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4-Methylphenol produced in freshwater sediment microcosms is not a bisphenol A metabolite

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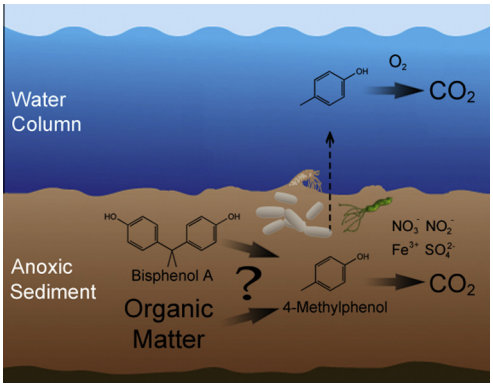
HIGHLIGHTS

- Partial disappearance of bisphenol A (BPA) observed in anoxic sediment microcosms.
- 4-Methylphenol (4-MP) detected as putative degradation intermediate.
- Experiments using ^{13}C-labeled BPA demonstrated that 4-MP was not derived from BPA.
- The formation of 4-MP to suggest BPA degradation must be carefully interpreted.

GRAPHICAL ABSTRACT



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ABSTRACT

4-Methylphenol (4-MP), a putative bisphenol A (BPA) degradation intermediate, was detected at concentrations reaching 2.1 mg L^{-1} in anoxic microcosms containing 10 mg L^{-1} BPA and 5 g of freshwater sediment material collected from four geographically distinct locations and amended with nitrate, nitrite, ferric iron, or bicarbonate as electron acceptors. 4-MP accumulation was transient, and 4-MP degradation was observed under all redox conditions tested. 4-MP was not detected in microcosms not amended with BPA. Unexpectedly, incubations with ^{13}C-labeled BPA failed to produce ^{13}C-labeled 4-MP suggesting that 4-MP was not derived from BPA. The detection of 4-MP in live microcosms amended with lactate, but not containing BPA corroborated that BPA was not the source of 4-MP. These findings demonstrate that the transient formation of 4-MP as a possible BPA degradation intermediate must be interpreted cautiously, as microbial activity in streambed microcosms may generate 4-MP from sediment-associated organic material.

1. Introduction

Bisphenol A, 2,2-bis(4-hydroxyphenyl)propane (BPA) is used to manufacture polycarbonate, epoxy resins, flame retardants, and

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lacquer coatings on food cans, as well as other products (Staples et al., 1998). The estimated global production exceeds 5.2 Mt per yr, making BPA one of the highest production volume chemicals in the world (The Dow Chemical Company, 2013). As a consequence of high-capacity use, BPA has been detected in environmental systems including surface waters, sediments, and groundwater (Bolz et al., 2001; Heemken et al., 2001). Bearing structural resemblance to estrogens, BPA can bind to the estrogen receptor and is considered weakly estrogenic. The fate of BPA in the environment has been studied in recent years. Several aerobic BPA degraders have been reported that use BPA as the sole source of carbon and energy (Lobos et al., 1992; Spivacks et al., 1994; Ike et al., 1995; Kang and Kondo, 2002; Kolvenbach et al., 2007; Fischer et al., 2010). Based on the identification of intermediates, a few different aerobic BPA degradation pathways have been proposed (Spivacks et al., 1994; Kolvenbach et al., 2007; Fischer et al., 2010). A recent survey of 107 soil samples demonstrated aerobic BPA degradation in 85 samples, and 26 BPA-degrading isolates were obtained belonging to the genera *Pseudomonas, Klebsiella, Pandoraea, Alcaligens, Enterobacter, Serratia, Bacillus, Bordetella,* and *Sphingomonas* (Matsumura et al., 2009). These findings suggest that diverse bacterial groups are capable of degrading BPA under oxic conditions.

A significant mass of BPA resides in anoxic sediments (Bolz et al., 2001; Heemken et al., 2001), but very little is known about the fate of BPA under anoxic conditions. Several studies investigating microbial BPA degradation under anoxic conditions have concluded that BPA is recalcitrant and undergoes "little or no" biodegradation in the absence of oxygen (Kang and Kondo, 2002, 2005; Voordeckers et al., 2002). No microbial BPA degradation was observed in anoxic microcosms established with freshwater sediment (Ying et al., 2003), marine sediments (Ying and Kookana, 2003), and soil (Ying and Kookana, 2005). Halogenated BPAs were reductively dehalogenated to BPA in estuarine sediment microcosms, but no further degradation was observed under different redox conditions (Voordeckers et al., 2002). Experimental evidence supporting anaerobic BPA degradation is scarce. Chiou (2010) reported a 14% of loss in BPA concentration after a 120 d incubation period with anoxic river sediment. Similarly, Kang and Kondo (2002) reported a 10% loss of initial BPA added to anoxic river water microcosms, and (Patterson et al., 2010) reported BPA removal under denitrifying conditions. At best, these studies demonstrated BPA disappearance but BPA degradation intermediates and end-products were not identified or quantified. To predict the environmental fate of BPA in anoxic environments, more detailed studies of BPA degradation under anoxic conditions are needed. This study detected 4-methylphenol (4-MP) in anoxic sediment microcosms as a possible BPA degradation intermediate but experiments using ^{13}C-labeled BPA implicated that 4-MP was derived from another source, presumably sediment-associated organic matter, emphasizing the need for careful results interpretation regarding the environmental fate of BPA or similar phenolic compounds.

2. Materials and methods

2.1. Chemicals

BPA (>99% purity) was obtained from Sigma Aldrich (St. Louis, MO), 4-MP (>99% purity) was purchased from Acros Organics (Fair Lawn, NJ), and L-tyrosine (99% purity) was purchased from MP Biomedicals (Chicago, IL). Specifically labeled versions of BPA, 2,2-bis(4-hydroxyphenyl)[1,3-$^{13}C_2$]propane ($^{13}C_2$-BPA) and 2,2-bis(4-hydroxyphenyl)[$^{13}C_3$]propane ($^{13}C_3$-BPA), were synthesized by condensation of [1,3-$^{13}C_2$] and [$^{13}C_3$]acetone, respectively, with five equivalents of phenol in the presence of a cation exchange resin catalyst, Amberlyst 15, which was purchased from Rohm and Haas (Philadelphia, PA) (Singh, 1992). The labeled acetone (>99% purity) was purchased from Cambridge Isotopes Laboratories (Andover, MA). Aromatic ring labeled BPA, 2,2-bis(4-hydroxy[$^{13}C_6$]phenyl) propane ($^{13}C_{12}$-BPA, >99% purity) was also purchased from Cambridge Isotopes Laboratories.

2.2. Microcosm setup

Microcosms were established in 60 mL serum bottles closed with black butyl rubber stoppers (Geo-Microbial Technologies, Ochelata, OK). Sediment samples were collected from four geographically distinct locations (latitude, longitude), including the Third Creek (35.949284, −83.939861), the Partnach Gorge (47.459198, 11.122777), the Neckar River (48.780193, 9.245422), and the Hainbach Creek (48.776559, 9.300439). The Third Creek location has a history of contamination of chlorinated solvents, the Neckar River flows along industrial areas, and the Partnach Gorge and Hainbach Creek are considered pristine water bodies. Each serum bottle received 5 g of sediment material (wet weight) and reduced (0.2 mM L-cysteine and 0.2 mM sodium sulfide) mineral salts medium to achieve a total volume of 30 mL in each vessel as well as a single addition of 2 mM of lactate as a readily fermentable substrate to ensure rapid establishment of anoxic conditions. BPA was added to the microcosms to a final aqueous phase concentration of 10 mg L^{-1} (44 μM). Autoclaved control microcosms with BPA, and live microcosms without BPA, electron acceptor, or lactate were established for each sediment sample. All manipulations were performed inside an anoxic chamber (Coy Laboratory Products, Ann Arbor, MI) containing a nitrogen/hydrogen (97/3; v/v) atmosphere. The microcosms were incubated at room temperature in the dark without shaking. To establish different redox conditions, electron acceptors including nitrate (2 mM), nitrite (0.5 mM), amorphous ferric oxyhydroxide (FeOOH, 10 mM, nominal concentration), ferric citrate (5 mM), sulfate (10 mM), and bicarbonate (30 mM) were added from anoxic, sterilized stock solutions at the concentrations indicated in parentheses. FeOOH was prepared following an established procedure (Lovley and Phillips, 1988). Electron acceptors were replenished by syringe when depleted. To test BPA degradation under oxic conditions, microcosms were established using the same mineral salts medium except that the bicarbonate buffer system was replaced with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer (10 mM, pH 7.0) and the reductants and lactate were omitted. These microcosms were incubated in the dark, and the headspace was purged with filter-sterilized air every second day. Additional microcosms were prepared with the Third Creek sediment to access the biodegradability of 4-MP. Ten mg L^{-1} of 4-MP and 2 mM of lactate were added and electron acceptors were replenished as above.

2.3. Analytical procedures

The amount of BPA associated with the sediments was determined after sampling by an ultrasonic solvent extraction method (Xu et al., 2008) with some modifications. In brief, 5 g of sediment was mixed with 10 mL of acetone/ethyl acetate (50:50, v/v), sonicated at 20 kHz for 15 min using a Branson Sonifier 250 (Branson Ultrasonic, Danbury, CT), and centrifuged at 5000*g* for 10 min. The extracts from three consecutive solvent extractions were combined and evaporated to dryness under a stream of filtered (0.25 μm) nitrogen. The residue was dissolved in 10 mL water/acetonitrile (50:50, v/v) and subjected to HPLC analysis. The adsorption capacity of BPA on Third Creek sediment was also evaluated. Triplicate 60 mL vessels that received 5 g of autoclaved sediment,