

## Influence of Incubation Temperature on the Microbial Reductive Dechlorination of 2,3,4,6-Tetrachlorobiphenyl in Two Freshwater Sediments

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We studied the impact of incubation temperatures on the dechlorination of 2,3,4,6-tetrachlorobiphenyl (2346-CB) in two sediments from different climates: polychlorinated biphenyl (PCB)-free sediment from Sandy Creek Nature Center Pond (SCNC) in Athens, Ga., and PCB-contaminated sediment from Woods Pond (WP) in Lenox, Mass. Sediment slurries were incubated anaerobically with 350  $\mu\text{M}$  2346-CB for 1 year at temperatures ranging from 4 to 66°C. Most of the 2346-CB was dechlorinated between 12 and 34°C in both sediments and, unexpectedly, between 50 and 60°C in WP sediment. This is the first report of PCB dechlorination at thermophilic temperatures. The data reveal profound differences in dechlorination rate, extent, and products as a function of sediment and temperature. The highest observed rate of dechlorination of 2346-CB to trichlorobiphenyls occurred at 30°C in both sediments, but the rate was higher for WP than for SCNC sediment (46 versus 16  $\mu\text{mol liter}^{-1} \text{day}^{-1}$ ). For SCNC sediment the rate of dechlorination dropped sharply below 30°C, but for WP sediments it was near optimal from 20 to 34°C and then dropped sharply below 20°C. In WP sediment most of the *meta* chlorines were removed between 8 and 34°C and between 50 and 60°C. *para* dechlorination was restricted from 18 to 34°C and was optimal at 20°C. *ortho* dechlorination occurred between 8 and 30°C, with optima around 15 and 27°C, but the extent was highly variable. In SCNC sediment complete *meta* dechlorination occurred from 12 to 34°C and *para* dechlorination occurred from 18 to 30°C; both were optimal at 30°C. No *ortho* dechlorination was observed. Dechlorination products were 246-CB, 236-CB, and 26-CB (both sediments) and 24-CB, 2-CB, and 4-CB (WP sediment). The data suggest that in SCNC sediment similar factors controlled *meta* and *para* PCB dechlorination over a broad temperature range (18 to 30°C) but that in WP sediment there were multiple temperature-dependent changes in the factors controlling *ortho*, *meta*, and *para* dechlorination. We attribute the differences observed in the two sediments to differences in their PCB-dechlorinating communities.

Reductive dechlorination of polychlorinated biphenyls (PCBs) under anaerobic conditions was discovered within the last decade and has become an important area of research because of its potential impact on the environment (1–5, 10–13, 20, 21). Reductive dechlorination is expected to reduce the potential toxicity of PCBs by eliminating the dioxin-like congeners (5, 11, 22) and by reducing bioaccumulation in humans (4, 9). Furthermore, many of the lower chlorinated biphenyls generated by reductive dechlorination under anaerobic conditions are biodegradable by aerobic PCB-degrading bacteria that are readily isolated from soil and sediments (6, 8, 14, 23). However, the degree to which these benefits are realized depends on both the specificity of dechlorination and the extent to which it occurs. Both of these parameters vary considerably at different PCB-contaminated sites. Extensive microbial dechlorination of PCBs has occurred in the Hudson River (N.Y.) via a nearly complete loss of *meta* and *para* chlorines (10, 12), but *meta* and *para* dechlorination of the PCBs in Woods Pond (Lenox, Mass.) has been much more limited (4).

Of necessity, all investigations of microbial PCB dechlorination have been carried out with sediment slurries both as the source of microorganisms and as the growth matrix, because attempts of isolating the PCB-dechlorinating microorganisms

have not been successful and because dechlorination activity is dependent on the presence of sediment (5). It is likely that PCB-dechlorinating microorganisms function in syntrophic communities, and they may be dependent on such communities for electron donors, micronutrients, and maintenance of an optimal hydrogen concentration (5). Furthermore, it appears that the PCB dechlorination observed in sediments may result from the actions of multiple PCB-dechlorinating populations that exhibit different substrate specificities (5, 12, 13, 20). Some of these populations may compete with each other for the same PCB substrates and produce PCB congeners with fewer chlorines that are not substrates for either population. The most extensive dechlorination occurs when several PCB-dechlorinating populations act simultaneously or sequentially to attack different suites of congeners (5, 13). For example, the terminal dechlorination products of one population may serve as substrates for a different PCB-dechlorinating population (5, 13), leading to more extensive removal of chlorines. It is reasonable to expect that incubation temperature might have profound effects on PCB dechlorination in such complex systems, yet this important area of research has not previously been explored.

Nearly all laboratory studies of microbial PCB dechlorination reported to date have been conducted at 22 to 30°C, yet PCB-contaminated sediments in the environment typically experience a different and much wider range of temperatures. The actual range of temperatures depends on the climate and on the depth of the water and the sediment itself. To gain a

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better understanding of the impact of temperature on PCB dechlorination, we studied the dechlorination of 2,3,4,6-tetrachlorobiphenyl (2346-CB) in two sediments incubated over a broad range of temperatures. This particular congener is an excellent choice for studying how temperature affects the microbial dechlorination of PCBs, because it is readily dechlorinated by microorganisms in a variety of different sediments (2a, 7) and because its various dechlorination products can be unequivocally identified. The first sediment is from an unpolluted pond in Sandy Creek Nature Center (SCNC) in Athens, Ga. The usual summer temperature of the sediment in this pond (at an 8-cm depth) is between 25 and 29°C and at the edge can be as high as 35°C in the summer. For short periods of a week or two, the temperature can drop close to freezing in the winter. The second sediment is a PCB-contaminated sediment from Woods Pond in Lenox, Mass. The summer temperatures of this sediment range from 15°C at a 45-cm depth to 18 to 20°C at a 10- to 15-cm depth. Winter temperatures drop to ~1 to 4°C at all depths.

Our studies showed that temperature had profound effects on the rate, extent, and products of dechlorination and that these effects differed substantially in the two sediments. These findings indicate a need for caution in using the results of studies conducted at 22 to 30°C or with another sediment to understand PCB dechlorination in sites that generally experience lower or higher temperatures.

#### MATERIALS AND METHODS

**Sediment collection and storage.** PCB-free sediments were collected from SCNC as described previously (18). The pond is a shallow (depth, up to 1.5-m) clay pit (25 acres) located in a wooded area without nearby agricultural areas that could lead to a runoff of chlorinated pesticides. The subsediment is red clay covered by a 5- to 20-cm-thick humus-rich layer of decomposing organic material (mainly from leaves) with pH values between 6.5 and 6.8. The overlying water has a pH of 6.3 to 6.5. All possible direct discharges of pollution into this pond have been prohibited since the Nature Center was established in 1976 (18). No chlorophenols or PCB congeners were present at detectable concentrations by our standard analysis procedures (detection limit, around 0.01 µg/g of Aroclors 1242, 1254, and 1260). However, the *ortho*-chlorophenol-dehalogenating organism *Desulfotobacterium dehalogenans* was isolated from this pond sediment (25). The total oil and grease content of the sediment was 0.02% on a dry weight basis (by Environmental Protection Agency method 413.1).

PCB-contaminated sediments were collected near the western shore of Woods Pond by repeated coring, transferred to 5-gallon buckets, and then topped with site water and sealed. They were briefly stored at 4°C and then transported to Athens, Ga., at ambient temperatures. Woods Pond is a shallow impoundment (60 acres) on the Housatonic River. The pond sediments have a pH of 6.9 to 7.2 and are composed of a mixture of black humic matter, silt, and sand overlying a clay subsediment. These sediments are contaminated with an unidentified hydrocarbon oil (0.5 to 3.2% on a dry weight basis) and with 15 to 180 µg/g (on a sediment dry weight basis) of a PCB mixture composed of tri- to octachlorobiphenyls, the residue from partially dechlorinated Aroclor 1260 (4). Sediments from both locations were stored in glass containers at 4 to 7°C until use (2 and 26 days later, respectively).

**Preparation of slurries and incubation.** The sediment was homogenized under a stream of O<sub>2</sub>-free nitrogen gas with a blender (Hamilton Beach 14-speed blender; A. Glen Dimplex Co., Washington, N.C.), and the slurries were prepared by mixing 8.5 volumes of wet sediment with 1.5 volumes of K<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> buffer at pH 6.9. This pH is close to the original pH of the Woods Pond sediment but higher than the natural pH of 6.5 to 6.8 of SCNC sediment. The final concentration of potassium phosphate was 10 mM. The dry weight of the sediment in the slurries was 0.15 g/ml. Aliquots of the slurries (30 ml) were dispensed into 50-ml serum bottles and allowed to stand overnight in an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lake, Mich.) in an atmosphere of nitrogen and 2 to 3% hydrogen. The next day, 100 µl of a stock solution of 2346-CB (105 mM in acetone) was added to each serum bottle to give a final concentration of 350 µM (micromole per liter of slurry). The bottles were crimp sealed with Teflon-lined butyl rubber stoppers (catalog no. 224100-175; Wheaton, Millville, N.J.). After being vigorously shaken by hand for 1 min, the samples were allowed to stand in the chamber overnight. Sterile controls were autoclaved twice for 1 h at 121°C on each of two consecutive days before the congener was added. Triplicate samples and controls were incubated in the dark without shaking at the following temperatures: 4, 8, 12, 15, 18, 20, 22, 25, 27, 30, 34, 37, 40, 45, 50, 55, 60, and 66°C (Woods Pond sediment samples) and 4, 12, 18, 25, 27, 30, 34, 40, and 50°C (SCNC sediment samples). Water baths were used for

most incubations. Thermometers in each water bath were calibrated with a certified Environmental Protection Agency water analysis thermometer (Fisher Scientific; a National Institute of Standards and Technique traceable certificate showed actual readings at seven calibration temperatures ± 0.02°C). Temperatures in the water baths were checked frequently and were maintained at the desired temperature with a variation of less than ±1°C. Woods Pond sediment samples incubated at temperatures from 15 to 34°C and at 50°C were respiked with 2346-CB (350 µM) when most of the congener had been dechlorinated.

**Sample extraction and analysis.** PCBs were analyzed at various time points for more than a year. Samples were withdrawn at intervals ranging from 2 days for highly active samples respiked with 2346-CB to more than 2 months for samples with extremely low levels of dechlorination activity. After vigorous shaking, aliquots (1 ml) of the slurries were sampled with a 1-ml micropipettor (Wheaton) in an anaerobic chamber. To facilitate sampling of the slurries, the first 2 to 3 mm were cut off the pipette tip. PCBs were extracted by vigorously shaking the samples on a G24 environmental incubator shaker (New Brunswick Scientific Co., Edison, N.J.) at 200 strokes per min for 24 h with diethyl ether (5 ml) in 8-ml vials sealed with Teflon-lined foam-backed screw caps (catalog no. C4801-8 and B7503-2; Baxter, McGaw Park, Ill.). Octachloronaphthalene (4 µg/ml) was added to the ether as an internal standard before extraction of the samples. After centrifugation at 1,570 × *g* for 30 min, the organic phase of each sample was transferred from the ether-sediment mixture into a fresh 8-ml vial and shaken with elemental mercury (0.1 ml) for 30 min to remove sulfur compounds. The extracts were analyzed with a gas chromatograph (Hewlett-Packard 5890 series II) fitted with a DB-1 (polydimethylsiloxane) capillary column (30 m by 0.25 mm [interior diameter] by 0.25 µm; J & W Scientific, Folsom, Calif.) and an Ni<sup>63</sup> electron capture detector. The detector temperature was 300°C, and the injector temperature was 250°C. The oven temperature was raised from 40 to 160°C at increments of 20°C per min, held for 3 min, and then raised to 200°C at increments of 2°C per min and finally to 250°C at increments of 8°C per min and held for 18 min. We initially used a reference standard composed of Aroclors 1242, 1254, and 1260 (70:20:10) and congener assignments reported by Bedard et al. (3) to identify all dechlorination products of 2346-CB and then subsequently confirmed their identities by matching their gas chromatography retention times with those of authentic standards (99% purity; AccuStandard). The calibration mixture contained 2346-CB, 236-CB, 246-CB, 24-CB, 26-CB, 2-CB, 4-CB, and biphenyl. Each congener was quantified by use of a third-order calibration curve (CA-Cricket Graph III 1.01; 1992) generated from reference standards at 15 calibration levels ranging from 0.1 to 150 µM.

**Reduction of experimental data to derive more general statements of observed trends.** The stoichiometric conversion of 2346-CB to products could be determined without serious interference from the PCBs that contaminate Woods Pond sediment, because the total concentration of 2346-CB and its dechlorination products (350 µM initially and 700 µM after respiking) was more than 20-fold higher than that of the Aroclor residue contaminating the sediment (total concentration of extractable PCBs, 15 µM). In order to derive general statements of observed trends, in several instances the variations observed within the data from the triplicate samples were ignored and a single averaged value for each congener at a given temperature and time point was calculated and used. Standard deviations were given where appropriate.

#### RESULTS

**Temperature ranges and optima for dechlorination.** Within a period of 1 year (375 to 385 days), we observed unequivocal dechlorination of 2346-CB to products in PCB-free sediment samples from SCNC at all tested temperatures except 4, 37, and 40°C but not in the sterile controls incubated at the temperatures between 4 and 66°C (data not shown). The best dechlorination occurred at temperatures between 12 and 34°C.

The principal product from the first dechlorination step at all tested temperatures was 246-CB. As an example, Fig. 1 shows the stoichiometric dechlorination of 2346-CB to 246-CB at two different temperatures in each sediment. Figure 2 illustrates the incubation time required to remove the first chlorine from 50% of the 2346-CB (*t*<sub>50</sub>) in each sediment at each temperature. This measurement includes the acclimation time, which may involve adaptation of the microbial community, enzyme induction, and growth of the PCB-dechlorinating population, and is therefore a useful way of quantifying the response of the indigenous microbial population to the addition of 2346-CB. In SCNC sediment the most rapid acclimation and dechlorination occurred at 25 to 34°C, yielding *t*<sub>50</sub> values ranging from 46 to 79 days. At 12°C the *t*<sub>50</sub> increased to 228 days. At 4 and at 37°C and above, only trace amounts of 246-CB were detected after more than 1 year, indicating a negligible

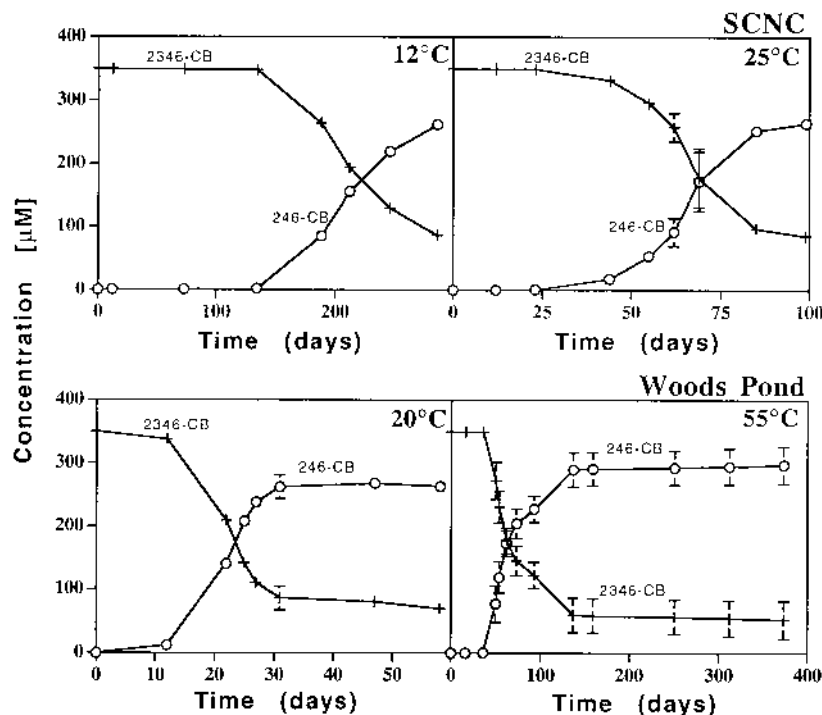


FIG. 1. Time course of dechlorination. The data are averaged values from triplicate samples. Standard deviations are represented by vertical bars; otherwise, the deviations were smaller than the size of the symbols.

dechlorination capability in this pond for 2346-CB at these temperatures.

In Woods Pond sediment samples we observed at least traces of dechlorination products from 2346-CB at all tested temperatures from 4 to 66°C. In all except one case (the first incubation at 18°C), the principal product from the first dechlorination step was again 246-CB. The most rapid acclimation and dechlorination occurred at temperatures from 20 to 30°C, yielding  $t_{50}$  values ranging from 16.1 to 23.5 days. At lower temperatures, the  $t_{50}$  increased sharply, reaching 147 days at 8°C (Fig. 2). The  $t_{50}$  value increased to 51 days at 34°C but could not be calculated at 37, 40, 45, and 66°C because of the minor or negligible dechlorination at those temperatures.

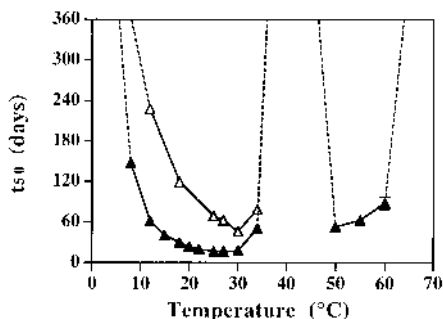


FIG. 2. Times required for loss of the first chlorine from 50% of the 2346-CB ( $t_{50}$  values) in SCNC ( $\Delta$ ) and Woods Pond ( $\blacktriangle$ ) sediment samples at various temperatures. At 4, 37, 40, 45, and 66°C in Woods Pond sediment samples and at 4, 37, 40, and 50°C in SCNC sediment samples, small amounts of dechlorination of 246-CB were observed after more than a year, but the  $t_{50}$  could not be determined because less than 50% of the congener had been dechlorinated. The data are averaged concentrations from triplicate samples. Standard deviations are represented by vertical bars; otherwise, the deviations were smaller than the size of the symbols.

In the thermobiotic range of 50 to 60°C, significant dechlorination occurred and  $t_{50}$  values ranged from 53 to 88 days.

**Dechlorination products of 2346-CB.** The final dechlorination products of 2346-CB (after 1 year) in the two sediment samples varied at different temperatures. In SCNC sediment samples, the 2346-CB was dechlorinated to various amounts of 236-CB, 246-CB, and 26-CB at all temperatures from 12 to 30°C. The highest formation of 26-CB was observed at 30°C. At 4 and at 34°C the sole product was 246-CB. No monochlorobiphenyls or biphenyl was detected at any of the tested temperatures.

In Woods Pond sediment samples, 2346-CB was dechlorinated to varying amounts of 236-CB, 246-CB, 24-CB, 26-CB, 2-CB, and 4-CB at temperatures from 12 to 30°C. The highest amounts of monochlorobiphenyls (56.5 mol%) were formed at 27°C. At 8 and 34°C, the major products were trichlorobiphenyls and only small amounts of dichlorobiphenyls were formed. Below 8°C and above 34°C the only products were trichlorobiphenyls. No biphenyl was detected at any temperature.

**Maximal rates of dechlorination of 2346-CB.** The highest dechlorination rate of 2346-CB (as measured by the disappearance of 2346-CB and concomitant appearance of the trichlorobiphenyl products) (Fig. 1) was observed at 30°C in both sediment samples ( $\approx 16 \mu\text{mol liter}^{-1} \text{day}^{-1}$  in SCNC samples and  $\approx 46 \mu\text{mol liter}^{-1} \text{day}^{-1}$  in Woods Pond samples) (Fig. 3). In SCNC sediment the maximal observed rate of dechlorination decreased by 46% at 27°C, 70% at 18°C, and 87% at 12°C. In Woods Pond sediment the maximal observed rate of dechlorination remained within 15% of the highest observed value over the broad temperature range of 20 to 34°C but then decreased by 72% at 15°C and by 93% at 8°C. The rate of dechlorination was negligible between 37 and 45°C and then rose to appreciable levels in the thermobiotic temperature range of 50 to 60°C ( $4.3$  to  $12.5 \mu\text{mol liter}^{-1} \text{day}^{-1}$ ).

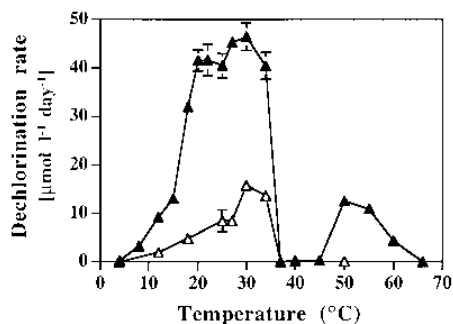


FIG. 3. Maximal observed rates of dechlorination of 2346-CB in SCNC ( $\Delta$ ) and Woods Pond ( $\blacktriangle$ ) sediment samples at various temperatures. The data are averaged values from triplicate samples. Standard deviations are represented by vertical bars; otherwise, the deviations were smaller than the size of the symbols.

**Extent of dechlorination of the 2346-CB.** In SCNC samples the highest overall dechlorination (lowest residual chlorine number) of 2346-CB after 1 year was observed at 30°C (Fig. 4A). Nearly complete *meta* dechlorination of 2346-CB occurred from 12 to 34°C, and nearly complete *para* dechlorina-

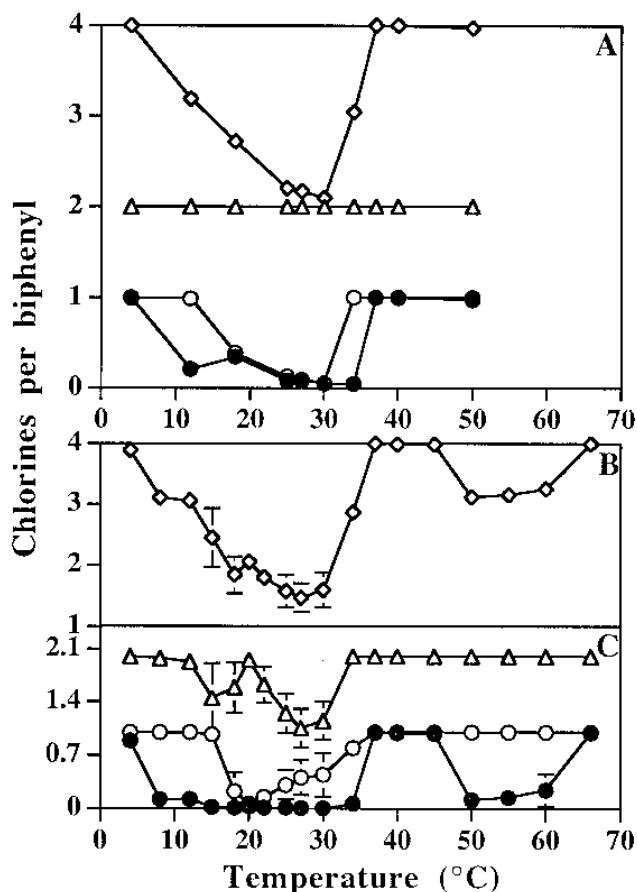


FIG. 4. Residual chlorines of 2346-CB after 1 year of incubation at various temperatures. (A) SCNC samples. (B and C) Woods Pond samples.  $\bullet$ , *meta* chlorines;  $\circ$ , *para* chlorines;  $\Delta$ , *ortho* chlorines;  $\diamond$ , total chlorines. The samples incubated between 15 and 34°C and those incubated at 50°C were respiked with the congener. The data are averaged values from triplicate samples. Standard deviations are represented by vertical bars; otherwise, the deviations were smaller than the size of the symbols.

tion occurred from 18 to 30°C. No *ortho* dechlorination was observed at any of the tested temperatures.

In Woods Pond sediment samples the maximal removal of chlorines after 1 year occurred at 27°C (Fig. 4B). This differs from the temperature (30°C) at which the maximal dechlorination rate for 2346-CB was observed (Fig. 3). Virtually all of the *meta* chlorines were removed from 2346-CB at temperatures between 8 and 34°C. Substantial *meta* dechlorination (75 to 89% removal) was also observed at thermobiotic temperatures (50 to 60°C). In contrast, *para* dechlorination was observed only from 18 to 34°C, and there was a clear optimum at 20°C with a steady decline above this temperature. *ortho* dechlorination was strongest at 25 to 30°C, but fewer than half of the *ortho* chlorines were removed. *ortho* dechlorination dropped to 5% at 20°C and then increased to a second maximum at 15°C. Little or no *ortho* dechlorination occurred below 15°C or above 30°C.

## DISCUSSION

**Effect of prior PCB contamination on the PCB-dechlorinating community.** It has been reported that PCB dechlorination can be detected in a number of PCB-free sediments upon the addition of PCBs in laboratory tests (1). Therefore, it was not surprising to find PCB-dechlorinating microorganisms as well as chlorophenol-transforming anaerobes (15, 18, 19) in PCB-free SCNC sediment. Our results indicate unequivocally that PCB dechlorination in these sediments occurred at reasonable rates, although at lower rates and to a lesser extent than in the PCB-contaminated sediment from Woods Pond. Our data support the previous observation (21) that the microbial communities present in PCB-contaminated sites are better adapted for PCB dechlorination than those in PCB-free sites.

**Temperature effects on reductive dechlorination: general observations.** Previous studies give little information about the specific effects of temperature on PCB dechlorination. PCB dechlorination was observed at 12°C in laboratory experiments with microorganisms from Hudson River sediments, but the dechlorination progressed only half as fast as at 25°C and the pattern of dechlorination was much more limited (5, 24). No dechlorination occurred at 37°C.

Temperature influences, to some extent, the adsorption and desorption kinetics of PCBs from soil particles (16, 17) and thus the availability of PCBs for microbial transformations. However, these effects are probably minor in comparison with the effects on biological activity. The temperature-dependent changes in PCB dechlorination that we observed in SCNC and Woods Pond sediments most likely result from the aggregate effects of temperature on the whole ecosystem, including the composition of the active community, the interactions between its members, and the enzyme-catalyzed dehalogenation reaction(s). PCB dechlorination in sediments probably results from the action of multiple distinct PCB-dechlorinating populations interacting with nondechlorinating microorganisms in syntrophic communities. Presumably each distinct variety of PCB-dechlorinating microorganism has its own temperature requirements, but the temperature requirements of the various nondechlorinating microorganisms in the community are also important because they may partially control the activity of the dechlorinators by affecting the supply of electron donors, electron acceptors, and micronutrients. Similar complex temperature effects have been reported for the reductive dechlorination of chlorophenols (29, 30) and chlorobenzoates (19). In the experiments reported here, temperature influenced the rate, extent, and products of PCB dechlorination and the influence of temperature differed substantially in the two sediments. The

two sediments differ in various ways, e.g., pH, average temperature exposure during the year, consistency, presence and absence of oil and PCB contamination, and seasonal changes of nutrients, and thus likely harbor communities with different PCB-dehalogenating members.

**Effect of temperature on the regiospecificity of dechlorination.** We observed substantial dechlorination at 12 to 34°C in SCNC sediment and at 4 to 34°C and 50 to 60°C in Woods Pond sediment. Maximal chlorine removal occurred at 30°C in SCNC sediment and at 25 to 30°C in Woods Pond sediment. *meta* dechlorination was the preferred initial dechlorination step in both sediments and was more robust than dechlorination from other positions: most of the *meta* chlorines were removed over a broad temperature range. The temperature optimum for *meta* dechlorination was 30°C for both sediments. Our finding that microorganisms from both sediments preferentially remove the doubly flanked *meta* chlorine from 2346-CB is consistent with Williams' observations (28) that microorganisms from three different freshwater sediments preferentially removed the doubly flanked central chlorine from 234-CB and 345-CB.

*para* dechlorination occurred over a narrower range than *meta* dechlorination in both sediments, but there were significant differences in the impact of temperature on *para* dechlorination in the two sediments. In SCNC sediment the extent of *para* dechlorination was equal to that of *meta* dechlorination from 18 to 30°C, and the temperature optimum of *para* dechlorination (30°C) was the same as for *meta* dechlorination. In Woods Pond sediment, *para* dechlorination was more variable among replicates and was more sensitive to small temperature changes than *meta* dechlorination. Furthermore, in Woods Pond sediment the optimum for *para* dechlorination was 20°C, significantly lower than that of *meta* dechlorination (Fig. 4B).

*ortho* dechlorination was observed only in Woods Pond sediment. It is striking that although this dechlorination occurred over nearly the same temperature range as *para* dechlorination, it dropped to a near minimum at the temperature at which *para* dechlorination was optimal (20°C) (Fig. 4B). This and the observed variation between replicates in the extent of *ortho* dechlorination suggest competition between *ortho* and *para* dechlorination. Our observations that the microorganisms in Woods Pond sediment can dechlorinate PCBs from the *ortho*, *meta*, and *para* positions are in agreement with previous reports of PCB dechlorination from the same location (4, 7, 26, 28). In addition, the differential impact of temperature on *ortho*, *meta*, and *para* dechlorination in Woods Pond sediment further supports the hypothesis that several different dechlorinating enzymes, and probably several different dechlorinating microorganisms, are involved in PCB dechlorination in this sediment (3–5, 7).

**Impact of temperature on the rate of dechlorination.** Temperature effects are frequently described by applying the Arrhenius equation. Although developed for a simple chemical reaction, the Arrhenius plot is very useful in describing temperature effects on more complex reactions as long as one does not interpret it in specific physicochemical terms. Linear graphs indicate that the same factor(s) is probably rate limiting over the temperature range, whereas breaks usually indicate a change in the factors limiting the rate of the reaction. Thus, the Arrhenius equation provides a method for incorporating the effect of temperatures into complex mathematical models for predicting the fate of organic compounds released into the environment. The results can be safely extrapolated over the temperature range for which the Arrhenius plot is linear; however, the boundaries of the linear relationship must be defined. The differences in the relationship of the maximal observed

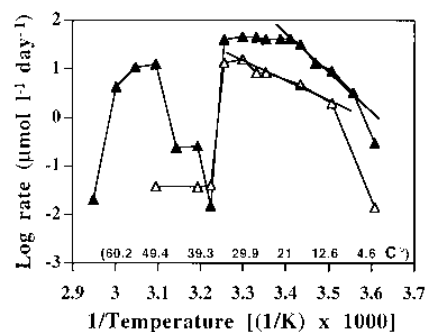


FIG. 5. Arrhenius plots of the 2346-CB dechlorination rates of SCNC ( $\Delta$ ) and Woods Pond ( $\blacktriangle$ ) sediment samples.

rate of removal of the first chlorine to different incubation temperatures for the SCNC and Woods Pond sediment samples are illustrated with an Arrhenius graph (Fig. 5).

In SCNC samples a linear relationship was observed in the temperature range from 12 to 30°C (Fig. 5). This suggests that the same factor(s) was probably rate limiting for the dechlorination of 2346-CB to 246-CB over that temperature range. However, the sharp drop above 34°C may indicate that a different enzyme or microorganism was responsible for the dechlorination observed at 50°C or, alternatively, that a different factor became rate limiting.

In Woods Pond samples, dechlorination of 2346-CB occurred between 4 and 66°C, but the Arrhenius plot was linear only for the narrow range from 8 to 20°C (Fig. 5). Beyond these ranges, the temperature dependence varied significantly, suggesting multiple changes in the factors limiting the rate of dechlorination. We speculate that in this instance the observed changes may reflect the involvement of more than one dechlorinating enzyme or dechlorinating microorganism as well as the impact of various temperature-dependent microbial interactions on individual 2346-CB-dechlorinating population(s). The two clearly distinct temperature optima illustrated in the Arrhenius graph (Fig. 5) suggest the presence of both mesophilic and thermophilic dechlorinating communities.

**Thermophilic PCB dechlorination activity.** This is the first demonstration of PCB dechlorination at temperatures suited for the growth of thermophiles. The dechlorination activity at 50 to 60°C was not expected for Woods Pond sediment samples, because the summer temperatures of the sediment do not exceed 22°C, but it is not unusual to find thermophilic microorganisms in areas that are not usually exposed to high temperatures at the macro level (27). It will be necessary to establish highly enriched or pure cultures to determine whether the microorganisms that dehalogenate 2346-CB at 50 to 60°C are truly thermophilic or thermotolerant and how much this relatively narrow temperature range is due to properties of and interactions with the other members of the microbial community.

**Implications.** It is tempting to make predictions about biodehalogenation of chlorinated organics in the environment based on laboratory experiments conducted at a single temperature. However, the data presented here clearly demonstrate that this practice can be highly misleading. The data from the Arrhenius graphs and the parallel temperature relationships for *meta* and *para* dechlorination in SCNC sediment suggest that the same factors control PCB dechlorination over a broad temperature range in this sediment. This is clearly not the case for Woods Pond sediment. The Arrhenius curve for Woods Pond sediment is essentially flat from 20 to 34°C, sug-

gesting multiple changes in the factors controlling the rate of dechlorination of 2346-CB in this temperature range. The distinct temperature optima for *ortho*, *meta*, and *para* dechlorination in Woods Pond sediment (Fig. 3 and 4B) further illustrate the complexity of PCB dechlorination in this sediment. If highly enriched or pure cultures of the PCB-dechlorinating microorganisms can be obtained, they will permit separation of direct temperature effects on the PCB dechlorinators themselves from those on carbon sources, electron donors, or micronutrients supplied by nondechlorinators. Nevertheless, the reality is that PCB dechlorinators in sediments exist in complex communities that exhibit complex responses to temperature. Thus, to obtain realistic predictions for bioremediation of specific sites, it is recommended that the biodegradation tests are performed with samples from those sites incubated at several environmentally relevant temperatures.

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