Biotransformation of polychlorinated biphenyls in St. Lawrence River sediments: reductive dechlorination and dechlorinating microbial populations

Y.-C. Cho, J. Kim, R.C. Sokol, and G-Y. Rhee

Abstract: Polychlorinated biphenyl dechlorinating microbial populations in St. Lawrence River sediments were fractionated and estimated based on the dechlorination pattern using a combination of serial dilution and most probable number techniques. Two distinctive dechlorination patterns were found in most probable number sediments spiked with Aroclor 1248. A high-dilution inoculum decreased the average number of chlorines per biphenyl from 4.0 to 3.4 but was unable to dechlorinate *meta*-substituted congeners consisting mainly of 2,5,2′,5′-, 2,4,2′,5′-, and 2,5,2′-chlorobiphenyl (pattern B). On the other hand, a low-dilution inoculum did dechlorinate the *meta*-rich congeners and reduced the average number of chlorines to 2.9 (pattern A). These results indicate that there are at least two populations. While pattern B was produced by pattern B producing dechlorinators, pattern A was produced by a combination of pattern B producers plus another population that dechlorinates the *meta*-substituted congeners. When the population size was calculated based on the frequency of respective dechlorination patterns, the populations yielding pattern B were approximately 2.4×10^6 cells·g dry weight sediment^{-1} whereas the dechlorinators of the *meta*-rich congeners were two orders of magnitude less at 3.5×10^4 cells·g dry weight sediment^{-1}. Despite lower numbers, these *meta*-dechlorinators in pattern A increased the overall dechlorination by almost twofold.

Résumé : Les populations de microorganismes utilisées pour la déchloration des polychlorobiphényles (PCB) contenus dans les sédiments du Saint-Laurent ont été fractionnées et estimées en fonction du profil de déchloration grâce à la combinaison des techniques de dilution en série et du nombre le plus probable (NPP). Deux profils de déchloration distincts ont été observés avec la technique NPP dans les sédiments auxquels on a ajouté de l'Arochlore 1248. Un inoculum de haute dilution a fait passer de 4,0 à 3,4 le nombre moyen d'atomes de chlore (Cl) par biphényle, mais il n'a pas pu déchlorer les congénères avec substitution en méta composés surtout des 2,5,2′,5′-, 2,4,2′,5′- et 2,5,2′-chlorobiphényles (profil B). Par ailleurs, un inoculum de faible dilution a déchloré les congénères riches en méta et a réduit à 2,9 le nombre moyen de Cl (profil A). Ces résultats montrent l'existence d'au moins deux populations. Le profil B a été obtenu par des agents de déchloration produisant le profil B, mais le profil A a été obtenu par une combinaison de producteurs du profil B plus une autre population qui déchlore les congénères avec substitution en méta. Lorsque l'effectif de la population a été calculé en fonction de la fréquence des profils respectifs de déchloration, les populations donnant le profil B étaient approximativement de 2,4 × 106 cellules·g de sédiment en poids sec-1 tandis que les agents de déchloration des congénères riches en méta donnaient des résultats inférieurs de deux ordres de grandeur, avec 3,5 × 104 cellules·g^{-1} de sédiment en poids sec^{-1}. Malgré leur plus faible nombre, ces agents de déchloration avec substitution en méta dans le profil A ont presque fait doubler globalement la déchloration.

[Traduit par la Rédaction]

Introduction

The St. Lawrence River has been heavily contaminated with polychlorinated biphenyls (PCBs) as a result of past waste disposal practices at the General Motors (GM) Power Train Division and the aluminum production plants of Reynolds Metals and ALCOA (Sokol et al. 1994*b*). PCBs at these facilities were used mainly as a component of hydraulic fluids and electrical equipment. Because of the hydrophobicity of these nonpolar halogenated organic compounds,

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their major sink in the aquatic environment is sediments. Once contaminated, sediments become a chronic source of PCBs to the aquatic food chain, with their release from sediments into the water column essentially governed by equilibrium partitioning.

In general, the partitioning of PCB congeners into the sediment (or nonaqueous phase), expressed as the octanol–water partition coefficient (K_{ow}), increases with the degree of chlorination. Their bioaccumulation in the food chain organisms also exhibits a similar trend (Evans et al. 1991; William et al. 1997). There is clear evidence in the St. Lawrence River sediments and elsewhere that the sediment microbial community in anaerobic layers has been transforming PCBs into lower-chlorinated congeners through reductive dechlorination (Sokol et al. 1994*b*, 1998; Bedard and May 1996; Kim and Rhee 1997, 1999). Such biotransformation would certainly alter the dynamics of exchange between sediments and the water column through increased desorption of lower-chlorinated congeners. This then exposes the food chain organisms to contaminants that are different in their physical properties from those in the original mixture. Lower-chlorinated congeners are less bioaccumulating and have lower vapor pressure. Therefore, they would also volatilize more readily across the air–water interface, reducing the overall PCB budget of the aquatic system. Because the biological activity of PCBs varies with congeners (Parkinson et al. 1983; Seegal 1996; Carpenter et al. 1998), the kind of final congeners produced by dechlorination in sediments may also have different ecological impacts.

Evidence indicates that dechlorinator populations are divergent and that they have distinct dechlorination pathways and products from the same Aroclor mixture (Ye et al. 1992, 1995; Sokol et al. 1994*a*, 1998). The final congener pattern of Aroclor 1248 dechlorination was markedly different between sediment microorganisms from the GM, Reynolds, and ALCOA sites in the St. Lawrence River (Sokol et al. 1994*b*), indicating that dechlorinating populations vary among different sites contaminated with the same PCBs. Such qualitative differences in dechlorination would also have different impacts on the aquatic ecosystem.

The present paper reports investigations of sediment microbial populations in St. Lawrence River sediments for different dechlorination competence and population sizes using the serial dilution and most probable number (MPN) techniques.

Materials and methods

Culture preparation

PCB-free sediments collected from Owasco Lake, New York, were air dried and sifted through a 150-μm sieve. These sediments were spiked with Aroclor 1248 in hexane to yield a concentration of 300 μg·g dry weight sediment^{-1} and hexane was removed by evaporation. The sediments were then made into slurries by adding reduced synthetic mineral medium (Balch et al. 1979) in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) with an N_2–CO_2–H_2 atmosphere (85:5:10). The slurry contained 20% sediments (w/v) and resazurin (0.0001% final concentration) as a redox indicator. Throughout this study, sediments were homogeneously mixed when they were dispensed or sampled.

For population fractionation, Aroclor 1248 spiked sediments inoculated with microorganisms eluted from the GM site in the St. Lawrence River were used after 9 weeks of incubation. The experimental protocols of preparing the inoculum and incubation were described previously (Kim and Rhee 1997). At the time of fractionation, dechlorination had reduced the average number of chlorines per biphenyl in these sediments from 4.0 to 2.8. These sediments were diluted from 10^{-1} to 10^{-9} by taking a 1-mL portion of sediment slurry immediately after mixing and serially transferring into a 9-mL portion of fresh slurry in a 30-mL serum vial. Each dilution was then inoculated into a series of five vials containing Aroclor 1248 spiked sediment slurries to determine the MPN of dechlorinators. The MPN vials were statically incubated at room temperature and analyzed after a 48-week incubation. To determine whether dechlorination patterns can be sequentially transferred, 1-mL portions of 10^{-3} and 10^{-6} dilutions were inoculated into fresh Aroclor 1248 spiked sediments and serially transferred twice after 19 weeks of incubation.

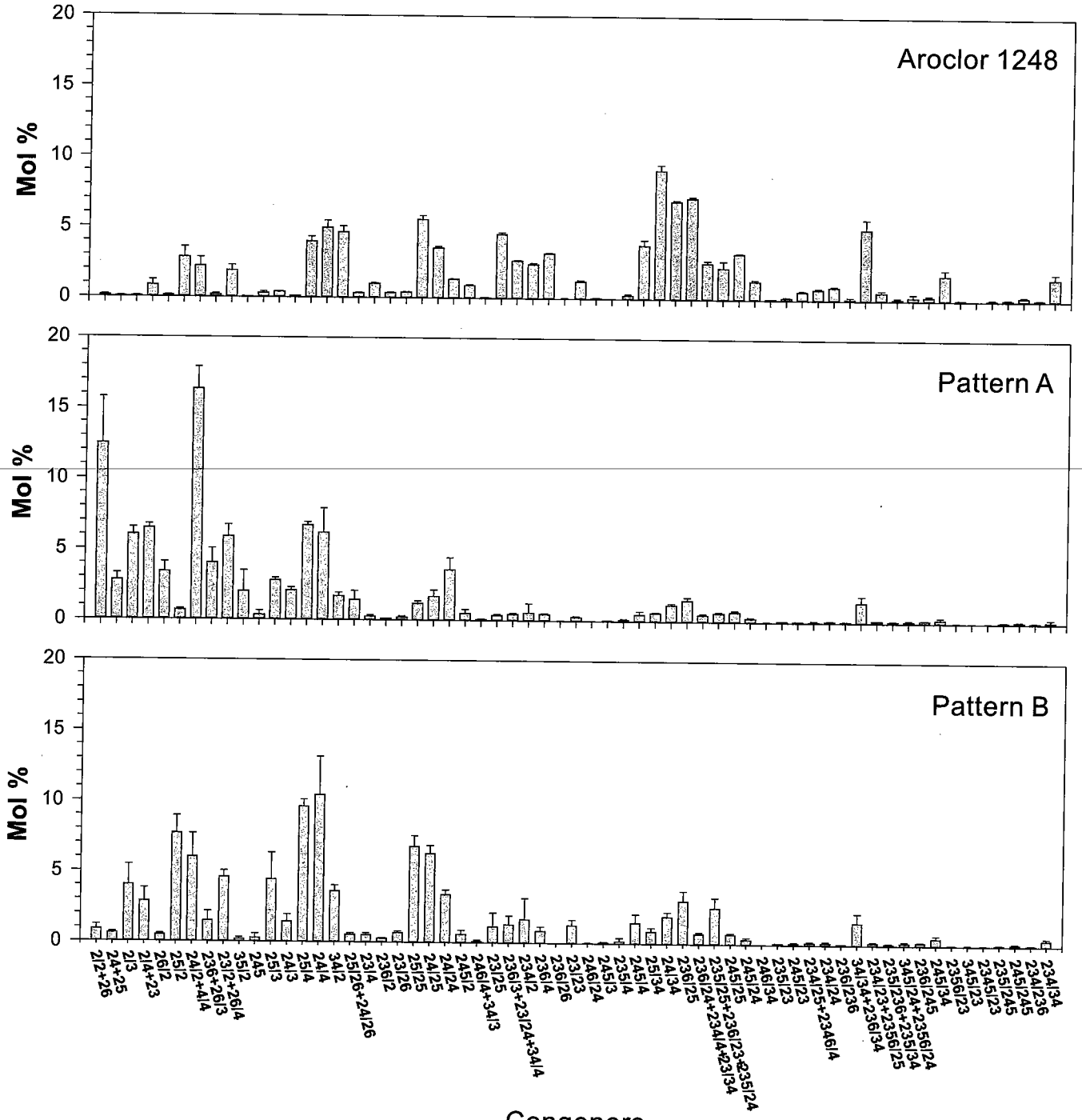
MPN estimation

After a 48-week incubation, MPN vials showing dechlorination were counted as positive when dechlorination removed greater than 5% of the total chlorines. Sulfate reducers were determined by the blackening of sediments in the sample vials with the production of FeS. Methanogens were determined by analyzing the head-space gas of MPN vials for methane on a gas chromatograph (GC) with a thermal conductivity detector (Kim and Rhee 1997). MPNs were calculated from the frequency of positive vials by using an MS-DOS QBASIC program (Koch 1993) and normalized to gram dry weight of sediment.

PCB extraction and analysis

Sediments from the culture vials were extracted with acetone and hexane by ultrasonication, as described previously (Rhee et al. 1993). Congener-specific PCB analysis was performed on a Hewlett-Packard 5890 GC equipped with a ^{63}Ni electron capture detector, autosampler, and splitless injector and interfaced with a computerized data acquisition system (Chrom Perfect, Justice Innovations, Mountain View, Calif.). Samples were analyzed on an Rtx®-5 fused silica capillary column (60 m × 0.25 mm inside diameter × 0.1 mm; Restek, Bellefonte, Pa.). The GC conditions used are described in Sokol et al. (1994*b*). The PCB congeners in the extract were identified and quantified using a calibration standard containing a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 μg·mL^{-1} in hexane). Peaks were identified and calibrated according to response factors published by Schulz et al. (1989), as previously described (Rhee et al. 1993; Sokol et al. 1994*b*, 1998; Kim and Rhee 1997). The calibration standards were run after every sixth sample for recalibration as part of quality assurance and 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. Autoclaved PCB-spiked sediment controls, set up at the beginning of the experiment, were used to monitor extraction efficiency. After a GC run, each calibration standard was checked to ensure proper congener peak assignment and quantitation. For a run to be accepted, calibration standards had to be within ±10% of one another. In addition, every GC chromatogram was manually reviewed to verify peak assignment and to ensure that the resolution, shape, and automatic baseline selection for each peak were appropriate. When required, manual processing of peaks was performed to rectify any problems. The PCB congeners in each sample were calculated and expressed as mole percent. The average number of total chlorines per biphenyl and the average number of *ortho*-, *meta*-, and *para*-chlorines 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 calibrations were based on the conservation of the

Fig. 1. Congener patterns A and B (mean ± SD).



biphenyl moiety and the assumption that coeluting congeners were present in equal proportions (Quensen et al. 1990; Sokol et al. 1994*b*).

Results

Dechlorination was positive in all five MPN vials in each dilution series from 10^{-1} to 10^{-5} and negative in dilutions below 10^{-7} after 11 months of incubation. The 10^{-6} dilution showed two positives and three negatives. The overall extent of dechlorination was identical for 10^{-1} to 10^{-3} dilutions, with the average number of chlorines per biphenyl decreasing to 2.91 from 4.01 in undechlorinated Aroclor 1248. In addition, the final congener pattern in each of these three dilutions showed little difference.

The extent of dechlorination in the 10^{-4} and 10^{-5} dilutions was significantly less than in the 10^{-1} to 10^{-3} dilutions (t test, $P < 0.05$), with an average number of chlorines per biphenyl of 3.36 versus 2.91. This difference was almost entirely due to a limited *meta*-dechlorination.

When the congener pattern was examined for each MPN vial, two different types emerged: one in which *meta*-substituted congeners primarily consisting of 2,5,2′,5′-, 2,4,2′,5′-,

Fig. 2. Difference in parent congeners (top panel) and products (bottom panel) between pattern A and pattern B dechlorination.

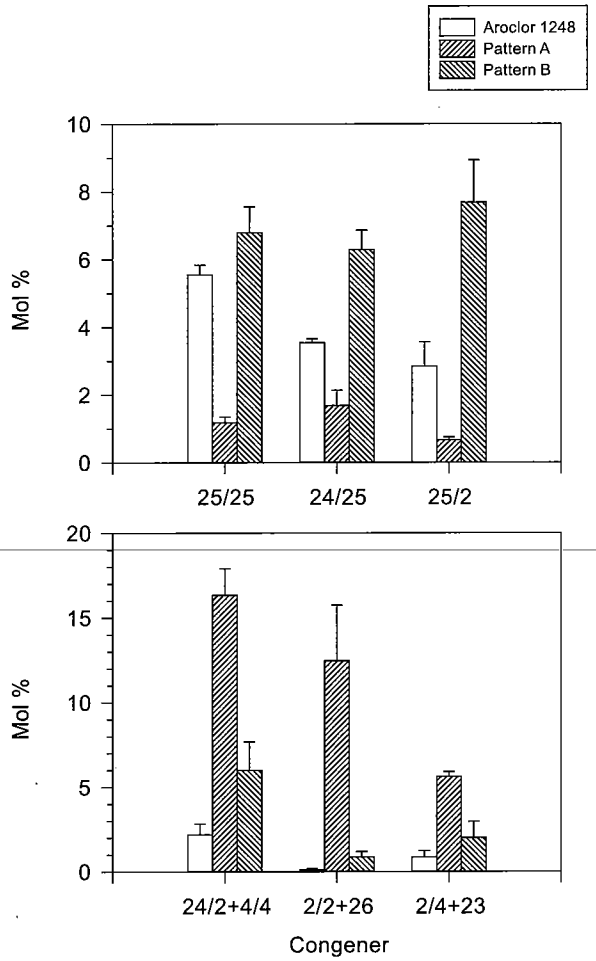
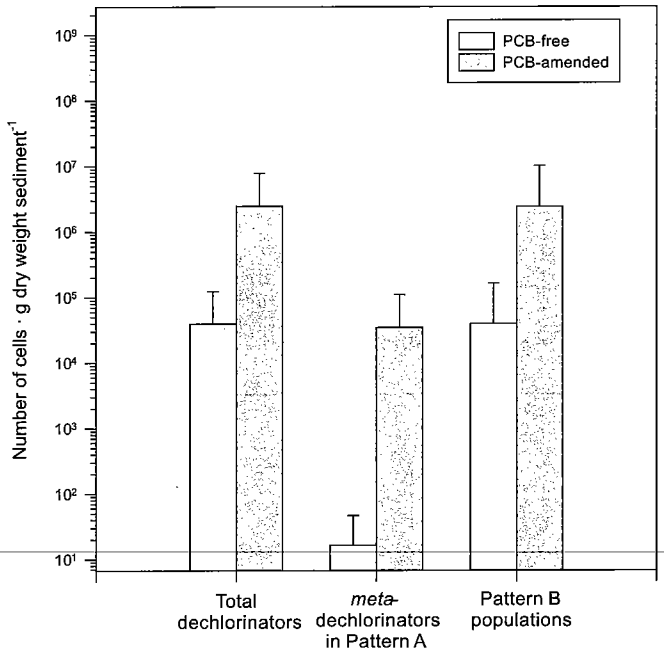


Fig. 3. *meta*-Dechlorinators in pattern A and dechlorinators producing pattern B in PCB-free and Aroclor 1248 amended sediments.



and 2,5,2′-chlorobiphenyls (CBPs) were dechlorinated (pattern A) and another in which they were not (pattern B) (Fig. 1). As a consequence, congener peaks for 2,4,2′- + 4,4′-, 2,2′- + 2,6-, and 2,4′- + 2,3-CBPs were significantly higher (t test, $P < 0.005$ for all congeners) in pattern A than in pattern B (Fig. 2).

All five MPN vials exhibited pattern A in the 10^{-1} to 10^{-3} dilutions. However, in the 10^{-4} and 10^{-5} dilutions, pattern A was found only in two vials and one vial, respectively, with the remainder being pattern B. Despite this difference between the two dilutions, overall dechlorination showed little difference (t test, $P = 0.988$), probably because any quantitative difference was not large enough to be analytically resolved. The two positives in the 10^{-6} dilution showed only pattern B.

A closer examination of the dechlorination characteristics disclosed that *meta*- as well as *para*-chlorines were dechlorinated in both patterns. However, there was a pronounced quantitative as well as qualitative difference in *meta*-dechlorination between the two patterns. In pattern A, the average number of *meta*-chlorines per biphenyl decreased by 54% from 1.39 to 0.64 whereas in pattern B, they were reduced by only 27% (1.39 to 1.02). The decrease in the average number of *para*-chlorines was much less than the reduction in *meta*-chlorines in both patterns; in pattern A, they decreased by 24% (1.02 to 0.78) whereas in pattern B, they were reduced by 17% (1.02 to 0.85). Overall, the average number of chlorines per biphenyl was reduced from 4.01 to 2.91 and to 3.41 in patterns A and B, respectively. These two types of dechlorination characteristics were maintained through two sequential transfers into fresh sediment medium (data not shown).

In pattern A, the most significant reduction in congener peaks occurred in 2,5,3′,4′-, 2,4,3′,4′-, 2,3,6,2′,5′-, 2,5,2′,5′-, 2,3,2′,5′-, 3,4,3′,4′- + 2,3,6,3′,4′-, and 2,4,5,4′-CBP, resulting in an increase in the peaks for 2,6,2′-, 2,3,6- + 2,6,3′-, 2,3,2′- + 2,6,4′-, 2,4′- + 2,3-, 2,3′-, 2,2′- + 2,6-, and 2,4,2′- + 4,4′-CBP (Figs. 1 and 2). In pattern B, the most significant decrease was found in the peaks for 2,5,3′,4′-, 2,4,3′,4′-, 2,3,6,2′,5′-, 3,4,3′,4′- + 2,3,6,3′,4′-, and 2,3,2′,5′-CBP, yielding an increase in the peaks for 2,4,2′- + 4,4′-, 2,3′-, 2,5,3′-, 2,5,2′-, 2,4,4′-, and 2,5,4′-CBP (Figs. 1 and 2).

The total number of dechlorinating microorganisms was about two orders of magnitude higher in PCB-spiked sediments than in the PCB-free control (2.5×10^6 versus 4.0×10^4 cells·g dry weight sediment^{-1}) (Fig. 3). This result confirms the earlier finding that dechlorinating microorganisms require PCBs for growth (Kim and Rhee 1997).

The existence of two dechlorination patterns indicates that there are at least two different dechlorinating subpopulations. Pattern A appears to involve the same population that produces the dechlorination of pattern B plus another one that is responsible for the additional dechlorination involving *meta*-substituted congeners such as those shown in Fig. 2. When the biomass of each subpopulation was calculated in the Aroclor 1248 sediments from the MPN data of each pattern, the number of dechlorinators for pattern B was approximately 2.4×10^6 cells·g dry weight sediment^{-1} (Fig. 3). The number of microorganisms in the population responsible for the additional dechlorination in pattern A was only a fraction of the total dechlorinating populations at about $3.5 \times$

10^4 cells·g dry weight sediment^{-1} (Fig. 3). However, this small subpopulation was responsible for the enhancement of dechlorination by nearly 50%.

In unamended control sediments, the size of each subpopulation was 4.0×10^4 and 1.6×10^1 cells·g dry weight sediment^{-1}, respectively (Fig. 3). Although the difference between the two populations seems greater in the control than in the PCB-amended sediments, it is difficult to determine its significance due to the sensitivity of the MPN technique.

When methanogens and sulfate-reducing microorganisms, the two major anaerobic respirators in sediments, were estimated from the MPN vials, there was no difference between PCB-free and PCB-spiked sediments. However, the number of sulfate reducers (1.1×10^8 cells·g dry weight sediment^{-1}) was about two orders of magnitude greater than that of dechlorinators whereas methanogens (2.3×10^6 cells·g dry weight sediment^{-1}) were of the same order of magnitude as dechlorinators.

Discussion

The presence or absence of the dechlorination of *meta*-substituted congeners in Aroclor 1248 such as 2,5,2′,5′-, 2,4,2′,5′-, and 2,5,2′-CBPs clearly demonstrates that there are at least two different types of dechlorinating populations in sediments at the GM site. Although the population dechlorinating *meta*-rich congeners in pattern A was small, it reduced tetra- and tri-CBPs further into tri- and di-chlorinated congeners and nearly doubled the overall extent of dechlorination. Thus, this population may further enhance the mobility of PCBs from sediments to the water column, exposing organisms there to higher PCB concentrations. However, these dechlorination products are lower-chlorinated congeners and would tend to bioaccumulate less and leave the system more rapidly through volatilization and hydraulic flushing because of their lower partition coefficients and higher Henry's Law constants. In addition, lower-chlorinated congeners are in general more readily degraded by aerobic microorganisms.

Different dechlorination patterns have also been reported in natural sediments (e.g., Bedard and Quensen 1995) as well as in laboratory incubation studies (e.g., Ye et al. 1992, 1995; Wu et al. 1997), which suggests that there may be many different dechlorinating microbial populations. However, it is unclear in these cases whether the congener patterns observed represented the terminal products. If dechlorination is carried out by a consortium of microorganisms, a different pattern may not always indicate a different dechlorinating population, since it is theoretically possible that the same dechlorinating microorganisms can yield a new pattern if they can form a different functional consortium.

Pattern B observed in the present investigations is remarkably similar to the congener pattern of final dechlorination products of Aroclor 1248 by St. Lawrence microorganisms from the GM site when methanogenesis was selectively inhibited by the metabolic inhibitor bromoethanesulfonate (Kim and Rhee 1999). However, in the present study, pattern B was found in the MPN vials that also contained methanogens. Nevertheless, it is still possible that dechlorinators may require interactions with specific strains of methanogens for the dechlorination of the *meta*-rich congeners, and these strains may be absent in those vials despite the occurrence of methanogenesis.

Studies of dechlorination kinetics showed that the maximum extent of dechlorination by St. Lawrence River sediment microorganisms was concentration dependent, as was the dechlorination rate (Sokol et al. 1998). This was due largely to the fact that at lower concentrations below 120 ppm, the same *meta*-substituted congeners that were not dechlorinated in pattern B in the present work also showed little dechlorination. If the same populations are also involved here in the present study, it appears that the *meta*-dechlorinating subpopulations can grow only at high PCB concentrations, and the final dechlorination products at low-concentration areas would be less dechlorinated.

Patterns A and B found in the present study were also observed in laboratory dechlorination studies of Aroclor 1248 by sediment microorganisms from different sites in the St. Lawrence River (Sokol et al. 1994*b*); the congener pattern of the final products by microorganisms from the Reynolds site was similar to pattern A, whereas the pattern by those from the GM and ALCOA sites was similar to pattern B. Thus, pattern A observed in the original sediments used for fractionation or in low dilutions in the present study was not the same as that observed in the previous study (Sokol et al. 1994*b*), even through the inoculum came from the same general site, albeit at different times. These results suggest that the composition of the microbial population varies spatially as well as temporally within the general site.

The present results clearly show that a combined action of populations of different competence resulted in greater removal of chlorines. It is therefore important to clearly understand the population dynamics and interactions of various dechlorinating microorganisms in sediment, since this information can be directly applied to bioremediate contaminated sediments, in situ or ex situ, and to remove the major barrier to complete destruction of PCBs through aerobic degradation. This information will also help us predict the fate of PCBs in contaminated natural sediments.

Recent investigations of sediment microorganisms in the St. Lawrence River suggest that the number of dechlorinators may be a good indicator for the status of in situ dechlorination or in situ dechlorination potential. A kinetic study of the reductive dechlorination of PCBs by these organisms has clearly shown that there is a threshold concentration of about 40 ppm below which no dechlorination can occur (Sokol et al. 1998). The reason for this threshold value is the fact that dechlorinating microorganisms cannot grow at concentrations below this value (G-Y. Rhee et al., unpublished). We have also found a significant correlation (linear regression analysis, $P < 0.05$, $n = 10$) between the initial number of dechlorinating microorganisms in historically contaminated St. Lawrence River sediments and the extent of dechlorination after a 1-year laboratory incubation (Y.-C. Cho et al., unpublished). When these findings are combined with the earlier report that dechlorinators require PCBs for growth (Kim and Rhee 1997), the number of dechlorinators may turn out to be a good indicator for the dechlorination potential for contaminated sites. In addition, determining the pattern of dechlorination exhibited by dechlorinators at a particular site may provide useful information about the progress of dechlorination in situ.

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References

Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., and Wolfe, R.C. 1979. Methanogens: reevaluation of unique biological group. Microbiol. Rev. **43**: 260–296.

Bedard, D.L., and May, R.J. 1996. Characterization of the polychlorinated biphenyls in the sediments of Woods Pond: evidence for microbial dechlorination of Aroclor 1260 in situ. Environ. Sci. Technol. **30**: 237–245.

Bedard, D.L., and Quensen, J.F., III. 1995. Microbial reductive dechlorination of polychlorinated biphenyls. *In* Microbial transformation and degradation of toxic organic chemicals. *Edited by* L.Y. Young and C. Cerniglia. John Wiley & Sons, New York. pp. 127–216.

Carpenter, D.O., Arcaro, K.F., Bush, B., Niemi, W.D., Pang, S., and Vakharia, D.D. 1998. Human health and chemical mixtures: an overview. Environ. Health Perspect. **106**: 1263–1270.

Evans, M.S., Noguchi, G.E., and Rice, C.P. 1991. The biomagnification of polychlorinated biphenyls, toxaphene and DDT compounds in a Lake Michigan offshore food web. Arch. Environ. Contam. Toxicol. **20**: 87–93.

Kim, J., and Rhee, G-Y. 1997. Population dynamics of polychlorinated biphenyl-dechlorinating microorganisms in contaminated sediments. Appl. Environ. Microbiol. **63**: 1771–1776.

Kim, J., and Rhee, G-Y. 1999. Interactions of polychlorinated biphenyl-dechlorinating microorganisms with methanogens and sulfate reducers. Environ. Toxicol. Chem. **18**: 2696–2702.

Kimbrough, D.E., Chin, R., and Wakakuwa, J. 1994. Wide-spread and synthetic errors in the analysis of soils for polychlorinated biphenyls. Part 3. Gas chromatography. Analyst, **119**: 1293–1301.

Koch, A.L. 1993. Growth measurement. *In* Methods for general and molecular bacteriology. *Edited by* P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg. American Society for Microbiology, Washington, D.C. pp. 248–276.

Parkinson, A., Safe, S., Robertson, L.W., Thomas, P.E., Ryan, D.E., Reik, L.M., and Levin, W. 1983. Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure–activity relationships. J. Biol. Chem. **258**: 5967–5976.

Quensen, J.F., III, Boyd, S.A., and Tiedje, J.M. 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microbiol. **56**: 2360–2369.

Rhee, G-Y., Bush, B., Bethoney, C.M., DeNucci, A., Oh, H.-M., and Sokol, R.C. 1993. Reductive dechlorination of Aroclor 1242 in anaerobic sediments: pattern, rate and concentration dependence. Environ. Toxicol. Chem. **12**: 1025–1032.

Schulz, D.E., Petrick, G., and Duinker, J.C. 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography – electron capture detection. Environ. Sci. Technol. **23**: 852–859.

Seegal, R.F. 1996. Epidemiological and laboratory evidence of PCB-induced neurotoxicity. Crit. Rev. Toxicol. **26**: 709–737.

Sokol, R.C., Bethoney, C.M., and Rhee, G-Y. 1994*a*. Effect of hydrogen on the pathway and products of PCB dechlorination. Chemosphere, **29**: 1735–1742.

Sokol, R.C., Kwon, O-S., Bethoney, C.M, and Rhee, G-Y. 1994*b*. Reductive dechlorination of polychlorinated biphenyls (PCBs) in St. Lawrence River sediments and variations in dechlorination characteristics. Environ. Sci. Technol. **28**: 2054–2064.

Sokol, R.C., Bethoney, C.M., and Rhee, G-Y. 1998. Effect of Aroclor 1248 concentration on the rate and extent of PCB dechlorination. Environ. Toxicol. Chem. **17**: 1922–1926.

William, E.J., Manchester-Neesig, J.B., and Armstrong, D.R. 1997. Influence of *ortho-* substitution on patterns of PCB accumulation in sediment, plankton and fish in a freshwater estuary. Environ. Sci. Technol. **31**: 3712–3718.

Wu, Q., Bedard, D.L., and Wiegel, J. 1997. Effect of incubation temperature on the route of microbial reductive dechlorination of 2,3,4,6-tetrachlorobiphenyl in polychlorinated biphenyl (PCB)-contaminated and PCB-free freshwater sediments. Appl. Environ. Microbiol. **63**: 2836–2843.

Ye, D., Quensen, J.F., III, Tiedje, J.M., and Boyd, S.A. 1992. Anaerobic dechlorination of polychlorobiphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediments. Appl. Environ. Microbiol. **58**: 1110–1114.

Ye, D., Quensen, J.F., III, Tiedje, J.M., and Boyd, S.A. 1995. Evidence of *para* dechlorination of polychlorobiphenyls by methanogenic bacteria. Appl. Environ. Microbiol. **61**: 2166–2171.