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Biodegradation of PCB congeners by *Paraburkholderia xenovorans* LB400 in presence and absence of sediment during lab bioreactor experiments*

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Abstract

Experiments were conducted to measure biodegradation of polychlorinated biphenyl (PCB) congeners contained in mixture Aroclor 1248 and congeners present in wastewater lagoon sediment contaminated decades earlier at Altavista, Virginia. A well-characterized strain of aerobic PCB-degrading bacteria, *Paraburkholderia xenovorans* LB400 was incubated in laboratory bioreactors with PCB-contaminated sediment collected at the site. The experiments evaluated strain LB400's ability to degrade PCBs in absence of sediment and in PCB-contaminated sediment slurry. In absence of sediment, LB400 transformed 76% of Aroclor 1248 within seven days, spanning all homolog groups present in the mixture. In sediment slurry, only mono- and dichlorinated PCB congeners were transformed. These results show that LB400 is capable of rapidly biodegrading most PCB congeners when they are freely dissolved in liquid but cannot degrade PCB congeners having three or more chlorine substituents in sediment slurry. Finally, using GC/MS-MS triple quadrupole spectrometry, this work distinguishes between physical (sorption to cells) and biological removal mechanisms, illuminates the process by which microorganisms with LB400-type congener specificity can selectively transform lower-chlorinated congeners over time, and makes direct comparisons to other studies where individual congener data is reported.

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Author statement

Thank you for the opportunity to submit this manuscript to *Environmental Pollution* following the revisions suggested by the reviewers. Their comments and those of the editorial staff were very helpful in improving our submission. Additionally, we appreciate the invitation to co-submit a "Data in Brief" article with the revised manuscript.

Data availability statement

The underlying data for this work (Bako et al., 2020) has been deposited in the Iowa Research Online (IRO) institutional data repository for future reuse under an Open Data Commons Attribution License (ODC-By). There are no registration or fee requirements to download the underlying dataset for this work.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2020.116364>.

Keywords

Polychlorinated biphenyls; *Paraburkholderia xenovorans* LB400; Lab bioreactor experiments; Bioremediation; Biodegradation; Bioavailability; Bioaccessibility; Biotransformation

1. Introduction

1.1. Biodegradation & bioavailability of PCBs

PCBs are a group of chlorinated hydrocarbons whose structural foundation is two aromatic rings that are linked by a single carbon-carbon bond with a total of 209 possible congeners. PCB congeners are subdivided into 10 homolog groups depending on the total number of chlorine substituents (Erickson, 1997). PCBs exist in soil and sediment as mixtures of high molecular weight (HMW) and low molecular weight (LMW) congeners. Complete mineralization of these complex mixtures can be achieved via biodegradation – the process by which microorganisms use their natural metabolic capabilities to break down relatively large compounds into smaller molecules to use for energy and growth.

PCB biodegradation often requires two sequential or concurrent microbial processes: (1) anaerobic reductive dechlorination and (2) aerobic oxidation (Abramowicz, 1995; Field and Sierra-Alvarez, 2008; Meggo et al., 2013). Anaerobic reductive dechlorination transforms HMW congeners to LMW congeners by removing chlorine substituents without destroying the biphenyl ring structure of the PCB molecule (Brown et al., 1987; Van Dort and Bedard, 1991). LMW congeners having four or fewer chlorine substituents are much more amenable to aerobic oxidation (Quensen III et al., 1988). Oxidation of PCB congeners proceeds by the upper and lower *bph* pathways mediated by enzymes expressed by the *bph* gene cluster (Passatore et al., 2014). The upper *bph* pathway biodegradation reaction begins when 2,3-dioxygenase (*bphAEFG*) enzymes introduce molecular oxygen into 2,3- or 3,4- sites along the aromatic ring (Haddock and Gibson, 1995). The aromatic ring is subsequently cleaved by dihydroxybiphenyl dioxygenase (DHBD; *bphC*) to yield upper pathway transformation products (Furukawa, 2000; Haddock et al., 1995). These products enter the lower *bph* pathway where they are further broken down to smaller compounds by other enzymes, which can be completely mineralized after entering the Krebs cycle (Dai et al., 2002; Martínez et al., 2007; Pieper and Seeger, 2008).

Lack of PCB bioavailability has been identified as a key parameter which prevents efficient bioremediation or natural attenuation of environmental PCB contamination (Ehlers and Luthy, 2003; Semple et al., 2004). Microorganisms are unable to biodegrade PCBs when PCB molecules are irreversibly (or slowly reversibly) bound to organic material in soil or sediment (Di Toro et al., 1982; Luthy et al., 1997; Ren et al., 2018; Semple et al., 2007). The degree to which PCBs are irreversibly or slowly reversibly bound to soil or sediment particles depends heavily on the total organic carbon (TOC) content or the fraction that can be defined as black carbon (BC) (Cipullo et al., 2018; Lohmann et al., 2005; Werner et al., 2010; Zhu et al., 2016). This work compares *Paraburkholderia xenovorans* LB400's ability to biodegrade a commercial PCB mixture (Aroclor 1248) when the mixture is freely dissolved (bioavailable) to LB400's ability to degrade a similar mixture strongly bound to

sediment after decades of environmental contamination. Strain LB400 was first isolated from a PCB-contaminated landfill soil in Moreau, New York and has become one of the best-characterized PCB degrading microorganisms ever studied (Bedard et al., 1986; Chain et al., 2006).

The purpose of the present work is to better understand the temporal, aerobic biodegradation process by which LB400 degrades a mixture of PCB congeners in presence and absence of sediment. Previous studies which examined the capacity of LB400 to biodegrade PCBs reported only selected congeners, certain homolog groups, or used analytical techniques where individual PCB congener peaks were poorly resolved within the entire mixture (Arnett et al., 2000; Bedard et al., 1986; Bopp, 1986; Gibson et al., 1993; Haddock et al., 1993; Payne et al., 2013, 2017, 2019; Rehmann and Daugulis, 2008a, 2008b, 2008c; Tehrani et al., 2012). This study represents a novel contribution to the scientific record by screening for all 209 congeners as 174 co-eluting peaks and making direct comparisons of biodegradation, by individual congener, to other known studies which investigated PCB biodegradation by LB400 where individual congener data was reported. Additionally, this work differentiates between physical removal (sorption) and biological transformation of PCBs within the bioreactor by comparison of live cell treatment to dead cell controls. Finally, by observation and comparison of complete PCB profiles, we demonstrate that strong sorption of PCBs to aged sediments severely limits LB400's ability to biodegrade them – except for the least chlorinated congeners ($\#Cl < 3$) – and briefly discuss the implications of this finding on risk assessment and remedial design.

2. Methods & materials

2.1. Site description & sampling

The sediment used in this study was taken from a PCB-contaminated emergency overflow lagoon located in Altavista, VA (37°06'52"N, 79°16'21"W). The lagoon became contaminated with PCBs prior to 1977. Sediment samples were taken from sampling location between F3 and F4 (Figure S1) using a steel headed transfer shovel. Four (4) 23 L coolers and one (1) 5 L cooler were filled with sediment, limiting headspace within containers. The coolers were chilled with icepacks, sealed with duct tape, transported back to University of Iowa laboratories, and stored in refrigerators at 4 °C prior to further analysis.

2.2. Sediment homogenization and characterization

Sediment was transferred to a 20gal drum and thoroughly homogenized using a mud mixer attached to a power drill before use in experiments. Three 1.5 kg test aliquots were transferred to red clay pots. Five sub-samples were taken from each pot. The first two sub-samples from each pot were analyzed as replicates and the last three sub-samples were combined to form a composite sample. The PCB profiles and concentrations of replicate sub-samples and composite samples from each pot were compared to confirm that sediment was properly homogenized to a degree such that further samples including sediment were representative. The average total PCB concentration (Σ PCB) was 6346 ng/g (6.346 mg/kg; 6.346 ppm; 5% RSD; n = 6). The PCB-congener profile of Altavista, Virginia sediment most

closely resembled Aroclor 1248 ($\text{Cos } \theta = 0.89$; Figure S2). Altavista sediments had slightly higher mass percent in the LMW range and at the very HMW range than the commercial mixture Aroclor 1248. These differences between the PCB profile of Altavista sediment and pure Aroclor 1248 are mostly likely due to the accumulation of lightly chlorinated (mono-, di-, and trichlorobiphenyls) resulting from reductive dechlorination of tetra- and pentachlorobiphenyls by native organohalide respiring bacteria (OHRB) within the lagoon sediment (Mattes et al., 2018).

Sediment texture characterization and physico-chemical analyses were conducted by Minnesota Valley Testing Laboratories (MVTL; www.mvtl.com; New Ulm, MN). We provided MVTL with 9×250 g sediment samples (triplicates representing each test aliquot from the homogenization procedure and air-dried sediment taken directly from the 20gal drum). Sediment was sandy loam consisting of 72.2% sand, 24.7% silt, and 3% clay. Soil pH was 6.4, total organic matter 4.2%, total organic carbon (TOC) 7.2%, and cation exchange capacity (CEC) $6.0 \text{ cmol}_c/\text{kg}$. Additional soil parameters can be found in Table S1.

2.3. Bacterial strain and growth conditions

Paraburkholderia xenovorans LB400 was grown aerobically at 30°C on a platform shaker in a 500 mL Erlenmeyer flask containing 250 mL of K1 medium and solid biphenyl crystal (5 mM; 0.19 g) as a sole carbon and energy source until mid-exponential phase ($\text{OD}_{600} = 0.8$). Cells were harvested by centrifugation ($5000 \times g$, 15 min), washed twice with sterile K1 medium, resuspended in K1 medium, and inoculated into bioreactors using a 1 mL pipette (Liang et al., 2014).

2.4. PCB quantification

GC-MS/MS (Agilent 7890 A GC system, Agilent 7000 Triple Quad, Agilent 7693 autosampler) in multiple reaction monitoring mode (MRM) was used for identification and quantification of 209 PCBs as 174 chromatographic peaks. The GC was equipped with a Supelco SPB-Octyl capillary column (50% n-octyl, 50% methyl siloxane, $30 \text{ m} \times 0.25 \text{ mm}$ ID, $0.25 \mu\text{m}$ film thicknesses) with helium as the carrier gas flowing at $0.75 \text{ mL}/\text{min}$ and nitrogen/argon as the collision gas. The GC operated in solvent vent injection mode at the following injection conditions: initial temperature 45°C , initial time 0.06 min, ramp $600^\circ\text{C}/\text{min}$ to inlet temperature 325°C at 4.4 psi. The GC oven temperature program was 45°C for 2 min, $45\text{--}75^\circ\text{C}$ at $100^\circ\text{C}/\text{min}$ and hold for 5 min, $75\text{--}150^\circ\text{C}$ at $15^\circ\text{C}/\text{min}$ and hold for 1 min, 150 to 280 at $2.5^\circ\text{C}/\text{min}$ and final hold 5 min (total run time 70.86 min). The triple quadrupole MS electron ionization source was set to 260°C . Additional details can be found in the supporting information (Tables S3–S13).

2.5. Quality assurance & quality control (QA/QC)

Extraction efficiency, reproducibility, and accuracy was assessed using surrogate standards, replicates of method blanks, development of QC acceptance criteria (Table S2), and analysis of standard reference materials. Standard reference material purchased from the National Institute of Standards and Technology (NIST SRM, 1944, New York, New Jersey Waterway sediment; Gaithersburg, MD, USA) was analyzed in quintuple. The mean percent difference between the measured and certified values (27 congeners) was $13\% \pm 14\%$ (Figure S3).

Mean and standard deviation percentage recoveries of PCB14, PCB65-d5 and PCB166 were $97 \pm 1\%$, $94 \pm 4\%$ and $114 \pm 4\%$, respectively. According to QC criteria, any sample having a recovery less than 50% was excluded from analysis. Percentage recoveries of surrogate standards were used to correct congener mass as follows: PCB14 recovery was used to correct PCB1 to PCB39, PCB65-d5 was used to correct PCB40 to PCB127 and PCB166 was used to correct PCB128 to PCB209 (sorted by IUPAC number). PCB congener masses were corrected for surrogate recoveries less than 100%. Samples were processed in batches of five along with one method blank per batch. All materials used in sample extraction had either been triple rinsed with solvent (methanol, acetone, and hexane) or combusted overnight at 450 °C to prevent background PCB contamination. Results from the method blanks were used to determine the limit of quantification (LOQ) as the upper limit of the 95% confidence interval (average mass plus two times the standard deviation).

2.6. Statistical analysis

The concentration data set was first dichotomized at the threshold of the congener specific LOQ: concentrations of congeners below the LOQ were treated as zero. Cosine theta ($\cos \theta$) was calculated to quantitatively describe and compare PCB profiles where $\cos \theta = 0$ describes two completely different profiles and $\cos \theta = 1$ describes identical profiles (Johnson et al., 2010). GraphPad Prism's two-sided, unpaired *t*-test with Welch's correction applied was used to test for significant differences between treatment conditions and controls.

2.7. Aroclor 1248 Biodegradation Assay with *Paraburkholderia xenovorans* LB400 in Absence of Sediment

An experiment was conducted with LB400 cells in an aqueous solution of Aroclor 1248 to test the ability of LB400 to degrade soluble, bioavailable PCB congeners (experimental design matrix shown in Figure S4). Bioreactors were 9 mL Pyrex® test tube with PTFE-lined screw caps. Live-cell treatments and dead-cell controls were added to the bioreactors. First, 1 mL of K1 bacterial medium was transferred to the bioreactor and spiked with Aroclor 1248 in acetone (3 μ L; 25 ng/mL final concentration). PCBs equilibrated with the aqueous phase by shaking on a platform shaker table at 150 rpm for 48 h prior to the addition of LB400 cells. The biodegradation reaction was started by adding 2 mL of live LB400 cells ($OD_{600} = 0.8$) suspended in K1 medium to the bioreactor. Dead-cell controls were inactivated by adding perchloric acid (20 μ L; final concentration 0.7%; Bedard et al., 1986). Time-zero samples were prepared to equal volume but did not contain LB400 cells. Bioreactors were incubated at room temperature (~ 25 °C) for one week on a platform shaker table at 150 rpm. All treatments were prepared in quintuplet ($n = 5$) but two samples were lost at $T = 48$ h due to a faulty equipment sensor which led to the complete evaporation of one sample each in the live and dead-cell treatments resulting in $n = 4$ at that time point.

2.8. PCB congeners

A stock solution of Aroclor 1248 suspended in acetone was created with analytical standard obtained from AccuStandard, Inc. (New Haven, CT). The final concentration of the stock solution used to spike the bioreactors was approximately 25 mg/L (25×10^3 ng/mL). Aroclor 1248 stock solution (3 μ L) was added to 1 mL K1 medium already inside the

bioreactors using a 10 μ L syringe for a final concentration of 25 ng/mL as Aroclor 1248. PCB congeners referenced in this work will be described by their congener number and chlorine substitution pattern, where necessary or helpful (US EPA, 2003).

PCBs were extracted from the aqueous bioreactor using a liquid-liquid extraction (LLE) with an equal volume of hexane (3 mL) added to each bioreactor. Prior to extraction, the bioreactor was spiked with surrogate standards PCB 14 (50.81 ng; 3,5-dichlorobiphenyl), deuterated PCB 65-d5 (52.5 ng; 2,3,5,6-tetrachlorobiphenyl-d5, deuterated) and PCB166 (52.56 ng; 2,3,4,4',5,6-hexachlorobiphenyl; Cambridge Isotope Laboratories, Inc.). The bioreactor was vortexed and centrifuged. The hexane layer containing the sample extract was transferred to a TurboVap (Biotage, Uppsala, Sweden) tube. The LLE process was repeated three times. The resulting volume of solvent containing the sample extract was concentrated to approximately 1 mL using a TurboVap II Concentration Workstation (Caliper Life Sciences).

The final hexane extract was passed through a Pasteur pipette filled with 0.1 g of combusted silica gel and 1 g of acidified silica gel (2:1 silica gel:sulfuric acid by weight) and eluted with approximately 10 mL of hexane (US EPA, 1996). Samples were concentrated to approximately 1 mL and transferred to a gas chromatography vial. The final sample was spiked with internal standards deuterated PCB 30-d5 (19.6 ng; 2,4,6-trichlorobiphenyl-2',3',4',5',6'-d5, deuterated) and PCB 204 (19.6 ng; 2,2',3,4,4',5,6,6'-octachlorobiphenyl; Cambridge Isotope Laboratories, Inc.).

2.9. Aroclor 1248 Biodegradation Assay with *Paraburkholderia xenovorans* LB400 in Sediment Slurry

An experiment was conducted with LB400 cells in a biphasic solution of K1 bacterial medium and PCB-contaminated sediment slurry to test the ability of LB400 to degrade PCB congeners from the contaminated sediment gathered from the Altavista, VA field site. The sediment was not spiked with any additional PCB congeners. The biodegradation reaction was started by adding 2 mL of live LB400 cells ($OD_{600} = 0.8$) in K1 medium to the incubation vessel containing Aroclor 1248-contaminated sediment slurry (1 mL K1 medium with 0.3 g PCB-contaminated sediment; experimental design matrix shown in Figure S5). Dead-cell controls were inactivated by adding perchloric acid (20 μ L; final concentration 0.7%; Bedard et al., 1986). Cells were incubated at room temperature (~ 25 $^{\circ}$ C) for one month ($T = 28$ days) on a platform shaker table at 150 rpm. All treatments were performed in quintuplet ($n = 5$).

The analytical method employed for sample extraction is a modification of U.S. EPA Method 3545 (US EPA, 2007). Sediment slurry samples were extracted using pressurized fluid extraction (Accelerated Solvent Extractor; Dionex ASE-200) with equal parts acetone and hexane. At the time of sampling, contents of the bioreactor were poured into a mortar and pestle containing ~ 7 g combusted diatomaceous earth. The mass of sediment remaining in the bioreactor after pouring was determined gravimetrically. The diatomaceous earth containing the sample was ground to a fine powder and transferred to a 33 mL ASE-200 cell containing approximately 3 g silica gel sorbent. The sample was spiked with surrogate standards PCB 14 (50.81 ng; 3,5-dichlorobiphenyl), deuterated PCB 65-d5 (52.5 ng; 2,3,5,6-

tetrachlorobiphenyl-d5, deuterated) and PCB166 (52.56 ng; 2,3,4,4',5,6-hexachlorobiphenyl; Cambridge Isotope Laboratories, Inc.). The sample extract resulting from pressurized fluid extraction was concentrated within the collection vial to approximately 1 mL and transferred to a 15 mL Pyrex® test tube with PTFE-lined screw cap.

Polar interferences and other compounds were removed by extraction with sulfuric acid. The final hexane extract was passed through a Pasteur pipette filled with 0.1 g of combusted silica gel and 1 g of acidified silica gel (2:1 silica gel:sulfuric acid by weight) and eluted with approximately 10 mL of hexane (US EPA, 1996). Samples were concentrated to approximately 1 mL and transferred to a gas chromatography vial. The final sample was spiked with internal standards deuterated PCB 30-d (19.6 ng; 2,4,6-trichlorobiphenyl-2',3',4',5',6'-d5, deuterated) and PCB 204 (19.6 ng; 2,2',3,4,4',5,6,6'-octachlorobiphenyl; Cambridge Isotope Laboratories, Inc.). All treatments were prepared in quintuplet (n = 5) but, due to surrogate recoveries below established QA/QC criteria (Table S2) in the time-zero measurement, two samples were excluded from the analysis, resulting in n = 3 at that time point. Additionally, one sample from the live cell treatment at the 1-month timepoint was excluded from analysis because it had low surrogate recoveries due to an instrumentation error during PCB extraction resulting in n = 4 at that time point, for that treatment.

3. Results & discussion

3.1. Aroclor 1248 biodegradation assay with *Paraburkholderia xenovorans* LB400 in absence of sediment

Results of an Aroclor 1248 biodegradation assay conducted in absence of sediment showed that LB400 grown on biphenyl as the sole carbon and energy source could biodegrade a wide spectrum of PCB congeners in the mixture. After one week of incubation at room temperature the live-cell treatment transformed 76% of total PCB concentration (Σ PCBs), relative to time-zero measurements (Fig. 1A). Significant differences were detected between live cell treatments and dead cell controls for all homolog groups present in the Aroclor 1248 mixture except for hexachlorobiphenyls, which make up a small fraction of the total mixture (Fig. 1B). In the dead-cell control, there was a 23% drop in PCB concentration within the first 48 h of the incubation period. Additionally, a linear relationship ($r^2 = 0.9072$) between the relative mass abundance of an individual congener (PCB_i) and its contribution to total biological removal was observed. Deviations from this relationship indicate congener preference of LB400 (Figure S6). In general, the observed magnitude of both biological and physical removal of an individual PCB congener was proportional to its relative abundance (by mass) in the Aroclor 1248 mixture. Findings from this experiment were consistent with other studies which demonstrate the broad PCB biodegradation capability of *Paraburkholderia xenovorans* LB400 (Table 1) but also provide new insight into how PCB biodegradation of Aroclor 1248 occurs with time (Bedard et al., 1986; Bopp, 1986; Payne et al., 2017).

Biodegradation of the Aroclor 1248 mixture was observed on a congener-by-congener basis throughout the 1-week incubation period (Fig. 2A–C). Di- and trichlorinated congeners with

the highest relative abundance (by mass) were the preferred substrate of LB400 over the first 48 h of the incubation period (Fig. 2A). Similarly, the most abundant tetrachlorobiphenyls PCB 44/47, 48, 49, and 52 were partially transformed during the first 48 h. To illustrate how LB400 transformed HMW congeners after preferentially depleting LMW congeners, we subtracted PCB_i removal after 48 h on a congener-by-congener basis from total PCB_i removal after 1-week ($\text{PCB}_i \text{ Removal}_{T=1\text{wk}} - \text{PCB}_i \text{ Removal}_{T=48\text{h}}$; Fig. 2B). By observation of Fig. 2A and B, we reason that transformation of pentachlorobiphenyls during the first 48 h was competitively inhibited because LB400 preferentially degraded di-, tri-, and a select few tetrachlorobiphenyls. In addition, the 23% drop in PCB concentration displayed during the first 48 h in the dead cell control was due to rapid sorption of PCB congeners to inactivated cell material. In the dead cell control, removal of individual congeners occurred proportionally to each congener's relative abundance in the mixture and regardless of molecular weight (Fig. 2A and B). In the live cell treatment, strain LB400 acted upon all congeners and demonstrated at least 20% transformation for each one, up to and including hexachlorobiphenyls (Fig. 2C; Table 1). The biodegradation pattern observed in the present work is consistent with reports that LB400 has broad congener specificity which encompasses congeners belonging to separate structural classes but is limited in its ability to degrade double-*para*-substituted congeners (Table 1; Bedard et al., 1986; Bopp, 1986; Gibson et al., 1993; Nadim et al., 1987). Most double-*para*-substituted congeners belong to the final two structural classes shown in Table 1.

LB400's limited ability to degrade double-*para*-substituted congeners results from extremely slight differences in the amino acid sequences of LB400's large subunit, relative to other PCB-degraders. These slight differences in amino acid sequence are also the reason for dramatic differences in substrate specificity, compared to other microorganisms with lesser PCB biodegradation capability, such as *Pseudomonas furukawaii* KF707 (Erickson and Mondello, 1992, 1993; Haddock and Gibson, 1995; Mondello et al., 1997). The dioxygenases of LB400 and KF707 share >98% sequence similarity and their large subunits differ by only 20 amino acids (Haddock et al., 1995; Mondello et al., 1997). Strains with LB400-type specificity can degrade a broad range of congeners due to relaxed regiospecificity of where their terminal oxygenase (*bphA*) inserts molecular oxygen to the aromatic ring structure of a PCB molecule (Arnett et al., 2000). That is, microorganisms with LB400-type specificity can initiate dioxygenase attack at an unchlorinated 2,3 or 3,4 site of a PCB congener but are relatively weak against di-*para*-substituted congeners (Bedard et al., 1986; Mondello et al., 1997). In contrast, strains with KF707-type specificity degrade a much narrower range of PCBs but have enhanced ability to degrade di-*para*-substituted congeners. For example, PCB 15 (4,4'-CB) is poorly degraded by LB400 compared to strains with KF707-type specificity (Mondello et al., 1997). Interestingly, LB400 degrades 2,4,4'-CB and 2,4,2',4'-CB (PCBs 28 and 47, respectively) much more effectively than other di-*para*-substituted congeners, suggesting that the additional *ortho*-chlorine assists with enzymatic binding to the dichlorinated ring (Table 1; Bedard et al., 1987, 1986; Erickson and Mondello, 1993; Gibson et al., 1993; Mondello et al., 1997).

To further observe the difficulty with which LB400 degrades double-*para*-substituted congeners we compared the transformation of two tetrachlorinated congeners that are abundant in Aroclor 1248: PCB 52 (2,2',5,5'-CB, 7.37% by mass) and PCB 66 (2,3',4,4'-

CB, 5.69% by mass; Frame, 1997; Koh, 2015). PCB 52 is a congener that is notoriously difficult to degrade for microorganisms without LB400-type specificity because it only has unchlorinated 3,4 sites available for enzymatic binding. Most microorganisms without LB400-type specificity can only attack PCB congeners at unchlorinated 2,3 sites (Bedard et al., 1987; Mondello et al., 1997). PCB 52 was one of the few tetrachlorinated congeners significantly degraded by LB400 within the first 48 h of the incubation period (Fig. 2A). Significantly greater ($p < 0.05$) biological transformation of PCB 52 was observed relative to PCB 66 even though the difference in relative abundance in Aroclor 1248 between the two congeners was small (1.68%, by mass). Presumably, each congener should display removal proportional to its relative abundance in the mixture. A linear relationship ($r^2 = 0.9072$) was observed by plotting the relative mass abundance of PCB_i and its contribution to total biological removal (Figure S6). PCB 52 falls above the best-fit line whereas PCB 66 falls below which indicates LB400's preference for PCB 52 and aversion for double-*para*-substituted PCB 66. It may be possible to overcome LB400's limited capacity to biodegrade double-*para*-substituted congeners in a bioremediation scenario by using bioaugmentation consortia containing strains with both LB400-type and KF707-type congener specificity.

It is unclear whether LB400 used PCBs for growth based on the results of this study. The Aroclor 1248 mixture was not 100% degraded perhaps due to the brevity of the 7 d exposure period or the possible accumulation of transformation byproducts such as chlorobenzoic acids (CBAs) and/or 2-hydroxy-6-oxo-6-phenyl-hexa-2,4-dienoic acids (HOPDAs, or *meta*-cleavage products) in the bioreactor, which can interfere with the *bph* pathway (Adebusoye et al., 2008; Guilbeault et al., 1994; Seah et al., 2000; Sondossi et al., 1992). Percent depletion of PCB_i is shown in Table 1. Table 1 represents the most detailed dataset of individual PCB congener biodegradation by LB400 to date (Bako et al., 2020). Direct comparisons are made between the present work and past studies that have reported individual congener data in biodegradation assays using LB400.

4. Aroclor 1248 biodegradation assay with *Paraburkholderia xenovorans* LB400 in sediment slurry

Results of a biodegradation assay in presence of PCB-contaminated sediment slurry show that LB400 could not significantly biodegrade PCB congeners with more than two chlorine substituents that are bound to sediment gathered from Altavista, VA. After one month of incubation ($T = 28$ days) at room temperature, the live-cell treatment did not significantly transform the total PCB concentration (Σ PCBs), relative to time-zero measurements or dead cell controls (Fig. 3A). Significant differences were detected between live cell treatments and dead cell controls only in the mono- and dichlorinated homolog groups (Fig. 3B). In the dead-cell treatment, there was a detected increase in PCB concentration over the course of the incubation period which could be attributed to sediment sampling heterogeneity between time-zero and subsequent samples. Findings from this experiment were consistent with the literature that indicates microorganisms capable of PCB biodegradation are unable to do so when congeners are irreversibly or slowly reversibly bound to soil or sediment particles (Cipullo et al., 2018; Ghosh et al., 2000; Harkness et al., 1993; Luthy et al., 1997; Ren et al., 2018).

Fig. 4 indicates transformation of PCB 1 (2-chlorobiphenyl), PCB 4 (2,2'-dichlorobiphenyl), and PCB 8 (2,4'-dichlorobiphenyl) in the live cell treatment. No biodegradation of double-*para*-substituted PCB 15 (4,4'-dichlorobiphenyl) was detected. In the dead cell control, there were detected increases in PCB_i concentration for nearly all congeners likely due to slight sediment sampling heterogeneity between time zero samples and T = 28 days samples. Results of this experiment show that LB400 is unable to degrade HMW PCB congeners in Altavista sediment because they are likely irreversibly or slowly-reversibly bound to sediment particles. LB400 still significantly ($p < 0.05$) biodegraded congeners that are most likely to become airborne (mono- and dichlorobiphenyls) – partially disrupting a significant pathway of human exposure to PCBs (Awad et al., 2016; Herkert et al., 2018; Jahnke and Hornbuckle, 2019; Kvasnicka et al., 2019; Marek et al., 2017; (Martinez et al., 2017), 2019; Shanahan et al., 2015). Volatilization of PCBs to the atmosphere has long been recognized as a significant mass transport process (Achman et al., 1993; Hafner and Hites, 2003; Jeremiason et al., 1994; Totten et al., 2001). As such, the inhalation exposure pathway of PCBs has been included as a major factor when making risk-based decisions regarding remediation. New methods for measuring PCB concentrations in air, over time, offer new insight into the inhalation exposure pathway which has traditionally been considered secondary to the consumption of PCB-contaminated foods ((Martinez et al., 2017), 2019).

Together, these two biodegradation assays clearly display how LB400 microorganisms possess a high degree of PCB biodegradation capacity but cannot use their ability to fully remediate PCB-contaminated environments unless PCBs are soluble and/or bioavailable to a greater extent. Substrate availability and cell viability also become limiting factors due to slow desorption kinetics of PCBs in the sediment environment (Ren et al., 2018; Semple et al., 2004, 2007).

5. Conclusion

For the first time by use of GC-MS/MS QQQ, we show that biodegradation by LB400 in aged, contaminated sediments is limited to degradation of the mono- and dichlorinated PCB congeners out of the entire 209 possible due to strong sorption to aged sediment. Mono- and dichlorobiphenyls are the most volatile substrates in the congener profile, so bioaugmentation with LB400 should help to decrease aquatic concentrations and airborne flux of these toxic LMW-PCBs thereby limiting inhalation exposure in communities where PCB contamination of nearby waterways is common (Ortega-Calvo et al., 2015). Our findings are also important because they inform strategies for bioaugmentation/biostimulation of legacy sediments. To biodegrade the highly chlorinated PCBs with LB400, the highly sorbed-compounds must be made more soluble and bioavailable to microorganisms through amendments such as biosurfactants, solvents, and/or novel sorbent materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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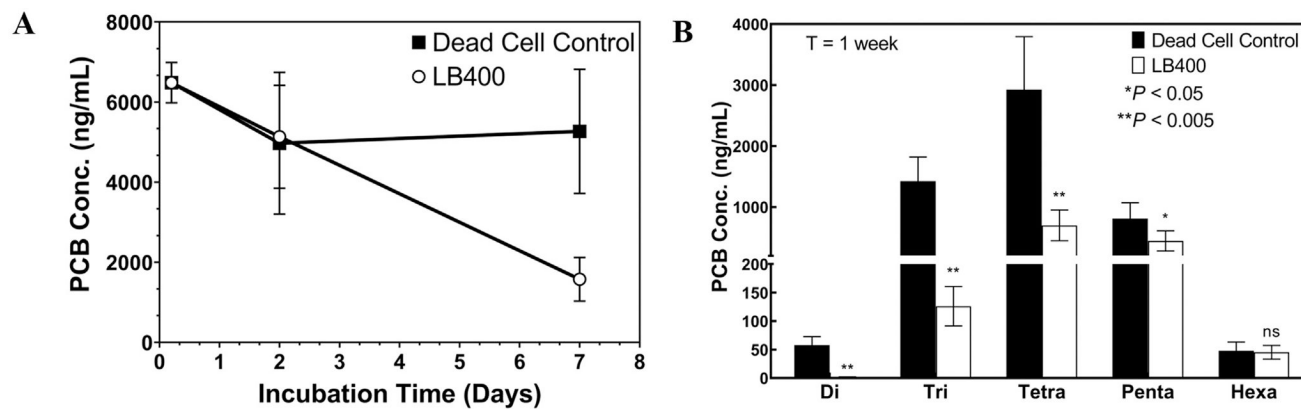


Fig. 1.

Panel A: PCB concentration in solution (ng/mL) over a 1-week incubation period. Mean and standard deviation of $n = 4$ replicates are shown for $T = 48$ h and $n = 5$ replicates are shown for time-zero and $T = 1$ wk. **Panel B:** PCB concentration in solution (ng/mL), by homolog group at $T = 1$ wk. Average per-congener depletion within each homolog group (%) in the live cell treatment relative to the dead cell control was as follows: Di – 93%; Tri – 88%; Tetra – 72%; Penta – 56%; Hexa – 27%. For all homolog groups, a two-sided, unpaired t -test with Welch’s correction applied was used to test for significant differences between live and dead-cell treatments. Mean and standard deviation for $n = 5$ replicates are shown, except for $T = 48$ where $n = 4$.

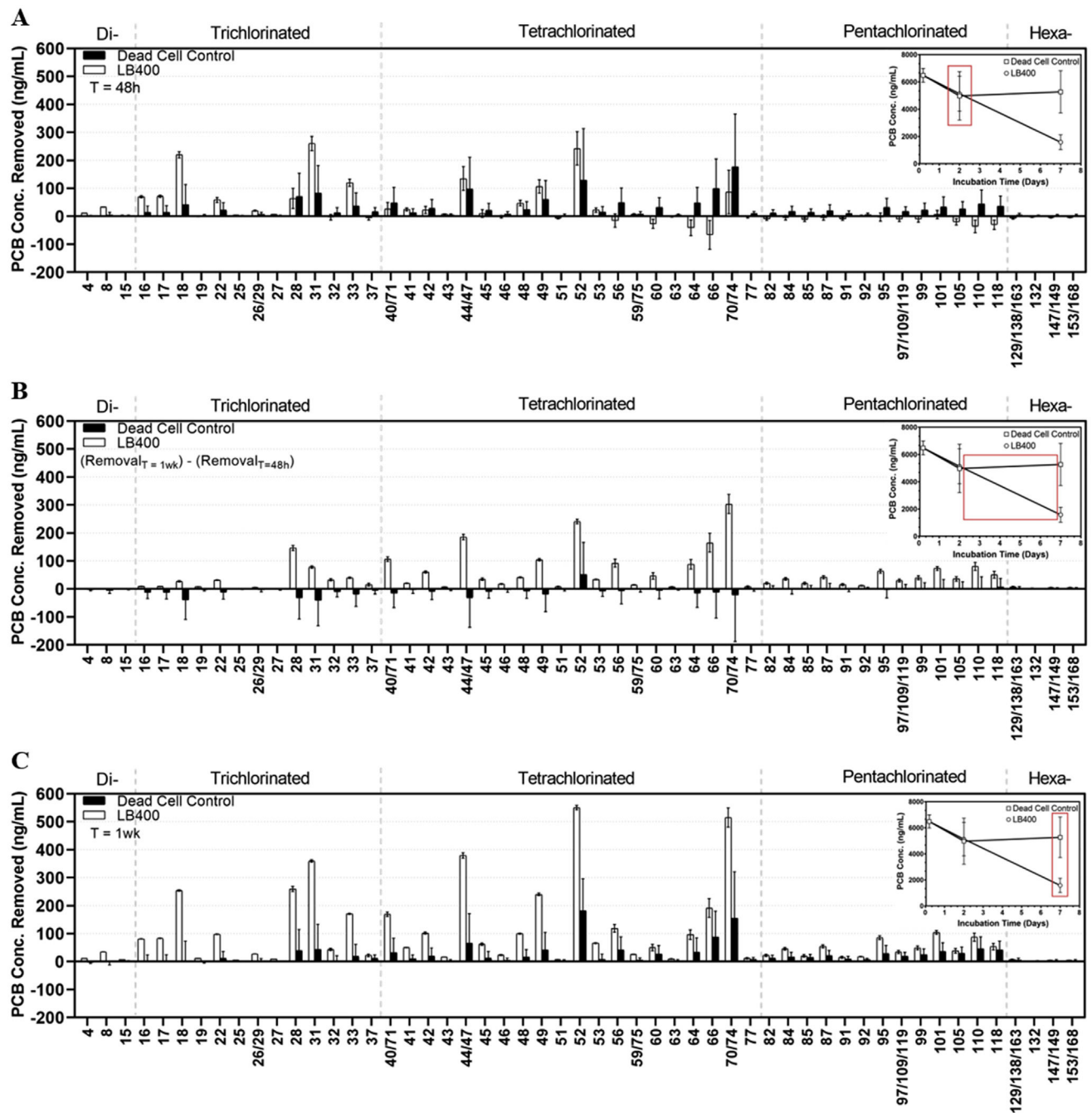


Fig. 2. –

Panel A: PCB removal from K1 medium, by individual congener, (ng/ml) over 48 h. In the live-cell treatment (white bars), LB400 demonstrates a clear preference for degradation and removal of the most abundant di-, tri-, and a select few tetrachlorobiphenyls (from PCB4 to PCB52) during the first 48 h of the incubation period, whereas pentachlorobiphenyls remain unaffected. In the dead-cell control (black bars), removal across the wide-range of congeners is assumed to be due to rapid sorption to inactivated cell material. Inset displays the time interval reflecting the congener-by-congener removal. **Panel B:** PCB removal from K1

medium, by individual congener (ng/ml) between T = 48 h and T = 1wk. At this time point, the live-cell treatment, LB400 transformed the most abundant di- and trichlorobiphenyls and shifted to tetra- and pentachlorinated congeners through PCB 118. In the dead-cell control, highly hydrophobic (pentachlorinated) PCBs were bound tightly to dead cell membranes with no further removal, whereas some more moderately chlorinated (tri-, and tetrachlorobiphenyls) became re-associated with the aqueous phase. **Panel C:** PCB removal, by individual congener, (ng/mL) after 1-week incubation in liquid K1 medium. LB400 was effective at removing di-, tri-, tetra-, and pentachlorinated congeners with limited ability to transform those belonging to the hexa-chlorinated homolog groups. Hexachlorobiphenyls make up a small portion of the Aroclor 1248 mixture. Mean and standard deviation for 5 replicates are shown for all time points except T = 48 h.

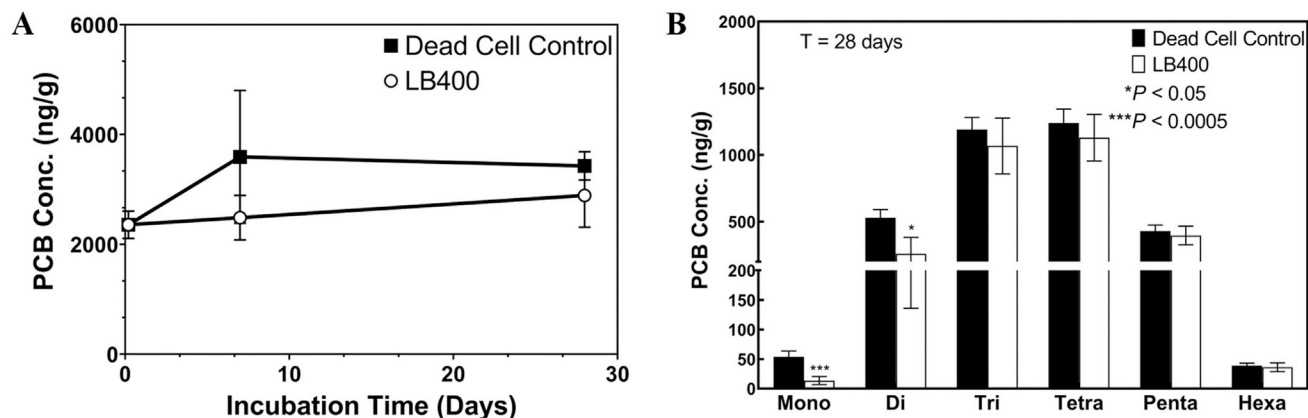


Fig. 3.

Panel A: PCB concentration (ng/g sediment) over a 28-day incubation period. Mean and standard deviation of $n = 5$ replicates shown. **Panel B:** PCB concentration (ng/g), by homolog group at $T = 28$ days. Significant differences ($p < 0.05$) were detected between the live cell treatment and dead-cell control for only mono- and dichlorinated homolog groups. For all homolog groups, a two-sided, unpaired t -test with Welch's correction applied was used to test for significant differences. Mean and standard deviation from $n = 5$ replicates shown for dead cell control. Mean and standard deviation from $n = 3$ replicates shown for both the dead cell control and LB400 at $T = 0$ days. Mean and standard deviation from $n = 4$ replicates shown for LB400 at $T = 28$ days.

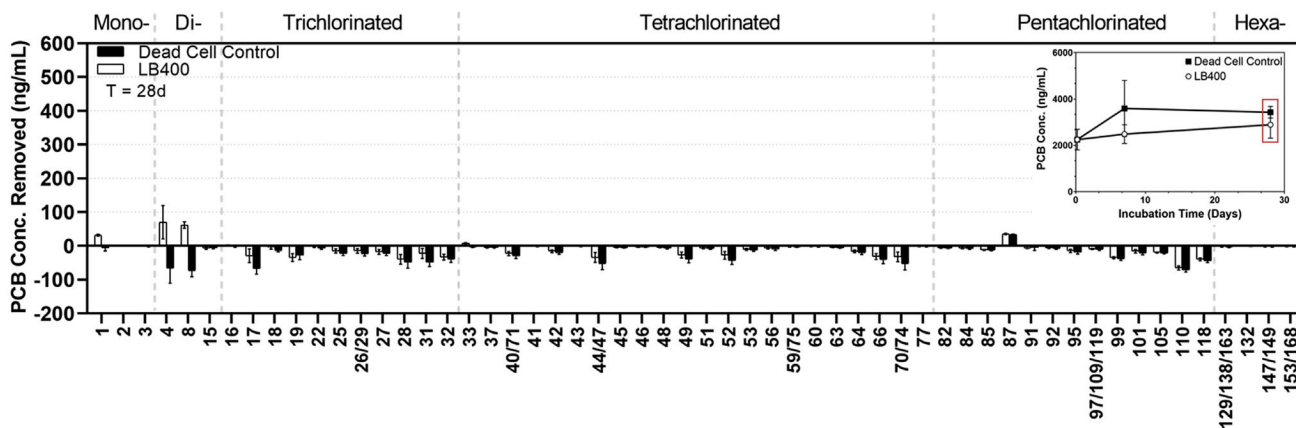


Fig. 4. PCB removal, by individual congener, due to biodegradation (ng/g) after 28-day incubation in liquid K1 medium. LB400 was only able to partially transform mono- and dichlorinated congeners. Inset shows the time point reflected by the congener-by-congener breakdown. Mean and standard deviation for 5 replicates for are shown. Although we screened for 209 congeners, only a condensed profile is shown for ease of profile comparison.

Table 1

Comparison of PCB_i congener depletion (%) by *Paraburkholderia xenovorans* LB400 between literature values and present work.

Congener Description		% Depletion of PCB _i						
Structural Class	BZ Congener#	Substitution Pattern	Present work	Bedard et al. (1986)	Bopp et al. (1986)	Nadim et al. (1987)	Gibson et al. (1993)	
Open 2,3 and 3,4 Sites	4	2,2'	100	80-100 ^d	100	100	100/100 ^e	
	5	2,3		80-100	100	100	100/100	
	8	2,4'	99	80-100	100	100	100/100	
	9	2,5				100		
	10	2,6				40		
	16	2,2',3	99					
	17	2,2',4	99					
	18	2,2',5	99	80-100	100	100	100/100	
	19^a	2,2',6	74					
	22	2,3,4'	97					
	25	2,3',4	97					
	26/29 ^b	2,3',5/2,4,5	98					
	27	2,3',6	98					
	30	2,4,6				25		
	31	2,4',5	98	80-100	100	100	98/100	
	32	2,4',6	63					
	33	2,3',4'	97					
	40	2,2',3,3'	81	80-100	100		100/100	
	41	2,2',3,4	94					
	42	2,2',3,4'	87					
	43	2,2',3,5	94					
	44/47 ^c	2,2',3,5'/2,2',4,4'	89	80-100/80-100	100/100	100/100	100/81/86	
	45	2,2',3,6	66					
	46	2,2',3,6'	70					
	48	2,2',4,5	95					
	51	2,2',4,6'	37					

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Congener Description		% Depletion of PCBs						
Structural Class	BZ Congener#	Substitution Pattern	Present work	Bedard et al. (1986)	Bopp et al. (1986)	Nadim et al. (1987)	Gibson et al. (1993)	
	53	2,2',5,6'	92			100		
	56	2,3,3',4'	92					
	<u>59/75</u>	2,3,3',6,2,4,4',6	81					
	63	2,3,4',5	64					
	64	2,3,4',6	46					
	<u>70/74</u>	2,3',4',5,2,4,4',5	73	80-100/NA	100/NA		100/NA	
	82	2,2',3,3',4	50					
	84	2,2',3,3',6	67					
	87	2,2',3,4,5'	67	80-100	100	100	97/96	
	91	2,2',3,4',6	41					
	<u>97/109/119</u>	2,2',3,4',5',2,3,3',4,6,2,3',4,4',6	53	40-59/NA/NA	90/NA/NA	100/NA/0	50/53	
	110	2,3,3',4',6	49					
	<u>129/138/163</u>	2,2',3,3',4,5,2,2',3,4,4',5',2,3,3',4',5,6	26					
	132	2,2',3,3',4,6	25					
	<u>147/149</u>	2,2',3,4',5,6,2,2',3,4',5',6	27					
Open 3,4 Sites	52	2,2',5,5'	96	80-100	100	100	100/100	
	54	2,2',6,6'				0 ^g		
	92	2,2',3,5,5'	80					
	95	2,2',3,5',6	70			75		
	101	2,2',4,5,5'	78	80-100	100	100	100/98	
	103	2,2',4,5,6'				35		
	135	2,2',3,3',6,6'				50	25/11	
<u>Open 2,3 Sites</u>	15	4,4'	81	60-79	47	100	81/86	
	28	2,4,4'	84	80-100	98	100	89/54	
	37	3,4,4'	41					
	47	2,2',4,4'	@44/47 ^f	80-100	100	100		
	60	2,3,4,4'	39					
	66	2,3',4,4'	48	40-59	64	65	43/34	
	75	2,4,4',6	@59/75			0		
	77	3,3',4,4'	42	40-59	41	0	6/11	

Congener Description		% Depletion of PCBs					
Structural Class	BZ Congener#	Substitution Pattern	Present work	Bedard et al. (1986)	Bopp et al. (1986)	Nadim et al. (1987)	Gibson et al. (1993)
	85	2,2',3,4,4'	42				
	99	2,2',4,4',5	56				
	100	2,2',4,4',6			0		
	118	2,3',4,4',5	40		0		
<i>Blocked 2,3 & 3,4 Sites</i>	121	2,3',4,5',6			0		
	153/168	2,2',4,4',5,5',2,3',4,4',5',6	30	40-59/NA	59/45	70/NA	[41/61]/NA
	155	2,2',4,4',6,6'				0	

^a PCB congeners with BZ# in bold have more than two *ortho*-chlorine substituents.

^b PCB congeners separated with a forward slash co-elute with each other.

^c PCB congeners underlined or in italics belong to the corresponding structural class separate from the PCB congener(s) it co-elutes with i.e. PCB congeners that are underlined belong to the structural class with open 2,3 sites and those in italics belong to that with blocked 2,3 and 3,4 sites.

^d No specific values were given by Bedard et al. (1986), only approximate ranges.

^e Gibson et al. (1993) assayed the biodegradation ability of LB400 using both whole cells and cell extracts from LB400. Depletion percentage by whole cells is given first, followed by that of cell extracts.

^f Since some congeners that are both present in the Aroclor 1248 mixture co-elute in the analytical method used in the present work, a determination cannot be made about exactly how much of either congener was depleted but the '@' symbol references the first entry in the table for the co-eluting congeners.

^g Zero values indicate that none of the PCB congener was depleted whereas a blank space indicates the congener was not detected in the corresponding work. Congeners not detected in the present work are not present in Aroclor 1248 but were screened for.