SETAC PRESS Environmental Toxicology and Chemistry, Vol. 15, No. 10, pp. 1738–1744, 1996

Printed in the USA
0730-7268/96 $6.00 + .00

AN INVESTIGATION OF FACTORS LIMITING THE REDUCTIVE DECHLORINATION OF POLYCHLORINATED BIPHENYLS

XIA LIU, ROGER C. SOKOL, O.-SEOB KWON, CHARLOTTE M. BETHONEY and G.-YULL RHEE*
School of Public Health, State University of New York at Albany and Wadsworth Center,
New York State Department of Health, Albany, New York 12201-0509, USA

(*Received* 27 *November* 1995; *Accepted* 22 *April* 1996)

Abstract—A study was conducted to determine whether the apparent limitation of dechlorination of Aroclor 1248 was due to bioavailability of polychlorinated biphenyls (PCBs) or an accumulation of metabolic products. After 15 weeks of laboratory incubation, sediment microorganisms from the St. Lawrence River reduced the total number of chlorines in Aroclor 1248-spiked sediments by 33%. However, dechlorination apparently leveled off with a significant number of *meta*- and *para*-chlorines still remaining, showing no further change out to 30 weeks. When these sediments were incubated an additional 18 weeks with either fresh medium or the original supernatant, no additional dechlorination was found in either treatment. Similarly, dechlorination was not inhibited in freshly spiked Aroclor 1248 sediment slurries made with the old supernatant. Addition of the nonionic surfactant Tween 20, at a concentration below the critical micelle concentration that increased PCB desorption, also failed to enhance dechlorination of the plateau sediments. The extent and final congener pattern in all treatments were nearly identical. Therefore, the termination of dechlorination at the plateau level was not due to PCB bioavailability or accumulation of inhibitory metabolic products. These results strongly suggest that the cessation of dechlorination at the plateau was due to the accumulation of daughter congeners with chlorine substitution patterns that were not amenable to further dechlorination by the present microbial consortium.

Keywords—Polychlorinated biphenyls Reductive dechlorination Lower limit Inhibition Bioavailability

INTRODUCTION

Polychlorinated biphenyls (PCBs) are degraded by sediment microorganisms in both aerobic and anaerobic environments through oxidation reactions and reductive dechlorination, respectively. Reductive dechlorination of PCBs is well documented in both natural and artificially spiked sediments [1–9]. Failure to isolate individual dechlorinating organisms suggests that PCB dechlorination may involve interactions of different sediment microbial populations. The biotransformation process generates lower-chlorinated products from higher-chlorinated congeners primarily through the selective removal of *meta*- and *para*-chlorines, which in turn is determined by the pattern of chlorine substitution [10]. The less chlorinated products are generally more amenable to aerobic biodegradation processes. However, a complete transformation to biphenyl has not been reported for Aroclor mixtures primarily because *ortho*-chlorines were not removed [2,4].

A typical time course of PCB dechlorination in the laboratory has an initial lag followed by a relatively rapid dechlorination and then a plateau showing no further change with continued incubation [2,7,9]. However, this process often stops at a point where a significant number of *meta*- and *para*-chlorines still remain. From a bioremediation point of view, it is important to understand what sets this apparent lower limit of dechlorination so that it can be further reduced.

Various factors can be postulated to explain the plateau; they include the bioavailability of sediment-bound PCBs and accumulation of metabolic products that are inhibitory to dechlorination. The degradation of hydrophobic organic compounds (HOCs) such as PCBs is often limited by their bioavailability to the degrading microorganisms. The HOCs tend to have high octanol–water partition coefficients and thus are strongly sorbed to sediment and soil particles. Such partitioning may make HOCs less available to microorganisms for degradation [11]. For example, the degradation of 1,2-dibromoethane was limited by interparticle diffusion [12] and the kinetics of α-hexachlorocyclohexane biodegradation were correlated to the retarded diffusion from inside solid particles [13]. Biodegradation of (2,4-dichlorophenoxy)-acetic acid [14], naphthalene [15], and other polycyclic aromatic hydrocarbons (PAHs) [16] was also reduced by the sorption of these compounds to sediment or soil particles. Bioavailability may also decrease with increasing age of sediment contamination [17–19]. However, addition of surfactants can increase the aqueous concentration of HOCs, thereby increasing bioavailability. Surfactant-induced desorption promoted the aerobic degradation of PCBs [20] and other HOCs such as phenanthrene and biphenyl [21,22].

In addition to bioavailability, the formation and accumulation of metabolites can decrease and even inhibit degradation. End product inhibition is well known for the degradation of many HOCs [23,24], including aerobic degradation of PCBs [25–27]. Biodegradation of HOCs may also involve a consortium of different microbial populations; during the degradation of chemical mixtures, one substrate could be converted to products that are inhibitory to a population acting on the second, as was found in the mineralization of a phenol and *p*-nitrophenol mixture by two different strains of *Pseudomonas* [28]. The anaerobic degradation of 2,4-dichlorophenol into methane and CO_2 by sediment microorganisms also required sequential actions involving at least five different types of organisms [29].

The purpose of the present study was to determine whether the apparent limitation of dechlorination of Aroclor 1248 was due to the bioavailability of PCBs or an accumulation of metabolic products. The current study examined the effect of sur-

* To whom correspondence may be addressed.

factant addition and exchanging the overlying media on dechlorination once the transformation process reached a plateau.

MATERIALS AND METHODS

Sediments free of PCBs collected from the Grasse River, a tributary of the St. Lawrence River near Massena, New York, were air-dried and passed through a 150-μm sieve. The sediments were coated by mixing with Aroclor 1248 (300 μg/g; AccuStandard, New Haven, CT, USA) in a hexane solution. After the hexane evaporated, the sediments were made into a slurry containing 10% sediment (w/v on a dry-weight basis) with reduced mineral media, as previously described [4]. Batch incubations were prepared by dispensing 25-ml portions of the slurry into serum vials (50 ml) and sealing them with Teflon®-coated stoppers and aluminum crimp caps. All vials were autoclaved and, except for the controls, inoculated with the supernatant of a slurry prepared from St. Lawrence River sediments collected adjacent to the Reynolds Metal site [8]. When the extent of dechlorination, measured as the total chlorines per biphenyl, reached a plateau showing no further change, the overlying media were removed and the sediments were divided into two sets. One set received freshly reduced mineral media and the second set of sediments had the original supernatant replaced. In addition, sediments newly coated with Aroclor 1248 received either fresh mineral media or the overlying media from the sediments that had stopped dechlorinating. These treatments were then inoculated with microorganisms eluted from the plateau sediments.

To investigate the effect of PCB bioavailability on dechlorination at the plateau, the nonionic surfactant Tween 20 (polyoxyethylene sorbitan monolaurate) was added (0.05% v/v) to a subset of plateau sediments after the overlying media were exchanged with an equal volume of fresh media. A dechlorination assay was used to test for the presence of competent microorganisms at the plateau. A slurry (0.5 ml) of sediments from the plateau vials was inoculated into 234-chlorobiphenyl (CBP)-spiked sediments, prepared in the same manner as described above. This congener was selected because it is readily dechlorinated and has been used to determine dechlorination activity in earlier studies [4,5,8].

Vials were sampled at regular intervals in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA). A 3-ml aliquot was removed using a Pasteur pipette with a cutoff tip while the slurry was continuously mixed on a magnetic stir plate. Immediately after sampling, the vials were recapped and returned for further incubation.

The aliquots were extracted with acetone and hexane by ultrasonication, treated with tetrabutylammonium hydrogen sulfate and sodium sulfite to remove elemental sulfur, and cleaned up on a 4% deactivated Florisil column [4]. Hexane extracts were analyzed with a gas chromatograph (Hewlett-Packard Model 5890 series II) equipped with an electron capture-detector and a Rtx®-5 fused-silica capillary column (60-m length, 0.1-μm film thickness, 0.25-mm i.d.; Restek Corp, Bellefonte, PA, USA). An equivalent mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 μg/ml each in hexane) was used as a calibration standard. This standard mix was also used for quality assurance/quality control to check instrument performance, reproducibility, and sensitivity. Chromatographic data were acquired and processed on a personal computer using ChromPerfect chromatography data system (Justice Innovations, Mountain View, CA, USA). The extent of dechlorination was expressed as the average number of total chlorines per biphenyl and calculated as the product of the number of chlorines and molar concentration for each peak divided by the total molar concentration summed over all peaks. Coeluting congeners were assumed to be present in equal proportions [2,8].

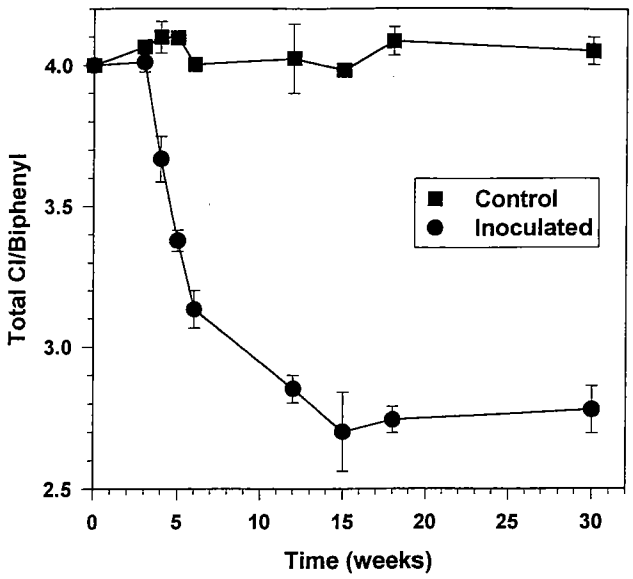

Fig. 1. Time-course of Aroclor 1248 dechlorination in control (■) and inoculated (●) sediments expressed as total chlorine/biphenyl.

RESULTS

The time course of dechlorination, expressed as the total chlorines (Cls) per biphenyl, showed that the Aroclor 1248-spiked sediments were rapidly dechlorinated after a 3-week lag period (Fig. 1). After 4 weeks of incubation, 8% of the total Cls were removed and by 6 weeks approximately 22% were removed. Analysis after 15 weeks showed that the total number of Cls per biphenyl was reduced to about 2.7 or an overall 33% reduction from the original Aroclor 1248. No further dechlorination was observed for up to 30 weeks of incubation. This dechlorination "plateau" appeared to represent the apparent endpoint of dechlorination.

Dechlorination occurred predominantly from the *meta*- position. After 4 weeks of incubation, the chromatographic pattern showed strong decreases in *meta*-rich parent congeners such as 25-2′, 25-2′5′, 23-2′5′, 236-3′+23-2′4′+34-4′, 23-2′3′, 25-3′4′, 236-2′5′ and 245-2′5′-CBP with a corresponding increase in the peaks for 2-2′+26, 26-2′, 2-4′+23, 24-2′+4-4′, 23-2′+26-4′ and 25-4′+24-4′-CBP (Fig. 2). As a result of this strong *meta*-dechlorination, several *para*-substituted congeners were predominant products, such as 25-4′+24-4′-CBP and 2-4′+23-CBP. As dechlorination proceeded there was an even greater reduction in the parent congeners such that by 18 weeks the major dechlorination products were 2-2′+26, 2-4′+23, and 25-4′+24-4′-CBP, which together comprised more than 57% of the total molar concentration (Fig. 2).

After 24 weeks of incubation the sediments showed no sign of further dechlorination. At this point the presence of active dechlorinating microorganisms was tested by inoculating a slurry of sediments from these plateau vials into sediments spiked with 234-CBP. Analysis of these assay sediments after 6 weeks of incubation demonstrated the production of 24-CBP from 234-CBP, indicating the presence of competent organisms. To determine whether this apparent lower limit was due to PCB bio-

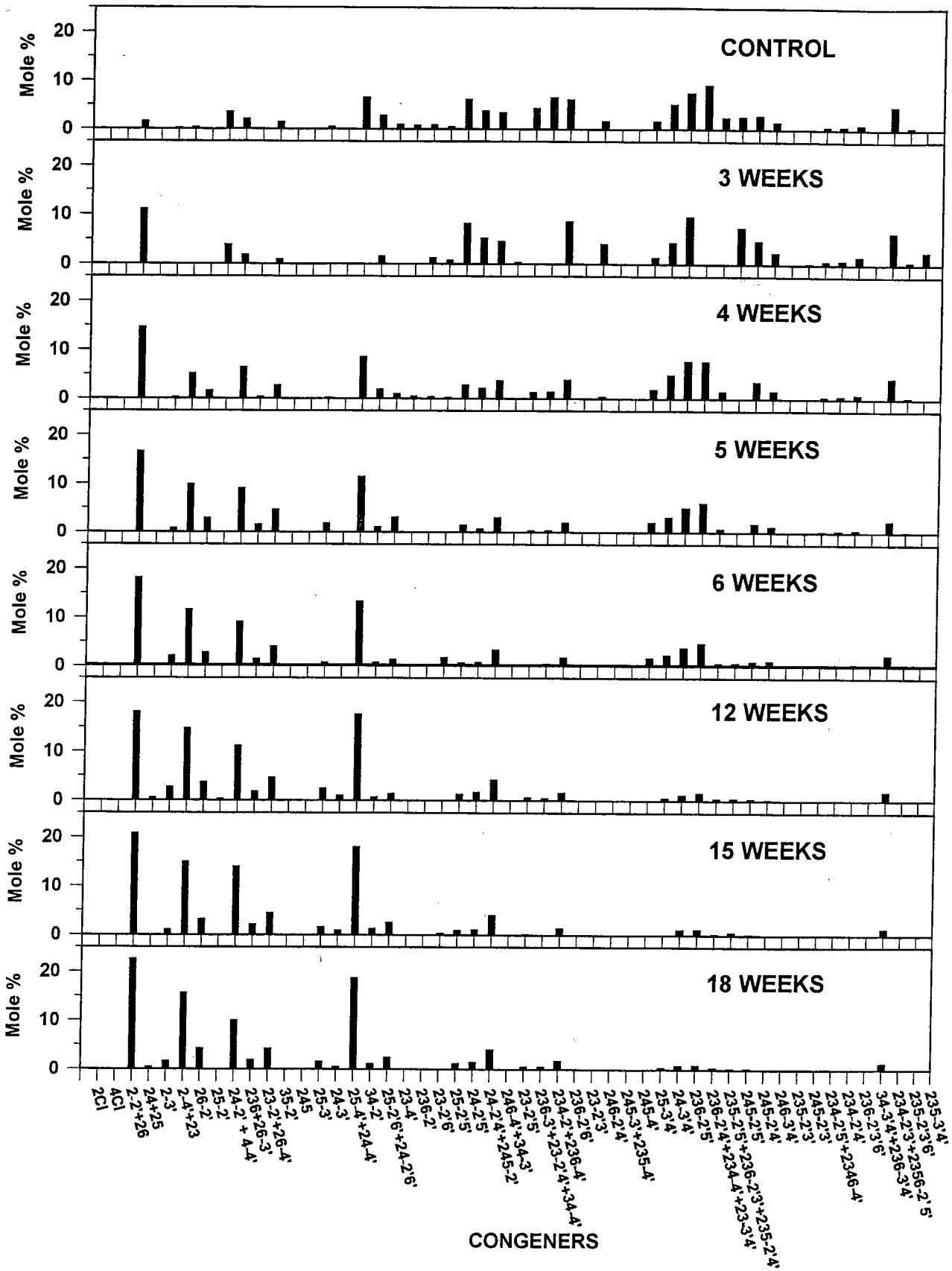

Fig. 2. Mole percentage of Aroclor 1248 congeners at each time point over the 18-week period of incubation.

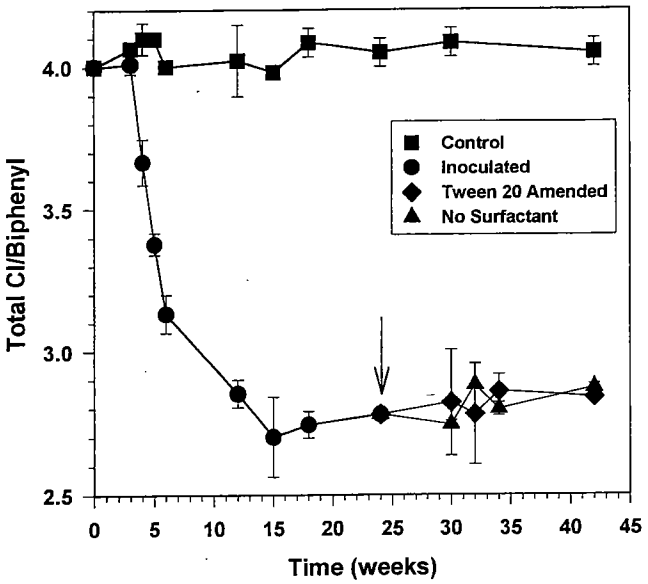

Fig. 3. Time-course of Aroclor 1248 dechlorination in control (■) and inoculated (●) sediments. The arrow indicates when the vials were divided and amended with a concentration of Tween 20 below the critical micelle concentration (0.05% v/v) (♦) or left untreated (▲).

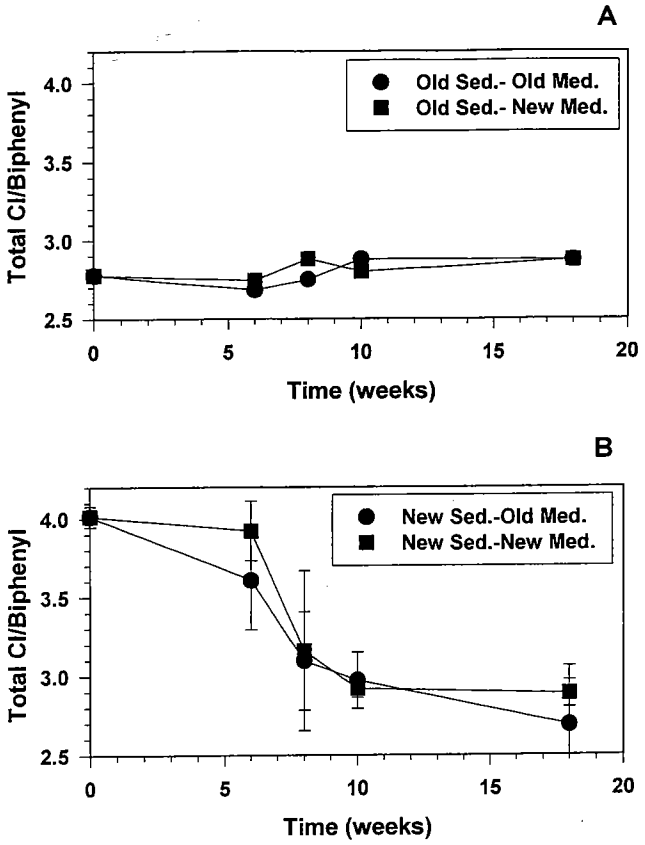

Fig. 4. Comparison of dechlorination between (A) plateau sediments with the addition of fresh mineral media (old sediment–new media) and the original supernatant from the 24-week-old sediments (old sediment–old media) and (B) fresh Aroclor 1248-spiked sediments prepared with the supernatant of 24-week-old plateau sediments (new sediment–old media) and new mineral media (new sediment–new media). The final congener pattern was the same in all treatments regardless of sediment–media combination.

availability, the nonionic surfactant Tween 20 was used. Preliminary experiments showed that Tween 20 concentrations below the critical micelle concentration (CMC) were not toxic to dechlorinating microorganisms up to 0.1% (v/v). Using [^{14}C]-labeled 245-2′4′5′-hexachlorobiphenyl (HCBP)-spiked sediments, PCB desorption was found to increase proportionally with increasing sub-CMC concentrations of Tween 20 up to 0.1%. With a 0.05% concentration of Tween 20, approximately 35% of 245-2′4′5′-HCBP was in the aqueous phase, whereas less than 1% was detectable without the addition of Tween (data not shown). We therefore chose a sub-CMC concentration of 0.05% (v/v) to determine whether surfactant addition could increase the extent of dechlorination. Tween 20 was added to plateau sediments with and without replacing the supernatants with fresh anaerobic media. Continued incubation for up to 18 weeks showed no enhancement of dechlorination when compared with unamended sediments (Fig. 3). Therefore, the cessation of dechlorination did not appear to be due to the bioavailability of PCBs to the dechlorinating microorganisms.

To determine whether further dechlorination was limited by the accumulation of any soluble metabolic products, the following experiment was set up. After 24 weeks of incubation, when dechlorination reached an apparent plateau, the supernatant from these vials was removed and the sediments split into two portions. One portion of the sediments received fresh mineral media and the second had the original 24-week-old supernatant replaced. Continued incubation of the 24-week-old sediments with addition of fresh media (old sediment–new media) and the supernatant of the 24-week-old sediments (old sediment–old media) did not result in any further dechlorination. Analysis of the sediments after 6, 8, 10, and 18 weeks showed that the total Cls per biphenyl remained constant at approximately 2.7 (Fig. 4A). A detailed examination of the congener profile also revealed that there was no change in congener pattern in either treatment with continued incubation. These results indicate that the limit of dechlorination was not set by the production of any soluble metabolites by the sediment microorganisms.

To determine whether the old supernatant might affect the dechlorination of fresh Aroclor 1248, unlike the residual congeners at the plateau, sediment slurries, spiked with fresh Aroclor 1248, were prepared with the 24-week-old supernatant and new anaerobic media and their dechlorination was compared. After 14 weeks, the total Cls per biphenyl in both treatments were reduced to 2.7, showing no further change out to the end of the experiment at 18 weeks (Fig. 4B). Both the time course and final extent of dechlorination were similar to that found in the initial experiment. Regardless of the media difference in the two treatments, both the pattern and extent of dechlorination were the same. These results show that there were no metabolic products in dechlorinated sediments at the plateau that would inhibit dechlorination of fresh Aroclor 1248.

A comparison of the dechlorination profiles between all four treatments after 18 weeks of incubation shows that the patterns were almost identical, regardless of the sediment–media combination (Fig. 5). This finding indicates that the plateau was due to the accumulation of daughter congeners that the microbial populations cannot further dechlorinate, rather than the limitation of bioavailability or inhibition by metabolic products. Our earlier findings [4,10] showed that PCB dechlorination was determined by the pattern of Cl substitution, not by the substitution position.

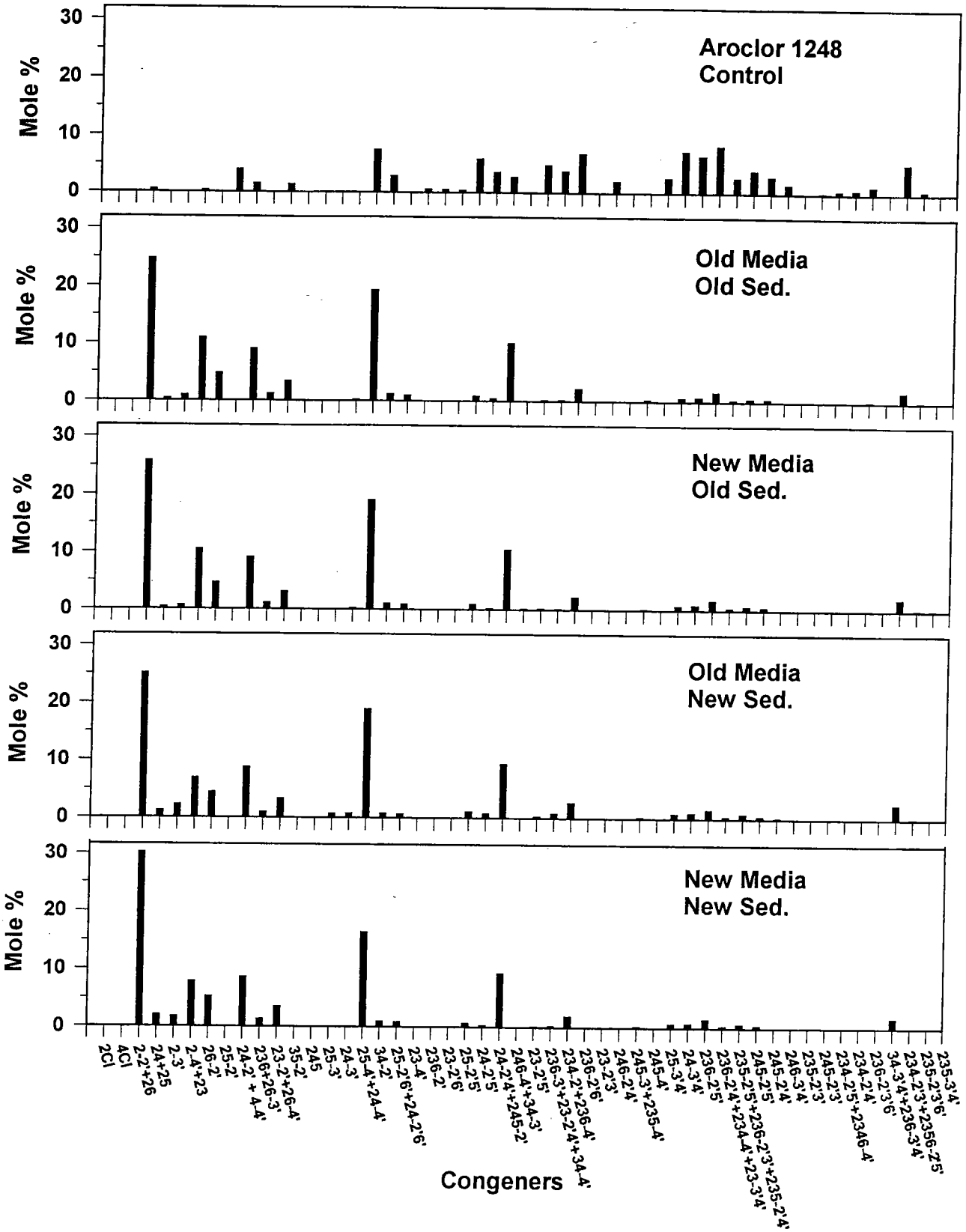

Fig. 5. Mole percentage comparison of congeners between old sediment–old media; old sediment–new media; new sediment–old media; and new sediment–new media treatments after 18 weeks of incubation.

DISCUSSION

The extent to which PCBs can be reductively dechlorinated appears to be limited, as shown here and elsewhere [2,7,9]; once dechlorination reached a plateau, no further change occurred within the incubation period examined. In an incubation lasting 11 months, no additional change was found after the first 2 months of dechlorination [7]. Partially dechlorinated St. Lawrence River sediments (originally contaminated by Aroclor 1248) showed similar results when incubated in the laboratory for approximately the same length of time. Furthermore, the extent of dechlorination and the final congener pattern at the plateau were also similar between the laboratory sediments inoculated with sediment organisms from the river and the natural river sediments (Rhee et al., unpublished manuscript).

The fact that no amendment was able to enhance dechlorination beyond the observed plateau level suggests that dechlorination has proceeded as far as possible with the present sediment populations. Previous studies have shown that dechlorination is congener-specific, being determined by the pattern of Cl substitution [4,10]. Thus, any further change may require different microbial populations to come along. It would then be interesting to find out whether we can artificially accelerate further dechlorination by inoculating with populations with different competence from other sites.

Bioavailability has often been proposed as an important factor that limits the biodegradation of HOCs such as PCBs [11,14,27,30,31]. Earlier studies have shown that sorption was responsible for decreasing the degradation of α-hexachlorocyclohexane, 1,2-dibromomethane, (2,4-dichlorophenoxy)-acetic acid, and naphthalene by reducing their bioavailability [12–15]. Recent kinetic studies of Harms and Zehnder [11] have also demonstrated that the biodegradation rate of 3-chlorodibenzofuran is correlated to desorption from particles into the aqueous phase. In other studies, the addition of surfactants also facilitated biodegradation, probably due to an increase in bioavailability [20–22,32–34].

Surfactants are most effective at concentrations above their CMC. However, most surfactants are toxic to microorganisms above this concentration. Recent investigations showed that the biodegradation of various sorbed compounds was also enhanced, even by sub-CMC concentrations of surfactants [21,22], probably because even at these levels HOCs can partition to non-ionic surfactant monomers, effecting an increase in their apparent aqueous phase concentrations [35,36]. In the present study, however, addition of a sub-CMC concentration of Tween 20 to sediments at the plateau did not bring about any further dechlorination even though the aqueous PCB concentration was enhanced. Therefore, the apparent cessation of dechlorination at this stage does not appear to be due to the bioavailability of PCBs. Two laboratory dechlorination studies of preexisting PCBs in sediments also suggested that bioavailability might not be a major limitation [6,7]. If the time scale for degradation is much longer than that for desorption, which is probably the case for PCB dechlorination, it is possible that bioavailability may not be an important factor because a maximum amount of PCBs would have been desorbed by equilibrium partitioning.

An earlier study showed that the lower limit of dechlorination did not appear to be the result of a carbon/energy limitation [37]. A major source of organic matter in sediments is algal cells sedimenting from the water column. When algal organic matter was added to a sediment-free system, it supported the dechlorination of the single test congener 234-CBP (Rhee et al., unpublished manuscript). When sediments at the plateau were amended with this algal carbon, however, no further dechlorination was detected even after 16 weeks of incubation. Amendments with a fatty acid mixture (acetate and formate) also failed to restart dechlorination in the plateau sediments after 8 weeks of incubation (Rhee et al., unpublished manuscript). Other attempts to enhance PCB dechlorination by adding various organic carbon substrates also failed to increase the overall extent of dechlorination; their addition only either shortened the lag period or increased the rate [3,7,38].

The limit of dechlorination was also not due to the loss or absence of competent dechlorinating organisms. Microorganisms eluted from the plateau sediments at 24 weeks showed active dechlorination of the 234-CBP assay congener. In addition, Aroclor 1248-spiked sediments inoculated with the populations at the plateau also demonstrated dechlorination, yielding the same final pattern as that at the old plateau (Fig. 5). Therefore, the cessation of dechlorination was not due to the death of competent species or loss of their competence.

Unlike the biodegradation of some HOCs (e.g., 3-chlorobenzoate, trichloroethylene, and phenol [23,24,28]), the production of metabolic products by consortium members does not appear to be responsible for the limitation of dechlorination in the present study. Although our experimental design only allows us to rule out soluble metabolic products, it is unlikely that any hydrophobic products are responsible because they would be tightly bound to the particulate fraction, and thereby unavailable to the microorganisms. Rather, the limitation appeared to be due to the molecular structure of the daughter congeners, which resisted further dechlorination. This pattern may be specific to sediment microbial populations with distinct physiologic competence [39]. In the present study, for example, the dechlorination products of Aroclor 1248 by St. Lawrence River microorganisms included congeners containing *para*-chlorines without neighboring (adjacent) *meta*-substituents. The observed congener distribution pattern at the plateau is similar to a pattern produced by a dechlorination process known as H′, in which only *meta*- and *para*-chlorines positioned adjacent to others are removed, but not isolated chlorines [40]. However, unlike H′, St. Lawrence River microorganisms in the present study were able to remove the isolated *meta*-chlorines from 25-2′ and 25-2′5′-CBP. However, if dechlorination is a dynamic process, albeit on a long-term scale, it is difficult to differentiate different dechlorination processes based on the congener pattern at a specific time point.

Acknowledgement—This work was supported by the National Institute of Environmental Health Science Superfund Basic Research Program, New York State Center for Hazardous Waste Management, and the Hudson River Foundation. We thank B. Michon and K. Hickey for their technical assistance.

REFERENCES

1. **Brown, J.F., Jr., R.F. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan** and **R.J. May.** 1987. Environmental dechlorination of PCBs. *Environ. Toxicol. Chem.* **6**: 579–593.
2. **Quensen, J.F., III, S.A. Boyd** and **J.M. Tiedje.** 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Appl. Environ. Microbiol.* **56**: 2360–2369.
3. **Nies, L.** and **T.M. Vogel.** 1990. Effects of organic substrates on dechlorination of Aroclor 1242 in anaerobic sediments. *Appl. Environ. Microbiol.* **56**:2612–2617.
4. **Rhee, G.-Y., B. Bush, C.M. Bethoney, A. DeNucci, H.-M. Oh** and **R.C. Sokol.** 1993. Reductive dechlorination of Aroclor 1242

in anaerobic sediments: Pattern, rate and concentration dependence. *Environ. Toxicol. Chem.* **12**:1025–1032.

5. **Rhee, G.-Y., R.C. Sokol, B. Bush** and **C.M. Bethoney.** 1993. A long-term study of the anaerobic dechlorination of Aroclor 1254 with and without biphenyl enrichment. *Environ. Sci. Technol.* **27**: 714–719.
6. **Abramowicz, D.A., M.J. Brennan, H.M. Van Dort** and **E.L. Gallagher.** 1993. Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson River sediments. *Environ. Sci. Technol.* **27**:1125-1131.
7. **Alder, A.C., M.M. Häggblom, S.R. Oppenheimer** and **L.Y. Young.** 1993. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. *Environ. Sci. Technol.* **27**:530–538.
8. **Sokol, R.C., O.-S. Kwon, C.M. Bethoney** and **G.-Y. Rhee.** 1994. Reductive dechlorination of polychlorinated biphenyls (PCBs) in St. Lawrence River sediments and variations in dechlorination characteristics. *Environ. Sci. Technol.* **28**:2054–2064.
9. **Pagano, J.J., R.J. Scrudato, R.N. Roberts** and **J.C. Bemis.** 1995. Reductive dechlorination of PCB-contaminated sediments in an anaerobic bioreactor system. *Environ. Sci. Technol.* **29**:2584–2589.
10. **Rhee, G.-Y., R.C. Sokol, C.M. Bethoney** and **B. Bush.** 1993. Dechlorination of PCBs by Hudson River sediment organisms: Specificity to the chlorination pattern of congeners. *Environ. Sci. Technol.* **27**:1190–1192.
11. **Harms, H.** and **A.J.B. Zehnder.** 1995. Bioavailability of sorbed 3-chlorodibenzofuran. *Appl. Environ. Microbiol.* **61**:27–33.
12. **Steinberg, S.M., J.J. Pignatello** and **B.L. Sawhney.** 1987. Persistence of 1,2-dibromoethane in soils: Entrapment in intraparticle micropores. *Environ. Sci. Technol.* **21**:1201–1208.
13. **Rijnaarts, H.H.M., A. Bachmann, J.C. Jumelet** and **A.J.B. Zehnder.** 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α-hexachlorocyclohexane in a contaminated calcareous soil. *Environ. Sci. Technol.* **24**:1349–1354.
14. **Ogram, A.V., R.E. Jessup, L.T Ou** and **P.S.C. Rao.** 1985. Effects of sorption on biochemical degradation rates of (2,4 dichlorophenoxy)-acetic acid in soils. *Appl. Environ. Microbiol.* **49**:582–587.
15. **Mihelcic, J.R.** and **R.G. Luthy.** 1991. Sorption and microbial degradation of naphthalene in soil–water suspensions under denitrification conditions. *Environ. Sci. Technol.* **25**:169–177.
16. **Weissenfels, W.D., H.J. Klewer** and **J. Langhoff.** 1992. Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: Influence on biodegradability and biotoxicity. *Appl. Microbiol. Biotechnol.* **36**:689–696.
17. **Pignatello, J.J., F.J. Ferrandino** and **L.Q. Huang.** 1993. Elution of aged and freshly added herbicides from a soil. *Environ. Sci. Technol.* **27**:1563–1571.
18. **Scribner, S.L., T.R. Benzing, S. Sun** and **S.A. Boyd.** 1992. Desorption and bioavailability of aged simazine residues in soil from a continuous corn field. *J. Environ. Qual.* **21**:114–120.
19. **Hatzinger, P.B.** and **M. Alexander.** 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol.* **29**:537–545.
20. **Aronstein, B.N.** and **J.R. Paterek.** 1995. Effect of nonionic surfactant on the degradation of glass-sorbed PCB congeners by integrated chemical–biological treatment. *Environ. Toxicol. Chem.* **14**:749–754.
21. **Aronstein, B.N., Y.M. Calvillo** and **M. Alexander.** 1991. Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. *Environ. Sci. Technol.* **25**:1728–1731.
22. **Aronstein, B.N.** and **M. Alexander.** 1993. Effect of a non-ionic surfactant added to the soil surface on the biodegradation of aromatic hydrocarbons within soil. *Appl. Microbiol. Biotechnol.* **39**: 386–390.
23. **Suflita, J.M., J.A. Robinson** and **J.M. Tiedje.** 1983. Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. *Appl. Environ. Microbiol.* **45**:1466–1473.
24. **Alvarez-Cohen, L.** and **P.L. McCarty.** 1991. Effects of toxicity, aeration, and reductant supply on trichloroethylene transformation by a mixed methanotrophic culture. *Appl. Environ. Microbiol.* **57**: 228–235.
25. **Sondossi, M., M. Sylvestre** and **D. Ahmad.** 1992. Effects of chlorobenzoate transformation on the *Pseudomonas testeroni* biphenyl and chlorobiphenyl degradation pathway. *Appl. Environ. Microbiol.* **58**:485–495.
26. **Lloyd-Jones, G., R.C. Ogden** and **P.A. Williams.** 1995. Inactivation of 2,3-dihydroxybiphenyl 1,2-dioxygenase from *Pseudomonas* sp. strain CB406 by 3,4-dihydroxybiphenyl (4-phenylcatechol). *Biodegradation* **6**:11–17.
27. **Barriault, D.** and **M. Sylvestre.** 1993. Factors affecting PCB degradation by an implanted bacterial strain in soil microcosms. *Can. J. Microbiol.* **39**:594–602.
28. **Murakami, Y.** and **M. Alexander.** 1989. Destruction and formation of toxins by one bacterial species affect biodegradation by a second species. *Biotechnol. Bioeng.* **33**:832–838.
29. **Zhang, X.** and **J. Wiegel.** 1990. Sequential degradation of 2,4-dichlorophenol in freshwater sediments. *Appl. Environ. Microbiol.* **56**:1119–1127.
30. **Guerin, W.F.** and **S.A. Boyd.** 1992. Differential bioavailability of soil-sorbed naphthalene to two bacterial species. *Appl. Environ. Microbiol.* **58**:1142–1152.
31. **Mihelcic, J.R., D.R. Lueking, R.J. Mitzell** and **J.M. Stapleton.** 1993. Bioavailability of sorbed- and separate-phase chemicals. *Biodegradation* **4**:141–153.
32. **Tiehm, A.** 1994. Degradation of polycyclic aromatic hydrocarbons in the presence of surfactants. *Appl. Environ. Microbiol.* **60**:258–263.
33. **Tsomides, H.J., J.B. Hughes, J.M. Thomas** and **C.H. Ward.** 1995. Effect of surfactant addition on phenanthrene biodegradation in sediments. *Environ. Toxicol. Chem.* **14**:953–959.
34. **Volkering, F., A.M. Breure, J.G. vanAndel** and **W.H. Rulkens.** 1995. Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. *Appl. Environ. Microbiol.* **61**:1699–1705.
35. **Kile, D.E.** and **C.T. Chiou.** 1989. Water solubility enhancements of DDT and trichlorobenzene by some surfactants below and above the critical micelle concentration. *Environ. Sci. Technol.* **23**:832–838.
36. **Edwards, D.A., Z. Liu** and **R.G. Luthy.** 1992. Interactions between nonionic surfactant monomers, hydrophobic organic compounds and soil. *Water Sci. Technol.* **26**:147–158.
37. **Kwon, O.-S., N. Tulve, R.C. Sokol, X. Liu** and **G.-Y. Rhee.** 1995. Effect of different sediment sources and bioavailability on PCB dechlorination. *Proceeding* Pacific Basin Conference on Hazardous Waste, Edmonton, AL, Canada, May 7–12.
38. **Morris, P.J., W.W. Mohn, J.F. Quensen III, J.M. Tiedje** and **S.A. Boyd.** 1992. Establishment of a polychlorinated biphenyl-degrading enrichment culture with predominantly *meta* dechlorination. *Appl. Environ. Microbiol.* **58**:3088–3094.
39. **Ye, D., J.F. Quensen III, J.M. Tiedje** and **S.A. Boyd.** 1995. Evidence for *para* dechlorination of polychlorobiphenyls by methanogenic bacteria. *Appl. Environ. Microbiol.* **61**:2166–2171.
40. **Bedard, D.L.** and **J.F. Quensen III.** 1995. Microbial reductive dechlorination of polychlorinated biphenyls. In L.Y. Young and C.E. Cerniglia, eds., *Microbial Transformation and Degradation of Toxic Organic Chemicals.* Wiley-Liss, New York, NY, USA, pp. 127–216.