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Reductive Dechlorination of Polychlorinated Biphenyls as Affected by Natural Halogenated Aromatic Compounds

Jongseol Kim[1,*], Ahmi Lee[1], Yong-Suk Moon[1], Jae-Seong So[2] and Sung-Cheol Koh[3]

[1]Division of Biological Sciences, University of Ulsan, Ulsan 680-749, Republic of Korea
[2]Department of Biological Engineering, Inha University, Incheon 402-751 Republic of Korea
[3]Division of Civil and Environmental Systems Engineering, Korea Maritime University, Busan 606-791, Republic of Korea

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We investigated the effects of halogenated aromatic compounds (HACs) including naturally occurring ones (L-thyroxine, 3-chloro-L-tyrosine, 5-chloroindole, 2-chlorophenol, 4-chlorophenol and chlorobenzene) on polychlorinated biphenyl (PCB) dechlorination in sediment cultures. A PCB-dechlorinating enrichment culture of sediment microorganisms from the St. Lawrence River was used as an initial inoculum. When the culture was inoculated into Aroclor 1248 sediments amended with each of the six HACs, the extent of dechlorination was not enhanced by amendment with HACs. The dechlorination patterns in the HAC-amended sediments were nearly identical to that of the HAC-free sediments except the 3-chloro-L-tyrosine-amended ones where no dechlorination activity was observed. When these sediment cultures were transferred into fresh sediments with the same HACs, the dechlorination specificities remained the same as those of the initial inoculations. Thus, in the present study, the substrate range of the highly selected enrichment culture could not be broadened by the HACs. It appears that HACs affect PCB dechlorination mainly through population selection rather than enzyme induction of single population.

Keywords: reductive dechlorination, polychlorinated biphenyls, halogenated aromatic compounds, dechlorinating microorganisms

Polychlorinated biphenyls (PCBs) are ubiquitous and recalcitrant pollutants especially in aquatic sediments. Reductive dechlorination of PCBs is a natural bioremedial process that transforms PCBs into lower-chlorinated congeners (Mohn and Tiedje, 1992; Bedard, 2003). Despite clear evidence for microbial reductive dechlorination of PCBs both in the laboratory and natural sediments, efforts to obtain a pure culture of PCB-dechlorinating microorganisms have been unsuccessful (see references in Bedard, 2003). Thus, their physiological characteristics and nutritional requirements for PCB-dechlorination still remain unclear.

Various halogenated aromatic compounds (HACs) are known to be reductively dechlorinated under anoxic conditions (Mohn and Tiedje, 1992). A few anaerobic microorganisms capable of dechlorinating chlorobenzoates, chlorophenols and chlorobenzenes have been isolated as pure cultures (Shelton and Tiedje, 1984; Cole *et al.*, 1994; Holliger *et al.*, 1998; Adrian *et al.*, 2000). Studies of these isolates have demonstrated that reductive dechlorination is not a co-metabolic but an energy generating respiratory process. It has also been proposed that dechlorinating microorganisms can use PCBs as electron acceptors in anaerobic respiration (Brown *et al.*, 1987; Kim and Rhee, 1997). Microbial dechlorination of PCBs in anaerobic sediment resulted in nearly a 200-fold increase in the number of PCB dechlorinators as measured by the most-probable-number technique, and this increase was concurrent with the dechlorination of the PCBs (Kim and Rhee, 1997). Priming with certain PCB congeners or non-PCB HACs could enhance certain types of PCB dechlorination possibly by stimulating the growth of PCB-dechlorinating microorganisms (Bedard *et al.*, 1997; Bedard *et al.*, 1998; Van Dort *et al.*, 1997; Deweerd and Bedard, 1999; Wu *et al.*, 1999).

There are reports of inducing PCB-dechlorinating activities through enrichment with non-PCB HACs in sediment microbial communities. Anaerobic mixed cultures enriched with 2,3,6-trichlorobenzoate, 1,2,4-trichlorobenzene and chlorophenol were able to carry out PCB dechlorination (Hartkamp- Commandeur *et al.*, 1996; Middeldorp *et al.*, 1997; Chang *et al.*, 1999). A recent study (Cho *et al.*, 2002) has shown that HACs

* To whom correspondence should be addressed.
(Tel) 82-52-259-2387; (Fax) 82-52-259-1694
(E-mail) jkim@mail.ulsan.ac.kr

could not only enhance PCB dechlorination, but also enrich PCB dechlorinators in PCB-free sediments. Such cross-enrichment and enhancement of PCB-dechlorinating activities with HACs is probably due to the fact that many dehalogenating enzymes are not specific to a single compound and HACs support the growth of dechlorinators possibly by acting as electron acceptors. Alternatively, it is still plausible that non-PCB HACs may induce the synthesis of PCB-dechlorinating enzymes.

The identification of effective and environmentally acceptable non-PCB HACs to induce certain PCB-dechlorinating activities would lead to the development of practical methods for *in situ* bioremediation of PCB-contaminated sediments. Since PCBs are xenobiotics, it is highly likely that there are natural substrates of PCB-dechlorinating enzymes.

In the present study, we investigated the effects of non-PCB HACs such as L-thyroxine, 3-chloro-L-tyrosine, 5-chloroindole, 2-chlorophenol, 4-chlorophenol and chlorobenzene on microbial dechlorination of a commercial PCB mixture Aroclor 1248.

Materials and Methods

Sediment slurry preparation

We collected uncontaminated sediments from Duhyun Reservoir near the University of Ulsan (Ulsan, Korea), and analyzed them before use to confirm the absence of PCBs. The sediments were air-dried, thoroughly mixed, and passed through a series of sieves with the final screen having a 150-μm opening. The dry PCB-free sediments were spiked with Aroclor 1248 in hexane to yield a concentration of 300 μg/g sediment (dry-weight basis) according to the procedures described previously by Rhee *et al.* (1993). Sediment slurries were established in 50-ml serum vials by adding 4 g of PCB-spiked sediment and 18 ml of reduced synthetic mineral medium (Balch *et al.*, 1979) in an anaerobic chamber (Coy Laboratory Products, USA) containing an atmosphere of N_2-H_2-CO_2 (85:10:5). The sediment vials were then capped with Teflon-coated butyl rubber stoppers and aluminum crimp seals, removed from the chamber, and autoclaved at 121°C for 40 min on three successive days. These vials were amended with each of the non-PCB HACs (L-thyroxine, 3-chloro -L-tyrosine, 5-chloroindole, 2-chlorophenol, 4-chlorophenol and chlorobenzene) in acetone stock solutions (0.5%, v/v) to yield a final concentration of 100 μg/g sediment (dry-weight basis). The chemicals were reagent grade and purchased from Aldrich and Sigma Chemical Co.

Inoculation and incubation

A 0.5-ml sediment slurry from a single batch of previous enrichment cultures was inoculated into each of the vials. The enrichment cultures had been started with microorganisms eluted from PCB-contaminated sediments collected from the St. Lawrence River near the General Motors site (NY, USA) (Kim and Rhee, 1999). After inoculation, the vials were incubated statically at room temperature in the dark. Every 24-week interval, 2-ml sediment slurry of each vial was sequentially transferred into fresh sediments containing the same type of amendments. All treatments were set up in duplicate. HAC-free Aroclor 1248 sediments with inoculation were used as positive controls, while autoclaved samples of the same sediments served as sterile controls.

PCB extraction and analysis

After each transfer, PCBs in the remaining sediment slurries were extracted twice, first with acetone-hexane (1:1, v/v) by shaking on an orbital shaker overnight and then by shaking for 4 h with hexane (Kim and Rhee, 1997). Distilled water was added to the solvent extract for phase separation and the hexane layer was placed into a flask with sodium sulfate. The hexane extracts were then reduced to a 10 ml volume using a Kuderna-Danish condensing apparatus, treated with a tetrabutylammonium hydrogen sulfite reagent to remove elemental sulfur, and cleaned up on a 4% deactivated Florisil column.

Congener-specific PCB analysis was performed on an Agilent 6890 gas chromatograph (GC; Agilent Co., USA) equipped with a ^{63}Ni electron capture detector, an autoinjector, and a 60-m HP-1 fused silica capillary column. The GC system used nitrogen as a carrier gas and argon-methane (95:5) as a make-up gas, and the injector and detector temperatures were both 300°C. The initial oven temperature of 100°C was maintained for 2 min and then increased at a rate of 10°C/min to 160°C with a 1-min hold, and finally to 250°C by 2°C/min. This final temperature was maintained for 21 min. The PCB congeners in the extract were identified and quantitated using a calibration standard containing equal amounts of Aroclors 1016, 1221, 1254 and 1260 (0.2 μg/ml in hexane). Peaks were identified and calibrated as described previously (Schulz *et al.*, 1989; Sokol *et al.*, 1994; Frame *et al.*, 1996). The analysis resolved 98 peaks representing 127 congeners.

The PCB congener numbering system in the text uses a slash to represent the separation of rings to permit an easier visualization of the chlorine substitution pattern (e.g., 2,3',4',5-chlorobiphenyl will be denoted as 25/34-CBP). The PCB congeners in each sample were calculated and expressed as mole percent.

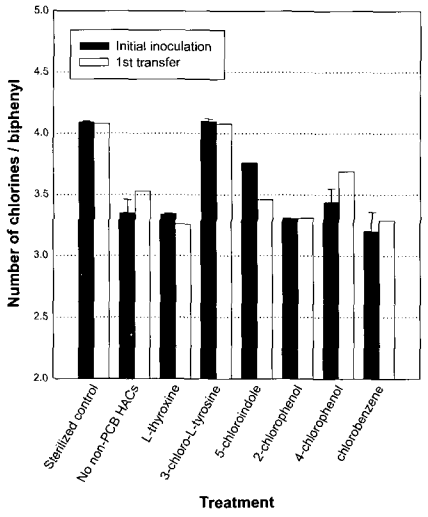

Fig. 1. Dechlorination of Aroclor 1248, expressed as the number of chlorine molecules per biphenyl, in sediments amended with non-PCB HACs after 24 weeks of incubation with PCB-dechlorinating microcosms. Each bar of initial inoculation represents the mean (± SD) of duplicate vials, while that of 1st transfer is from single vial.

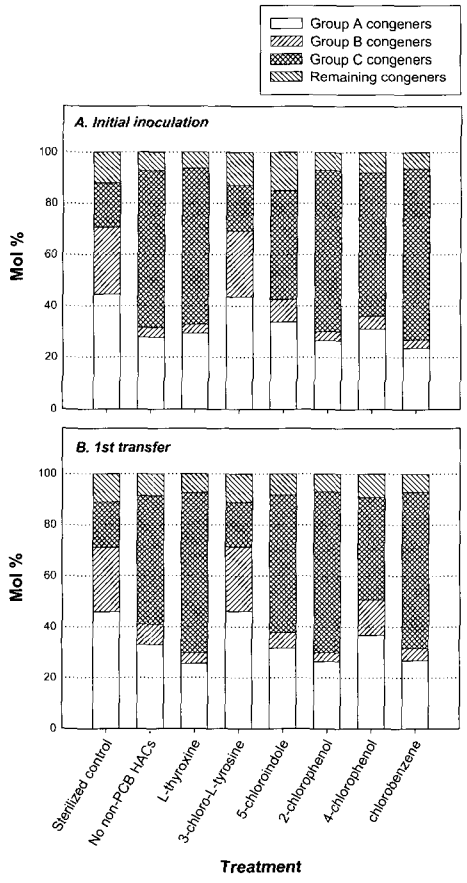

Fig. 2. Mole percent of Aroclor 1248 congener groups (Cho *et al.*, 2003) in sediments amended with non-PCB HACs after 24 weeks of incubation with PCB-dechlorinating microorganisms.

Results

To investigate the effect of non-PCB HACs on PCB dechlorination, sediments spiked with Aroclor 1248 were amended with each of these non-PCB HACs: L-thyroxine, 3-chloro-L-tyrosine, 5-chloroindole, 2-chlorophenol, 4-chlorophenol, and chlorobenzene. When the dechlorination of Aroclor 1248 was compared between HAC-free sediments and sediments containing various non-PCB HACs, amendments with the six HACs did not enhance the extent of Aroclor 1248 dechlorination by the PCB-dechlorinating microorganisms (Fig. 1). However, there was a quantitative difference in the overall dechlorination among the sediments amended with the HACs. Analysis of HAC-free sediments after 24 weeks showed that the average number of chlorine molecules per biphenyl was reduced by 18.1% from original Aroclor 1248 levels at the initial time of inoculation with microorganisms (Fig. 1). In sediments with L-thyroxine, 2-chlorophenol and chlorobenzene, the levels of dechlorination were rather similar to that seen in HAC-free sediments, showing 18.3 to 21.8% reduction of the number of chlorines per biphenyl after 24 weeks from initial inoculation (Fig. 1). On the other hand, dechlorination was less extensive in cultures amended with 5-chloroindole and 4-chlorophenol, with the former removing only 8.1% of the chlorines and the latter taking out 13.4% of the chlorines. No dechlorination was evident in 3- chloro-L-tyrosine sediments after 24 weeks, indicating complete inhibition by the HAC. Sequential transfers into fresh sediments with the same amendments did not enhance the level of Aroclor 1248 dechlorination either (Fig. 1). In all of the sediments, the level of Aroclor 1248 dechlorination at 24 weeks was an apparent end point because no further changes of congener patterns were observed up to 36 weeks of incubation (data not shown).

Recently, the congeners of Aroclor 1248 could be divided into three groups, A, B and C, based on kinetic characteristics of dechlorination (Cho *et al.*, 2003). Group A represents congeners with low threshold concentrations of dechlorination and group B indicates those with high threshold concentrations (Table 1). Group C congeners are the final products of dechlorination. In sterilized controls, congeners of groups A accounted for 45.2 mol% of Aroclor 1248, B made up 25.8%, and C was responsible for 17.5% (Fig. 2). Dechlorination of Aroclor 1248 reduced the molar concentration of groups A and B, and increased the concentration of group C. Analysis after 24 weeks showed that the concentrations of group A decreased to 27.7 mol% (a 37.8% reduction) and group B decreased to 4.0 mol% (a 85.0% reduction) of total congeners (Fig. 2). The concentration of group C in-

Table 1. Aroclor 1248 congeners in groups A, B, and C[a]

Group A	Group B	Group C
23/4	25/2	2
236/4 + 234/2	23/3 + 25/26 + 34/2	2/2 + 26
245/4	23/26	24 + 25
25/34 + 245/26	25/25	2/3
24/34 + 236/25	24/25 + 235/2	2/4 + 23
236/24	23/25	26/2
23/34 + 234/4 + 235/25	236/3 + 23/24 + 34/4	24/2 + 4/4
236/23	23/23	236 + 26/3
245/25 + 234/26 + 235/24		25/3
245/24		24/3
34/34 + 236/34		25/4
245/34		24/4
234/34 + 234/236		24/26
		24/24 + 246/4

[a] Modified from Cho *et al.*, 2003.

creased to 61.1 mol% (a 253.6% increase) of total congeners in initially inoculated HAC-free sediments (Fig. 2). Similarly, in sediments with L-thyroxine, 2-chlorophenol, and chlorobenzene, the concentrations of group A were in the ranges of 23.6 ~ 29.3 mol% and group B made up 3.3 ~ 3.7 mol% of total congeners, with concomitant increases in group C between 60.8 ~ 66.8 mol% after 24 weeks from initial inoculations (Fig. 2). On the other hand, the concentrations of group A congeners in sediments amended with 5-chloroindole were 39.5 mol% and 4-chlorophenol showed group A concentrations of 29.1 mol%. Concentrations of group B were 12.5 mol% with 5-chloroindole and 4.4 mol% of total congeners were with 4-chlorophenol after 24 weeks from initial inoculations (Fig. 2). The relative concentration of each congener group in sediments amended with 3-chloro- L-tyrosine was little different from that in sterilized controls. Analysis after one sequential transfer into fresh sediments did not show any significant

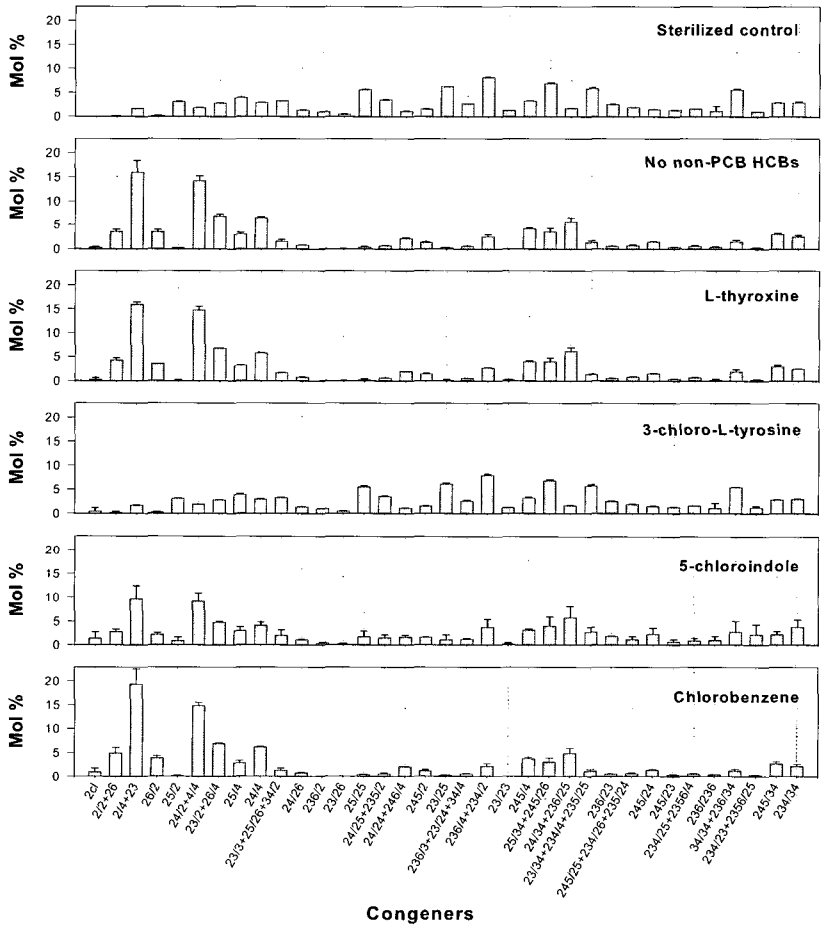

Fig. 3. Mole percent (mean ± SD) of individual Aroclor 1248 congeners in sediments of initial inoculations after 24 weeks of incubation with PCB-dechlorinating microorganisms. Congeners of which the moles percent were higher than 1% at any treatments were selected for the graphs.

changes in the relative proportions of congener groups (Fig. 2).

In sediments where dechlorination was evident, the congeners involved in dechlorination were similar regardless of the type of amendments and transfers. A closer examination of Aroclor 1248 congeners revealed a major reduction in the peaks 34/34- + 236/34-, 23/34- + 234/4- + 235/25-, 25/34- + 245/26-, 23/23-, 236/4- + 234/2-, 236/3- + 23/24- + 34/4-, 23/25-, 24/25- + 235/2-, 25/25-, 236/2-, and 25/2-chlorobiphenyls (CBPs) with a corresponding increase in the chromatographic peaks for 2/2- + 26-, 2/4- + 23-, 26/2-, 24/2- + 4/4-, 23/2- + 26/4-, and 24/4-CBPs in HAC-free sediments of initial inoculations (Fig. 3). Among those congeners dechlorinated, group A congeners such as 34/34- + 236/34-, 23/34- + 234/4- + 235/25-, 25/34- + 245/26-, and 236/4- + 234/2-CBPs have *para*-substituted chlorines, while group B congeners such as 23/23-, 236/3- + 23/24- + 34/4-, 23/25-, 24/25- + 235/2-, 25/25-, and 25/2-CBPs are largely *meta*-rich ones (Table 1). It is interesting to note that although the initial concentration of group B was about 57% of group A, the overall removal was much higher. This was because group B exhibited a 85.0% reduction compared to 37.8% reduction in group A in HAC-free sediments (Fig. 2). The accumulating congeners were mostly group C, which represented the final products of dechlorination (Table 1, Fig. 3). The congener pattern in sediments amended with 3-chloro-L-tyrosine was nearly identical to that of the sterilized controls (Fig. 3).

Discussion

Unfortunately, we did not determine whether the non-PCB HACs were dehalogenated by sediment microorganisms. Anyhow, in the present study, none of the six HACs could enhance the extent of Aroclor 1248 dechlorination, and the substrate range or the dechlorination specificity of the microorganisms could not be broadened by the amendments. Contrary to these observations, the results of other researchers (Bedard *et al.*, 1998; Deweerd and Bedard, 1999; Wu *et al.*, 1999; Cho *et al.*, 2002) have shown the enhancement of PCB-dechlorinating activities with non-PCB HAC-amendments. However, this discrepancy is not surprising because the effects of HACs can vary with the nature of microbial community as well as the kinds of non-PCB HACs (Sokol *et al.*, 1994; Deweerd and Bedard, 1999; Kim and Rhee, 1999; Cho *et al.*, 2002). Also it is still possible that these HACs may reduce the lag period or enhance the rate of dechlorination, since we did not analyze with the time course of incubations but at the plateau.

At the time of inoculation, the enrichment culture we used as the inoculum source had been maintained over five years through sequential transfers into fresh sediments spiked with Aroclor 1248. These transfers had been performed every 16 to 24 weeks and might have selected certain populations, limiting the diversity of the microorganisms in the experiment. On the other hand, earlier cross-enrichment studies (Hartkamp -Commandeur *et al.*, 1996; Middeldorp *et al.*, 1997; Cho *et al.*, 2002) usually began with amending HACs to microbial communities directly obtained from natural sediments, better conserving the pool of dechlorinators.

As far as we know, no reports have been published regarding the effects of L-thyroxine, 3-chloro-L-tyrosine, or 5-chloroindole on microbial PCB dechlorination. L-thyroxine is a major hormone derived from thyroid gland and 3-chloro-L-tyrosine is a derivative of the aromatic amino acid, L-tyrosine (Murray *et al.*, 2000). 5-Chloroindole contains an indole group, which is a motif for such compounds as L-tryptophan, serotonin, auxin and indigo (Murray *et al.*, 2000). Among these three naturally occurring HACs, no dechlorination was found in the presence of 3-chloro- L-tyrosine. At the concentration used, the compound may have been toxic to PCB-dechlorinating microorganisms or inhibitory to dechlorinating enzymes.

PCB dechlorination was enhanced by chlorophenols and chlorobenzenes with two or three substituted chlorines (Cho *et al.*, 2002), whereas these HACs with one substituted chlorine did not show such enhancement in the present study. It is unclear whether this discrepancy is due to the number of chlorines on the HACs. In a study with various brominated aromatic compounds, there was no clear correlation between partition coefficients of the HACs and their aptitude for priming PCB dechlorination (Deweerd and Bedard, 1999). The relationship between HAC structure and activity on dechlorination need to be defined to utilize HACs as stimulants for PCB dechlorination (Bach *et al.*, 2005).

The results of this study strongly indicate that the effect of non-PCB HACs on PCB-dechlorinating activities is mainly through the selection of dechlorinating populations rather than the induction of dechlorinating enzymes from single population. Different dechlorination specificities have been attributed to different dechlorinating microbial populations in sediments (see references in Bedard, 2003; Cho and Oh, 2005). Thus, for the successful application of HACs, it is important to understand the diversity of dechlorinating populations in nature and the composition of microbial communities in PCB-dechlorinating enrichment cultures. The highly enriched culture of the present study may be a good source for obtaining a pure culture of PCB-dechlorinating microorganisms.

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