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Use of Halogenated Benzoates and Other Halogenated Aromatic Compounds To Stimulate the Microbial Dechlorination of PCBs

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We recently reported that certain polychlorinated biphenyl (PCB) congeners and bromobiphenyl congeners activate or "prime" dechlorination of the Aroclor 1260 in Housatonic River sediments. In this study, we tested the ability of halogenated benzoates and other halogenated aromatic compounds to prime PCB dechlorination in the same sediments. We found that none of the fluorinated or chlorinated benzoates primed PCB dechlorination, but several brominated and iodinated benzoates initiated this activity. Of the halogenated benzoates tested, 4-bromobenzoate, 4-iodobenzoate, and 2,5-dibromobenzoate primed the most extensive PCB dechlorination, decreasing the hexa- through nonachlorobiphenyl fraction of Aroclor 1260 by 40−70%, 10−50%, and 10−50%, respectively. None of the halogenated benzoates were as effective at priming PCB dechlorination as 2,6-dibromobiphenyl, which primed a 60−80% decrease of the hexa- through nonachlorobiphenyl fraction of Aroclor 1260. Several other brominated aromatic compounds were also tested for their ability to prime PCB dechlorination. Monobrominated isomers of acetophenone, phenol, or toluene did not prime PCB dechlorination, but all monobrominated isomers of benzonitrile, 2-bromo-, 4-bromo-, and 2,5-dibromonitrobenzene, 4-bromobenzamide, 4-bromobenzophenone, 4-bromobenzoic hydrazide, 4-bromobenzoic methyl ester, and 2,5-dibromobenzene sulfonate primed PCB dechlorination in Housatonic River sediments. All of the compounds primed PCB Dechlorination Process N (primarily flanked *meta* dechlorination) except 4-bromonitrobenzene, which primed Dechlorination Process P (flanked *para* dechlorination). These results indicate that halogenated aromatic compounds that are not structural analogues to PCBs can prime PCB dechlorination.

Introduction

Industrial mixtures of polychlorinated biphenyls (PCBs) were used for decades in electrical equipment, heat exchangers, hydraulic fluids, and compressor fluids. Unfortunately, the chemical and thermal stability that made PCBs so useful have resulted in their persistence in many aquatic sediments. PCBs bioaccumulate and are potentially toxic to humans and wildlife; hence, their persistence in the environment is a widespread concern. Microbial dechlorination of PCBs has been observed in many anoxic sediments (*1*) and can play an important role in natural restoration because it decreases the toxicity of PCBs and increases their degradability (*1*−*4*). Consequently, one goal of our research was to investigate novel approaches for enhancing or accelerating microbial PCB dechlorination in aquatic sediments.

Previous studies indicated that PCB dechlorinating microorganisms were present in Woods Pond sediments (a shallow impoundment on the Housatonic River in Lenox, MA), but that their PCB dechlorination activity under the intrinsic conditions was limited (*2, 5*−*7*). However, these microorganisms could be activated or "primed" to dechlorinate the PCBs that have persisted for years in these sediments. Several PCB congeners, especially 2,3,4,5,6-pentachlorobiphenyl (23456-CB), 2,3,4,6-tetrachlorobiphenyl (2346-CB), and 2,3,6-trichlorobiphenyl (236-CB), were shown to prime extensive and sustained *meta* dechlorination of the Aroclor 1260 in Housatonic River sediment, whereas the PCB congener 245-CB primed *para* dechlorination of PCBs in the same sediments (*6*). The different PCB dechlorination patterns suggested that specific chlorobiphenyls prime dechlorination by enriching distinct microbial populations that exhibit unique PCB dechlorination specificities.

The success of priming PCB dechlorination with specific chlorobiphenyl congeners prompted further investigation of PCB priming using individual bromobiphenyl congeners. All of the tested bromobiphenyl congeners were completely dehalogenated to biphenyl, and most required a relatively short acclimation period of 1−2 weeks, which was considerably less time than the corresponding chlorobiphenyl isomers (*8*). In addition, specific bromobiphenyl congeners such as 2,6-dibromobiphenyl (26-BB), 2,5,3′-tribromobiphenyl (25−3-BB), 25-4-BB, and 245-BB primed more extensive PCB dechlorination in Woods Pond than was observed with the best results from 23456-CB (*9*). The complete dehalogenation of the bromobiphenyl primers, the shorter lag times, and the more extensive PCB dechlorination showed great promise for the use of compounds other than chlorobiphenyls to stimulate PCB dechlorination in situ.

We hypothesize that the reason bromobiphenyls are so effective at priming PCB dechlorination is that they enrich for bacteria that can use halobiphenyls as electron acceptors. Reductive dehalogenation reactions have been calculated to be energy yielding reactions for a variety of halogenated aromatic compounds including PCBs, and there is evidence that they can supply sufficient energy as a respiratory process to support the growth of halorespiring bacteria (*10, 11*; for a review, see ref *12*). Although no microorganisms capable of dechlorinating PCBs have been isolated, two studies have shown by most probable number (MPN) methods that the number of PCB dechlorinating microorganisms increased 200−1000-fold as a result of either PCB or bromobiphenyl dehalogenation (*13, 14*). These results suggest that halogenated substrates could potentially be used to increase the population size of dehalogenating microorganisms and influence the bioremediation of habitats contaminated with chlorinated compounds.

The ability of the microbial populations that are enriched using halogenated substrates as nutrients to also dehalogenate specific targeted contaminants is considered a type of cross-acclimation. Cross-acclimation of dehalogenation activity has been observed previously in sediments and soils and with isolated microorganisms (*2, 5, 6, 9, 15*−*22*). In some cases, specific halogenated and nonhalogenated compounds that were not transformed were still able to induce the dehalogenation of structural analogues (*18*). In addition, some

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chlorinated substrates co-induced the dechlorination of compounds that were not structural analogues; for example, perchloroethylene (PCE) and trichloroethylene (TCE) were dechlorinated by *Desulfomonile tiedjei* after induction with 3-chlorobenzoate (*15, 20*). These results suggest that some microorganisms may possess enzymes or cofactors that have a relaxed specificity for halogenated substrates and perform fortuitous dehalogenation of a variety of halogenated compounds.

In this study, we describe the stimulation of PCB dechlorination in sediment microcosms by a variety of halogenated compounds that are not biphenyl analogues. The compounds tested contain a benzene nucleus, a halogen substituent, and a functional moiety. All of the compounds that primed microbial dechlorination of PCBs, including several bromo- and iodobenzoates, were dehalogenated in the sediment microcosms. The mineralization of halobenzoates in anaerobic habitats is well documented (*18*), whereas biphenyl was a terminal product of bromobiphenyls used to prime PCB dechlorination (*9*). Therefore, halobenzoates could potentially offer an advantage over halogenated biphenyls for priming PCB dechlorination.

Materials and Methods

Microcosms: Preparation and Incubation. Sediment slurry microcosms were set up using sediment and water collected near the western shore of Woods Pond (Lenox, MA). Multiple core samples were taken and pooled into glass jars, topped with site water, and stored at 4 °C until experimental setup. On a dry weight basis, the sediment used in these experiments contained 30−150 μg/g of partially dechlorinated Aroclor 1260 and approximately 5−7 mg/g of weathered hydrocarbon oil (*7*). Sediment slurries were prepared inside an anaerobic glovebox under an atmosphere of approximately 98−99% nitrogen and 1−2% hydrogen. Wet sediment (2 vol) was combined with pond water (3 vol) in a large beaker, mixed vigorously, and dispensed into serum bottles or crimp seal test tubes in aliquots of 30 or 15 mL, respectively. Slurries were amended with halogenated compounds from concentrated stock solutions prepared in acetone. Halogenated benzoic acid stock solutions were also prepared in acetone prior to their addition to slurries. Final concentrations of the halogenated compounds in the slurry were 500 μmol/L of slurry unless indicated otherwise, and the final acetone concentration was 0.5% (v/v) of the slurry. The majority of experiments also contained either sodium malate or sodium fumarate, pH 7.0, at 10 mmol/L since previous experiments showed that these compounds enhance dehalogenation when added with a primer (*8, 9*). The serum bottles or tubes were crimp sealed with Teflon-lined butyl rubber stoppers. Sterilized controls were prepared by pasteurization (75 °C, 20 min), followed by incubation (22−25 °C, 48 h) and autoclaving (121 °C, 3 h). Sterile controls contained the same amendments as the experimental samples, whereas live controls contained only malate or fumarate (10 mM) and acetone (0.5%). Experiments were performed in either duplicate or triplicate. Most experiments were performed with 2,6-BB (500 μmol/L) as a positive control. The microcosms were incubated statically in the dark at room temperature (22−25 °C). Although no bicarbonate or CO_2 was added, all incubations became methanogenic within 1−2 weeks.

Chemicals. All PCBs and 2-bromobiphenyl were purchased from AccuStandard, Inc. (New Haven, CT). 26-BB (99.93% purity by GC-FID) was synthesized by chemists at GE. The sources for all other halogenated aromatics are given in Tables 1 and 2. All nonhalogenated aromatic compounds were 99+% purity and were purchased from Aldrich Chemical Co. (Milwaukee, WI). L-Malic acid (98% purity by enzyme assay) was purchased from Sigma Chemical Co. (St. Louis, MO). Fumaric acid (99% purity) was purchased from Janssen Chimica (Geel, Belgium). OmniSolv grade acetone(99.99% pure), acetonitrile (99.95% pure), and copper filings (GR, fine powder) were purchased from EM Science (Gibbstown, NJ). Sodium bicarbonate was from J. T. Baker, Inc. (Phillipsburg, NJ).

Sample Analysis. Aliquots (0.5 mL) of the slurries were removed periodically in the anaerobic chamber, combined with a 1 M solution of bicarbonate (1 vol) and extracted in vials with Teflon-lined screw caps by vigorous shaking (24 h) with anhydrous diethyl ether (5 vol). The bicarbonate solution was added to convert any halobenzoic acid to the halobenzoate salt prior to ether extraction. We used elemental mercury (0.25 vol) or acid-reduced copper filings (~0.4 g) to remove sulfur from extracted material. Quantitative comparisons of the mole percent (mol %) PCB congener distributions for samples extracted by this simple procedure and by a rigorous Soxhlet procedure (EPA 3540) (*23*) demonstrated no significant differences (*24*); hence, we routinely used the simple one-step extraction procedure. The progress of PCB dechlorination was monitored by gas chromatography (GC) with a Ni^{63} electron capture detector (ECD). The ether portion was removed and analyzed for PCBs by GC-ECD (Hewlett-Packard 5890) and for aromatic metabolites by GC−mass spectrometry (MS) (Hewlett-Packard 5890/5971A). Both GCs were equipped with a DB-1 poly(dimethylsiloxane) fused silica capillary column (length, 30 m; i.d., 0.25 mm; phase thickness, 0.25 μm; J&W Scientific, Folsom, CA) and operated using methods as described by Bedard et al. (*8, 9*). PCB congeners measured by GC-ECD were identified and quantified by comparison to a customized PCB standard (Aroclor 1260 supplemented with PCB congeners known to be dechlorination products) using a quadratic fit of the data to a four-point external standard calibration curve forced through zero (*9, 24*). Mole percent distributions of the PCB congeners and homologues and the distribution of *ortho*, *meta*, and *para* chlorines per biphenyl were calculated using a spreadsheet program (Microsoft Excel) (*24*). Aromatic metabolites were identified by comparison of retention times and mass spectra to those of authentic standards or to spectra in the National Bureau of Standards Library.

The halobiphenyls used to prime PCB dechlorination, 2346-CB and 26-BB, did not seriously interfere with the quantitation of the PCBs, albeit a few minor PCB components could not be quantified due to the coelution with the primer. These were peaks 14 and 15 (25-2-CB/4-4-CB and 24-2-CB) for samples primed with 26-BB. 2346-CB was dechlorinated primarily to 246-CB, but trace amounts of 236-CB and 26-CB were also produced. The latter two compounds interfered with potential PCB dechlorination products with which they coelute, 26-3-CB and 2-2-CB, respectively. However, in each case it was estimated from experiments with other primers that the components of the obscured peaks constitute less than 1.25 mol % of the total PCBs before and after dechlorination (*5, 6*). The polar compounds, such as the benzoates or benzene sulfonates, were not extracted by ether, but most of the other brominated aromatic compounds that were tested were present in the ether phase. All of the tested compounds migrated ahead of the PCB congeners and therefore did not interfere with PCB quantitation.

Samples were taken periodically and analyzed for benzoates and halide ions. The samples were centrifuged at 15000*g* for 5 min, and the supernatant was removed. The aqueous portion was analyzed using a Waters 840 high-performance liquid chromatography system (HPLC, Waters Corporation, Milford, MA) equipped with either a reverse-phase C18 column (4.6 × 110 mm, Whatman Partisphere) for benzoates or a Waters IC-Pak A HR column (4.6 × 7.5 mm) for halide ions. The benzoates were eluted using a linear

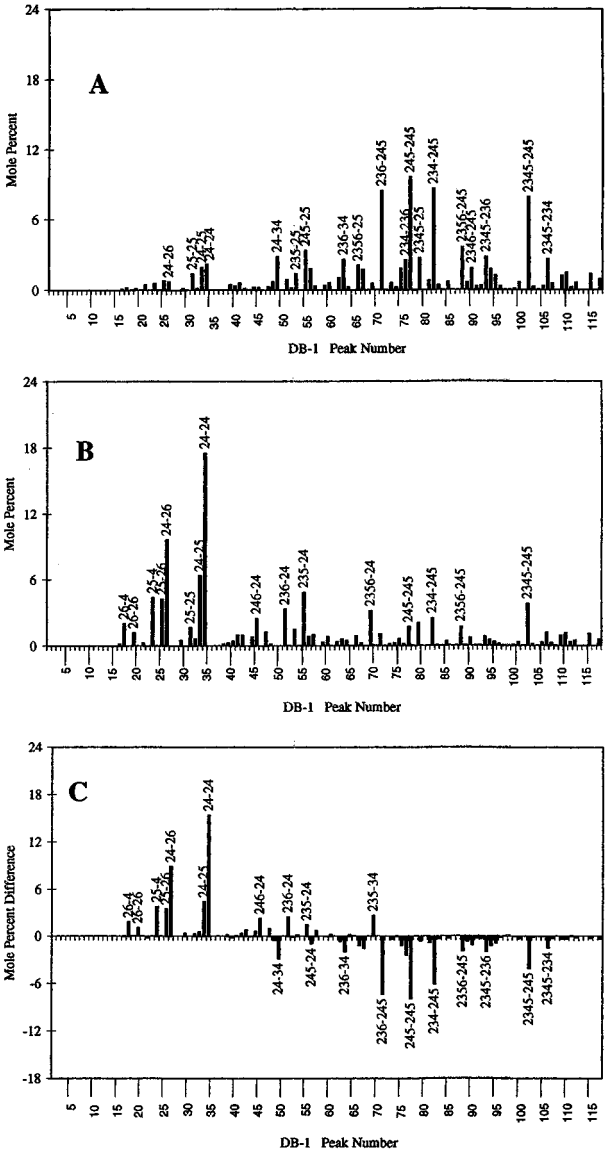

FIGURE 1. Effect of PCB dechlorination primed by 4-BBz. (A and B) PCB congener distribution of sediments at 0 and 163 days, respectively, after priming with 4-BBz. (C) Difference plot showing the changes in the congener distribution between days 0 and 163. For a complete list of PCB congener assignments, see ref *6*.

gradient of two solvents. Solvent A was 50 mM sodium acetate, pH 4.5. Solvent B was acetonitrile. The gradient ran from 90% A/10% B to 75% A/25%B in 9 min. Benzoates were identified using a photodiode array detector (Waters model 996) by comparing retention times and absorbance spectra of peaks to those of authentic standards. Halobenzoates were quantified at 230 and 254 nm after extrapolation from linear standard curves of external standards. Millennium 2010 Chromatography Manager software (Waters Corporation) was used to obtain and process data. The halide ions were eluted with an aqueous mixture of acetonitrile (24% v/v), 1-butanol (4% v/v), glycerin (0.38% v/v), boric acid (16.5 mM), lithium hydroxide monohydrate (6.9 mM), and gluconic acid (1.3 mM) and were measured using a conductivity detector (Waters model 430).

Results

Using Halobenzoates to Prime Dechlorination of the Aroclor 1260 Residue in the Sediment. Several isomers of iodinated and brominated benzoic acids were tested for their

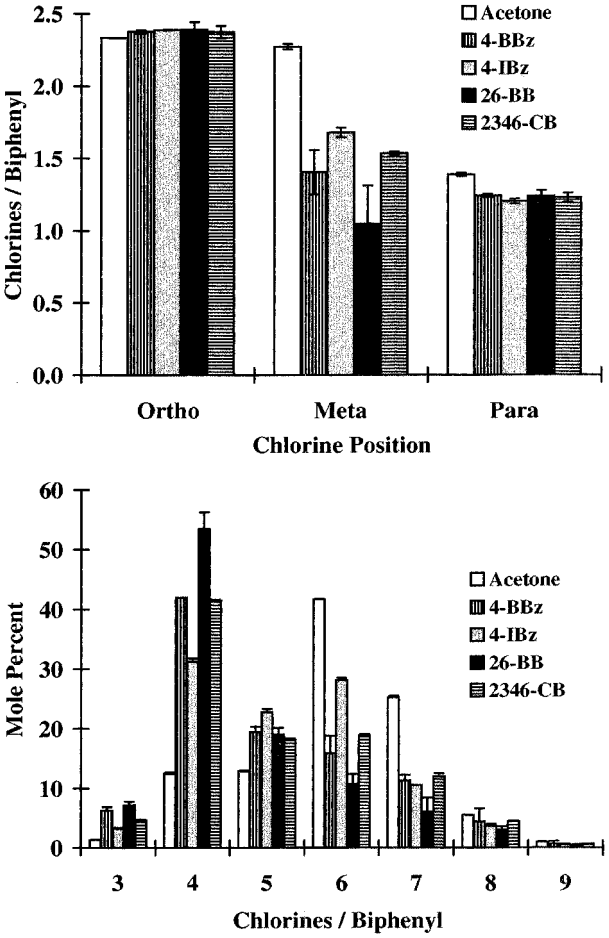

FIGURE 2. Comparison of the extent of PCB dechlorination in Housatonic River sediment incubated for 163 days after priming with different priming substrates. The order of the compounds compared is acetone, 4-BBz, 4-IBz, 26-BB, and 2346-CB, respectively for both graphs. (A) The chlorine position on the biphenyl nucleus that underwent dechlorination. (B) The distribution of chlorobiphenyl homologues 163 days after priming with the same compounds. Error bars represent 2 SD from the mean of duplicate samples.

ability to prime PCB dechlorination in the sediment slurries. Microcosms amended with 4-iodobenzoic acid (4-IBz) started to show increases in the PCB congeners 24-24-CB, 24-25-CB, and 24-26-CB at the first sampling (~3 weeks) indicating that dechlorination of more highly chlorinated congeners had occurred. Extensive dechlorination of the Aroclor 1260 residue in the sediment was evident after several months in slurries amended with 4-bromobenzoic acid (4-BBz) (Figure 1). The PCB congener distribution profile resulting from the addition of either 4-BBz or 4-IBz showed the same dechlorination process that was observed in Housatonic River sediments primed with either 2346-CB or 26-BB. This type of dechlorination, known as Process N, results in the removal of flanked *meta* chlorines from the penta- through nonachlorobiphenyls (*1*, *6*, *25*) as is illustrated in Figures 1 and 2. The kinetics of Process N PCB dechlorination can be monitored by measuring the hexa- through nonachlorobiphenyl fraction of the PCB mixture over time since these highly chlorinated biphenyls initially constitute ~70 mol % of the total PCBs in the sediment and are converted to tri- and tetrachlorobiphenyls by dechlorination (*9*). PCB dechlorination was previously found to closely follow the dehalogenation of 2346-CB and 26-BB (*6*, *9*, *22*). A comparison of PCB dechlorination primed by 4-BBz, 4-IBz, 26-BB, and 2346-CB shows that the halobenzoates were just as effective at priming PCB dechlorination as the tetrachlorobiphenyl but

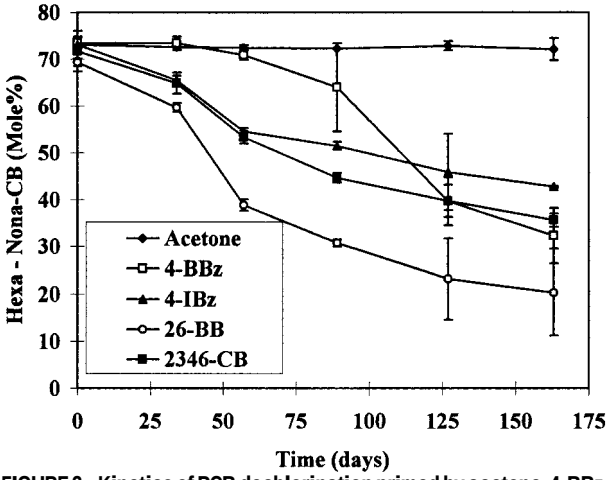

FIGURE 3. Kinetics of PCB dechlorination primed by acetone, 4-BBz, 4-IBz, 26-BB, and 2346-CB. Hexa- through nona-CB refers to the hexa- through nonachlorobiphenyl fraction of PCBs. Error bars represent 2 SD from the mean of duplicate samples.

not as effective as the dibromobiphenyl (Figures 2 and 3). PCB dechlorination primed with both halobiphenyls and 4-IBz was apparent at 34 days, but PCB dechlorination primed by 4-BBz was not obvious until 89 days (Figure 3).

Figure 4 shows the temporal relationship between dehalogenation of 4-BBz or 4-IBz and dechlorination of the hexa- through nonachlorobiphenyl fraction of the Aroclor 1260 in the sediment when primed by these compounds. 4-BBz dehalogenation showed a lag phase lasting at least 30 days followed by a fairly rapid dehalogenation, whereas dehalogenation of 4-IBz started almost immediately. The PCB dechlorination primed by 4-BBz showed a longer lag, but it eventually surpassed the amount of dechlorination primed by 4-IBz. No PCB dechlorination was observed until after substantial dehalogenation of the primer. Slurries that were amended with either acetone or benzoic acid plus halide ions instead of the halobenzoic acids did not stimulate PCB dechlorination, indicating that neither the carrier nor the products of the priming substrate contributed to the priming of PCB dechlorination.

The ranges in the total amount of PCB dechlorination primed by all of the tested halobenzoates are summarized in Table 1. None of the fluorinated or chlorinated benzoates or benzoates containing both a chloro and fluoro substituent primed PCB dechlorination in any of the experiments tested. However, specific isomers of brominated or iodinated benzoates were effective primers of PCB dechlorination. The priming activity of some compounds, especially 2-BBz, 2-IBz, 4-IBz, 25-BBz, and 25-IBz, was highly variable. When PCB dechlorination was primed by these compounds, the extent of dechlorination did not progress significantly beyond 130 days even though the experiments were monitored for at least 240 days. 4-BBz was the most reliable of any of the halobenzoates at priming PCB dechlorination. Without exception, the halobenzoates that acted as primers appeared to be dehalogenated before PCB dechlorination was activated. However, some halobenzoates did not prime PCB dechlorination even though they were dehalogenated. Unfortunately, none of the halobenzoates primed PCB dechlorination as extensively or as reproducibly as the positive control, 26-BB, which was previously shown to reliably prime extensive PCB dechlorination in Housatonic River sediments (*9*, *14*).

Priming of PCB Dechlorination with Other Brominated Aromatic Compounds. Because 4-BBz effectively primed PCB dechlorination in Housatonic River sediments, we also tested the ability of other brominated aromatic compounds to prime

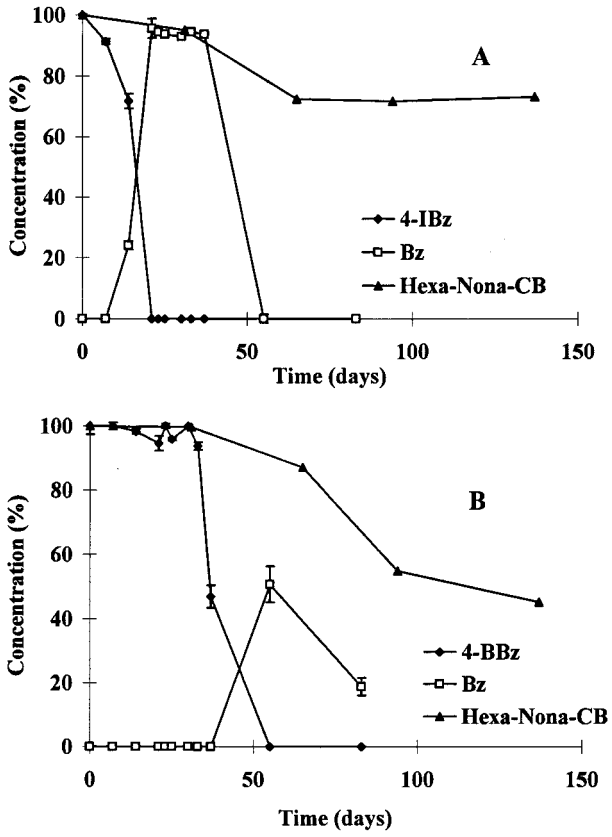

FIGURE 4. Dehalogenation of 4-bromobenzoate (4-BBz), 4-iodobenzoate (4-IBz), and PCBs in Housatonic River sediments. (A) Dehalogenation of 4-IBz, appearance of benzoate (Bz), and dechlorination of the hexa- through nonachlorobiphenyl fraction of the PCBs. (B) Dehalogenation of 4-BBz, appearance of benzoate, and dechlorination of the hexa- through nonachlorobiphenyl fraction of the PCBs. The initial concentrations of 4-BBz and 4-IBz were 612 and 630 μM, respectively. 4-BBz, 4-IBz, and benzoate are plotted as mol % of the initial concentrations of the halogenated benzoates. Dechlorination of the PCBs is shown as percent of hexa- through nonachlorobiphenyl fraction of the PCBs remaining. The initial value of hexa- through nonachlorobiphenyls was 73 mol % of the total PCBs. Error bars in both graphs represent the standard deviations of duplicate samples. The symbols for the hexa- through nonachlorobiphenyl fraction of the PCBs were larger than the error bars.

PCB dechlorination and compared the extent of PCB dechlorination primed by these compounds to the amount primed by 4-BBz and 26-BB. The aromatic compounds that were tested contained a benzene nucleus, a functional group, and typically a single bromine substituent. The results of several experiments are summarized in Table 2. We tested all of the mono-brominated isomers of many aromatic compounds, but only the *para* substituted isomers of others. Because our studies were directed at stimulating PCB dechlorination, we did not always follow the fate of the added compounds, but in some cases we used GC−MS to look for intermediates of the added compounds. In most cases, we observed dehalogenated intermediates of the priming compounds after 2 weeks (Table 2).

None of the brominated acetophenones, phenols, or toluenes primed PCB dechlorination whereas all tested isomers of the brominated benzonitriles and nitrobenzenes primed dechlorination. 4-Bromobenzamide, 4-bromobenzoic hydrazide, and 4-bromobenzophenone primed small amounts of PCB dechlorination. 4-Bromobenzene sulfonate failed to initiate dechlorination, but 2,5-dibromobenzene sulfonate did prime PCB dechlorination.

TABLE 1. Summary of the Effect of Various Halogenated Benzoates on PCB Dechlorination in Housatonic River Sediment Slurries

halobenzoate tested	halobenzoate source[a]	haobenzoate dehalogenation[b]	identified products[c]	% dechlorination of hexa−nonachlorobiphenyls[d]
		Monohalobenzoates		
2-F-	Ald	−		0
3-F-	Ald	−		0
4-F-	Ald	−		0
2-Cl-	Ald	−		0
3-Cl-	P&B	+	benzoate	0
4-Cl-	Ald	−		0
2-Br-	P&B	+	benzoate	0−50
3-Br-	P&B	+	benzoate	0
4-Br-	Ald	+	benzoate	40−70
2-I-	Lan	+	benzoate	10−50
3-I-	Lan	+	benzoate	0−30
4-I-	Lan	+	benzoate	10−50
		Dihalobenzoates		
2-Cl-4-F-	Ald	−		0
4-Cl-2-F-	TWC	−		0
2-Cl-5-Br-	Lan	+	2-chlorobenzoate	0
2-Br-5-Cl-	Lan	+	3-chlorobenzoate, benzoate	0
4-Br-2-Cl-	Lan	+	2-chlorobenzoate, benzoate	10−20
2,5-Cl-	Ald	−		0
2,5-Br-	Lan	+	3-bromobenzoate, benzoate	10−50
3,5-Br-	Lan	+	3-bromobenzoate, benzoate	0
2,5-I-	TWC	+	2-iodobenzoate, benzoate	10−50
		Trihalobenzoates		
2,3,6-Cl-	P&B	−		0
2,4,6-Cl-	Ald	−		0
2,4,6-Br-	TCI	ND[e]		10−20
2,3,5-I-	Lan	ND[e]		10−20
3,4,5-I-	P&B	ND[e]		0
		Halosalicylates (Halogenated 2-Hydroxybenzoates)		
4-Cl-	Lan	−		0
5-I-	P&B	+	salicylate	10−20
3,5-Cl-	Ald	+	3-chlorosalicylate (Ald)[a]	0−10
3,5-Br-	Lan	+	3-bromosalicylate [f], salicylate	0−10

[a] Sources of chemicals were as follows: Ald, Aldrich Chemical Co., Milwaukee, WI; Lan, Lancaster Synthesis, Inc., Windham, NH; P&B, Pfaltz and Bauer, Inc., Waterbury, CT; TCI, TCI America, Inc., Portland, OR; TWC, Trans World Chemicals, Inc., Rockville, MD. [b] (+) Halobenzoate disappearance and appearance of products were observed using HPLC. (−) Halobenzoate concentrations unchanged and no products identified using HPLC. [c] Products were identified by HPLC analysis using a photodiode array detector by matching their elution times and absorbance spectra with those of authentic standards. [d] Data show the range obtained from at least two separate experiments and represent the percent dechlorination of the hexa- through nonachlorobiphenyl fraction of PCBs measured following at least 130 days of incubation after addition of the priming substrate. PCB dechlorination was confirmed by the appearance of PCB dechlorination products in all samples showing ≥10% dechlorination. [e] ND, not determined, although disappearance of substrate and appearance of unidentified products were observed by HPLC. [f] 3-Bromosalicylate was not commercially available for confirmation, but we deduced that the metabolite was 3-bromosalicylate from the following evidence. The appearance and disappearance of the metabolite corresponded with the removal of 3,5-dibromosalicylate and the formation of salicylate, respectively, but the metabolite had a different retention time than 5-dibromosalicylate (Lancaster).

Mass spectral data of the metabolites of the nitrobenzenes suggested that the parent compounds were reduced to the corresponding anilines prior to the debromination step. For example, 4-bromoaniline was identified as an intermediate of 4-bromonitrobenzene, and PCB dechlorination was not apparent until aniline was observed by GC−MS, hence it is likely that the bromoaniline primes the dechlorination activity. However, this has not been verified by adding the bromoanilines as priming substrates.

The methyl ester of 4-BBz was just as effective as 4-BBz at priming PCB dechlorination, but the *ortho* and *meta* brominated isomers did not initiate the dechlorination activity. It is likely that the PCB dechlorination primed by 4-bromobenzoic methyl ester occurred after its hydrolysis to 4-BBz since this compound was identified as a metabolite only a few hours after adding the compound to sediment slurries (data not shown).

All of the compounds except 4-bromonitrobenzene primed Process N PCB dechlorination. 4-Bromonitrobenzene primed Process P, a limited *para* dechlorination (*5*). 4-BBz primed the most extensive PCB dechlorination of all the brominated compounds tested containing the benzene nucleus.

Discussion

Several brominated and iodinated benzoates primed PCB dechlorination, but none of the chlorobenzoate or fluorobenzoate isomers had any effect on this process. Both 3-fluoro and 3-chlorobenzoate were previously shown to have much longer acclimation periods (~120 days) before dehalogenation than either 3-bromo- or 3-iodobenzoates in anaerobic lake sediment (*26*). It is possible that the fluorinated and chlorinated benzoates that were tested in this study did not have sufficient time to be dehalogenated and prime PCB dechlorination in these experiments.

Several halobenzoates that were dehalogenated did not prime PCB dechlorination. However, every halogenated benzoate that successfully primed PCB dechlorination was dehalogenated as the initial step in its biodegradation. The same observation was made previously about the bromobiphenyls that were used to prime PCB dechlorination (*9*). In those experiments, the priming of PCB dechlorination with bromobiphenyls was achieved by stimulating the growth of PCB dechlorinating microorganisms (*14*). Therefore, a plausible explanation for our results is that a microbial population was enriched by using the halobenzoate as an

TABLE 2. Brominated Aromatic Compounds Tested for Ability to Prime PCB Dechlorination in Woods Pond Slurries

aromatic compound	functional group	bromo isomer[a]	identified products[a,b]	hexa−nona-CB dechlorination[c]	relative PCB dechlorination[d]	type of primed dechlorination
acetone (negative control)				0[e,f,g,i]	0[e,f,g]	–
acetophenone	$COCH_3$	2-Br	NA[h]	0[g,i]	0	–
		3-Br	NA	0[g]	0	–
		4-Br	NA	0[g]	0	–
2,6-dibromobiphenyl (positive control) C_6H_5	C_6H_5	2,6-Br	2-bromobiphenyl, biphenyl	38[e]**, 54 [f]**, 66[g]***	100[e,f,g]	N
benzamide	$CONH_2$	4-Br	benzamide	11[e]*	30	N
benzene sulfonate	SO_3^-	4-Br	NA	0 [f]	0	–
		2,5-Br	ND [j]	16 [f]	29	N
benzoate	CO_2^-	4-Br	benzoate	22[e]*, 37 [f]**, 61[g]***	84[e], 68 [f], 86[g]	N
benzoic hydrazide	$CONHNH_2$	4-Br	ND	17[e]*	44	N
benzoic methyl ester	CO_2CH_3	2-Br	NA	0[e]	0	–
		3-Br	NA	0[e]	0	–
		4-Br	4-bromobenzoate, benzoate	64[g]***	96	N
benzonitrile	CN	2-Br	benzonitrile	4[e], 11 [f]***	11[e], 20 [f]	N
		3-Br	benzonitrile	11[e]***, 18 [f]***	28[e], 34 [f]	N
		4-Br	benzonitrile	15[e]***, 36 [f]***	39[e], 66 [f]	N
benzophenone	COC_6H_5	4-Br	NA	3[e]**	8	N
nitrobenzene	NO_2	2-Br	2-bromoaniline, aniline	14[e]***, 34 [f]***	47[e], 63 [f]	N
		4-Br	4-bromoaniline, aniline	3[e]*, 7 [f]***	8[e], 13 [f]	P
		2,5-Br	2,5-dibromoaniline, aniline	9 [f]***	16	N
phenol	OH	2-Br	NA	2[g]	6	–
		3-Br	NA	3[g]	8	–
		4-Br	NA	2[g]	6	–
toluene	CH_3	2-Br	NA	3[g]	8	–
		3-Br	NA	2[g]	6	–
		4-Br	NA	2[g]	6	–
trifluorotoluene	CF_3	4-Br	NA	0[e]	0	–

[a] All compounds were tested at 500 μmol/L of slurry. 26-BB was synthesized by GE; 2-BB was from AccuStandard. The 4-Br- and 2,5-Br isomers of benzene sulfonate were purchased from Chem Service (West Chester, PA) and Eastern Chemical, United-Guardian, Inc. (Smithtown, NY), respectively. The methyl esters of all three bromobenzoic acids were purchased from Lancaster Synthesis, and 4-bromotrifluorotoluene was purchased from TCI America. All other halogenated aromatic compounds were obtained from Aldrich. [b] Products of the transformation of the added brominated compounds were identified by comparing GC−MS data to spectra in the library of the National Bureau of Standards or to authentic standards analyzed under the same conditions. [c] The amount of PCB dechlorination that was stimulated by the addition of the brominated compound in three separate experiments (*e*−*g*) after incubating for at least 110 days after addition of the priming substrate. The values listed are the percent of the hexa- through nonachlorobiphenyls that were dechlorinated at specified times in each experiment as compared to the acetone controls. The initial values of the hexa- through nonachlorobiphenyl fraction of PCBs ranged from 63 to 68% of the total chlorobiphenyls. [d] The amount of PCB dechlorination relative to the negative control (acetone) set at 0% and the positive control (2,6-dibromobiphenyl) set at 100%. [e,f,g] Three separate experiments in which the halogenated aromatic compounds were tested for the ability to prime PCB dechlorination. [h] NA, not analyzed. [i] Level of significance using a two sample *T*-test. $df = 2$. Significant decreases are designated by asterisks; *, significant at $P = 0.1$; **, $P = 0.05$; ***, $P = 0.01$. [j] ND, none detected.

energy source and was then able to dehalogenate specific chlorobiphenyls. In most cases, PCB dechlorination continued long after the halobenzoates were transformed, indicating that the dehalogenating population survived long after the halobenzoates were gone.

The bromobiphenyl and chlorobiphenyl priming compounds are substrate analogues to the PCBs; therefore, enrichment of a microbial population that attacks both the halogenated biphenyl primer and the residual PCBs in the sediment was not surprising. However it appears from our data that halogenated compounds which are not chlorobiphenyl analogues may also enrich for microorganisms capable of dechlorinating PCBs. There is some precedent for this kind of cross-acclimation. Both PCE and TCE dechlorination activities in *D. tiedjei* are co-induced with 3-chlorobenzoate dehalogenation activities even though these compounds are not chlorobenzoate analogues (*15, 18, 20*).

Halobenzoate dehalogenating microorganisms appear to be fairly common, and their activities are routinely found in anaerobic habitats such as sediments, soil, and sewage sludge (reviewed in ref *18*). The specificity of chlorobenzoate dechlorination can vary at different sites within sediments, suggesting that multiple dechlorinating populations can coexist in these habitats (*27*). In addition, brominated and iodinated benzoates appear to be more easily dehalogenated than either chlorinated or fluorinated benzoates as evidenced by their faster dehalogenation rates and shorter lag times in anaerobic habitats (*26, 28*). On the basis of this evidence and our data showing the variability of halobenzoates to prime PCB dechlorination, we propose that multiple halobenzoate dehalogenating populations were present in the Housatonic River sediments. We also hypothesize that competition between halobenzoate dehalogenating populations affected both the extent and the reliability of PCB dechlorination primed by halobenzoates. Priming of PCB dechlorinating microorganisms with halobiphenyls has been very reliable, apparently because these compounds are PCB analogues. However, the priming of PCB dechlorinating microorganisms with halobenzoates may be more difficult if multiple halobenzoate dehalogenating populations are present, and only a subset of these can also dechlorinate the PCBs.

4-IBz primed dechlorination of PCBs, but not as consistently or to the same extent as 4-BBz. Since the carbon−iodide bond is very labile, 4-IBz is potentially more susceptible to reductive dehalogenation by a greater number of sediment microorganisms. This decreases the likelihood that a microbial population capable of dehalogenating both 4-IBz and the PCBs will be enriched by this compound. When 4-IBz did prime PCB dechlorination, there was much less of a lag time in the PCB dechlorination as compared to that primed by 4-BBz. However, following a long lag, the 4-BBz consistently primed more rapid and extensive PCB dechlorination than was primed by 4-IBz.

All of the halobenzoates that primed PCB dechlorination produced Process N dechlorination, and the *para* substituted isomers primed the most extensive dechlorination. In

contrast, *para* substituted bromobiphenyls activated Process P (*para*) dechlorination while *ortho* and *meta* substituted bromobiphenyls primed Process N (*meta*) dechlorination of PCBs (*9*). These results suggest that either the same PCB dechlorinating population was enriched with a variety of different halogenated substrates or that there are multiple microbial populations in Woods Pond that carry out Process N PCB dechlorination.

Experiments with other brominated aromatic compounds illustrated that a variety of brominated aromatic compounds can elicit PCB dechlorination in Housatonic River sediments. The inability of the brominated acetophenones, phenols, and toluenes to prime PCB dechlorination may be due to toxicity of these compounds at the concentrations used. The compounds that successfully primed PCB dechlorination, such as the *para* brominated isomers of benzamide, benzoic hydrazide, benzoate, and benzonitrile, all have an electron-withdrawing functional group *para* to the bromine substituent. However, 4-bromoacetophenone and 4-bromosulfonate both failed to stimulate PCB dechlorination although they also have electron-withdrawing functional groups *para* to the bromine substituent. There does not appear to be any correlation between priming ability and partition coefficients of the various brominated aromatic compounds that were tested.

The use of halogenated benzoates instead of halogenated biphenyls to prime PCB dechlorination offers several advantages over 26-BB. The mineralization of benzoate under anaerobic conditions is well documented in the literature, but the removal of biphenyl under anaerobic conditions has not been reported. The halogenated benzoates are also less expensive than the brominated biphenyls and are commercially available in bulk quantities. Potential disadvantages of using halobenzoates include their solubility and the variable and less extensive PCB dechlorination resulting from their addition. Optimization would be required.

The use of halogenated aromatic compounds as primers of dechlorination is an important finding. This approach may prove useful, particularly with respect to stimulating the dechlorination of chlorinated chemicals that are slowly biodegraded in anoxic habitats. The use of brominated or iodinated compounds that are easily mineralized under anaerobic conditions may enrich for dehalogenating populations as well as provide necessary carbon and electron flow in nutrient-starved environments.

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