Potential Risk Reduction of Aroclor 1254 By Microbial Dechlorination in Anaerobic Grasse River Sediment Microcosms

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Abstract

Aroclor 1254 was the second most produced commercial PCB mixture and is found in soils, sediments and sewage throughout the globe. This commercial PCB mixture is considered particularly toxic because of the relatively high concentrations of congeners with dioxin-like properties. The potential for risk reduction by microbial reductive dechlorination of Aroclor 1254 (A1254) was investigated in sediment microcosms from Grasse River (GR), Massena, NY. The specificity of A1254 dechlorination was doubly- and singly-flanked chlorines in *meta* positions and to a less extent doubly-flanked *para* chlorines of 2345-substituted chlorobiphenyl rings. The average dechlorination rate of A1254 was 0.0153 Cl⁻/biphenyl/day, and dechlorination rates of single congeners ranged between 0.001 and 0.0074 Cl⁻/biphenyl/day. Potential risk associated with A1254 based on the toxic equivalency factors of the dioxin-like congeners was reduced by 83%. Additional potential risk associated with bioaccumulation in fish was reduced by 35% based on biota-sediment accumulation factor estimates for all detected congeners. Finally, the dechlorination end-products were tri- and tetra-chlorobiphenyls with unflanked chlorines, all of which are susceptible to further degradation by aerobic microorganisms. The combined results indicate that microbial reductive dechlorination has the potential for reducing risk associated with toxicity and bioaccumulation in fish in sites contaminated with A1254.

Keywords: Aroclor 1254, anaerobic dechlorination, Grasse River, polychlorinated biphenyls, dioxin TEF, BSAF.

1. Introduction

Polychlorinated biphenyls (PCBs) belong to the halogenated aromatic group of environmental contaminants found in diverse environmental matrices. Thousands of metric tons of commercial PCBs, manufactured under the trade name Aroclor in the U.S., persist in aquatic sediments and continue to be a threat to human and ecosystem health.

PCBs were widely used in transformers and capacitors due to their high flash points and electrical resistance [1]. Transformer and capacitor oils commonly contained A1254 or 1260 [2]. According to Monsanto U.S., which accounts for over half of worldwide PCB production, sales records between 1957 and 1974 show that A1254 was the second most produced/sold Aroclor mixture after Aroclor 1242 [3]. In the U.S., Lake Hartwell was contaminated with an estimated 200 metric tons of mainly Aroclor 1016 and 1254, and the entire Twelve Mile Creek watershed and the Seneca River arm of Lake Hartwell were placed on the National Priorities List [4]. In Turkey, A1254 was identified as one of the most common PCB mixtures detected in sludge and sediment samples [5].

A1254 consists of mostly penta- and hexa-chlorinated PCB congeners in proportions of approximately 49 and 29%, respectively [6]. Aroclors, especially Aroclors 1254 and 1260, present a particularly difficult biodegradation challenge because these complex mixtures consist of dozens of highly chlorinated congeners. Despite their recalcitrance to biodegradation, microbial-mediated anaerobic reductive dechlorination of PCBs has been reported in a variety of anaerobic sediments [7-16]. The process consists of the sequential reduction of highly chlorinated congeners into lesser chlorinated congeners that generally have lower toxicity, lower bioaccumulation potential and greater susceptibility to subsequent

mineralization by aerobic bacteria [17, 18]. Dechlorination under anaerobic conditions occurs due to halorespiration, by which halogenated compounds are used as terminal electron acceptors in the presence of small molecular weight electron donors such as hydrogen or organic fermentable substrate as electron donors [13, 19]. The degree of chlorination as well as the position of chlorines on the biphenyl rings influence the degradation potential of individual congeners [20].

Microbial PCB dechlorination reduces the risk of human exposure to PCBs by decreasing the potential for PCB bioaccumulation and persistence in the environment. Known correlations with octanol/water partitioning coefficients show that each chlorine loss decreases the log K_{ow} by 0.5 with a corresponding reduction in bioaccumulation potential. The depletion of highly chlorinated congeners may reduce the exposure level of PCBs, thereby, reducing the potential risk associated with toxicity of PCBs [7, 8, 21, 22]. In addition, aerobes such as LB400 can subsequently cleave the aromatic ring of congeners having less than five chlorines with biphenyl dioxygenase after anaerobic reductive dechlorination in the 2,3- or 3,4-positions [23]. As a result, this degradation mechanism has significant implications for risk assessment and remediation strategies.

Although there have been prior reports on the microbial reductive dechlorination of Aroclor 1254 in sediment microcosms [13, 24-29], this is the first report to examine the potential reduction in risk as a result of microbial dechlorination of dioxin- like congeners and the potential for bioaccumulation in fish. A1254 dechlorination pathways and rates were determined in sediment microcosms from Grasse River shown previously to have robust halorespiring activity. The results of this study indicate that microbial reductive dechlorination has the potential for reducing toxicity and bioaccumulation in sites

contaminated with A1254 as well as generating less-toxic dechlorinating products that are susceptible to complete degradation by aerobic microorganisms. Assessing microbial dechlorination from the perspective of toxicity and bioaccumulation of A1254 has important implications for risk assessment and remediation strategies.

2. Materials and Methods

2.1. Chemicals

All PCBs (99-100% purity) were purchased from AccuStandard. PCE was purchased from Sigma-Aldrich. All other chemicals were reagent grade.

2.2. Sediment sampling

GR sediments used in this study were collected during Spring 2008 with a Ponar grab sampler from GR site located in the lower GR in the Village of Massena, NY, US, as described previously [9]. Sediments were stored anaerobically in glass jars sealed with Teflon lined tops at 4°C in the dark. Sediments were black in color and had a sulfide odor indicative of reduced anoxic conditions. The sampling site was contaminated primarily with Aroclor 1248 that was used for aluminum production at this site since the 1930s [30].

2.3. Sediment microcosms

Low-sulfate (<0.3mM) estuarine medium [31], prepared with the exclusion of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, was anaerobically dispensed as 24-mL aliquots into 60-mL serum bottles with individual PCB congeners or as 50-mL aliquots into 160-mL serum bottles with A1254. The vials were sealed under $\text{N}_2:\text{CO}_2$ (80:20) with 20-mm Teflon-coated butyl rubber stoppers (West Co., Lionville, PA) secured with aluminum crimp seals. The medium was autoclaved at 121°C for 20 minutes. The final pH was 6.8. All subsequent additions were performed in an anaerobic

glove box (Coy Laboratory Products, Ann Arbor, Michigan, USA) containing N₂:CO₂:H₂ (75:20:5). A1254 and individual PCB congeners were solubilized in 10 µl acetone and added to triplicate microcosms to a final concentration of 100 mg/L and 50 mg/L, respectively, as indicated. Only the initial A1254 microcosm was inoculated with GR sediment (10 mL wet wt.) and tetrachloroethylene (200 µM) to stimulate growth of halorespiring bacteria. Thereafter, dechlorination pathways were determined by transferring 1-mL of homogenized slurry from an A1254 microcosm into medium containing 0.5% (w/v) dry, sterile sediment prepared according to Fagervold *et al.* [10]. Prior to inoculation, a fatty acid mixture containing sodium salts of acetate, propionate and butyrate was supplied at a final concentration of 2.5 mM each as electron donors and carbon sources. Individual A1254 congeners were selected from among the most abundant congeners in A1254 [32] that were dechlorinated in the initial microcosms including: tetra-chlorobiphenyls: 44, 52, 70, and 74, penta-chlorobiphenyls: 85, 87, 91, 95, 97, 99, 101, 105, and 110, hexa-chlorobiphenyls 128, 132, 138, 141, 149, 153, 156, and 163, and hepta-chlorobiphenyl: 180. Control microcosms contained acetone without PCB. Sterile controls were prepared for only three congeners (PCBs 44, 87, 101) in the same way except that the bottles were autoclaved twice for 1 h at 121°C on two consecutive days prior to PCB addition. All cultures were prepared in triplicate and incubated statically at 30°C in the dark.

2.4. Analytical techniques

2.4.1. PCB extractions and analytical procedures

Microcosms were sampled every 30 days. Triplicate samples were analyzed for PCBs by extracting 1-mL samples with (5 mL) hexane on a wrist action shaker (Burrell Corp., PA) for 16 h. The organic phase was passed through copper/Florisil (Sigma Aldrich, St. Louis, MO) (1:4) in a glass column and PCB congeners were analyzed with an Agilent 6890 GC/ECD as

described previously [10]. PCB congeners in a mixture containing 250 µg/L Aroclor 1232, 180 µg/L Aroclor 1248 and 180 µg/L Aroclor 1262 [33] were used together with 55 additional congeners absent in the Aroclor mixture that were potential dechlorination products [22] to create a 10-point calibration curve.

PCBs 30 and 204 were added as internal standards. Recovery of PCB-166 added as a surrogate was 85±3%. Co-eluting congeners were designated by slashes separating their congener numbers. The PCB concentrations were measured as µg PCB/mL of microcosm slurry and converted to mol%. Total chlorines per biphenyl was calculated as the product of the average number of chlorines and molar concentration of each congener divided by the sum of the total molar concentration of all congeners [34]. The dechlorination rate was calculated within the linear slope of the dechlorination curve by dividing total chlorines removed per biphenyl with the time elapsed in days [10]. Since most dioxin-like congeners co-elute with other congeners, PCBs extracted from A1254 microcosms were re-analyzed for toxicity reduction calculations with a Hewlett Packard 5890 GC/ECD equipped with a 60-m HT8-PCB 8% phenyl capillary column (SGE, 0.25 mm i.d., 0.25 µm film thickness), which separates all twelve congeners. GC parameters were as described above except that PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 181 were used to create a 10-point calibration curve. Reduction in the total toxic equivalent (TEQ) was calculated based on the toxic equivalent factor for each dioxin-like congener relative to 2,3,7,8-tetrachlorinated dibenzo-p-dioxin as defined by the World Health Organization [35, 36]. Risk reduction based on potential for bioaccumulation in fish was based on fish/trap lipid-organic carbon accumulation factors (BSAFs) versus the k_{ow} for individual congeners using the quadratic regression equation: $\log BAFs = -0.38 \log k_{ow}^2 + 5.16 \log k_{ow} - 15.85$ [37].

2.4.2. DNA extraction

DNA was extracted (in triplicate) from microcosm samples and was used for enumeration and community analysis of PCB dechlorinating bacteria. DNA was extracted by adding 0.25 mL of slurry samples from each microcosm to a 96-well bead-beating plate for homogenization (MOBIO Laboratories, Inc.). Total DNA was isolated from the 96-well plates according to the manufacturer's directions. DNA was eluted in 100 μ L of Tris buffer and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). Extracted DNA samples had an A₂₆₀/A₂₈₀ ratio of ≥ 1.6 and an A₂₆₀/A₂₃₀ ratio of ≥ 2.0 .

2.4.3. Enumeration of PCB halorespiring bacteria by quantitative PCR

Putative dechlorinating Chloroflexi were enumerated by PCB amplification with 16S rRNA gene primers Chl-348F and Dehal-884R [10] as described by Payne *et al.* [22]. Amplification efficiencies of standards were $92.5 \pm 8.4\%$ with $R^2 = 0.999$.

2.4.4. Community analysis of PCB dechlorinating bacteria

PCR amplified 16S rRNA gene analysis was conducted by denaturing high pressure liquid chromatography (DHPLC) using a WAVE 3500 HT system (Transgenomic, Omaha, NE) equipped with a fluorescence detector (excitation 490 nm, emission 520 nm). Primers Chl-348F and Dehal-884R were used for DHPLC following PCR or qPCR products were used where indicated. PCR reactions were performed as described previously [9]. PCR products of the correct length (ca. 500 base pairs) were confirmed by gel electrophoresis using a 1.2% (w/v) high-melt agarose gel prior to analysis by DHPLC.

2.4.5. Sequencing and analysis

Each DHPLC fraction was sequenced in the 5' and 3' direction with 100 pmols of primer 348F or 884R using the BigDye Terminator v3.1 kit (Applied Biosystems, Foster City, CA) per the manufacturer's instructions. Sequencing of purified DNA was performed on an ABI 3130 XL automated capillary DNA sequencer (Applied Biosystems, CA) by the BioAnalytical Services Lab (IMET, Baltimore, USA). Sequences were examined for errors and assembled using the software Finch TV software package [38]. Alignments were then refined manually. Chimera formation was examined using Chimera Check [39]. The sequence alignments were done by using the Ribosomal Database Project (RDP) [39, 40]. A phylogenetic tree was generated based on published chloroflexi sequences over 1200 base pairs by the neighbor joining approach using default settings in RDP [40]. The Genbank accession numbers for the 16S rRNA sequences reported in this paper are KX859302-KX859310.

3. Results and Discussion

3.1. Dechlorination of A1254

Dechlorination of A1254 lagged approximately 30-days, then the average number of Cl⁻ molecules per biphenyl was reduced from 5.03 to 3.88 after 120 days of incubation (Figure 1a). Approximately 24% total chlorine of A1254 was removed at a rate of 0.0153 Cl⁻/biphenyl/day. The average dechlorination rate in this study is similar to the rate of 0.0122 Cl⁻/biphenyl/day observed in upper Hudson River (HR), NY, sediment microcosms, which resulted in a total of 63% *meta* plus *para* chlorine removal [24]. The rate is about 29 and 49 times greater than those observed in HR (Fort Miller, NY), for non-enriched and biphenyl-enriched treatments, respectively, which resulted in about 30% total chlorine removal of the same Aroclor mixture at a higher initial concentration of 500 ppm [41]. On the other hand,

the rate in this study was lower than the rate of 0.054 and 0.024 Cl⁻/biphenyl/day observed in A1254 (500 ppm) microcosms inoculated with sediments from two locations in the Twelve Mile Creek arm of Lake Hartwell located on the state line between South Carolina and Georgia [4], which resulted in about 40% total chlorine removal. The differences in the rates and extent of dechlorination of A1254 observed in different studies indicates the results are site specific, which suggests possible differences in the dechlorinating population from site to site.

Dechlorination of A1254 was achieved by the removal of chlorines mostly from 88% penta-, followed by 69% hexa- and 51% hepta-chlorinated congeners (Figure 1b) resulting in 66% tetra-, and 95% tri-chlorinated congeners as the predominant dechlorination products. In a prior study [42], A1254 was dechlorinated by a coplanar PCB-dechlorinating microbial community enriched from PCB impacted marine sediment from a Venice lagoon. Similarly, those cultures dechlorinated about 74% of the penta- through hepta-chlorinated biphenyls to tri- and tetra-chlorinated congeners, however, dechlorination required 30 weeks compared with 16 weeks to dechlorinate 88% of penta-chlorinated congeners in the current study. In another study [25], dechlorination of A1254 in the presence and absence of sulfate started after 4 months of incubation and resulted in only 14-15% *meta* and 9-10% of *para* chlorine removal over a period of 1 year with the accumulation of mainly tetra-chlorobiphenyls; the extent of dechlorination was 7-8%, which is significantly lower than the current study.

The ten most predominant A1254 congeners that were dechlorinated and their respective products are shown in Figure 2. As can be seen from this figure, the most predominant dechlorination products after 120 days of incubation were PCBs 53, 49, 47, 28, and 15/17 with mol% ranging between 10.34 and 4.61. GR culture resulted in about 39 mol% increase

in the mole percentages of congeners in Aroclor 1254 with three or less *ortho* chlorines combined with open 2,3 and 3,4 positions. These congeners are less hydrophobic and generally more susceptible to complete mineralization by aerobic bacteria with genes encoding the biphenyl degradation pathway, which attack the biphenyl ring in unsubstituted 2,3, or the 3,4-positions [43]. Most of the tri- and tetra-chlorinated dechlorination products detected in this study are substrates for ring cleavage by a variety of aerobic microorganisms [44]. The products of aerobic degradation, primarily chlorobenzoates and chlorinated aliphatic compounds, are ultimately mineralized via a variety of pathways [45]. The combined results indicate that microbial reductive dechlorination of A1254 is effective for promoting the complete degradation of A1254 by generating congener products are susceptible to complete degradation by aerobic microorganisms.

3.2. Pathways of A1254 dechlorination

The dechlorination pathways were determined by monitoring the reductive dechlorination of 22 individual congeners predominant in A1254. The positions of the target chlorines, the rate of each reaction, and the final mol% for each of the starting congeners, intermediate, and daughter products at day 180 are presented in Table 1. The dechlorination rates of the parent compounds were higher than the dechlorination rates of the daughter compounds with the exception of PCB 141 (2345-25), which was dechlorinated less rapidly than the dechlorination product PCB 101 (245-25).

Based on the observations of the individual congeners, the general sequence of dechlorination in GR microcosms was: doubly flanked *meta* > doubly flanked *para* of 2345-substituted chlorobiphenyl rings > doubly flanked *meta* of 234-substituted chlorobiphenyl rings > *ortho*-flanked *meta* of 236-substituted chlorobiphenyl rings > *ortho*-flanked *meta* of 23-substituted

chlorobiphenyl rings > *para*-flanked *meta* of 245-substituted chlorobiphenyl rings > *para*-flanked *meta* of 34-substituted chlorobiphenyl rings > *ortho*-flanked *meta* of 235-substituted chlorobiphenyl rings (Figure 3). This was confirmed with PCB 180 (2345-245), where all the dechlorination occurred in the double-flanked *meta* position, and with PCB 156 (2345-34) and PCB 141 (2345-25), where $80\pm0.1\%$ and $91\pm0.6\%$, respectively, of total dechlorination resulted from the doubly flanked *meta* chlorine removal. The predominant dechlorination products observed within 180 days of incubation in single PCB congener experiments were PCB 53 (26-25), PCB 51 (24-26), PCB 47 (24-24), PCB 49 (24-25), PCB 32 (26-4), PCB 18 (25-2), PCB 17 (24-2), PCB 27 (26-3), which is consistent with the results observed for dechlorination of A1254. In all microcosms with individual PCB congeners the end products of dechlorination were congeners with unflanked chlorine substitutions. Dechlorination was not detected in sterile controls.

The pattern of A1254 dechlorination in GR sediment microcosms resembles Process N and Process H' [24]. In process N, exclusive dechlorination in the *meta* position for both flanked and doubly flanked chlorine substitutions, with a characteristically high accumulation of 2,2',4,4'-CB (PCB 47) has been demonstrated [7]. This pattern has been previously observed in sediment microcosms from several PCB impacted freshwater sources, including Woods Pond [46], Hudson River [41], sediment-free microcosms developed from the Housatonic River [47], and Baltimore Harbor sediment microcosms enriched with Aroclor 1260 [11]. Also, several observations were consistent with the dechlorination pathways for PCB 101, 132, 138, 153, and 180 in Process N [7]. Process H' is the removal of both *meta* and *para* chlorines, but only if these chlorines are flanked. PCB 163 and PCB 141 were dechlorinated to PCB 32 and 49, respectively, by Process H', which is the removal of both *meta* and *para* chlorines, but only if these chlorines are flanked (Figure 3). Alder *et al.* [48] reported

dechlorination pattern H' for preexisting Aroclor 1242 PCBs in New Bedford Harbor sediments. Other studies have also reported pattern H/H' as a frequently observed dechlorination pattern [28, 42].

Overall, the ability to attack flanked *meta* and *para* substituted chlorines positions would result in extensive dechlorination potential for highly chlorinated A1254. Indeed, all 22 major parent congeners, which account for about 85% by mass of A1254, were dechlorinated each to a different extent. The dechlorination rates of PCB 70 and 74 (Table 1) were among the lowest, whereas, PCBs 91, 99, and 105 showed slightly greater dechlorination rates. The reason for elevated dechlorination rates of the latter congeners in A1254 might be due to “priming” by the presence of other congeners in the mixture, which possibly have an overall stimulatory effect [7, 49, 50].

Among the individual PCB congeners, only PCB 52, which does not have flanked chlorines, did not dechlorinate. This is consistent with prior studies in which unflanked chlorines are have been shown to be the terminal products of Aroclor dechlorination [11, 51]. Although removal of an unflanked chlorine was demonstrated by process LP [52], it is rarely observed.

All singly or doubly-flanked congeners either added individually or as A1254 incubated with GR sediment exhibited reductive dechlorination preferentially in *meta* position and to a less extent in *para* position, as observed during the dechlorination of PCBs 180, 156, 141, and 110. This is in agreement with the findings of Kjellerup *et al.* [9] in which the analysis of GR sediments showed a high level of *ortho*-chlorinated congeners and unflanked chlorines at the *ortho* position, indicating preferential removal of chlorines from *meta* and *para* positions. The same pattern of *meta* and *para* dechlorination was also previously reported for

dechlorination of A1254 in Hudson River sediment [41, 53], in Venice Lagoon [28], in methanogenic and sulfate-amended laboratory cultures enriched from marine sediments Puget Sound, WA [25], but with much lower extent of chlorine removal compared to this study.

The abundance of highly chlorinated congeners decreases while those of lower chlorinated, *ortho*-substituted, congeners increases by the preferential removal of *meta*- and *para*-substituted chlorines. Since the *meta* and *para* chlorines are removed with anaerobic dechlorination, the concentration of coplanar congeners with dioxin-like toxicity is reduced [21, 54, 55]. Moreover, all of the tri- and tetra-chlorinated dechlorination products detected in this study are substrates for ring cleavage by a variety of aerobic microorganisms [7, 18, 44, 45], indicating that reductive dechlorination of A1254 has the potential to promote further degradation by aerobic microorganisms.

3.3. Enumeration of dechlorinating phylotypes

Microorganisms were enumerated in microcosms by quantitative PCR using specific primer sets [12] for phylotypes closely related to known PCB dechlorinators in order to determine whether dechlorination of A1254 and its selected congeners is growth-linked. The number of 16S rRNA gene copies of putative dechlorinators per mL microcosm slurry dechlorinating A1254 increased nearly 100-fold, from 4.14×10^5 to 1.03×10^7 indicating that dechlorination was linked to growth of halorespiring bacteria (Table 1). This result is about 4-times higher and occurred within a 3-fold shorter timeframe than in a previous study with Baltimore Harbor sediment microcosms incubated with Aroclor 1260, which suggests that the GR halorespiring community has more robust dechlorinating activity [11]. The no-PCB control culture incubated with PCB-free acetone showed only a slight increase over 180 days, which

could be possibly due to PCB carried over in the transfer from the A1254 microcosm and the presence of indigenous PCBs in GR sediments.

Growth-linked halorespiration was confirmed during incubation with individual PCB congeners typically found in A1254 with populations of putative PCB dechlorinating microorganisms increasing about 1-2 orders of magnitude. No change was observed in sterile controls. However, the extent of growth varied with different congeners (Table 1). The highest increase was observed during the dechlorination PCB 180 (2345-245) with a 38-fold increase while the lowest increase occurred during the dechlorination of PCB 156 (2345-34) at about 4.6-fold. The number of 16s rRNA gene copies (Figure S1) increased for PCBs 180, 163, 110, and 44 and both growth and dechlorination reached a plateau, after 90 days of incubation (Figure S2). Similar observations were observed for other PCBs congeners indicating a direct relationship between microbial growth and dechlorination rate as reported previously [10, 11, 56]. The results suggest that A1254 impacted sediments will support populations of halorespiring bacteria that are either indigenous to the site or added as a bioamendment.

3.4. Community analysis of PCB dechlorinating bacteria

DNA was extracted from the sediment microcosms and analyzed by denaturing high pressure liquid chromatography (DHPLC) to characterize changes in community profiles of putative dechlorinating phylotypes during dechlorination of A1254. Initial screening results from DHPLC analysis for day zero and day 120 (final) of A1254 microcosms are shown in Figure S3. Initial diversity of phylotypes was relatively high and decreased after incubation for 120 days, which indicates that dechlorinating phylotypes were enriched during the incubation of A1254 with GR sediments. Although not all of the putative dechlorinating bacteria identified

are necessarily involved in PCB dechlorination, the identified phylotypes in the indigenous dechlorinating phylotypes of GR sediments spiked with A1254 showed high 16S rRNA sequence similarities ranging between 98-100% (Figure 4) to *Dehalococcoides mccartyi* strains [57, 58].

The chromatograms for microcosms enriched with individual PCB congeners, were nearly identical after 180 days indicating the likely presence of the same or similar phylotypes (Figure S4). Comparative sequence analysis of fractions 36 to 49 (Figure S5) showed high 16S rRNA sequence similarities to *Dehalococcoides* spp. ranging mostly between 95-100% except for fragment 36, which had only 85% similarity to previously reported phylotypes within the Chloroflexi. Fractions 41, 44, 46 and 48, did not produce a discrete band on an agarose gel and were not sequenced.

A phylogenetic tree (Figure 4) shows the relationships between the phylotypes of this study and the closest dechlorinating species within the dechlorinating *Chloroflexi* group. Sequence of Phylotype G1 was excluded from the phylogenetic tree because of its low query coverage [40]. Phylotypes G2-G7, showed 16S rRNA gene sequence similarities greater than 97% to all three subgroups (i.e., Cornell, Victoria and Pinellas) of *Dehalococcoides mccartyi*. The remaining three phylotypes (G8, G9, and G10) were not closely related to either *Dehalococcoides* spp. or the o-17/DF-1 clade (Figure 4), but they were within the phylum of Chloroflexi.

DHPLC chromatograms obtained for GR sediment culture in the current study (day 0, Figure S3) were different than those previously reported [9]. Table S1 presents the similarity percentages between the sequences of this study and those obtained in the study of Kjellerup

et al. [9] for GR. The highest similarities, 92-98%, were found between G2-G6. However, the prior study surveyed indigenous phylotypes within the Chloroflexi without enrichment. The results suggest that high levels of A1254 enriched for a specific group halo-respiring phylotypes. Community analysis and quantitative PCR confirmed that halo-respiring phylotypes within the phylum *Chloroflexi*, including strains of *Dehalococcoides mccartyi*, catalyzed growth-linked reductive dechlorination of A1254 in Grasse River sediment microcosms.

3.5. Effect of reductive dechlorination on potential risk reduction

The reduction in health risk as a result of microbial dechlorination was examined based on the changes in the potential toxicity and bioaccumulation of individual congeners in A1254. The most toxic PCB congeners are coplanar PCB-77, 81, 126, and 169 and mono-*ortho* coplanar PCB-105, 114, 118, 123, 156, 157, 167 and 189 [35, 36]. The toxicity of PCB mixtures is a major concern and assessments of the toxic potentials of PCBs calculated through their TEFs in male Wistar rats showed that A1254 was the most toxic mixture compared to the other Aroclor mixtures (1248, 1242, 1260, and 1232) in terms of ethoxyresorufin O-deethylase induction [59]. The GR microcosm reduced the potential toxicity of A1254 by about 83% based on combined TEQs of dioxin-like congeners PCB-77, 105, 114, 118, 123, 156, 157, and 167 that were detected in the A1254 sediment microcosms.

The terminal products of A1254 dechlorination were *ortho*-chlorinated tetra- and tri-chlorinated biphenyls with unflanked chlorines. Although non-coplanar *ortho* substituted PCB congeners generally have low affinity for Ah receptors, many of these congeners have the potential to effect biological systems by disrupting Ca^{++} translocation. PCBs 28, 47 and 53 show slight to very active disruption of protein kinase C translocation in rat cerebellar

granule cells, however, none of the terminal dechlorination products detected are reported to have specific effects on humans [60].

Another potential risk to human health is the potential for bioaccumulation in finfish. As A1254 is dechlorinated from congeners with greater to fewer chlorine substitutions the relative k_{ow} of the congeners decreases, which has a direct effect on the potential for bioaccumulation in aquatic animals. The potential for A1254 bioaccumulation in fish before and after dechlorination was estimated based on BSAFs versus k_{ow} for individual congeners in detritivorous fish [37]. The results show the potential risk associated with bioaccumulation in fish was reduced by 35% (Table S2) as a result of microbial dechlorination based on BSAF estimates for all detected congeners. These overall results of this study suggest that natural attenuation by indigenous populations of halorespiring *Chloroflexi* or bioremediation with halorespiring *Chloroflexi* has the potential to reduce the potential health risks associated with A1254 in contaminated sludge and sediments. The mechanisms of risk reduction include reduction of highly chlorinated congeners to less chlorinated congeners that are susceptible to aerobic degradation and have reduced potential for bioaccumulation, and reduction in levels of dioxin-like coplanar congeners.

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5. Supporting Material available

Supplementary data related to this article can be found. Figure S1 presents Changes in 16S rRNA gene copy numbers of individual PCB congeners and Aroclor 1254 throughout the incubation period. Figure S2 shows mol% distributions of 22 PCB congeners of A1254 and their products during 180 days of incubation, Figure S3 presents DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in GR-1254 microcosm at day 0 (D0) and day final (D120), Figure S4 shows DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in GR individual PCB congeners microcosms, Figure S5 demonstrates DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in PCB-1254 microcosms at day 120 and Table S1 shows Percent similarities between the sequences of this study and those obtained in the study of Kjellerup *et al.* [9]. Table S2 displays Risk reduction based on the A1254 bioaccumulation potential in fish before and after dechlorination estimated based on BSAFs versus k_{ow} for individual congeners in detritivorous fish [32].

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Table 1. Final products and rates of reductive dechlorination for individual PCB and enumeration of halorespiring phylotypes.

Parent	Final Product	End Mol %	Rate (x10 ⁻³) ^a	16S rRNA gene copies (x10 ⁴) per ml of sample	
				Day 0	Day 180
PCB 180 (2345-245)	PCB 49 (25-24) PCB 47 (24-24)	74.6±0.8	4.4±0.2	20.4±10.8	778±194
PCB 163 (2356-34)	PCB 32 (26-4) PCB 25 (24-3)	72.8±5.4	5.1±0.7	59.7±50.6	589±223
PCB 156 (2345-34)	PCB 33 (2-34) PCB 28 (24-4) /PCB 31 (25-4)	81.8±1.0	1.5±0.1	44.8±13.1	207±47.6
PCB 153(245-245)	PCB 47 (24-24)	89.1±0.8	1.0±0.1	26.2±5.53	289±28.2
PCB 149 (236-245)	PCB 51(26-24) PCB 49 (25-24)	62.5±2.8	3.1±0.7	25.6±16.5	312±115
PCB 141 (2345-25)	PCB 52 (25-25) /PCB 43(235-2)	68.9±12.2	3.9±0.5	28.7±4.09	386±21.1
PCB 138 (234-245)	PCB 47 (24-24)	64.4±1.1	3.6±0.4	33.9±4.04	401±17.4
PCB 132 (234-236)	PCB 51 (26-24)	71.5±1.5	2.9±0.2	32.7±4	426±56.5
PCB 128 (234-234)	PCB 47 (24-24) PCB 32 (26-4)	88.0±0.2	1.1±0.1	20.5±9.74	256±59.4
PCB 110 (236-34)	PCB 27 (26-3) /PCB 24 (236-)	21.6±3.7	7.4±0.7	34.8±9.23	324±79.9
PCB 105 (234-34)	PCB 28 (24-4)	83.8±0.8	1.0±0.1	34.2±8.7	238±38.1
PCB 101 (245-25)	PCB 49 (25-24)	51.3±19.6	4.5±0.9	20.3±4.43	314±125
PCB 99 (245-24)	PCB 47 (24-24)	83.6±1.9	1.0±0.1	22.8±2.18	404±107
PCB 97 (23-245)	PCB 42 (24-23)	41.0±4.8	4.5±0.2	28.5±5.16	293±31.7
PCB 95 (236-25)	PCB 53 (26-25)	37.0±1.3	4.2±0.1	24±3.09	238±1.32
PCB 91 (236-24)	PCB 51 (26-24)	66.1±0.8	1.8±0.1	19.8±9.81	337±88.4
PCB 87 (234-25)	PCB 49 (25-24)	54.6±1.5	3.2±0.4	19.1±2.29	250±60.9
PCB 85 (234-24)	PCB 47 (24-24)	71.3±2.8	1.5±0.1	22.4±3.72	257±15.5
PCB 74 (245-4)	PCB 28 (24-4) /PCB 31 (25-4)	98.1±0.5	0.1±0.0	17.4±9.41	196±28.9
PCB 70 (25-34)	PCB 31 (25-4) PCB 26 (25-3)	95.7±1.3	0.3±0.0	38.1±6.22	217±31.1
PCB 52 (25-25)	PCB 18 (25-2)	99.3±0.0	0.01±0.0	21.7±6.28	148±9.4
PCB 44 (23-25)	PCB 18 (25-2)	24.04±0.7	6.7±0.1	25.6±11.4	291±102
PCB 44-Sterile	ND	ND	ND	2.3±2.4	2.2±1.1
PCB 87-Sterile	ND	ND	ND	1.3±1.4	1.2±2.9
PCB 101-Sterile	ND	ND	ND	2.1±1.7	2.3±0.8
No PCB control	ND	ND	ND	31.2±4.7	101±76.6

^a (Cl/biphenyl/day), ND: Not detected

FIGURE LEGENDS

Figure 1. (a) Changes in total chlorine per biphenyl values of first set of GR microcosms spiked with A1254 and (b) Homolog chlorine distributions of A1254 in GR (D=day).

Figure 2. Changes in mol% of A1254 congeners after 120 Days (D120) of incubation in GR microcosms spiked with A1254. Congeners less than 0.2 mol% at any time point were excluded.

Figure 3. PCB dechlorination pathways of the predominant PCB congeners in A1254. Parent congeners are shown in red. Solid arrows indicate main pathway and clear arrows indicate minor pathways. The predominant end products are boxed.

Figure 4. Phylogenetic tree showing the relationships between the phylotypes (G2-G10) identified at the final day (day 120) of A1254 microcosm and previously reported halorespiring species within the Chloroflexi group (Accession numbers are shown in parenthesis).

Figure 1.

Figure 2.

Figure 3.

Figure 4.