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Kinetics and Threshold Level of 2,3,4,5-Tetrachlorobiphenyl Dechlorination by an Organohalide Respiring Bacterium

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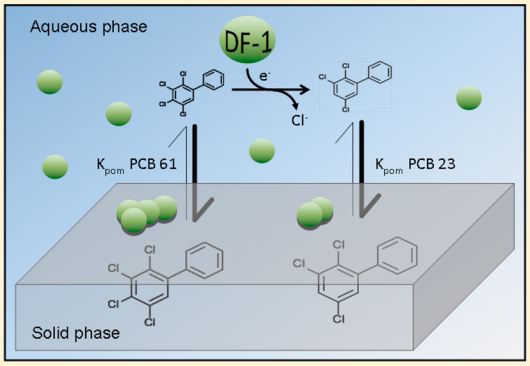
Supporting Information

ABSTRACT: The time required for a PCB-contaminated site to recover cannot yet be predicted due in part to lack of quantitative information on rates of PCB dechlorination in the porewater phase. We developed a method to measure rate of dechlorination in the aqueous phase at very low PCB concentrations. This approach utilizes a polymer functioning concurrently as a passive dosing system for maintaining a steady-state PCB substrate concentration in the water phase and as a passive equilibrium sampler to monitor the dechlorination product. Rates of dechlorination of 2,3,4,5-tetrachlorobiphenyl (PCB 61) to 2,3,5-trichlorobiphenyl (PCB 23) by an organohalide respiring bacterium, *Dehalobium chlorocoercia* DF-1, were measured over an environmentally relevant range of 1 to 500 ng L^{-1} in sediment-free medium using a high concentration of cells (>10^6 cells mL^{-1}). The results indicate that rate of dechlorination is a linear function of PCB substrate concentration below the maximum aqueous solubility of PCB 61 and occurs at concentrations as low as 1 ng L^{-1}. Demonstration of PCB 61 dechlorination at environmentally relevant concentrations suggests that low numbers of organohalide respiring bacteria rather than bioavailability accounts for low rates of dechlorination typically observed in sediments. Using passive samplers to measure the concentration of dissolved PCBs in the porewater combined with knowledge of congener-specific rates for organohalide respirer(s), it will be possible to project the in situ rate and final concentration of PCBs for a specific site after treatment by bioaugmentation.



INTRODUCTION

The extensive use of polychlorinated biphenyl (PCB) mixtures from 1929 to the 1970s and their release in the environment has led to ubiquitous and persistent distribution of these toxic compounds, even three decades after their manufacture was banned in the USA and other countries. They are found in air, water, sediment, and soil and bioaccumulate in organisms.[1,2] These compounds can be degraded by microbial communities naturally present in the environment through the combination of two processes: anaerobic reductive dechlorination (organohalide respiration) of higher chlorinated congeners and aerobic oxidative degradation of lower chlorinated congeners.[3] Natural attenuation of PCBs by reductive dechlorination is observed in the environment,[4−7] but the process is slow and factors affecting rates are not well understood. Since many commercial PCB mixtures such as Aroclors are highly chlorinated, microbial reductive dechlorination is often a rate-limiting step for PCB degradation in the environment.

There have been several efforts to identify factors affecting dechlorination and degradation activities in laboratory microcosms[8−12] with the goal of accelerating the natural processes in the environment. Enhanced dechlorination activity has been observed after biostimulation of indigenous populations by addition of electrons donors or electrons acceptors and/or bioaugmentation with isolates or enriched microbial consortia.[13−18] The first in situ stimulation of PCB degradation was reported almost 20 years ago,[19] and sequential anaerobic−aerobic bioaugmentation has been successfully applied at the laboratory scale.[20,21] More recent characterization and isolation of anaerobic dechlorinators has led to successful anaerobic dechlorination of PCBs by bioaugmentation in microcosms and mesocosms.[16,17] Inoculation of sediment mesocosms with an organohalide respiring bacteria *Dehalobium chlorocoercia* DF-1 showed that bioaugmentation not only stimulated PCB dechlorination of weathered Aroclor but also had an apparent synergistic effect on the indigenous organohalide respiring community.[17]

These results support the feasibility of using in situ bioremediation to treat PCB-contaminated sediments, but the time required for a PCB-contaminated site to recover cannot yet be predicted due in part to lack of quantitative information on rates of dechlorination, threshold PCB concentrations for dechlorination, and extrapolation of laboratory measured rates

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to field conditions. Although rates of dechlorination in sediments depend upon the specific activities and abundance of organohalide respiring microbes, in situ activity will also be influenced by the freely dissolved concentration of the PCBs. In previous studies, attempts to estimate dechlorination rates and the minimal threshold concentrations for organohalide respiration of PCBs involved adding high concentrations of Aroclors in the mg kg^{-1} range to sediment microcosms and assaying the rates of reductive dechlorination.[22−25] Results from these studies were not consistent as some reported a minimum concentration threshold of 40 mg kg^{-1} sediment,[25] which contrasts with recent reports that demonstrated dechlorination of 1.3 mg kg^{-1} weathered PCBs in sediments.[17] Most published evidence suggested that substrates in nonaqueous phase solids or liquids are unavailable for direct microbial uptake.[26] Therefore, one major challenge with relating dechlorination rate to PCB concentration in sediment is accounting for bioavailability differences caused by the association of PCBs to different organic matter types.[27] Recent studies have indicated the freely dissolved concentration of PCBs in the porewater may be a more appropriate metric that accounts for bioavailability to organisms.[28,29] Thus, a more relevant approach to understand the impact of chemical availability on dechlorination would be to measure dechlorination rates within a range of freely dissolved PCB concentrations typically observed in the environment. Accurate measurement and steady-state dosing of low aqueous concentrations of hydrophobic PCBs at ng L^{-1} levels has been challenging in the past. However, with recent advances in the use of polymer-phase passive samplers for measurement[30−33] and for passive dosing of compounds,[34] it is now possible to measure dechlorination rates for low, environmentally relevant aqueous concentrations.

In this study, we measured the dechlorination rate of the tetrachlorobiphenyl congener 2,3,4,5-tetrachlorobiphenyl (PCB 61) in the ng L^{-1} range, which is less than the aqueous solubility of 20 μg L^{-1}.[35] The range of concentrations in this study is lower than the average aqueous solubility range of 2.4−3000 μg L^{-1} reported for hepta- through monochlorinated homologue groups and is within the range that would be observed typically in contaminated sediment porewater.[36] Polyoxymethylene (POM) sheets were used as the passive dosing medium to deliver a known starting dissolved aqueous concentration of the congener and also as a passive sampling device to measure the concentration of the PCB dechlorination product as it was formed over time. The use of a well-characterized polymer with high PCB partitioning similar to that of sediment organic matter enabled us to work at very low concentrations that are difficult to detect directly in the aqueous phase. A similar principle was used previously with silicon O-rings to measure biotransformation rates of PAHs.[34] Here, we tested POM as a delivery polymer with the *Dehalobium chlorocoercia* DF-1, an anaerobic dechlorinator grown in sediment-free medium.[37] PCB 61, which was only dechlorinated to 2,3,5-trichlorobiphenyl (PCB 23), was tested to simplify the development and validation of the system. The dechlorination rate over a wide range of PCB concentrations (1.15−493 ng L^{-1} or 4−1689 pmol L^{-1}) was measured in order to determine the lower dechlorination limits for this bacterial model.

MATERIALS AND METHODS

Media and Growth Conditions. *Dehalobium chlorocoercia* DF-1 was grown anaerobically in mineral medium (E-Cl) as described previously[10] with modifications described below. Sodium formate (10 mM) was added as the electron donor, and 2,3,4,5-tetrachlorobiphenyl (PCB 61, purity >99%, Accustandard) or perchloroethene (PCE, purity >99%, Fluka) was diluted in acetone and added to medium (0.1% vol/vol) as the electron acceptor at final concentrations of 173 and 100 μM, respectively. *Desulfovibrio* sp. extract (1% vol/vol) was added as a growth factor,[37] titanium(III) nitrilotriacetate (0.5 mM) was added as a reductant,[37] and the sodium sulfide concentration was reduced to 0.01 mM. Cultures prepared for inoculum were grown statically at 30 °C in 160 mL serum bottles containing 50 mL medium sealed under N_2:CO_2 (80%:20%) with 20 mm Teflon-coated butyl stoppers (West Pharmceutical, Inc.) secured with aluminum crimp seals. PCE cultures were periodically purged (~20 days) with N_2/CO_2 for 2−3 s per mL headspace and replenished with PCE between transfers. Growth was monitored by sampling 100 μL of headspace with a gastight glass syringe (SGE, Inc.) every 10 days to measure the reductive dechlorination of PCE to trichloroethene (TCE) and dichloroethene (DCE). The headspace was analyzed on a HP6890 GC-FID (Agilent Technologies) equipped with a HP-5MS capillary column (30 m × 0.320 mm × 0.25 μm, Agilent Technologies). Chloroethene (CE) congeners were quantified using a 6-point calibration curve (0.4 to 200 mM) composed of PCE, TCE, *trans*-DCE, *cis*-DCE, and 1,1- DCE.

Preparation of Polyoxymethylene. Polyoxymethylene (POM, 77 μm; CS Hyde Co.) was cut into 50 mg strips (50.0 ± 0.6 mg, 3.5 cm × 1.4 cm) or punched into disks of 3 mg (3.3 ± 0.1 mg, diameter 6.23 mm). All POM was cleaned by sequential Soxhlet extraction with hexane followed by methanol, air-dried, and sterilized by autoclaving before use.[38]

Microcosm Preparation and Sampling. To prepare PCB-free inoculum for kinetic experiments, DF-1 grown with PCB 61 was serially transferred twice (4% vol/vol) in medium containing PCE, which could be purged from the culture prior to harvesting due to its high vapor pressure. One POM strip of 20−50 mg was added to absorb any residual PCB transferred with the inoculum. The culture was then inoculated into 500 mL (10% vol/vol) and periodically replenished with PCE as described above to an estimated cell density of 1.8×10^6 mL^{-1} (n = 3, sd = 7.8×10^6 mL^{-1}) based on enumeration of 16S rRNA gene copy number as described below. The culture was purged with N_2/CO_2 using a sterile gassing cannula to remove PCE and transferred to a sterile 250 mL Oakridge centrifuge bottle inside an anaerobic glovebox. The bottle was sealed under N_2/CO_2 prior to centrifugation at 26000g for 20 min. In an anaerobic glovebox, the supernatant was partially decanted and cells were resuspended in approximately 70 mL of supernatant. This PCB-free inoculum contained 5.9×10^7 (n = 4, sd = 3.9×10^7) 16s rRNA gene copies mL^{-1}.

Six concentrations of PCB 61_{pom} were tested in triplicate: 1.8×10^{-3}, 1.8×10^{-4}, 4.5×10^{-5}, 1.8×10^{-5}, 4.5×10^{-6}, and 1.8×10^{-6} mol kg^{-1} POM to achieve calculated aqueous concentrations of 2.9, 2.9×10^{-1}, 7.2×10^{-2}, 2.9×10^{-2}, 7.2×10^{-3}, and 2.9×10^{-3} nmol L^{-1}, respectively. A negative control without PCB was also included. POM strips were first pre-equilibrated in 50 mL of modified E-Cl medium in separate vials, each containing PCB 61 levels required for the six equilibrium aqueous concentrations described above. For each culture, two 50 mg POM strips each cut into 14 3.6 mg rectangular pieces and four 3 mg POM disks were added to 50 mL of sterile medium. After 30 days on an orbital shaker at 150

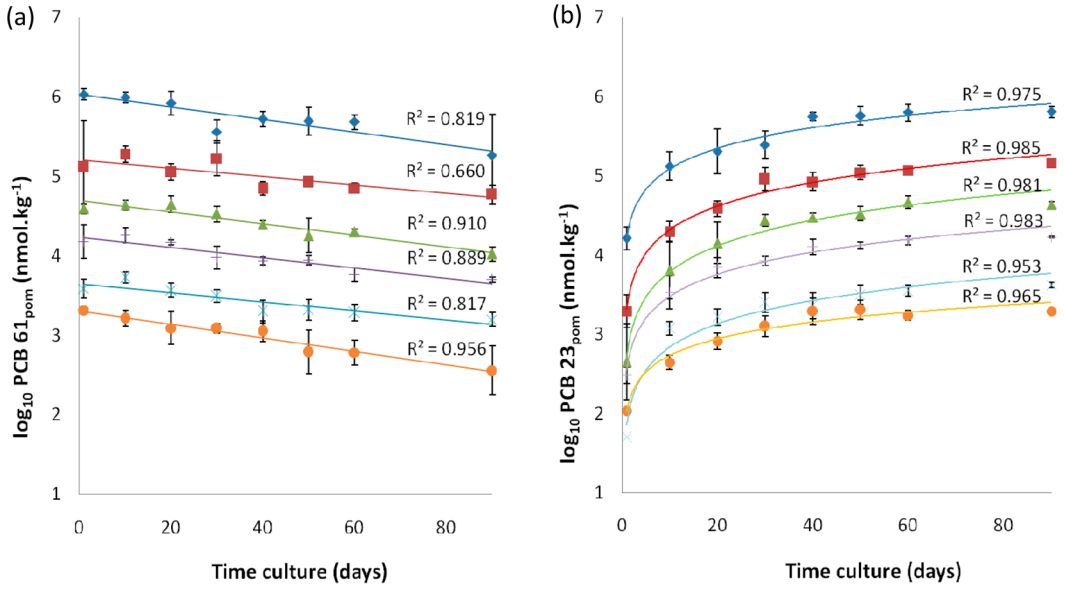

Figure 1. Dechlorination activity of PCB 61 measured in POM strips at different concentrations: (a) log PCB 61 (substrate) and (b) log PCB 23 (product) concentration measurements in POM strips, data points fitted with a logarithmic trendline. PCB 61 concentration in mol kg^{-1} POM: (♦) 1.0×10^{-3}, (■) 2.0×10^{-4}, (▲) 5.6×10^{-5}, (+) 2.1×10^{-5}, (×) 5.3×10^{-6}, (●) 2.4×10^{-6}.

rpm and 30 °C to achieve equilibrium of PCB 61 between POM and medium, the POM strips were transferred to 50 mL sterile medium without PCB 61. After equilibration for 30 days, 1 mL of DF-1 PCB-free culture was inoculated into each bottle containing the POM. The cultures were incubated in the dark at 30 °C on an orbital shaker at 150 rpm. During 60 days of incubation, a POM strip was removed every 10 days for PCB extraction and analysis (Supporting Information), a POM disk was removed every 30 days for DNA extraction, and 1 mL of culture was sampled every 30 days for DNA extraction. Additional POM strips were removed at days 90 and 150. At the end of the experiment, approximately 70% of POM remained (POM: medium (wt/vol) ratio decreased from 2:1 to 1.4:1).

DF-1 Enumeration by Quantitative PCR (qPCR). A 100 μL portion of culture was diluted into 1 mL of water Milli-Q Plus (Millipore) and then centrifuged at 18000*g* for 10 min at room temperature. After decanting, the cell pellet was resuspended in 100 μL of Instagene matrix (Bio-Rad Laboratories), and DNA was purified following manufacturer's instructions. Extracted DNA (50 μL) was stored in 1.5 mL microfuge tubes at −20 °C prior to enumeration by qPCR. To extract DNA from the surface of POM strips, a POM disk was added directly to 100 μL of Instagene matrix, mixed on a Vortex for 15 s, and then extracted as described above. DF-1 was enumerated by qPCR with IQ Sybr Green mix (Bio-Rad Laboratories) using primers 348F/884R[39] as described previously[17] with the following modifications. Each 25 μL reaction volume contained 1X iQ SYBR Green Supermix, 400 nM forward and reverse primers, and 2 μL of sample DNA. A plasmid containing the 16S rRNA gene of DF-1 was used for the standard curve (6 orders of magnitude: 3.4×10^3 to 3.4×10^9 16S rRNA gene copies mL^{-1}).

Bacterial Visualization on POM Strips. A POM strip and 2 mL of culture were aseptically transferred from cultures to a 5 mL crimp-neck serum glass vial and sealed with a Teflon septum. The glass vial was stored at room temperature prior to processing for fluorescence in situ hybridization (FISH) analysis. The bacterial cells present on the POM strips were fixed in 4% paraformaldehyde for 2 h, hybridized with the EUB-338 probe[40] as described previously[41] and observed with a Nikon Eclips E400 epifluorescent microscope (Nikon Corp.).

Estimation of Dechlorination Rates. The calculation of PCB dechlorination rate from a passively dosed system must take into account the sorption of PCB to the polymer phase and is described in the Supporting Information. In summary, the rate of change in the aqueous phase can be expressed as follows:

$$\frac{\mathrm{dPCB61_{aq}}}{\mathrm{d}t}\left(1 + \frac{m_s . K_d}{V_w}\right) = \mathrm{PCB61_{aq}} . k_b \quad (1)$$

where V_w is the volume of the aqueous phase, m_s is the mass of the solid phase (polymer or sediment), PCB 61_{aq} is the water phase concentration of PCB 61, k_b is the first-order rate constant (with respect to aqueous concentration), and K_d (K_{pom} 61) is the partition constant between the solid phase and water. The second term in parentheses represents the buffering capacity of the solid phase that attenuates the observed rate of dechlorination by suppressing the available PCB concentration in the freely dissolved phase. Previous work by Zhang et al.[26] recognized this influence of sorption on biodegradation kinetics and called it a bioavailability factor. Key assumptions in the model include constant biomass concentration, and faster exchange between the polymer and water compared to dechlorination rate. To predict dechlorination rates at other cell concentrations, the dechlorination rate was assumed to scale linearly with cell concentrations.

RESULTS

Validation of Approach for Measuring Kinetics of Dechlorination with POM. The sum of PCB 61 and 23 adsorbed to POM strips (PCB 61_{pom} and PCB 23_{pom}) remained relatively constant throughout the incubation period (coefficient of variation <0.4), with a decrease of PCB 61_{pom} and an increase of PCB 23_{pom} as expected (Figure 1 and Figure S1, Supporting Information). Background noise, caused by trace

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contaminants with retention times similar to PCB 23, was observed in the negative control without PCB ($(1.16 \pm 1.2) \times 10^{-7}$ mol kg^{-1}) but was negligible, averaging 5% of the lowest PCB concentration tested.

In separate partitioning experiments (data not shown), the experimental values obtained for the partition coefficient of PCB 61 between POM and media (K_{pom} 61) were similar to the predicted values obtained using eq S3 (Supporting Information) of Hawthorne et al.[42] over the range of concentrations tested. Therefore, the published K_{pom} values (K_{pom} 61 and K_{pom} 23) were utilized in this study to estimate PCB aqueous concentration (PCB 61_{aq} and PCB 23_{aq}) from the concentration in the POM strips (PCB 61_{pom} and PCB 23_{pom}). To test the accuracy of the estimated aqueous PCB concentration, PCBs were extracted directly from 1 mL of culture containing the highest initial PCB 61_{pom} concentration (1.8×10^{-3} mol kg^{-1}) at day 90, and the measured PCB_{aq} concentrations were compared with the calculated PCB_{aq} from PCB_{pom} (eq S2, Supporting Information). PCB 23_{aq} was detected at a concentration of $(2.05 \pm 0.5) \times 10^{-9}$ mol L^{-1}, which is equivalent to 5.40×10^{-4} mol kg^{-1} of PCB 23 in POM when eq S2 (Supporting Information) is applied. The average value of PCB 23_{pom} detected in POM was $(6.45 \pm 1.00) \times 10^{-4}$ mol kg^{-1}, which is within 20% of the value directly measured from the medium. The expected concentration of PCB 61_{aq} in medium at day 90 was 4.41×10^{-10} mol L^{-1}, which is beyond the detection limit for direct extraction.

Growth of *D. chlorocoercia* DF-1 Using POM as a PCB Reservoir. One milliliter of DF-1 containing 5.9×10^7 (n = 4, sd = 3.9×10^7) 16S rRNA gene copies mL^{-1} was inoculated into each bottle. The total number of 16S rRNA gene copies detected 24 h after inoculation was 4.3×10^7 (n = 21, sd = 3.2×10^7, rsd = 73%, $CI_{95\%} = [2.9 - 6.6 \times 10^7]$). For each PCB 61 concentration tested, total 16S rRNA gene copies detected in medium or on POM were plotted against time (Figure 2), and the slope of the linear function was recorded (Tables S1 and S2, Supporting Information). The slope ranged from −0.0042 to +0.0058 with a mean value of 0.00063 log 16S rRNA gene copies day^{-1} (n = 7, sd = 0.00373). No growth was observed except a slight increase (slope of 0.0058 log 16S rRNA gene copies day^{-1}, r^2 = 0.77) at the highest PCB 61 concentration and a slight decrease (slope of −0.0042 log 16S rRNA gene copies day^{-1}, r^2 = 0.73) at the lowest concentration tested. However, the relative standard deviation from the mean total 16S rRNA gene copies per concentration tested over time (n = 12, rsd = 60% for the highest, and rsd = 50% for the lowest concentration tested) did not exceed the relative standard deviation observed from the mean total 16S rRNA gene copies detected after 24 h (mean 16S rRNA gene copies for all PCB 61 concentrations, n = 21, rsd = 73%). The results indicate no significant growth of DF-1 over the course of each experiment. Interestingly, no change in DF-1 numbers was observed in the control without PCB, indicating that the microorganisms either survived without PCB or DNA persisted in the medium.

The numbers of attached cells on POM were compared to the planktonic cells in medium. Total DF-1 detected on POM strips was normalized to the total mass of POM in the culture and the number of planktonic cells mL^{-1} was normalized to the total volume of medium. Generally, greater numbers of cells were detected in medium than attached to POM strips with an average ratio media/POM of 10:1 (n = 21, sd = 8.8) at day 1. For all concentrations tested, a decrease of DF-1 on POM was observed over time that is partly attributed to POM removal

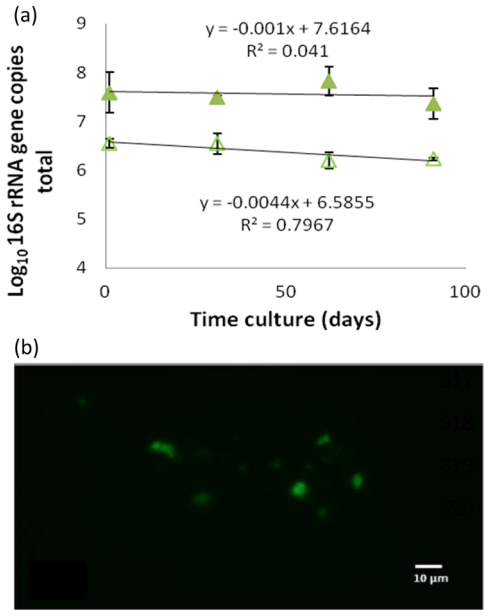

Figure 2. Growth of DF-1. (a) Monitoring over time in media and on POM strips at the intermediate PCB 61_{pom} concentration of 4.5×10^{-5} mol·kg^{-1} of POM. Genes copies of 16S rRNA gene copies were first estimated in medium and on POM by calculating the number of molecules detected, respectively, per milliliter of medium and per milligram of POM. Total 16S rRNA gene copies in media and on POM were then estimated by multiplying the value 16S rRNA gene copies·mL^{-1} to the amount of medium (mL) or POM (mg) remaining at the time point. Similar results have been obtained for the other concentrations tested: ▲, aqueous phase; △, on POM. (b) FISH image of DF-1 observed on POM strips at day 150 showing no contiguous distribution of cells (stained green) as a biofilm.

(30% POM removal; mean 16S rRNA gene copies decrease of 74% ± 33%). Attachment of DF-1 to POM was also monitored by FISH (Figure 2). Although some cell aggregation of 2−7 cells was observed on the POM surface, there was no evidence of contiguous biofilm formation with <1% of the total POM surface area covered by cells. An overall estimate of DF-1 population per 50 mL microcosm for the duration of the experiment was $(5.1 \pm 3.0) \times 10^7$ 16SrRNA gene copies.

DF-1 Dechlorination Activity (mol %). The concentration of PCB 23_{pom} product detected over time in POM strips was calculated as mol % following eq S1 (Supporting Information), and mol % of PCB 23_{pom} was plotted against time (Figure 3). Interestingly, the same dechlorination activity of 1.39% (n = 18, sd = 0.16%) mole of PCB 23 production per day was observed for all PCB 61 concentrations. No lag time was observed. There was an apparent plateau in activity observed at day 90 for the highest PCB concentration. For the other concentrations, the rate of dechlorination started decreasing at day 70 and a plateau in activity was not observed until day 150. For the highest PCB concentration, the maximum threshold of dechlorination did not exceed 70%. For other concentrations, 90% dechlorination activity was observed at day 150.

Estimation of Kinetics and Threshold Concentration for Dechlorination Activity by DF-1. The PCB 23_{pom} values were used to calculate PCB 23_{aq} values based on equilibrium partitioning between POM and the water phase as described above. PCB 23_{aq} was plotted against time after inoculation. Since no lag time was observed (Figure 3), the rate of

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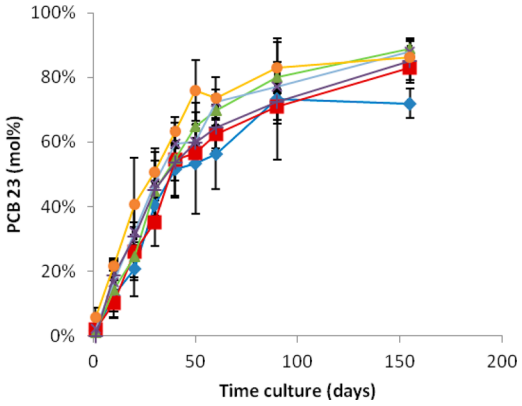

Figure 3. Dechlorination activity by DF-1 (mol %). Concentrations of PCB 61_{pom} in mol of PCB 61 kg^{-1} POM: (♦) 1.0×10^{-3}, (■) 2.0×10^{-4}, (▲) 5.6×10^{-5}, (+) 2.1×10^{-5}, (×) 5.3×10^{-6} (●) 2.4×10^{-6}.

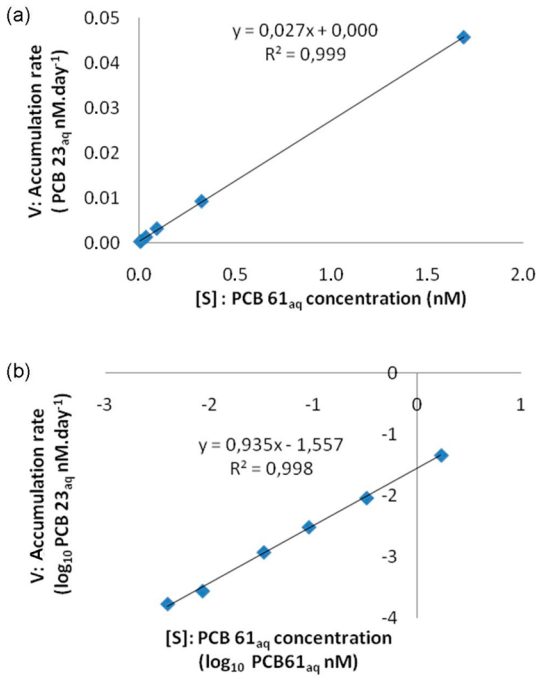

Figure 4. Accumulation rate of product PCB 23_{aq} plotted against concentration of the substrate PCB 61_{aq}: (a) normal scale, (b) logarithmic scale.

accumulation of PCB 23_{aq} (v) was determined as the slope of accumulation from day 1 to the beginning of the plateau phase (Figure S1, Supporting Information). The slopes had an average r^2 of 0.92 ± 0.06. The accumulation rate decreased as the initial concentration of PCB 61 decreased (Table 1). Nevertheless, an accumulation of product was observed even at the lowest PCB 61 concentration tested. When rates of accumulation were plotted against the concentration of PCB 61_{aq} substrate, a linear relation with r^2 of 0.99 was observed (Figure 4). No rate plateau was observed at the highest tested concentration of PCB 61_{aq} in the aqueous phase.

The aqueous phase dechlorination rates calculated based on eq S9 (Supporting Information) are shown in Table 1. The rate constants were within a factor of 2 for initial aqueous phase PCB 61 concentration, which ranged nearly 3 orders of magnitude. The true dechlorination rate (at a cell concentration of 10^6 cells mL^{-1}) was high (42 day^{-1}), indicating an average half-life of PCB 61 of 24 min when only freely dissolved PCB is present (no solid phase).

DISCUSSION

Dynamic passive dosing has been reported recently to measure biotransformation of hydrophobic organic chemicals (phenanthrene and fluoranthene) at low concentrations using a silicone O-ring.[34] Here, the approach was modified to provide both passive dosing of the substrate and passive sampling of the dechlorination product for measuring the dechlorination rate in anaerobic conditions at ultralow concentrations of a PCB using the polymer POM. Using this approach dechlorination of a tetrachlorobiphenyl in the aqueous phase was successfully monitored at subsaturation concentrations of 1.69×10^{-9} to 3.95×10^{-12} mol L^{-1}.

Impact of Population Density on Dechlorination Rate. Previous studies were conducted with a growing population of organohalide respiring microorganisms that dechlorinated a mixture of congeners (i.e., Aroclors). The dechlorination rates reported were the combined rates for different PCB congeners and organolide respiring microorganisms within the indigenous population. While providing valuable information on dechlorination rates from a given environment, dechlorination rates between environments cannot be compared, and simplified systems are necessary to elucidate rate of dechlorination per organohalide respirer and per congener. Such simplified systems are important to understand the global/apparent rates of dechlorination measured in the environment. Moreover, the prior studies were conducted with sediment particles likely to contain fractions with different partition coefficients and only the total PCB concentrations were measured. Since the aqueous PCB concentration was unknown the kinetics of

Table 1. Accumulation Rates of PCB 23_{aq} for Different Initial PCB 61_{aq} Concentrations[a]

initial PCB 61_{aq} concn (nM)	PCB 23_{aq} mean accum rate (nM day^{-1})	standard dev	data points	r^2	r^2 range	apparent dechlorination rate kb′ (day^{-1})	true aqueous phase dechlorination rate kb (day^{-1})[b]
1.69	4.58×10^{-2}	3.90×10^{-3}	4	0.89	0.69–0.97	2.71×10^{-2}	3.39×10^{-1}
3.23×10^{-1}	9.19×10^{-3}	8.08×10^{-5}	4	0.89	0.49–0.98	2.85×10^{-2}	3.56×10^{-1}
9.01×10^{-2}	3.08×10^{-3}	1.69×10^{-4}	4	0.97	0.77–0.86	3.42×10^{-2}	4.27×10^{-1}
3.33×10^{-2}	1.20×10^{-3}	6.66×10^{-5}	4	0.98	0.78–0.94	3.60×10^{-2}	4.51×10^{-1}
8.56×10^{-3}	2.78×10^{-4}	6.05×10^{-5}	4	0.84	0.26–0.91	3.25×10^{-2}	4.06×10^{-1}
3.95×10^{-3}	1.71×10^{-4}	2.50×10^{-5}	4	0.97	0.89–0.99	4.33×10^{-2}	5.41×10^{-1}

[a]PCB 61_{aq} and PCB 23_{aq} were both calculated from PCB 61_{pom} and PCB 23_{pom} respectively with eqs S2 and S3, Supporting Information. The rate of accumulation of PCB23_{aq} was determined by plotting PCB 23_{aq} against time and performing linear regression to calculate the rate. Each datum point is the mean of three replicates. [b]Conversion between apparent and true dechlorination rate is based on eq. S10 in the Supporting Information. The conversion factor value was 960 for the dechlorination experiment.

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dechlorination for bioavailable PCBs per (organolide respirer) cell could not be determined.

When dechlorination rates versus PCB 61 initial concentration in the current study were plotted, the relationship was linear suggesting first order kinetics as observed in previous studies,[22−25] but the rates observed were higher (up to 1000 fold) than rates reported previously (Table S3, Supporting Information). These rate variations can be explained in part by differences in number and types of dechlorinating microorganisms. Indeed, Cho et al.[25] reported that a 5-fold difference in rates observed between two independent studies was negligible after normalizing the rates with the number of dechlorinating microorganisms. When rates were normalized to the number of microorganisms, slope variations/differences could then be attributed to the cell (or more specifically the enzyme) affinity for their specific substrates. However, rate differences might also be explained by large differences in buffering capacity of the associated solids. Since only total PCBs were measured in these earlier studies and the aqueous PCB concentrations were unknown, the kinetics of dechlorination for bioavailable PCBs could not be determined.

In the current study, the system was simplified by using POM with well-known partitioning characteristics and a single organohalide respiring strain that was maintained at a steady-state concentration throughout the incubation period. Although organohalide respiration with PCBs is usually linked to growth,[37,43] dechlorination by DF-1 is decoupled from growth at high cell densities with no decrease in the dechlorination rate.[37] In the current study DF-1 was inoculated at a cell density of approximately 1×10^6 16S rRNA gene copies mL^{-1}, and no net population growth was observed either on the POM surfaces or in the aqueous phase throughout the incubation period. The thermodynamic cell yield of DF-1 based on the estimated cell yield from oxidation of formate[44] predicts that 2.4×10^{-8} mol of PCB 61 reduction is required to support one doubling of 6×10^7 DF-1 in a 50 mL microcosm. At the highest PCB 61 concentration tested only 3.3×10^{-11} mol of PCB 61 was reduced, which is consistent with the lack of detectable growth. Since no contiguous biofilms were observed on POM strips and the majority of cells were planktonic, the results indicate that the dechlorination activity observed in this study was the result of cells interacting directly with PCBs dissolved in the aqueous phase and were not influenced by localized activity on POM.

For a fixed number of dechlorinating microorganisms, the same mol % dechlorination rate was observed at all PCB 61 concentrations tested. Based on prior studies, PCB dechlorination rate would depend on cell number, since growth rate, PCB 61 concentration, and PCB dechlorination rate are tightly linked.[23] In this study, we did not observe a net decrease of cell numbers with a decrease of PCB concentration. DNA persistence of dead cells could explain the detection of constant number of dechlorinating microorganism. However, this latter explanation is unlikely since free DNA is rapidly degraded after cell lysis and although it can persist if adsorbed onto surfaces [45] no increase in DNA was detected on POM strips. Further experiments on actively dechlorinating microorganisms would be needed to determine whether the dechlorination rate is regulated by PCB concentration on a single cell level or as a population by a mechanism such as quorum sensing.

Dechlorination Rate and PCB Aqueous Concentration. A minimum concentration threshold for PCB 61 dechlorination was not detected with the size of inoculum used. The specific dechlorination rate was not related to PCB 61 concentration by a saturation function as reported previously[23] but was related to the aqueous PCB concentration by a linear function from the lowest to the highest PCB concentration tested. In a prior study the authors interpreted the saturation function as a rate-limiting step in dechlorination due to the PCB concentration dependent growth rate of dechlorinating microorganisms.[23] However, the earlier study included the combined effects of unknown electron donor concentrations, a range of dechlorination rates for different congeners in Aroclor 1242 and multiple desorption kinetics and aqueous concentration for different congeners and sediment fractions in the microcosms. In this study, the microcosms were sediment-free, the electron donor was not limiting (10 mM sodium formate) and a high cell density was used. The only rate-limiting step for reductive dechlorination of PCB 61 by DF-1 was PCB concentration in the aqueous phase, which was supplied at six starting concentrations below aqueous saturation using POM. Under these conditions dechlorination activity was observed at concentrations as low as the detection limit of 1.15 ng L^{-1}. This is the first confirmation of PCB dechlorination at such a low aqueous phase concentration. This freely dissolved PCB 61 value is equivalent to an estimated 0.015 mg kg^{-1} sediment for this congener assuming a 3% organic carbon fraction and using a standard correlation of $\log(K_{oc}) = \log(K_{ow}) - 0.21$ (PCB 61 log K_{ow} = 5.9).[46] In prior kinetic studies, dechlorination activity was reduced significantly or was undetectable at Aroclor 1242 or 1248 concentrations in the range of 10−40 mg kg^{-1} in sediment mesocosms,[22−25] where >45% of the estimated total mass most susceptible to dechlorination (i.e., flanked *meta* and *para* chlorines) were composed of individual congeners at concentrations greater than the estimated minimum of 0.015 mg kg^{-1} sediment tested in this study.[47] Rhee et al. [23] and Cho et al. [24] showed that inhibition of dechlorinating activity was linked to the inability of the organohalide respiring population to grow at the lowest PCB concentrations. Others have reported that stimulation of PCB organohalide respiring activity is only observed in microcosms containing Aroclors or other organohalides (i.e, "priming") at concentrations above the threshold level for growth[14,23,24,48] or by bioaugmenting sediment with a critical mass of PCB respiring bacteria.[17] The combined results of these studies suggest that bioavailability was not a factor in the apparent inhibition of activity at high PCB concentrations observed in earlier studies, but rather was due to low numbers of indigenous organohalide respiring microorganisms. Although dechlorination likely occurs with low cell numbers, the rates would be too low for short-term detection in many environments. Higher PCB concentrations would be required for sustained growth of the organisms to reach population levels where substantial dechlorination can be observed. The results of the current study support the feasibility of *in situ* bioremediation by inoculation of bacteria to PCB-impacted sediments to enhance abundance of the organohalide respiring bacterial population, which has the potential to treat porewater PCB concentrations down to 1.15 ng L^{-1}.

PCB Degradation in the Sediment Environment. Major challenges in translating PCB dechlorination observations in the laboratory to potential in situ remediation scenarios in the sediment environment have been the lack of data at environmentally relevant aqueous concentrations, adequate quantification of intrinsic rates of dechlorination in the aqueous phase, and accounting for the influence of sorptive solids. This

study demonstrates that by measuring the concentration of PCBs in the aqueous phase, the dechlorination rates can be determined and used in models to predict dechlorination rates in the sediment environment. Assuming a typical fine grained organic sediment matrix containing 30% solids, 3% organic carbon, and K_{oc} as described earlier, the solid phase buffering capacity term in eq 1 is 8.7×10^3 giving an apparent dechlorination rate for PCB 61 in sediment of 3.9×10^{-3} day^{-1}. This estimation assumes a cell density of 10^6 cells mL^{-1}, and the rates can be expected to decrease with decreasing cell densities as illustrated in Figure 5. While dechlorination rates at

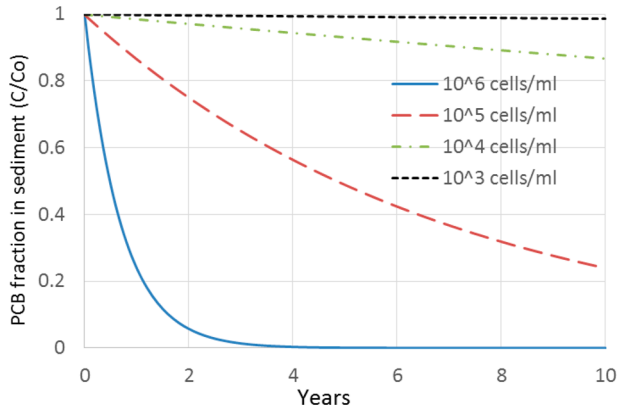

Figure 5. Simulation of dechlorination profiles for bioavailable PCBs in sediment for different cell densities based on aqueous phase dechlorination rates for PCB 61 experimentally determined in this study.

indigenous population densities of 10^1–10^3 cells mL^{-1} are typically low, which reflects the apparent environmental recalcitrance of PCBs, significantly greater rates of dechlorination can be achieved by bioaugmenting with densities of 10^5–10^6 cells mL^{-1} in sediment. Although these predictions are based on measurements of aqueous phase dechlorination rates of a single congener performed in the absence of sediment, they are consistent with the observed stimulation of dechlorination rates after bioaugmentation of sediment mesocosms containing a low concentration of weathered PCBs.[17,49]

The results of this study indicate that PCB organohalide respiring bacteria are capable of dechlorinating PCB 61 at environmentally relevant concentrations if present in sufficient numbers. This approach can be used to determine the rates of respiratory reduction for other PCB congeners within all homologue groups at environmentally relevant concentrations. Using passive sampling to measure the freely dissolved concentrations of PCBs in the porewater, rates of PCB desorption from the sediment matrix and distribution of PCB congeners combined with knowledge of the congener specificity of the organohalide respirer(s) used for bioaugmentation, it will be possible finally to project the rate and threshold levels of PCB dechlorination for a specific sediment site.

■ ASSOCIATED CONTENT

Supporting Information

Extraction and analysis of PCBs, calculations for determining dechlorination rates, tables showing growth rates of planktonic and attached DF1, table showing rates of dechlorination conversion and comparison with other studies, and figures showing rates of accumulation of PCB 23_{aq} for indicated initial concentrations of PCB 61_{aq}. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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