KINETICS OF POLYCHLORINATED BIPHENYL DECHLORINATION AND GROWTH OF
DECHLORINATING MICROORGANISMS

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Abstract—The present study has investigated a correlation between the kinetics of polychlorinated biphenyl (PCB) dechlorination and the growth of dechlorinating microbial populations. Microorganisms were eluted from Aroclor® 1248-contaminated St. Lawrence River (NY, USA) sediments and inoculated into clean sediments spiked with Aroclor 1248 at 10 concentrations ranging from 0 to 3.12 μmol/g sediment (0–900 ppm). The time course of PCB dechlorination and population growth were concurrently determined by congener-specific analysis and the most probable number technique, respectively. The specific growth rate was a saturation function of PCB concentrations above the threshold concentration (0.14 μmol/g sediment, or 40 ppm), below which no dechlorination or growth of dechlorinations were observed. The maximum growth rate was 0.20/d with a half-saturation constant of 1.23 μmol/g sediment. The yield of dechlorinating microorganisms showed a peak at 0.70 μmol/g sediment (200 ppm), with a value of 10.3 × 10^3 cells/mol Cl removed, and decreased below and above this concentration. The dechlorination rate (μmol Cl removed/g sediment/d) was a linear function of Aroclor concentration. Both the log of this rate and the maximum level of dechlorination were significantly correlated with growth rate. The biomass-normalized dechlorination rate (μmol Cl removed/g sediment/cell/d) was first order because of the exponential manner of the population growth. The first-order rate constant was a saturation function of Aroclor concentrations, with a maximum of 0.24/d (a half-life of 2.9 d) and a half-saturation constant of 1.18 μmol/g sediment, which are similar to the constants for growth. These results indicate that the dechlorination rate is tightly linked to the population growth of dechlorinating microorganisms.

Keywords—Polychlorinated biphenyl dechlorination kinetics

INTRODUCTION

The microbial reductive dechlorination of polychlorinated biphenyls (PCBs) is concentration-dependent [1–5]. Our recent detailed kinetic investigation [6] showed a linearity between dechlorination rate and the concentration of Aroclor® 1248 up to 200 ppm, the highest concentration employed, when expressed as mol Cl removed/g sediment/d. The 58-week study also revealed a clear threshold concentration (~0.14 μmol/g sediment; ~40 ppm) below which no dechlorination occurred. It also showed that the maximum extent of dechlorination was determined by the initial Aroclor concentration. Recent evidence suggests that a similar correlation may also exist between in situ sediment PCB concentrations and the extent of dechlorination in the Hudson River, USA [7].

However, the mechanisms underlying the concentration dependence are not clearly understood because the biomass of dechlorinating microorganisms was not determined in these studies. Since dechlorination took place over a period of weeks in these laboratory kinetic experiments, the size of the population may have also changed concurrently and at different rates with PCB concentrations. Our earlier studies of dechlorinating populations showed that the time course of dechlorination followed the population growth [8]. Without biomass information, therefore, it is not possible to determine whether the threshold concentration and the concentration dependence of the dechlorination rate simply reflect a difference in the number of dechlorinating microorganisms or enzyme affinity. It is also unclear whether the correlation between the initial PCB concentration and the maximum level of dechlorination is related to different population sizes or growth rates.

Therefore, the present study has investigated the dechlorination of Aroclor 1248 at various concentrations with a concurrent measurement of population size changes to determine any correlation between the kinetics of population growth and dechlorination.

MATERIALS AND METHODS

Slurry preparation and incubation

We collected uncontaminated sediments from the Grasse River, a tributary of the St. Lawrence River (NY, USA), and analyzed them before use to confirm the absence of PCBs. These sediments were air-dried, thoroughly mixed, and passed though a series of sieves with the final screen having a 150-μm opening. Individual batches of this sediment (40 g each) were coated with Aroclor 1248 in hexane to yield 10 different concentrations ranging from 0 to 3.12 μmol/g sediment (on a dry-wt basis; 0–900 μg Aroclor 1248/g sediment [ppm]) according to the procedures described previously [4]. The coated sediments were then weighed out in 20-g portions and placed into 100-ml serum vials (Wheaton, Millville, NJ, USA), sealed with Teflon®-lined stoppers (The West Company, Lionville, PA, USA), crimped with aluminum seals (Wheaton), and then purged for 20 min with N₂. The vials were brought into an

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anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA; N₂:H₂:CO₂ [85:10:5] atmosphere), where 90 ml of reduced and glass-fiber filtered (GF/C 4.25 cm; Whatman, Clifton, NJ, USA) St. Lawrence River water, containing sodium sulfide (1 mM) and resazurin (0.0001%), was added. The vials were removed from the chamber and autoclaved at 121°C for 40 min on 3 successive days. A 2-ml sediment slurry from a single batch of previous enrichment cultures was inoculated into each of the vials. Uninoculated vials of sediment at concentrations of 0.24 and 1.04 µmol/g sediment Aroclor 1248 served as controls. After inoculation, the vials were incubated statically at room temperature in the dark. All concentrations were set up in duplicate. The initial enrichment culture had been started with microorganisms eluted from sediments collected near the Reynolds Metals site in the St. Lawrence River and showed evidence of active PCB dechlorination at the time of transfer.

**Most probable number determination**

The number of PCB-dechlorinating microorganisms was enumerated at each concentration from one of the duplicate culture vials using a five-tube most probable number (MPN) procedure [8]. We analyzed only one of the duplicates because of the logistic difficulties of sample analysis. Even with MPN analysis on only one of these replicates, each time point produced 250 sediment vials to incubate and analyze. The MPN reflects the total number of phenotypic expressions regardless of genetic differences among dechlorinating microorganisms. Although it is a coarse estimate, it is the only method currently available for measuring the abundance of total PCB dechlorinators, with no universal molecular probes or biochemical markers yet available. Our previous investigations also have demonstrated that the population growth of microorganisms in PCB-contaminated sediments can be followed by this method [8].

The MPN test and dilution vials were prepared in 20-ml headspace vials (Wheaton) with 0.25 g of Grasse River sediments and 9 ml of reduced synthetic minimal media [9]. No sodium sulfide was added to the MPN vial media. The sediments in the MPN test vials were spiked with a mixture of 2,3,4- and 2,5,3'-chlorobiphenyls (150 µg/g sediment of each congener), while the sediments in the dilution vials were unspiked. A dilution series (10⁻¹ to 10⁻⁸) of each sample was prepared by serially transferring a 1-ml portion of sediment slurry using a sterile syringe with a 20-gauge needle immediately after vortexing. From each dilution within the 10² to 10⁸ range, five MPN test vials were inoculated with 1 ml of the sediment slurry. The vials were then incubated statically at room temperature in the dark for 10 weeks.

After 10 weeks of incubation, the entire contents of each of the 250 vials used for the MPN series at a given time point was extracted to determine PCB dechlorination (see below). Vials were considered positive for dechlorinators if test congeners were dechlorinated. The MPN of PCB dechlorinators was calculated from the number of positive vials in consecutive dilutions using an MS-DOS QBASIC program [10] and normalized per gram dry weight of sediment.

The specific growth rate (µ) was calculated using the formula

\[ \mu = \ln(x_1/x_2)/dt \]

where \( x_1 \) and \( x_2 \) are the cell number at times (t) 1 and 2, respectively.

**Sampling and PCB analysis**

To follow the time course of dechlorination at each concentration, the batch incubation vials were sampled every two weeks. The vials were brought back into the anaerobic chamber, decapped, and a 3-ml sample was removed while the slurry was being continuously stirred. The sediment samples were then mixed with 2.0 g of Celite® 521 (Aldrich Chemical, Milwaukee, WI, USA) and loaded into a stainless steel extraction cell. Extractions were performed with acetonitrile:methane (1:1 v/v) for 15 min using an Accelerated Solvent Extractor system (Dionex, Sunnyvale, CA, USA) operating at 100°C and 2,000 psi (U.S. Environmental Protection Agency Method 3545). Distilled water (10 ml) was added to the solvent extract for phase separation and the hexane layer was placed into a flask with sodium sulfate. The hexane extracts were then treated with a tetrabutylammoniumhydrogen sulfite reagent to remove elemental sulfur [11] and cleaned up on a 4% deactivated Florisil® column (ITC, Hunt Valley, MD, USA). To monitor dechlorination in the MPN vials, the entire content of each vial in the dilution series was extracted as described above.

Congener-specific PCB analysis was performed on one of two Hewlett Packard 5890 gas chromatographs (GC; Hewlett-Packard, Avondale, PA, USA), each equipped with a 63Ni electron-capture detector, autosampler, splitless injector, and a computerized data acquisition system (Chrom Perfect, Justice Innovations, Mountain View, CA, USA), following the procedures outlined in Sokol et al. [12]. Aroclor 1248 was separated on a 60-m Rtx®-5 capillary column (Restek, Bellefonte, PA, USA) while an Apiezon-L column (Restek) was used to analyze the 2,3,4- and 2,5,3'-chlorobiphenyl samples from the MPN vials. Each GC system used nitrogen as the carrier gas and argon:methane (95:5) as the make-up gas; the injector and detector temperatures were both 300°C. On the GC containing the Rtx-5 column, the initial oven temperature of 100°C was maintained for 2 min and then increased at a rate of 10°C/min to 160°C with a 1-min hold, and finally to 250°C by 2°C/min. This final temperature was maintained for 21 min. The PCB congeners in the extract were identified and quantitated using a calibration standard containing a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 µg/ml of each in hexane). Peaks were identified and calibrated as previously described [4–6,8,12–14]. The analysis resolves 98 peaks representing 127 congeners. The temperature program for the Apiezon-L column consisted of an initial oven temperature of 90°C, which was increased at a rate of 10°C/min to 150°C, then at a rate of 3°C/min to 220°C, which was maintained for 8.67 min, for a total run time of 40 min. The PCB extracts run on the Apiezon-L column were identified and quantitated with a calibration standard composed of a mixture of 47 individually weighed authentic single congener standards (AccuStandard, New Haven, CT, USA; 99% purity).

The calibration standards were run after every sixth sample for recalibration as part of quality assurance/quality control. A dilute to match procedure [15] was used to ensure that all samples were analyzed within the linear range of the calibration standard. Uninoculated PCB-spiked sediment controls, set up at the beginning of the experiment and sampled at every time point, were used to monitor extraction efficiency. Method blanks were included with each set of samples extracted. Precision on the GC was also ensured by running duplicate samples. After a GC run, each calibration standard was checked to assure proper congener peak assignment and quantitation. For a run to be accepted, standards had to be within ±10% of one another. In addition, every GC chromatogram was manually reviewed to verify peak assignment and to ensure that the resolution, shape, and automatic baseline selection for each
PCB dechlorination and dechlorinating microorganisms

Fig. 1. (A) The time course of dechlorination and growth of dechlorinators at 1.04 μmol/g sediment (300 ppm) Aroclor® 1248. The time course at other concentrations also followed the same pattern. No growth was observed below the threshold concentration for dechlorination (0.14 μmol/g sediment or 40 ppm). (B) A Michaelis–Menten fit of the specific growth rate of dechlorinating microorganisms to Aroclor 1248 concentrations (p < 0.05).

peak were appropriate. When required, manual processing of peaks was performed to rectify any problems.

The PCB congeners in each sample were calculated and expressed as mole percent. The average number of total Cl per biphenyl and the average number of ortho, meta, and para Cl were individually calculated from the product of the average number of chlorines and the molar concentration for each peak divided by the total molar concentration summed over all peaks. All calculations were based on the conservation of the biphenyl moiety and the assumption that coeluting congeners were present in equal proportions [12,16].

RESULTS

Growth of dechlorinating microorganisms

To determine the relationship between dechlorinating microorganisms and dechlorination rate, the time course of their growth was determined concurrently with the measurement of dechlorination using the MPN technique. The dechlorinating populations exhibited exponential growth that mirrored the time course of dechlorination. Figure 1A shows a typical time course of population growth and dechlorination at a concentration of 300 ppm (1.04 μmol/g sediment).

No PCB dechlorination was detected in sediments with Aroclor concentrations below 0.14 μmol/g sediment (40 ppm), a threshold level. Below this threshold, there was also no growth of dechlorinating populations (Fig. 1B). At these sub-threshold concentrations, the MPN number remained at or below the inoculation level of 6.6 × 10^4 cells/g sediment throughout the incubation period. However, at concentrations above this threshold level, population growth was a function of PCB concentrations, with the number increasing one to two orders of magnitudes (5.8 × 10^5 cells/g sediment to a maximum of 1.08 × 10^7 cells/g sediment).

The semilog plot of MPN numbers (specific growth rate) was linear (p < 0.05) at all concentrations above the threshold similar to that shown in Figure 1A. This linear slope, or the specific growth rate (d^-1), increased with Aroclor concentrations up to a concentration of 1.04 μmol/g sediment (300 ppm) and appeared to level off at higher concentrations of 2.08 (600 ppm) and 3.12 μmol/g sediment (900 ppm), although the error range at these high concentrations is relatively large (Fig. 1B). The Michaelis–Menten equation was fit to the growth curve (nonlinear fit; p < 0.05), and the maximum growth rate was 0.20 (±0.062)/d and the half-saturation constant was 1.23 (±0.81) μmol/g sediment.

When yield (the number of dechlorinators per mol Cl removed) was estimated using the maximum population size and the maximum amount of Cl removed at each PCB concentration, it was approximately 3.2 × 10^12 cells/mol Cl removed at concentrations of 0.24 and 0.42 μmol/g sediment (70 and 120 ppm) and increased threefold to a maximum of about 10.3 × 10^12 cells/mol Cl removed at a concentration of 0.70 μmol/g sediment (200 ppm) (Fig. 2). The yield (9.3 × 10^12 cells/mol Cl removed) and growth rate at 1.04 μmol/g sediment Aroclor 1248 were very close to that found at this concentration in our previous study [8]. Interestingly, the yield then decreased with the concentration to 2.8 × 10^12 and 1.1 × 10^12 cells/mol Cl removed at concentrations of 2.08 and 3.12 μmol/g sediment (600 and 900 ppm), respectively.

Growth rate versus dechlorination rate and the final extent of dechlorination

Similar to our previous study [6], no dechlorination was detected below 0.14 μmol/g sediment Aroclor 1248 (40 ppm) even after one year of incubation. Above this threshold, the time course of dechlorination at each concentration consisted of an initial lag phase, followed by a relatively rapid dechlorination and a plateau with little further change (Fig. 3). The dechlorination rate determined as the slope of the rapid phase (μmol Cl removed/g sediment/d) was a linear function of con-
concentration ($r^2 = 0.99; p < 0.0001$), but the linear relationship extended beyond 0.70 μmol/g sediment (200 ppm), the highest level in the earlier investigation [6], up to 3.12 μmol/g sediment (900 ppm) (Fig. 4). In both studies, the line intercepted the PCB concentration near 0.14 μmol/g sediment (40 ppm).

When the log of dechlorination rate (ln[μmol Cl removed/g sediment/d]) was plotted against the growth rate, it was linearly related to growth rate ($p < 0.05$) (Fig. 5A). This relationship and the absence of growth below the threshold concentration mentioned above strongly indicate that dechlorination is growth linked.

The final extent of dechlorination, represented as the plateau in Figure 3, was also significantly correlated to growth rate (Fig. 5B). As in our previous study [6], the final extent of dechlorination increased with the initial Aroclor 1248 concentrations in a saturation manner.

**Biomass-normalized dechlorination rate**

Since the dechlorinator populations grew during the rapid phase of dechlorination, the amount of Cl removed was normalized by the number of dechlorinators at each time point to determine the biomass-normalized kinetics. The time course of the dechlorinator-normalized dechlorination (μmol Cl/g sediment/cell) was first order. This exponential decrease in dechlorination per dechlorinating microorganism is almost entirely due to the exponential pattern of dechlorinator growth. When the reaction constant ($k$) was calculated, it increased with increasing PCB concentration from 0.024 to 0.196/d (with a half-life [$t_{1/2}$] of 29 and 3.5 d, respectively) and was related to PCB concentration by a saturation function. A fit of the Michaelis–Menten function (nonlinear fit; $r^2 = 0.90; p = 0.03$) yielded a maximum $k$ value ($k_{max}$) of 0.24 (±0.07)/d ($t_{1/2} = 2.9$ d) and a half-saturation concentration ($K_c$) of 1.18 (±0.77) μmol/g sediment (Fig. 6). These kinetic constants are not significantly different from those for the growth rate. Therefore, the rate-limiting step in dechlorination is the concentration-dependent growth rate of dechlorinating microorganisms.
One explanation for the absence of population growth below the threshold concentration is that the bioavailable PCBs in this concentration range may have been below the minimum level required to support growth. Since the bioavailable fraction is determined by many factors, such as organic content, sediment particle size distribution, aging, and the solubility of contaminants [25–28], it is not possible to determine what the actual minimum bioavailable concentration for growth is. Nonetheless, it seems that the biomass of dechlorinating microorganisms may indicate the dechlorination potential of a given site.

The growth of dechlorinators is very slow in absolute terms. Under laboratory conditions, the maximum specific rate was 0.20/d, with a doubling time of 3.47 d. The half-life of biomass-normalized dechlorination also ranged from 3.5 to 29 d, depending on the PCB concentration. However, when considered in terms of the historical time scale of PCB pollution in the St. Lawrence River (the original source of these dechlorinating microorganisms), these values seem rather fast. There is no data available on the in situ dechlorination rate during the stage corresponding to the rapid phase in the laboratory cultures. Even if this value were available, it is also important to know whether the dechlorination process in contemporary sediments is in the rapid or plateau stage in order to predict the significance of this natural attenuation.

The in situ dechlorination rate in the Thompson Island Pool of the Hudson River, determined recently by McNulty [29], appears to be extremely slow; the half-life of dechlorination (mol Cl/mol PCB) ranged from 29 to 100 years when it was calculated between 1983 and 1991 using 137Cs-dated cores. Another recent investigation of Hudson River sediments also suggested that dechlorination might have reached an end point [7]; a regression analysis of more than 250 sediment samples showed that there was no correlation between the age of the sediment and the level of dechlorination.

It is interesting to note that, although the growth rate increased with PCB concentrations in a hyperbolic manner, the yield of dechlorinating microorganisms per mol Cl removed showed a peak at between 200 and 300 ppm and dramatically decreased at higher concentrations. Although the reason is unclear, the decrease could be the toxic effect of PCBs at high concentrations, which does not manifest in growth responses.

Our previous study [8] demonstrated that, when dechlorination reached a plateau, the size of the dechlorinating population declined, probably because the dechlorinatable congeners had been exhausted. When we determined the size of the PCB dechlorinating populations in contaminated sediments from the Hudson and St. Lawrence Rivers, their numbers were generally lower than those found below the threshold concentration for dechlorination in the present kinetic study (G.-Y. Rhee, unpublished data). It was also noteworthy that the number in the lower Hudson River (below Poughkeepsie), where in situ dechlorination is not as extensive as upriver, was less than the sensitivity limit of the technique (<10^2 cells/g sediment).

As in our earlier study [6], the plateau level in the dechlorination curve, an apparent end point of dechlorination, is concentration dependent in the present study (data not shown). This concentration dependence of the plateau appears to be due at least in part to the fact that different subpopulations of dechlorinators are selected by PCB concentrations; since certain meta-dechlorination was observed at high concentrations but not low concentrations [6], it seemed that microorganisms
for this type of dechlorination require high concentrations for growth. When PCB-dechlorinating microorganisms were fractionated by the dilution technique in sediments contaminated with a high level of Aroclor 1248 (~300 ppm), meta-dechlorinators were found only in low dilutions, indicating that these subpopulations were present in lower numbers than other dechlorinators [30].

The dechlorination rate before biomass normalization was significantly higher in the present study when compared with our earlier results [6]. This difference could stem from various factors. For example, the inoculum, a mixed culture of sediment microorganisms, may well have been different between the two investigations although sediments from the same site were used. The core was kept at 2 to 4°C in a cold room and the present inoculum was taken approximately 8 months after the start of the earlier study. Second, the rate difference could be due to a difference in the population size. However, it is interesting to note that there was no significant difference in the maximum extent of dechlorination between the two studies. In other words, the plateau level at a given concentration was the same. The threshold concentration has also turned out to be identical at about 40 ppm [6].

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