



Microbial PCB dechlorination in dredged sediments and the effect of moisture

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Abstract

Evidence of reductive dechlorination of polychlorinated biphenyls (PCBs) in sediments was investigated in Hudson River sediments dredged and encapsulated in 1978 at Moreau, NY. The effect of different moisture contents in dredged sediments on dechlorination and dechlorinating microorganisms was also determined using PCB-spiked sediments in which the moisture level was adjusted by simulating a dewatering process. The congener pattern of PCBs indicated that the dechlorination in the dredged sediments was far less advanced than that in the river sediments collected from the general area of the dredged site (Ft. Edward site). Dechlorination in encapsulated sediments at the Moreau site appeared to have stopped soon after dredging. When microorganisms eluted from the encapsulated sediments were inoculated in clean sediments spiked with Aroclor 1242, an extensive dechlorination was observed, indicating that the encapsulated sediments still harbored dechlorinating microorganisms. However, the same inoculum failed to further dechlorinate residual congeners in the dredged sediments. On the other hand, an inoculum obtained in 1990 from the dredged site in the Hudson River dechlorinated the residual congeners further. In simulated dredged sediments, the maximum level of dechlorination was lower at reduced moisture contents. The population size of dechlorinating microorganisms, as determined by the most probable number (MPN) technique, was also smaller at the lower moisture levels. There was a significant correlation between the maximum extent of dechlorination and the specific death rate of dechlorinating populations. These results indicate that the underlying mechanism of the moisture-dependent maximum dechlorination is the moisture-dependence of the death rate of dechlorinating microorganisms. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The remediation of polychlorinated biphenyl (PCB) contaminated sediments in situ poses many technological and logistical problems. Heavily contaminated sediments are frequently dredged and disposed of in contained landfills as a preferred remediation method (Fraser, 1993; Gullbring and Hammar, 1993). Some of

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the most heavily contaminated sediments in the Hudson River were also dredged and encapsulated in 1978 at Moreau, NY. Preliminary analysis of these sediments taken in 1989 showed congener patterns that were far different from those in sediments taken from the general dredging site in the river. Visual observation indicated a very low moisture level in the dredged sediments (G.-Y. Rhee, unpublished data).

The importance of moisture content in biodegradation is well known. Both microbial activity and community size decrease as the moisture content decreases below the optimum level (Chen and Alexander, 1973; Orchard and Cook, 1983; Clein and Schimel, 1994; Barros et al., 1995) and the degradation of several different types of pesticides decreases with the moisture level (Parker and Doxtader, 1983; Ou, 1984; Chapman et al., 1986; Choi et al., 1988). The low moisture level in dredged sediments may then also hamper PCB dechlorination by changing the size and composition of the dechlorinating microbial community. Earlier investigations have shown evidence that PCB dechlorination is closely linked to the growth of dechlorinating microorganisms (Kim and Rhee, 1997). Recent studies have also revealed a significant correlation between the specific growth rate of dechlorinators and the rate of chlorine removal (Rhee et al., 1999; G.-Y. Rhee, unpublished data).

The present study investigated the level and pattern of PCB dechlorination in the dredged sediments and the effects of moisture content on PCB dechlorination and dechlorinating microbial populations.

2. Materials and methods

2.1. Sediment samples

Sediment cores were collected from the upper Hudson River below Ft. Edward at river mile (RM) 189 in 1990 using a core sampler. These cores were sectioned at 5 cm intervals and the total PCB concentration and congener patterns were determined for each section. For the present studies, we selected sections at a depth between 30 and 50 cm of a core with a total PCB concentration of approximately 200 ppm ($\mu\text{g g sediment}^{-1}$) and these sections were then integrated by mixing in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) with an $\text{N}_2:\text{CO}_2:\text{H}_2$ atmosphere (85:5:10). These integrated sediments were then sieved through a 150 μm sieve and made into slurries using reduced synthetic mineral medium (Balch et al., 1979). A portion of the same sediments was used to elute dechlorinating microorganisms that were subsequently used to determine further dechlorination with the restoration of moisture content to a saturation level (5 g dry weight

sediments in 50 ml water). Sediment cores of dredged river sediments were taken from the Moreau encapsulation site in 1989 by the New York State Department of Environmental Conservation. This site was built in 1978 to contain dredged sediments from the Hudson River in a clay capsule.

PCB-contaminated sediments were also taken in 1990 from the remnant site on the Hudson River bank (near RM 189) to determine the congener patterns. These exposed sediments were originally under water until the water level dropped with the removal of the Fort Edward Dam in 1973. Because of the exposure to air, they had very low moisture content at the time of sampling. (This site has since been capped to prevent PCB volatilization.)

2.2. Preparation of PCB-spiked sediments and dechlorinating inoculum

PCB-free sediments were collected from the Grasse River, NY, air-dried, and sifted through a 150 μm sieve before use. These clean sediments were spiked with PCBs by mixing with Aroclor 1242 or 1248 (AccuStandard, New Haven, CT) in hexane to yield a final concentration of 300 ppm (sediment dry weight basis). The hexane was removed by evaporation. These PCB-spiked sediments were then made into slurries by adding reduced synthetic mineral medium (Balch et al., 1979) in an anaerobic chamber. Resazurin (0.0001% final concentration) was added to the slurries as a redox indicator. Subsamples of this mixture were prepared by dispensing 50 ml aliquots of this sediment slurry into 100 ml serum vials. All vials were crimp-sealed with Teflon-lined rubber septa and aluminum crimp, removed from the anaerobic chamber, and autoclaved.

In order to determine the presence of dechlorinating microorganisms in the dredged sediment (Moreau site) and the river sediment (Ft. Edward site), an inoculum was eluted from these sediments. This inoculum was added into sediment slurries amended with Aroclor 1242 or slurries of autoclaved Moreau sediments to determine the dechlorinating activity. To study the effect of the moisture content on dechlorination, an inoculum was eluted from the sediments from the Reynold site in the St. Lawrence River (for site description, see Sokol et al., 1994) which were primarily contaminated by Aroclor 1248. This inoculum was used to investigate the effect of moisture content on PCB dechlorination in clean sediments spiked with Aroclor 1248. This Aroclor was chosen rather than Aroclor 1242 because there are more steps of dechlorination to reveal different pathways than with Aroclor 1242 which has fewer Cl substitutions. Inoculated sediment vials were incubated statically in the dark at room temperature.

2.3. Moisture content adjustment

To investigate the effect of moisture content, the sediment slurries, spiked with Aroclor 1248 and inoculated with sediment microorganisms from the St. Lawrence River, were incubated until active dechlorination was under way. When dechlorination removed about 9% of total chlorines after 8 weeks of incubation, all the sample vials were transferred into an anaerobic chamber. The slurry samples were mixed in a 1 l glass beaker and divided into five portions. The moisture was adjusted by gravity drain to appropriate levels (95%, 70%, 45%, and 15%). The gravity-drain apparatus (Fig. 1) was made with a 1 l beaker filled with glass beads (5 cm depth) as a support and covered with GF/C filter paper (Whatman, Clifton, NJ). The edge of the filter was sealed with Teflon tape. We monitored the moisture content (see below) by taking subsamples of sediment cores using glass tubing. It took 2 days to reach a moisture level of 95%; 6 days for 70%; 9 days for 45%; and 10 days for 15%. Once the moisture level reached the desired level, sediments from the top layer well above the filter paper were transferred into 100 ml serum vials in triplicate.

Sediment samples for time-course analysis from these vials were taken with a spatula or a Pasteur pipette, depending on moisture content. A portion of the sample was used to determine dry weight after drying in an oven at 150°C for 2 h. The remainder was used to make a dilution series to count the number of dechlorinating microorganisms by the most probable number (MPN) technique, a statistical method based on the serial dilution of microorganism that permits the estimation of population density by measuring a characteristic transformation in a given dilution.

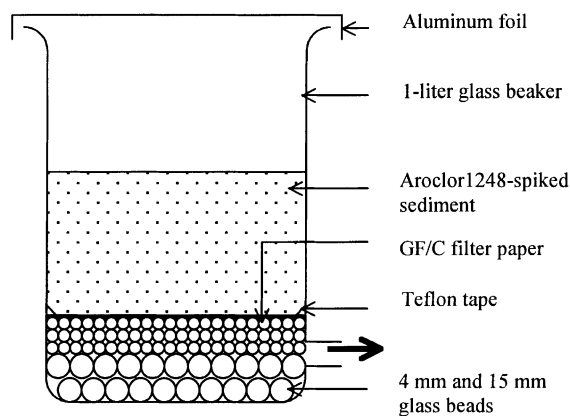


Fig. 1. Diagram of the gravity-drain apparatus used to adjust moisture level. The bold arrow indicates the water drain.

2.4. MPN estimation

The number of PCB-dechlorinating microorganisms was estimated by assaying the dechlorination of 2,3,4- and 2,5,3',4'-chlorobiphenyls (CBPs) in MPN vials after 15 weeks of incubation (Kim and Rhee, 1997). The preparation of MPN vials and procedures were as described by Kim and Rhee (1997). After 15 weeks, MPN vials showing dechlorination were counted as positive when dechlorination removed greater than 5% of the total chlorines. MPNs were calculated from the frequency of positive vials by using a Microsoft Excel program (Briones and Reichardt, 1999) and were normalized to gram dry weight of sediment.

2.5. PCB extraction and analysis

For congener-specific analysis, sediments were extracted with acetone and hexane by ultrasonication, sulfur was removed by tetrabutylammonium hydrogen-sulfate, and extracts were cleaned through a Florisil column as described previously (Rhee et al., 1993). PCBs in the MPN test vials were extracted with acetone and hexane by shaking in an orbital shaker as described by Kim and Rhee (1997). Congener-specific PCB analysis was performed on one of the two Hewlett Packard 5890 gas chromatographs (GCs), each equipped with a ^{63}Ni electron-capture detector, autosampler, splitless injector, and a computerized data acquisition system (Chrom Perfect, Justice Innovations, Mountain View, CA). Aroclor 1242 or Aroclor 1248 was separated on a 60 m Rtx[®]-5 capillary column (Restek, Bellefonte, PA). The gas chromatography conditions used to analyze Aroclor 1242 and 1248 were described elsewhere (Sokol et al., 1994). The PCB congeners in the extract were identified and quantitated using a calibration standard containing a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 $\mu\text{g ml}^{-1}$ of each in hexane). Peaks were identified and calibrated as previously described (Rhee et al., 1993; Kim and Rhee, 1997; Sokol et al., 1994, 1998a, 1998b). The analysis resolves 98 peaks representing 127 congeners. An Apiezon-L column (Restek) was used to analyze the samples from the MPN vials. The temperature program for the Apiezon-L column was described by Sokol et al. (1998a). PCB extracts run on the Apiezon-L column were identified and quantitated with a calibration standard composed of a mixture of 47 individually weighed authentic single congener standards (Accu-Standard; 99% purity).

The calibration standards were run after every sixth sample for recalibration as part of quality assurance/quality control. A "dilute to match" procedure (Kimbrough et al., 1994) was used to ensure that all samples were analyzed within the linear range of the calibration standard. Uninoculated PCB-spiked sediment controls, setup at the beginning of the experiment and sampled at

every time point, were used to monitor the extraction efficiency. Method blanks were included with each set of samples extracted. Precision on the GC was also ensured by running duplicate samples. The PCB congeners in each sample were calculated and expressed as mole percent. The average number of total Cl per biphenyl (total Cls/BP) and the average number of *ortho*, *meta*, and *para* Cl were individually calculated from the product of the average number of chlorines and the molar concentration for each peak divided by the total molar concentration summed over all peaks. All calculations were based on the conservation of the biphenyl moiety and the assumption that coeluting congeners were present in equal proportions (Quensen et al., 1990; Sokol et al., 1994).

2.6. Statistical analysis

Differences between final congener dechlorination patterns were quantified and compared by calculating the Euclidean distance (E_d) between samples. A sample is considered to occupy a point in multidimensional space in which each congener as the fraction of total PCB mass comprises a dimension and each axis is orthogonal. The distance between two samples indicates differences between them, with longer distances indicating greater dissimilarities. The distance was calculated as

$$E_d = \sqrt{\sum (A_i - B_i)^2}, \quad (1)$$

where A_i and B_i are the mole fractions of each congener (i) in samples A and B (Litten et al., 1993).

3. Results

3.1. PCB dechlorination in dredged sediments from the Hudson River

The PCB congener pattern in sediments dredged from the Hudson River and encapsulated in a confined disposal facility at Moreau, NY, in 1978, was clearly different from that in the river sediments taken in 1990 from the same general site (Fig. 2). These sediments were contaminated mainly by Aroclor 1242 although evidence of other Aroclors was also found. If we assume for the purpose of comparison that the site was contaminated only by Aroclor 1242, then only 9% of the total Cls were removed in the dredged sediments, whereas 41% were removed in the river sediments at the site of the previous dredging. The encapsulated sediments showed significant decreases primarily in 2,5,3',4'-, 2,3,6,4'-, 2,3,2',5'- and 2,3',4'-CBP with the accumulation of 2,2'- + 2,6-CBP. The river sediments, on the other hand, showed an extensive decline in many more congener peaks (2,5,2'-,

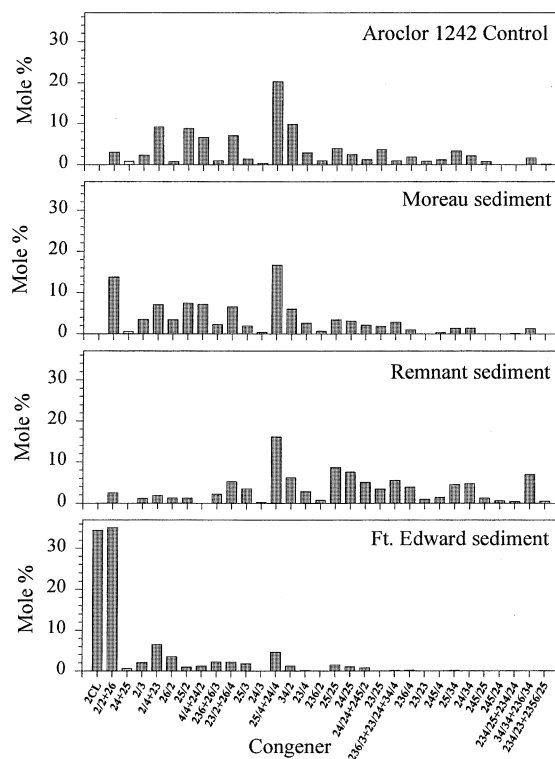


Fig. 2. The congener pattern of PCBs in sediments at the encapsulation (Moreau) and remnant sites and in the Hudson River at the dredged site (Ft. Edward) in 1990. Each value is the mean of three replicate samples.

4,4'- + 2,4,2'-, 2,3,2'- + 2,6,4'-, 2,5,4'- + 2,4,4'-, 3,4,2'-, 2,3,2',5'-, and 2,5,3',4'-CBP) with the accumulation of primarily mono- and di-CBP, which accounted for approximately 69 mol% of the total PCBs (Fig. 2).

The congener pattern of the encapsulated sediments was much closer to those in sediments at the remnant site on the river bank, which became exposed to air in 1973 with the removal of the Fort Edward Dam, than that in river sediments. These results seem to indicate that there was little dechlorination after dredging.

To investigate whether the encapsulated sediments still harbored dechlorinating microorganisms, two kinds of sediment slurries were prepared: one using clean sediments spiked with Aroclor 1242 (300 ppm) and the other using dredged sediments taken from the encapsulation site with a PCB contamination level of about 200 ppm. These slurries were sterilized by autoclaving and inoculated with microorganisms eluted from the encapsulated sediments. An analysis after 12 weeks of incubation demonstrated clear evidence of dechlorination in the Aroclor 1242-spiked sediments, with significant decreases in tetra- and tri-chlorinated homologs and concomitant increases in di- and mono-chlorinated ones (Fig. 3(a)). However, the same inoculum failed to de-

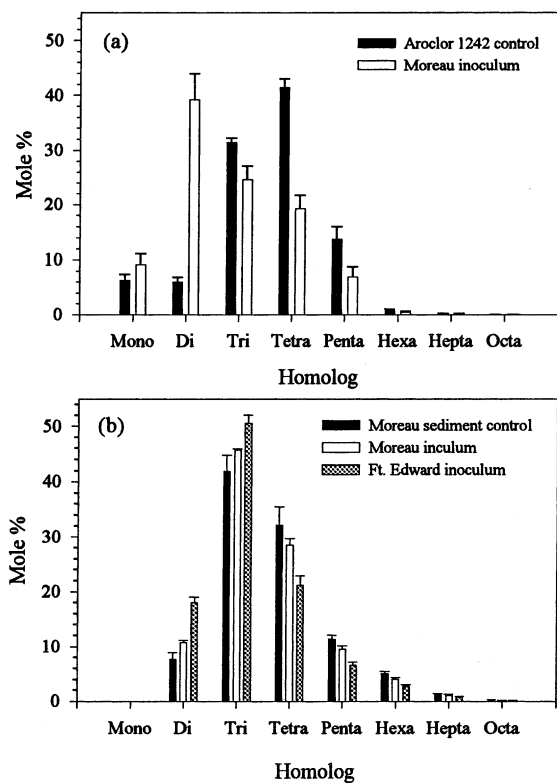


Fig. 3. (a) Relative concentrations of homologs (\pm S.D.) in sediments spiked with Aroclor 1242 and incubated with microorganisms eluted from dredged sediment after 4 months. (b) Relative concentrations of homologs (\pm S.D.) in Moreau sediments incubated with microorganisms eluted from the sediments or river sediments at Ft. Edward after 12 months. Each value represents the mean of three replicate sediment vials.

chlorinate PCBs in the slurries of the dredged sediments even after 12 months of incubation (Fig. 3(b)). On the other hand, when a subset of the same slurries were inoculated with microorganisms eluted from the Hudson River sediments taken at Ft. Edward, NY, greater dechlorination was observed. A plot of homolog distribution after 12 months of incubation showed a clear shift from highly chlorinated to lightly chlorinated ones (Fig. 3(b)). These results indicate that although the dredged sediments still harbored dechlorinating microbial populations, they might be quite different from those in the river sediments.

3.2. Moisture content versus PCB dechlorination

We investigated the effect of moisture content on dechlorination using sediments spiked with Aroclor 1248 (300 ppm) and inoculated with sediment microorganisms from the St. Lawrence River, which was contaminated by the same Aroclor. With tetra-chlorinated

congeners as the major component, there is a greater number of dechlorinating steps or pathways with this Aroclor than with Aroclor 1242 which is primarily comprised of congeners with three Cls or less.

When dechlorination removed about 9% of total chlorines after 8 weeks, the sediment slurry was divided into five portions and the moisture level was adjusted to 95%, 70%, 45%, and 15%. Dechlorination continued without a lag at all moisture levels. In the original slurries, about 29% of the total Cls were removed after 17 weeks, reducing the total Cls/BP from 3.95 to 2.82. Since no further dechlorination was observed during the subsequent 21 weeks of incubation (Fig. 4), this average number was considered a plateau level. The extent of Cl removal in sediments with 95% moisture content was not significantly different from the control slurry sediments, Cls/BP 2.85, with 28% reduction of the total Cls. However, at the lower moisture levels of 45% and 15%, dechlorination ceased when the Cls/BP levels reached 3.03 and 3.07 or when 23% and 22% of the total Cls were removed.

A comparison of the final congener patterns showed differences between the slurry and each sediment with different moisture contents. These differences are shown by the Euclidean distance, which in this case indicates differences between congener patterns with longer distances indicating greater dissimilarities (Fig. 5(a)). However, these dissimilarities were not apparent when the final pattern of each moisture content was compared to the pattern in the slurry at different time points

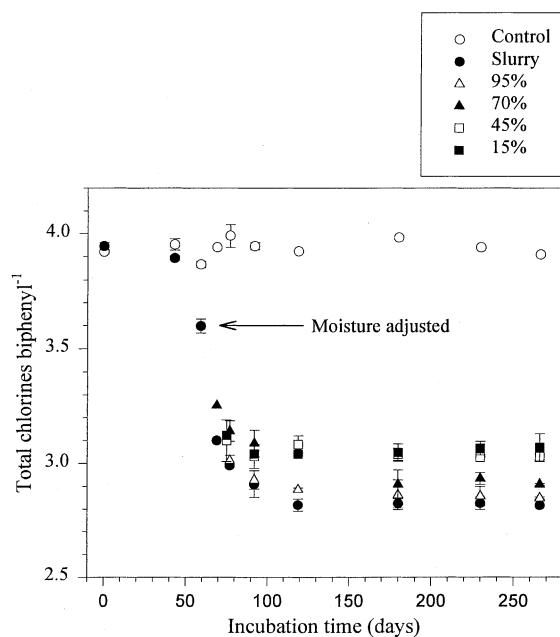


Fig. 4. Time course of dechlorination of Aroclor 1248 at various moisture levels.

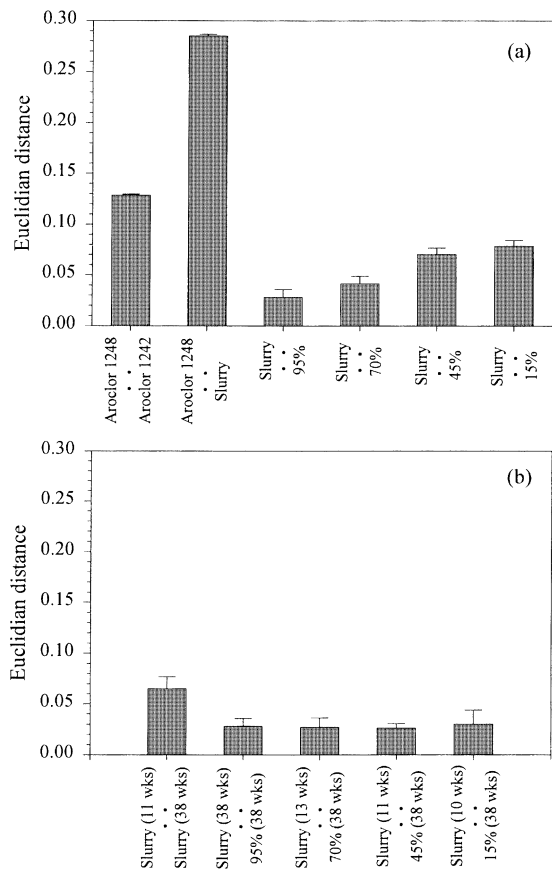


Fig. 5. The Euclidean distance (\pm S.D.) calculated from the mole fractions of each congener. (a) Between Aroclor 1248 and Aroclor 1242; between undechlorinated Aroclor 1248 and dechlorination products of the same Aroclor in sediment slurries after a 38 week incubation; and between dechlorination products in sediment slurries and those in sediments with moisture content of 95%, 70%, 45%, or 15%, respectively, after a 38 week incubation. (b) Between sediment slurries at 11 and 38 weeks of incubation; and between sediment slurries at various time points and sediments of different moisture contents at the end of incubation at 38 weeks.

(Fig. 5(b)). The Euclidean distances between slurry samples at 38 weeks and 95% moisture at 38 weeks, the slurry at 13 weeks and 70% moisture at 38 weeks, the slurry at 11 weeks and 45% moisture at 38 weeks, and the slurry at 10 weeks and 15% moisture at 38 weeks, had similar low values, indicating little difference between these pairs. (As a comparison, a significant change in the congener pattern is represented by the distance between slurry samples at 11 weeks and 38 weeks.) In other words, the final pattern at 95% moisture level after 38 weeks was similar to that of the slurry at 38 weeks, whereas the final pattern at 70%, 45%, and 15% was similar to that of the slurry at 13, 11, and 10 weeks, respectively. These results indicate that varying levels of

maximum dechlorination with moisture represent different stages of dechlorination in the same pathway, rather than differences in the pathway.

3.3. Growth of PCB dechlorinating microorganisms

When the time course of change in the population size of PCB dechlorinators was estimated by the MPN technique, the size of the sediment slurries increased about 4 orders of magnitude from an initial level of 3.6×10^3 to 2.9×10^7 cells g sediment⁻¹ by 9 weeks and maintained the level up to 13 weeks. After dechlorination ceased, the population began to decline as observed in the earlier study (Kim and Rhee, 1997). Although the initial number of dechlorinators in the present study was 2 orders of magnitude lower, the final population size was little different between the two investigations. (The MPN number in the earlier study (Kim and Rhee, 1997) was overestimated by an order of magnitude because of a calculation error.)

The dechlorinating populations decreased exponentially as the moisture level was reduced. The specific rate of population decrease, or the specific death rate calculated as $(d \ln(X_2/X_1)/dt)$, was $0.102 (\pm 0.018)$ day⁻¹ in the sediment slurry as determined by a four-point regression. The values at moisture levels of 95%, 70%, 45%, and 15% were higher: $0.581 (\pm 0.17)$, $0.717 (\pm 0.19)$, $0.819 (\pm 0.18)$, and $1.19 (\pm 0.16)$ day⁻¹, respectively. There was a significant correlation (linear regression; $P < 0.05$) between these values and the apparent maximum level of dechlorination (Fig. 6). These results indicate that the underlying mechanism of the moisture-

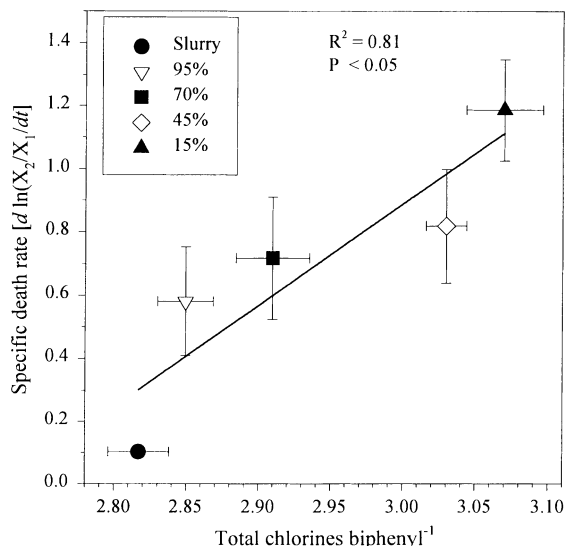


Fig. 6. Correlation between the specific death rate of dechlorinating microorganisms and the maximum level of dechlorination.

dependent maximum dechlorination is the moisture-dependence of the death rate of dechlorinating microorganisms.

4. Discussion

The congener patterns in sediments at the remnant site, the disposal facility at Moreau, and the dredged site in the river, exhibit progressive stages of dechlorination. The sediments at the Moreau encapsulation site were dredged from the river approximately 5 years after the removal of the Fort Edward Dam, and the river sediments analyzed in this study were taken approximately 13 years after the dredging. These results suggest that there has been little microbial dechlorination in sediments at the remnant site and at Moreau; however, active dechlorination was taking place in situ in the Hudson River during these time periods. A recent study of in situ dechlorination in the Thompson Island Pool of the Hudson River shows evidence of active dechlorination, albeit at very slow rates. When the rate was calculated between 1983 and 1991 using ^{137}Cs dated cores, the half-life of dechlorination (mol Cl/mol PCB) was greater than 30 years (McNulty, 1997).

The correlation between the specific death rate of dechlorinating population and the maximum level of dechlorination indicates that dechlorination is closely linked to population growth. This finding is consistent with the results of recent kinetic studies in which the dechlorination of Aroclor 1248 was measured concurrently with the population growth at 10 different PCB concentrations (Rhee et al., 1999; G.-Y. Rhee, unpublished data). In this study, the dechlorination rate (mol Cl removed $\text{g sediment}^{-1} \text{ day}^{-1}$) was found to be significantly correlated to the population growth rate. Therefore, the underlying mechanisms for the moisture-dependent maximum level of dechlorination found in the present investigation appears to be related to the moisture-dependent survival of dechlorinating microorganisms.

It is interesting to note that the microorganisms eluted from the Moreau sediments were unable to further dechlorinate the residual PCBs in these sediments, yet they were capable of dechlorinating Aroclor 1242. Although the reason is unclear, one may speculate with two hypotheses. If dechlorinating microbial populations include obligate and facultative anaerobes, most obligate anaerobes might have died during the dredging and encapsulation process due to exposure to oxygen. Any surviving dechlorinators may be incapable of dechlorinating the residual congeners while still being able to dechlorinate Aroclor 1242. However, this hypothesis cannot explain the observation that the extent of dechlorination of Aroclor 1242 is far more advanced than the level found in the dredged sediments. An alternate hypothesis is that the size of the surviving dechlorinating

populations in the dredged sediment slurries were unable to grow because of an insufficient amount of “dechlorinatable” congeners. Earlier investigations (Kim and Rhee, 1997) showed that dechlorinating microorganisms require PCBs for growth, possibly as alternate electron acceptors, and that when dechlorinatable congeners are exhausted, these microorganisms are unable to grow. Our kinetic study has also revealed the existence of the threshold concentration below which no dechlorination occurs (Sokol et al., 1998b) and the reason for the absence of dechlorination was found to be caused by the inability of dechlorinating microorganisms to grow in this concentration range (Rhee et al., 1999; G.-Y. Rhee, unpublished data). Therefore, the dredged sediment slurries may not have had enough dechlorinatable congeners for population growth, whereas fresh Aroclor 1242 provided such congeners.

The exposure age of the dredged sediments to PCBs, thus the bioavailability of sorbed PCBs, could be a potential reason for the absence of dechlorination in the dredged sediment slurries. If bioavailability is determined by equilibrium partitioning, however, an incubation period of nearly 1 year should have been sufficient to induce significant dechlorination.

The results of the present study indicate that a restoration of moisture level in the dredged sediments at the Moreau site will not restart dechlorination; in addition to an increased level of moisture, it may be necessary to re-inoculate with microorganisms from fresh river sediments. These results also indicate that for any ex situ remediation of PCBs in a confined disposal site using reductive dechlorination, it is essential to maintain an optimum moisture level.

Acknowledgements

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