a Hewlett-Packard 5971 mass-selective detector, and a Hewlett-Packard 5MS analytical column (cross-linked 5% PH ME Siloxane, 30 m length, 0.25 mm i.d., 0.25 μ m film thickness). The injection volume was 2 μ L into a helium carrier gas stream at a flow rate of 1 mL/min. The injector and detector temperatures were 280 and 350 $^{\circ}C$, respectively. The oven temperature was held at 100 $^{\circ}C$ for 1 min, ramped to 200 $^{\circ}C$ at 20 $^{\circ}C$ per min, then ramped to 300 $^{\circ}C$ at 15 $^{\circ}C$ per min, and then held at 300 $^{\circ}C$ for 5 min.

Analysis of Methane, Nitrate, and Sulfate. Production of methane and reduction of sulfate and nitrate were verified by gas chromatographic analysis and ion chromatography, respectively, as described previously (31).

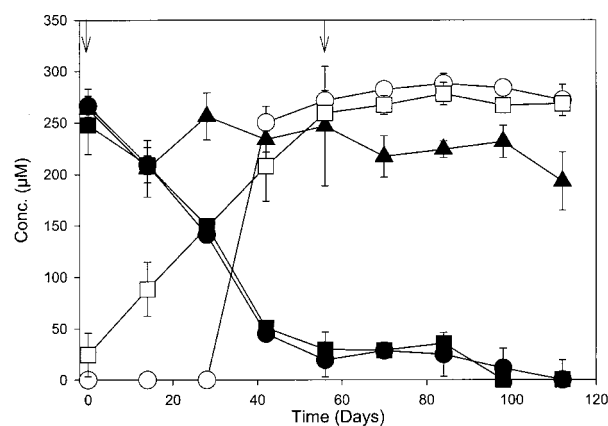
Results

Debromination of TBBPA in Sediment Enrichments. Under methanogenic conditions, initial TBBPA loss was observed within 14 days (Figure 1a). TBBPA was dehalogenated nearly concomitantly to a stoichiometrically equivalent amount of BPA (Figure 1a). Near complete loss of TBBPA was seen within 55 days. BPA was detected sooner in enrichments that received the analogue 2,6DBP as a cosubstrate. Production of methane (not quantified) confirmed that methanogenic conditions were promoted. Because no exogenous electron donor was added, reducing equivalents for the dehalogenation originated from the turnover of the organic matter in the sediment, and, in the case of the enrichments amended with 2,6DBP, from the degradation of the phenol produced through debromination.

In sediment enrichments to which sulfate was added, biotransformation of TBBPA commenced after a lag period of 28 days and was virtually complete within 112 days (Figure 1b). In contrast to the methanogenic enrichments, BPA was not detected until after there had been substantial loss of TBBPA (after 70 days). The final molar concentration of BPA was approximately equivalent to the amount of TBBPA depleted (Figure 1b). Sulfate loss was observed in the live enrichments, however no methane was produced, verifying that sulfate-reducing conditions were promoted. The onset of BPA production was not linked to the depletion of sulfate, which was present throughout the incubation period. In the methanogenic, and to a greater extent in the sulfidogenic enrichments, transient intermediates were detected by HPLC (the retention times were approximately 5.5 and 4.2 min). These compounds were assumed to be lesser-halogenated BPA compounds, however, identification of the transient intermediates in these enrichments was not pursued. No loss of TBBPA was observed in the autoclaved controls, indicating that the transformation was biologically mediated.

We were able to maintain dehalogenation activity in the methanogenic TBBPA enrichments through subculturing (1:3 into fresh media). An additional TBBPA spike (150 μ M) was stoichiometrically dehalogenated to BPA within 150 days.

a. Methanogenic



b. Sulfidogenic

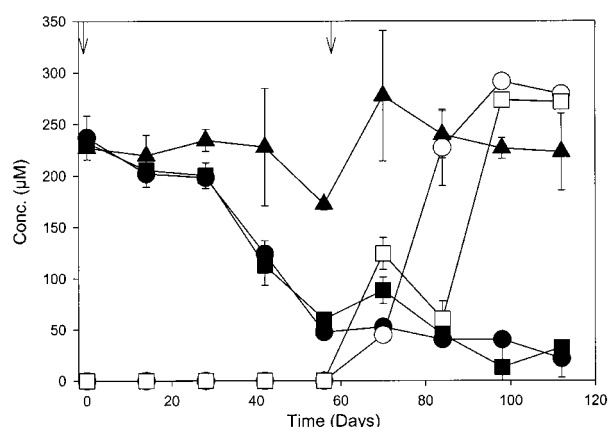


FIGURE 1. Anaerobic transformation of TBBPA under (a) methanogenic and (b) sulfidogenic conditions. Symbols: (●) TBBPA, (■) TBBPA (+ 2,6DBP), (▲) TBBPA (sterile control), (○) BPA (TBBPA), (□) BPA (TBBPA + 2,6DBP). 2,6DBP (200 to 240 μ M) was added on days 0 and 55 as indicated by an arrow.

Dechlorination of TCBPA in Sediment Enrichments. Biotransformation of TCBPA in the methanogenic sediment enrichments commenced following an initial lag period of 56 days (Figure 2a). Complete loss of TCBPA occurred within 112 days. TCBPA was converted to a metabolite that was detected by HPLC at a retention time of 3.9 min. GC–MS analysis (Figure 3a) of the acetylated derivative indicated that the metabolite was a dichlorinated compound with a mass spectrum consistent with that expected for a dichlorobisphenol A (DCBPA) isomer. The mass spectrum shows characteristic isotope distribution of two chlorines and the fragmentation of two acetyl groups (m/z 380 \rightarrow 338, 296). The position of the chlorine substituents, one on each of the phenol rings in DCBPA, was confirmed by the fragmentation pattern seen in the spectrum of DCBPA. The fragments of m/z 153 and 155 (at a ratio of 3.2:1, consistent with the theoretical chlorine 35:37 isotope ratio) are consistent with the presence of one chlorine on a 4-hydroxyphenyl ethane fragment. [Note that if dechlorination had occurred on one aromatic residue only, the mass spectrum of the dichlorinated bisphenol A intermediate would have 4-hydroxyphenyl ethane fragments with an m/z of 187/189/191, and 119, for the dichlorinated and nonchlorinated fragments, respectively].

GC–MS analysis also revealed a second metabolite (Figure 3b) at a lower concentration (approximately 5% of that of metabolite 1) that was not detected by HPLC. The acetylated derivative indicated that the second metabolite was a monochlorinated compound with a mass spectrum consis-

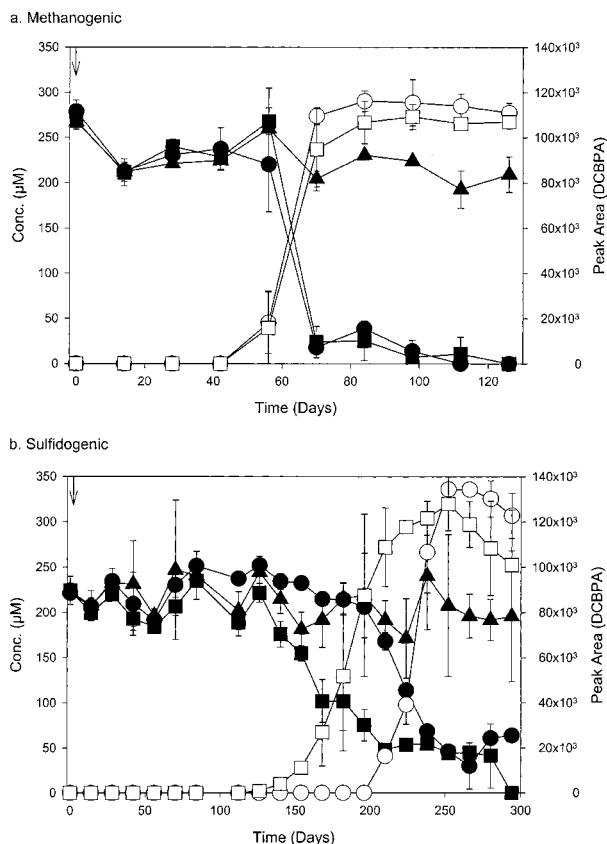


FIGURE 2. Anaerobic transformation of TCBPA under (a) methanogenic and (b) sulfidogenic conditions. Symbols: (●) TCBPA, (■) TCBPA + 2,6DCP, (▲) TCBPA (sterile control), (○) DCBPA (TCBPA), (□) DCBPA (TCBPA + 2,6DCP). 2,6DCP (260 μ M, methanogenic and 460 μ M sulfidogenic) was added on day 0 as indicated by an arrow.

tent with that expected for monochlorobisphenol A (MCBPA). The fragments at 153/155 and 119 are characteristic of the monochlorinated and nonchlorinated 4-hydroxyphenyl ethane fragments, respectively. Because there are no commercially available DCBPA and MCBPA standards, we were unable to confirm the concentration in the enrichments. The ultimate peak area observed during HPLC analyses of DCBPA was similar to the initial peak area of TCBPA, suggesting a near stoichiometric conversion, assuming equivalent extinction coefficients. BPA was not detected in any TCBPA enrichments. This indicates that dechlorination of TCBPA proceeds with removal of one chlorine from each ring to DCBPA, with slow dechlorination further to MCBPA.

In the sulfate-reducing sediment enrichments, TCBPA persisted for 156 days (Figure 2b). Thereafter, slow dehalogenation of TCBPA to the same dichlorinated DCBPA metabolite (3.9-min retention time) was observed, with the TCBPA concentration leveling out at approximately 60 μ M after 294 days. Enrichments with 2,6DCP as a cosubstrate exhibited dehalogenation of TCBPA approximately 70 days earlier than those without 2,6DCP. Again, the onset of dehalogenation was not linked to the depletion of sulfate, which was present in the bottles throughout the incubation.

No loss of TCBPA was observed in autoclaved controls. The methanogenic TCBPA cultures were successfully subcultured and exhibited continued dehalogenation of TCBPA to DCBPA.

Fate of Substrate Analogues 2,6DBP and 2,6DCP. Complete dehalogenation and degradation of 2,6DBP, with transient detection of 2BP, occurred within 28 days under both methanogenic and sulfate-reducing conditions. As with

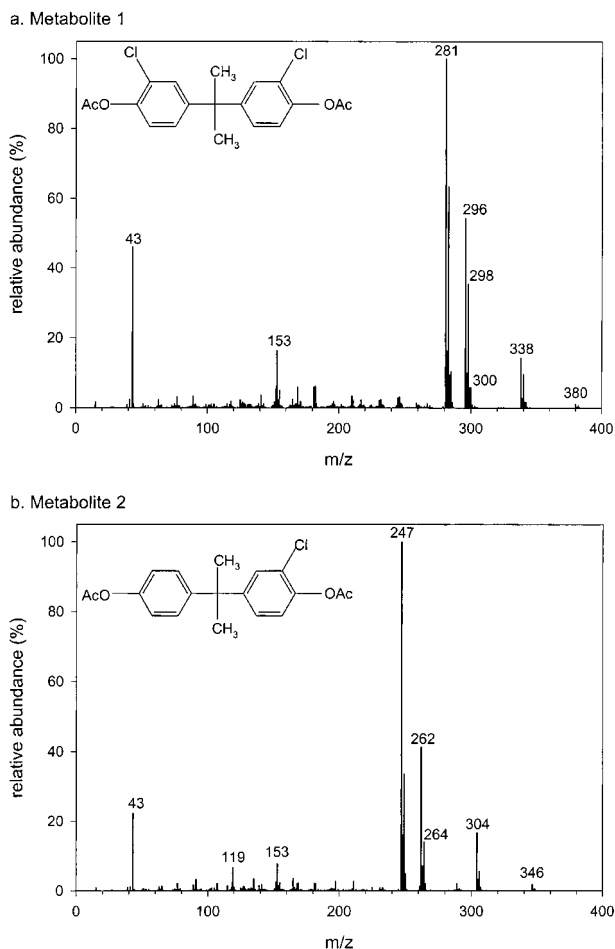


FIGURE 3. Mass spectra of acetylated metabolites of TCBPA.

TCBPA there was some difference in the onset of dehalogenation of 2,6DCP between methanogenic and sulfate-reducing conditions. Under sulfate-reducing conditions, there was a much longer lag period before dehalogenation of 2,6DCP than that observed under methanogenic conditions.

Dehalogenation of TBBPA and TCBPA in 2BP-Enrichment Cultures. Results of TBBPA and TCBPA dehalogenation in cultures pre-enriched on 2BP (data not shown) were not substantially different from that observed for unacclimated sediment. Under methanogenic conditions, TBBPA was dehalogenated stoichiometrically to BPA within 60 days. Dehalogenation of TCBPA commenced after a lag period of 56 days, and the lesser-chlorinated intermediate, DCBPA, was the only daughter product observed. In the presence of sulfate, TBBPA was converted to BPA; however, no loss of TCBPA was observed.

BPA Degradation Study. Because BPA accumulated in the methanogenic and sulfidogenic cultures after dehalogenation of TBBPA, we investigated the potential for anaerobic biodegradation of BPA in more detail. Anaerobic cultures with Arthur Kill sediment were amended with either nitrate, sulfate, Fe(III), or bicarbonate as terminal electron-acceptors and spiked with BPA. After 162 days of monitoring, no significant BPA loss in any of the live cultures or autoclaved controls was observed (Figure 4a). In a separate set of cultures, 4OHB (a structural analogue of BPA) was degraded readily under all electron-acceptor amended conditions. Under conditions promoting methanogenesis or sulfate-reduction, phenol accumulated transiently as 4OHB was degraded. After 2 doses of 4OHB (200 μ M each) were consumed, a third dose of 4OHB (200 μ M) was added, along with 200 μ M BPA.

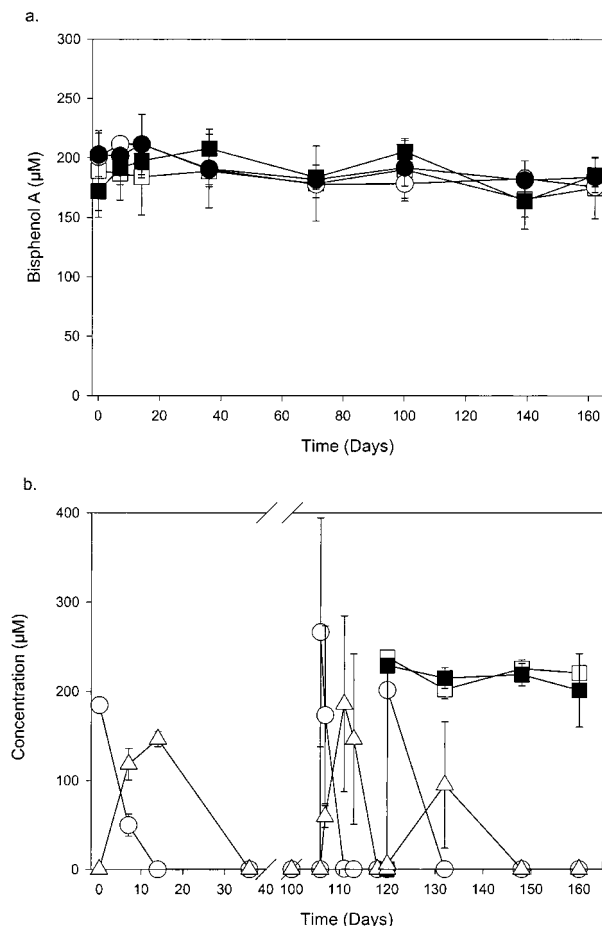


FIGURE 4. (a) Fate of BPA under nitrate-reducing (○), sulfate-reducing (■), Fe(III)-reducing (●), and methanogenic conditions (□). (b) Fate of co-amended 4OHB (○), phenol (△), and BPA (□) under methanogenic conditions compared to BPA sterile control (■).

Although 4OHB continued to be degraded with transient accumulation of phenol, no BPA loss was observed during an additional 40 days of monitoring in any of the incubations. An example of typical results (for the methanogenic cultures) is shown in Figure 4b.

Onset of the specific terminal electron-accepting processes was confirmed by the production of methane (ca. 100 µmol/bottle) in live methanogenic enrichments and its absence in sterile controls and in bottles amended with either sulfate, nitrate, or Fe(III). Likewise, nitrate depletion was noted in live nitrate-amended enrichments (with no methane production). In live sulfate-reducing enrichments, approximately 2 mM sulfate was consumed in those bottles that

were amended with 4OHB (consistent with the expected stoichiometric amount); however, no significant sulfate reduction occurred in bottles amended with BPA or in the sterile control bottles. Analysis for Fe(II) production was not performed, however, methanogenesis was not observed in bottles amended with Fe(III).

Discussion

Anaerobic biotransformation of the flame retardants TBBPA and TCBPA was shown to occur in anaerobic sediment slurries. Under methanogenic and sulfidogenic conditions, complete dehalogenation of TBBPA to BPA, with no further degradation of BPA, and partial dehalogenation of TCBPA to DCBPA was observed. In separate experiments, BPA was not degraded with either nitrate, sulfate, iron(III), or carbonate as an electron acceptor either with or without the addition of a readily degradable substrate analogue, 4OHB. These biotransformation pathways are summarized in Figure 5.

We theorized that co-amendment with 2,6DBP and 2,6DCP would stimulate dehalogenation of TBBPA and TCBPA by more easily enriching for dehalogenating bacteria in the sediments than TBBPA or TCBPA. Additionally, these substrates could provide a source of reducing equivalents upon the degradation of their debromination product, phenol.

Under methanogenic conditions, co-amendment with 2,6DBP had no significant effect on the relative time of onset of the dehalogenation of TBBPA. However, the completely dehalogenated daughter product, BPA, was detected approximately 25 days earlier in enrichments amended with 2,6DBP as compared to those without the co-amendment (Figure 1a). Additionally, under sulfate-reducing conditions, 2,6DCP co-amendment apparently shortened the lag period prior to dehalogenation of TCBPA by approximately 70 days over that of enrichments amended with TCBPA alone (Figure 2b). Therefore, some evidence was obtained to suggest that addition of halogenated analogues did contribute to stimulation of dehalogenation of the target substrates and their partially dehalogenated intermediates. This phenomenon has also been observed with PCB and dioxin dehalogenation (34, 35).

The debromination of 2,6DBP and 2,6DCP to the lesser halogenated intermediates 2BP and 2CP was transiently observed; however, the ultimate dehalogenation product, phenol, did not persist in any of the slurries or enrichments and was likely readily degraded to CO₂ as shown in previous studies (30–33). Although the degradation of phenol would supply reducing equivalents for dehalogenation, it is apparent from results from enrichments that did not receive co-amendment that the organic matter in the sediment supplied sufficient reducing equivalents for dehalogenation of TBBPA and TCBPA.

Dehalogenation of TBBPA to BPA was much slower in the sulfate-reducing enrichments than in the methanogenic

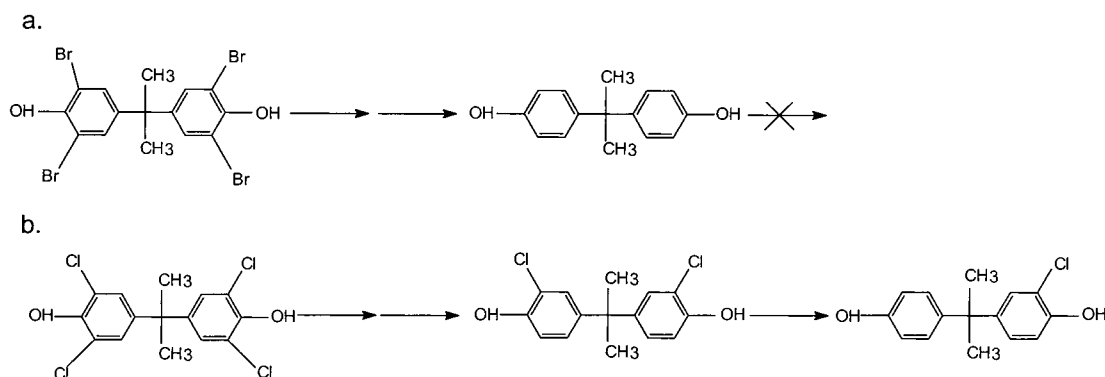


FIGURE 5. Proposed anaerobic biotransformation pathways of TBBPA and TCBPA.

enrichments. Some transient accumulation of lesser-brominated intermediates in the sulfate-reducing enrichments was observed prior to the final production of BPA. This reduction in the apparent rate of the overall process is interesting because the onset of dehalogenation or the eventual production of BPA was not linked to the depletion of sulfate. It is possible that in the presence of ongoing sulfate reduction lower levels of hydrogen (or other source of reducing equivalents) prevailed than in the methanogenic enrichments. Lower concentrations of electron donor may have resulted in the slower observed rates of dehalogenation. Alternately, presence of sulfate may select for entirely different dehalogenating populations that exhibit different rates of dehalogenation.

The dehalogenation of TCBPA was significantly retarded compared to dehalogenation of TBBPA. BPA was never produced. Instead, the dehalogenation resulted in the production of a dichlorinated intermediate with trace dehalogenation to a monochlorinated product. Also, in the presence of sulfate reduction, onset of dechlorination was later than onset of debromination. That debromination occurs more readily than dechlorination under various conditions was observed previously with brominated and chlorinated phenols in a variety of sediments (33).

Although TBBPA and TCBPA have not been shown to be degraded under aerobic conditions, TBBPA seems to be readily dehalogenated to BPA under both methanogenic or sulfate-reducing conditions (9, this study). This is fortuitous, as anoxic sediments are a potential sink for at least part of the enormous amounts of this flame retardant that are currently in use throughout the world. The persistence of BPA in anoxic sediments under a variety of electron-accepting conditions is thus a concern. BPA has been detected in anoxic marine sediments (16), and our data suggest that it will likely persist for an extended period of time. Formation of BPA through the dehalogenation of TBBPA could potentially increase concentrations of this compound in anoxic environments. Combined, the concerns regarding the potential estrogenic effects of BPA (15), the largely unknown effects of TBBPA and TCBPA, and the potential for the accumulation of BPA under anoxic conditions justify vigilance and the continued study of the environmental fate of this class of compounds.

Acknowledgments

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