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REDUCTIVE DECHLORINATION OF POLYCHLORINATED BIPHENYLS IN SEDIMENT FROM THE TWELVE MILE CREEK ARM OF LAKE HARTWELL, SOUTH CAROLINA, USA

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Abstract—Lake Hartwell is a U.S. Army Corps of Engineers reservoir system located on the state line between South Carolina and Georgia, USA. The lake was contaminated with an estimated 200 metric tons of polychlorinated biphenyls ([PCBs]; mainly Aroclor 1016 and 1254), and the entire Twelve Mile Creek watershed and the Seneca River arm of Lake Hartwell were placed on the National Priorities List. Monitored natural attenuation was chosen as a remedy for the contaminated sediment. The relatively warm temperature of Lake Hartwell and lack of significant cocontaminants along with the PCBs distinguish this site from others that have been studied for microbially mediated reductive dechlorination. Microcosm studies were conducted with sediment from two locations in the Twelve Mile Creek arm and confirmed the presence of indigenous microorganisms capable of reductively dechlorinating Aroclor 1254, which contains predominantly tetra-, penta-, and hexachlorobiphenyl. The average number of total chlorines per biphenyl decreased from 4.8 to 4.9 to 2.9 to 3.0, following 250 to 260 d of incubation. The maximum observed dechlorination rates were 0.29 to 0.87 μg-atoms Cl^- per gram sediment dry weight per week. The onset of dechlorination activity correlated strongly with maximum methanogenesis, which occurred without a lag in samples from the site that showed signs of in situ fermentation activity. Dechlorination occurred primarily at the *meta* and *para* positions (58–63% removal), with no apparent decrease in *ortho* chlorines. This most closely resembles pattern M, characterized by preferential removal of unflanked and flanked *meta* chlorines. The microcosm results are consistent with sediment cores analyzed from the same locations, which indicate accumulation with depth of the same *ortho*- and *para*-substituted congeners. It therefore appears that the success of monitored natural attenuation for Lake Hartwell will hinge on covering the recalcitrant PCBs with a sufficient amount of uncontaminated sediment to isolate them from the food chain.

Keywords—Aroclor 1254 Pattern M Monitored natural attenuation Methanogenesis Polychlorinated biphenyls

INTRODUCTION

Reductive dechlorination of PCBs has been studied extensively at numerous locations, including the Hudson River (NY, USA), Silver Lake (MA, USA), Sheboygan River (WI, USA), Waukegan Harbor (IL, USA), New Bedford Harbor (MA, USA), and Acushnet Estuary (MA, USA) [1]. Documentation of in situ PCB dechlorination activity prompted an increased interest in applying monitored natural attenuation (MNA) as a treatment approach for many of these sites, especially in light of the substantial cost savings that this strategy affords [2,3]. However, recent decisions to require dredging of the upper Hudson River [4] and Fox River (WI, USA) [5] have signaled a shift away from relying on MNA for cleanup of PCB-contaminated sediments. At this point in time, Lake Hartwell is the only location in the United States where MNA has been chosen as a remedy for PCB-contaminated sediment. Lake Hartwell is a U.S. Army Corps of Engineers reservoir system located on the state line between South Carolina and Georgia, USA, on the Savannah, Tugaloo, and Seneca Rivers (Fig. 1). The lake was contaminated with an estimated 200 metric tons of PCBs (mainly Aroclor 1016 and Aroclor 1254) from Sangamo Weston, a capacitor manufacturing company in Pickens, South Carolina, that operated between 1955 and 1978 [6,7]. As a result of PCB contamination, the entire Twelve Mile Creek watershed and the Seneca River arm of Lake Hartwell were placed on the National Priorities List in 1990 [8].

The principal basis for selecting MNA as the record of decision for Lake Hartwell in 1994 was the expectation that ongoing deposition of clean sediment would cap the PCB-contaminated material and thereby isolate it from the food chain [9]. Microbially mediated reductive dechlorination was not considered in this strategy, in part because of the lack of compelling evidence for in situ reductive dechlorination in Lake Hartwell sediment. This is a potential shortcoming since deposition may not occur in all parts of the lake, while in other areas where it does occur, environmental conditions may become more favorable for dechlorination. Several features of this ecosystem make it distinct from other PCB-contaminated sites, suggesting that the rate, extent, and pattern of PCB dechlorination may not be the same as what has been observed previously. First, Lake Hartwell is considerably warmer than other freshwater environments in which in situ dechlorination has been evaluated, having an annual water temperature ranging from 14 to 30°C. Laboratory studies with contaminated and spiked sediments have shown that temperature can exert an impact on the nature and extent of PCB dechlorination [10–13]. Second, PCBs are the only major contaminant in Lake Hartwell sediment [14] in contrast to other sites where PCBs are present with other contaminants, such as oils or polynuclear aromatic hydrocarbons [1,15–17]. The presence of cocontam-

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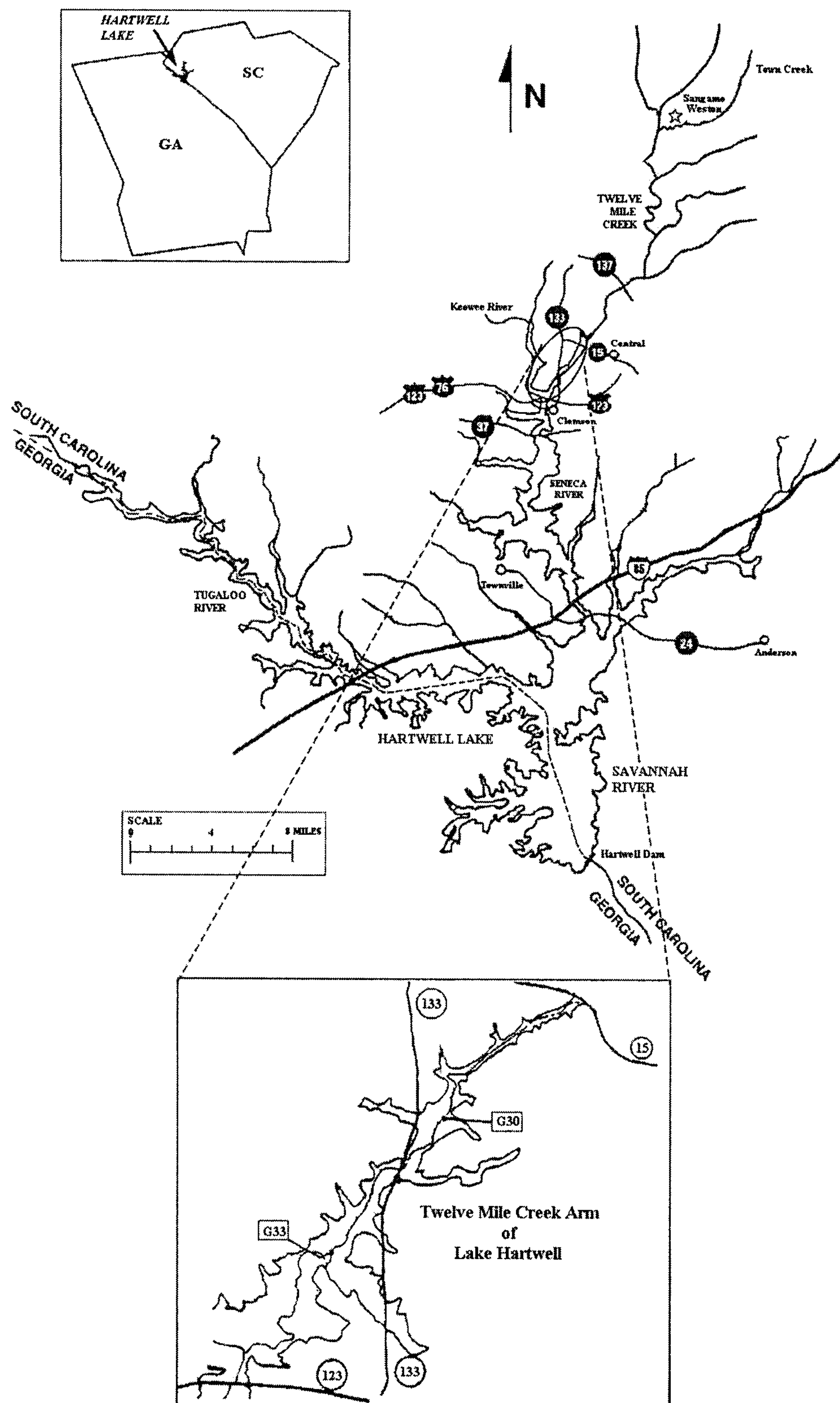

Fig. 1. Location of Lake Hartwell, the Twelve Mile Creek arm of the lake, and sampling points for this study (G30 and G33), South Carolina and Georgia, USA.

inants affects the rate and extent of PCB dechlorination [17] in part because of a decrease in PCB bioavailability [1].

The objective of this study was to determine if Lake Hartwell sediments contain microbial communities capable of mediating Aroclor 1254 dechlorination and, if so, to compare the rate, extent, and pattern of PCB dechlorination in Lake Hartwell sediments with colder sites that also contain significant levels of cocontaminants.

MATERIALS AND METHODS

Sediment collection

Sampling locations (G30 and G33) are shown in Figure 1. Sediment cores were collected in July 1998 using a Wildco sediment corer containing a Lexan™ tube (5-cm diameter, 76 cm long; Polymer Plastics, Mountain View, CA, USA) and 5.1-cm eggshell core catcher. The Lexan tubes were washed in a hot detergent bath, rinsed with hot water, and rinsed several times with distilled water. The cores were transported to the L.G. Rich Environmental Research Laboratory (Clemson University, Clemson, SC, USA) and stored in the dark prior to sectioning. The top layer (about 5 cm) of the core samples was removed, and the remaining portion was transferred to a glass jar, topped with site water, and stored at 4°C prior to use.

Microcosms

Triplicate microcosms for each treatment were set up in an anaerobic chamber (Coy Laboratory, Grasslake, MI, USA) with a 95% N_2 and 5% H_2 atmosphere. The sediment from the cores was first homogenized with a blender (Hamilton Beach 8-speed, Richmond, VA, USA) and then mixed with revised anaerobic minimal medium [18] at a 15:50 ratio (wet wt, g: volume, ml). Resazurin (1 mg/L) was added to the medium as a redox indicator. While continuing to mix, 50 ml of sediment slurry (containing 5.0–5.5 g of sediment, dry wt) were transferred to sterile serum bottles (125 ml total volume), which were then crimp sealed with Teflon®-faced butyl rubber septa. The bottles were removed from the anaerobic chamber, purged (1 min) with a 70% N_2 and 30% CO_2 gas mixture (to remove H_2 and equilibrate bicarbonate in the minimal medium with CO_2, resulting in a pH of 7.0), and resealed.

A concentrated stock solution of Aroclor 1254 (AccuStandard, New Haven, CT, USA) dissolved in acetone was added to the sediment slurry (using a syringe) to a final concentration of approximately 500 μg/g (sediment dry wt). Aroclor 1254 was chosen instead of Aroclor 1016 because its higher chlorine content afforded the greatest opportunity to determine the potential for reductive dechlorination. To ensure homogeneous distribution of Aroclor 1254, the serum bottles were shaken overnight. They were then incubated in an inverted position in the anaerobic chamber at room temperature (22–24°C). Killed controls were prepared identically except that the bottles were autoclaved (121°C, 15 min) for three consecutive days and then spiked with Aroclor 1254. Two live controls were also established in triplicate. One contained sediment, medium, and acetone but no Aroclor 1254, and the other contained medium, acetone, and Aroclor 1254 but no sediment.

Sediment slurry was sampled at various time points to analyze for PCBs. After headspace analysis for CH_4 and H_2 (see the following discussion), serum bottles were returned to the anaerobic chamber, mixed vigorously, and opened, and 1 ml of slurry was withdrawn with an autoclaved wide-bore pipette. The aliquots were transferred to 4-ml sample vials capped with Teflon-faced septa and stored at 4°C until extraction and analysis. Another 1 ml of slurry was transferred to a 3-ml syringe equipped with a 0.45-μm filter (Nylon Acrodisc®, Pall Gelman, Ann Arbor, MI, USA) to remove the suspended solids. Filtrate was stored in 2-ml vials for acetone and organic acid analysis. The serum bottles were resealed, purged with the N_2:CO_2 gas mixture, and returned to the anaerobic chamber for incubation.

PCB extraction and analysis

Polychlorinated biphenyls were extracted from sediments samples with acetone and isooctane under ultrasonication (Fisher 300 Sonic Dismembrator, Fisher Scientific, Atlanta, GA, USA) using previously developed methods [19], modified to minimize the volume of solvents used [7,20]. Samples were spiked with octachloronaphthalene as a recovery standard to determine the extraction efficiency, which averaged 79% (±17.4%).

Polychlorinated biphenyl analysis was performed on a gas chromatograph (Hewlett-Packard 6890, Palo Alto, CA, USA) equipped with a ^{63}Ni electron-capture detector (325°C), autosampler, splitless injector (250°C), a computerized data acquisition system (HPChem Station, Hewlett-Packard), and a 30-m fused silica capillary column with a 0.25-mm diameter and a film thickness of 0.25 μm (ZB5, Phenomenex®, Torrance, CA, USA). Helium (2 ml/min) was used as a carrier gas, with the same temperature program as described in Dunnivant and Elzerman [19], except the initial hold time was changed to 2.5 min.

The PCB congeners were identified and quantified using aldrin as an internal standard [19] in order to minimize run time [7]. Daily working standards contained a 1:1 mixture of Aroclor 1016 and 1254. Peaks were identified and calibrated according to relative response factors assigned by Germann [20]. The analysis resolves 63 peaks representing 96 PCB congeners. Individual PCB congeners are referred to by listing the substituted position on each ring and using a hyphen to separate both rings; for example, 2,2′,3,3′,4 chlorobiphenyl (IUPAC 82) is represented by 234-23 chlorobiphenyl. Calibration standards (250 pg/μl in isooctane) were run after every 12 samples to confirm the response factors for each peak as part of a quality assurance/quality control program. When response factors for an individual congener varied more than ±15%, recalibration was conducted.

Reductive dechlorination was quantified on the basis of the weight percentage of each identified PCB-containing peak and the homologue distribution or relative proportion of di- through heptachlorobiphenyl. The average number of *meta+para, ortho,* and total chlorines per biphenyl was calculated as the product of the average number of chlorines and molar concentration of each peak divided by the total molar concentration summed over all peaks [21]. Coeluting congeners were assumed to be present in equal proportions, and the biphenyl moiety was assumed to remain intact [15,16,21].

Other analytical methods

Filtered samples were analyzed for acetone with a gas chromatograph (Hewlett-Packard 5890 series II) equipped with a flame ionization detector and a 60-m fused silica capillary column (0.53-mm diameter and 3-μm film thickness; ZB-624, Phenomenex®). The column, injector, and detector temperatures were held at 40, 200, and 250°C, respectively; *tert*-butanol was used as an internal standard.

Acetic acid was monitored by high-performance liquid chromatography (Waters 717, 600E, Milford, MA, USA) using an Aminex® HPX-87H column (300 × 7.8 mm, BIO-RAD, Hercules, CA, USA) and a Micro-Guard cartridge (30 × 4.6 mm; BIO-RAD), a 0.005 M H_2SO_4 mobile phase, and an ultraviolet-Vis detector (210 nm; Waters 490E). The detection limit was approximately 2 mg/L. Acetoacetic, propionic, and other organic acids were also detectable with this method, but none were identified.

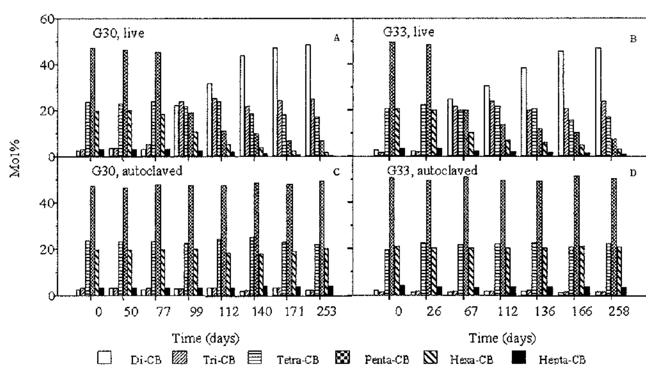

Fig. 2. Homologue distribution of Aroclor 1254 (AccuStandard, New Haven, CT, USA) in microcosms prepared with sediments from sites G30 (**A** live, **C** control) and G33 (**B** live, **D** control). Results are averages of triplicate microcosms.

Total methane formation per bottle was monitored by gas chromatographic analysis (Hewlett-Packard, 5890 series II) of headspace samples (0.5 ml, obtained with a gas-locking syringe; Supleco, Bellefonte, PA, USA), using a flame ionization detector and a Supleco 1% SP 1000 60/80 Carbopack B column (2.4 m × 3.2 mm; Supleco) held at 60°C. The injector and detector were held at 200°C. The detection limit was approximately 2 μmol per bottle (8.0×10^{-4} atm).

Hydrogen was also monitored by gas chromatographic analysis of headspace samples, using a high-sensitivity thermal conductivity detector and a 10% SP1000 100/120 Carbosieve column (3.0 m × 3.2 mm; Supleco) held at 105°C, with the injector and detector set at 200°C. The detection limit was approximately 0.4 μmol per bottle (1.6×10^{-4} atm).

RESULTS AND DISCUSSION

Rate and extent of dechlorination

Microcosm results with sediment from the G30 and G33 sites in the Twelve Mile Creek arm of Lake Hartwell confirmed the presence of indigenous microorganisms capable of reductively dechlorinating Aroclor 1254, which contains predominantly tetra-, penta-, and hexachlorobiphenyl (Fig. 2). After two to three months of incubation, a marked shift was observed toward lesser chlorinated congeners in the live treatments (Fig. 2A and B). Notably, a decrease was observed in the hexa- and pentachlorobiphenyls with a corresponding increase in tri- and dichlorobiphenyls. Additional incubation further increased the relative proportion of lower chlorinated biphenyls, especially dichlorobiphenyls. The congener distribution remained unchanged in autoclaved controls (Fig. 2C and D).

In the live treatments, a corresponding decrease was observed in the average number of total chlorines, from 4.8 ± 0.01 to 2.9 ± 0.05 in the G30 bottles (Fig. 3A) and from 4.9 ± 0.05 to 3.0 ± 0.16 in the G33 bottles (Fig. 3B), resulting in an overall removal of approximately 40%. The extent of dechlorination in the G30 and G33 microcosms was statistically equivalent (Student's t test, $\alpha = 0.05$). No change in chlorine content occurred in the autoclaved controls. A mass balance on the total moles of PCBs indicated recoveries of 93.4 ± 19.0% and 100.1 ± 14.2% in the live and autoclaved controls, respectively, when comparing initial and final amounts over the period of incubation.

The maximum observed dechlorination rate for the G30 microcosms was 0.87 ± 0.14 μg-atoms Cl^- per gram sediment dry weight per week, calculated on the basis of linear regression of the data from days 77 to 112 (nine data points). The rate for G33 bottles was 0.29 ± 0.064 μg-atoms Cl^- per gram sediment dry weight per week, based on the interval of days 26 to 67 (five data points), which is statistically lower than for the G30 bottles (Student's t test, $\alpha = 0.05$). Previous results have indicated that the rate and extent of dechlorination is site specific, suggesting possible differences in the dechlorinating population from site to site [1,15,16]. Nevertheless, the rates for G30 and G33 are within the same order of magnitude and are similar to the maximum rate of 0.22 ± 0.02 μg-atoms Cl^- per gram sediment dry weight per week observed in Hudson River microcosms exposed to the same Aroclor mixture [15].

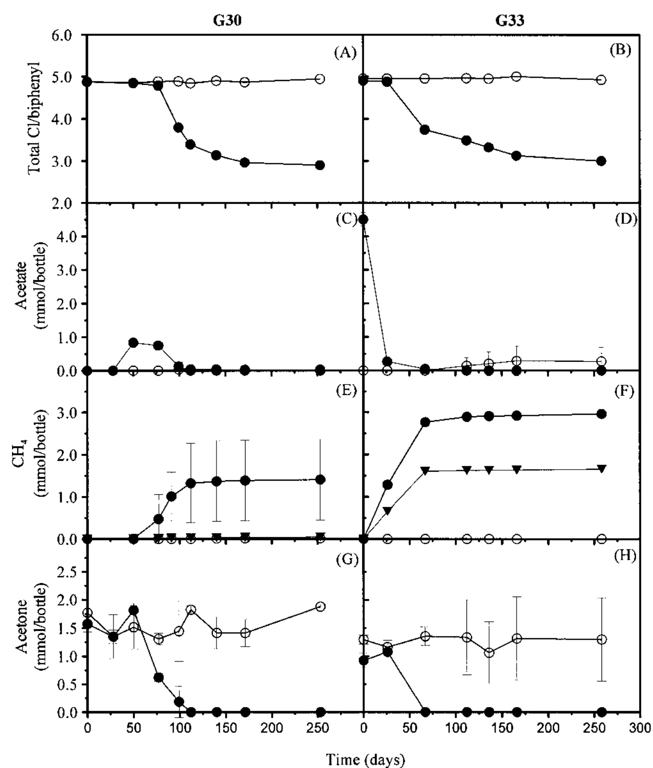

Fig. 3. Comparison of G30 and G33 microcosms in terms of the average number of total Cl per biphenyl (**A** and **B**), acetate levels (**C** and **D**), methanogenesis (**E** and **F**), and acetone consumption (**G** and **H**); (○), autoclaved controls with Aroclor 1254 (AccuStandard, New Haven, CT, USA); (●), live treatments with Aroclor 1254; (▼), live treatments without acetone or Aroclor 1254. Error bars are standard deviation of triplicate bottles; where not shown, the deviations were smaller than the size of the symbols.

The G30 microcosms required a longer acclimation time than the G33 microcosms before the onset of PCB dechlorination (Fig. 3A and B). Although the reasons for this are not yet known, the onset of dechlorination did correlate well with the initial concentration of acetate. No other organic acids were detected. The G33 sediment contained 5,300 ± 240 mg/L of acetate (Fig. 3D), while initially none occurred in G30 (Fig. 3C). The presence of acetate is indicative of fermentative activity and the availability of a readily biodegradable electron donor. The start of PCB dechlorination also correlated strongly with methanogenic activity (Fig. 3E and F), which took several months to initiate in the G30 microcosms but started immediately in the G33 bottles. The G33 live controls (no PCB or acetone added) also exhibited rapid and significant levels of methane formation, while the G30 live controls produced almost none. Sediment typically supplies the electron donor needed by PCB dechlorinators as well as substrates for non-

dechlorinators that likely provide the dechlorinators with growth factors [22]. Variations in sediment movement and deposition history leads to differences in composition in terms of carbon and energy sources, especially in a water body such as Lake Hartwell, where sediment movement varies greatly with location. These differences appear to extend to the initial population of Aroclor dechlorinators, possibly resulting in the different lag periods for the onset of PCB dechlorination.

Although methanogenesis and PCB dechlorination activity were strongly correlated in this study, the specific role of methanogens in PCB dechlorination is not yet known. Concomitant methane production has been observed with dechlorination of PCBs in previous studies [23–26], and methanogens are among the physiological groups of anaerobic PCB-dechlorinating organisms in Hudson River sediments [27].

Acetone, which was used as the PCB carrier in this study, was consumed in both sets of microcosms (Fig. 3G and H). Use of acetone as an electron donor during reductive dechlorination of PCBs has been noted previously [25]. In the G30 microcosms, acetate accumulated during acetone consumption. This is consistent with one of the known fermentation pathways for acetone, which is initiated by carboxylation to acetoacetate, followed by cleavage to acetate, without release of hydrogen [28]. Acetate consumption was accompanied by methane formation in bottles from both sites (Fig. 3C and D). Hydrogen never accumulated noticeably in either set of microcosms, with only occasional levels observed just above the detection limit. This suggests that significant amounts were not formed during fermentation of acetone or degradable organics in the sediment, or the rate of H_2 consumption equaled or exceeded its rate of formation. Hydrogen does not appear to be a ''universal'' electron donor for PCB dechlorination, in contrast to its central role in halorespiration of chlorinated alkenes [29]. However, the effect of hydrogen on the rate of PCB dechlorination and the pathway used has been noted [30,31].

The net amount of methane formation attributable to acetone was similar in both sets of live microcosms (total methane formed minus the methane from the live sediment controls), an average of 1.4 mmol per bottle. Net methane formation was also equivalent in live controls fed acetone but no PCBs (data not shown), indicating that the presence of PCBs did not inhibit methanogenesis. This amount of methane formation accounts for approximately 50% of the electron equivalents from the acetone consumed. Less than 1% of the electron equivalents available from acetone were used for PCB dechlorination (based on an average decrease of 2 mol of Cl per mol of PCB and 2 electron equivalents consumed per mol of Cl removed). The disposition of the remaining electron equivalents from oxidation of acetone is not yet known. Some was undoubtedly consumed for biosynthesis, although this typically represents less than 15% of the donor used in methanogenic systems [32]. The balance may reside with fermentation products. However, the concentrations of acetate, acetoacetate, and propionate were below detection at the point when methane accumulation leveled off. It is possible that other fermentation products (such as alcohols and longer-chain organic acids) accumulated but were not measured.

Dechlorination pattern

Dechlorination of Aroclor 1254 in the G30 and G33 microcosms occurred primarily at the *meta* and *para* positions. About 63 and 58% of the average number of *meta*+*para* chlorines were removed in the live G30 and G33 microcosms, respectively, with no dechlorination observed in autoclaved controls (Fig. 4A and B). No dechlorination was apparent at the *ortho* positions in live microcosms from either site (Fig. 4C and D).

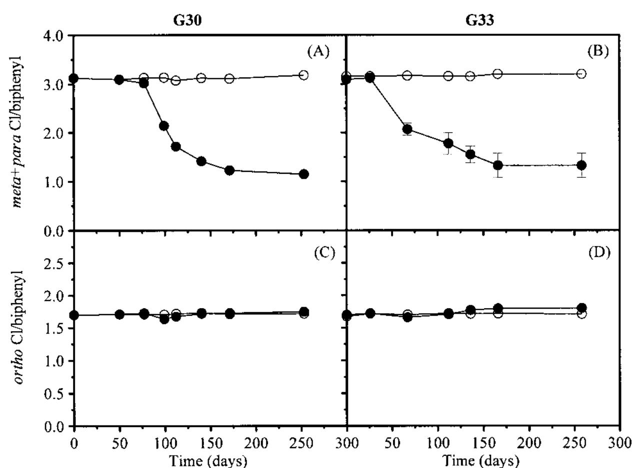

Fig. 4. The average number of *meta+para* Cl per biphenyl (**A** and **B**) and the average number of *ortho* Cl per biphenyl (**C** and **D**) in G30 and G33 microcosms; (○), autoclaved controls with Aroclor 1254 (AccuStandard, New Haven, CT, USA); (●), live treatments with Aroclor 1254.

The dechlorination pattern for Aroclor 1254 was similar in terms of the parent congeners that underwent dechlorination—234-245 (138) +2346-34 (158), 234-236 (132) +245-245 (153) +234-34 (105) +246-345 (168) +345-35 (127), 236-245 (149) +2345-3 (106) +245-34 (118), 234-24 (85) +245-35 (120) +236-236 (136), 234-25 (87), 245-23 (97) +345-4 (81), 236-35 (113) +245-24 (99) +34-35 (79), 245-25 (101), 235-25 (92) +236-23 (84), and 246-35 (121) +236-24 (91) +234-3 (55) chlorobiphenyls—and in terms of the daughter congeners that accumulated in G30 (Fig. 5) and G33 microcosms (Fig. 6). The major products were 2-2 (4) +26 (10)-chlorobiphenyls and 26-2 (19) +35 (14)-chlorobiphenyls. In addition, accumulation of *para*-substituted PCB congeners, such as 24-24 (47) +246-4 (75) +245-2 (48), 24-4 (28), 4-4 (15) +24-2 (17), and 2-4 (8) chlorobiphenyls, suggests that *para* chlorines were more resistant to dechlorination than *meta* chlorines. The dechlorination activity observed in these microcosms resembles pattern M, which is characterized by preferential removal of unflanked and flanked *meta* chlorines from the biphenyl molecule [1]. Pattern M was selected rather than N based on the disappearance of 25-25 (52) chlorobiphenyl. While process N is characterized by the removal of flanked and doubly flanked *meta* chlorines, depletion of 25-25 chlorobiphenyl suggests that dechlorinating organisms from these sediments are capable of removing unflanked *meta* chlorines, which is a characteristic of process M [1].

CONCLUSIONS

The results of this study confirm that Lake Hartwell sediments in the vicinity of the highest levels of PCB contamination contain microbial communities capable of mediating *meta* and *para* dechlorination. While the lag time prior to PCB dechlorination and the maximum observed rates differed between the two sites tested, the extent and pattern of dechlorination were similar. Furthermore, the rate, extent, and pattern of dechlorination were within the range of what has been ob-

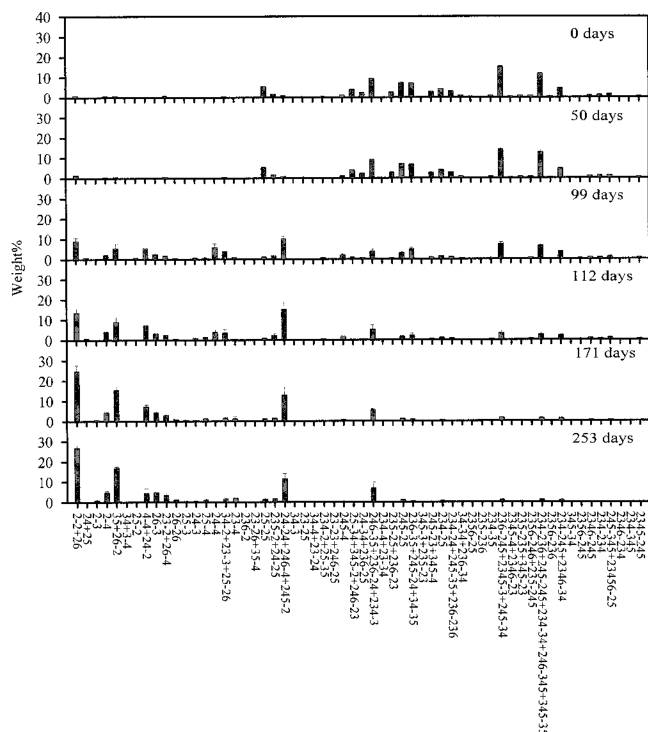

Fig. 5. Weight percentage of Aroclor 1254 (AccuStandard, New Haven, CT, USA) congeners in G30 microcosms. Error bars are standard deviations of triplicate bottles; where not shown, the deviations were smaller than the size of the symbols.

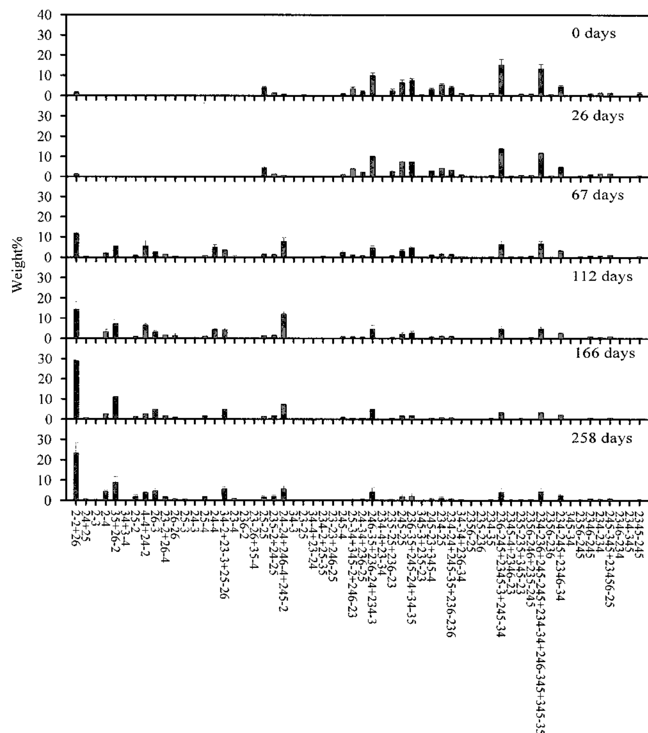

Fig. 6. Weight percentage of Aroclor 1254 (AccuStandard, New Haven, CT, USA) congeners in G33 microcosms. Error bars are standard deviations of triplicate bottles; where not shown, the deviations were smaller than the size of the symbols.

served for other freshwater ecosystems located in colder climates and containing cocontaminants [15]. The microcosm results are consistent with analysis of sediment core samples collected from the G30 and G33 sites, which indicates accumulation with depth of the same *ortho-* and *para*-substituted PCB congeners (U. Pakdeesusuk, Clemson University, Clemson, SC, USA, unpublished data). Taken together, the microcosm and field results have implications for the use of MNA as a remediation strategy for PCBs in Lake Hartwell. Since dechlorination appears to have stalled with the accumulation of *ortho*-substituted congeners, the success of MNA hinges on covering the remaining PCBs with a sufficient amount of uncontaminated sediment to isolate them from the food chain.

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