The Journal of Microbiology, April 2005, p.166-171 Vol. 43, No. 2

Effects of Sulfate Concentration on the Anaerobic Dechlorination of Polychlorinated Biphenyls in Estuarine Sediments

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(Received January 26, 2005 / Accepted March 2, 2005)

In order to determine the effects of sulfate concentration on the anaerobic dechlorination of polychlorinated biphenyls, sediments spiked with Aroclor 1242 were made into slurries using media which had various sulfate concentrations ranging from 3 to 23 mM. The time course of dechlorination clearly demonstrated that dechlorination was inhibited at high concentration of sulfate due to less dechlorination of *meta*-substituted congeners. When the dechlorination patterns were analyzed by the calculation of Euclidean distance, the dechlorination pathway in the 3 mM sulfate samples was found to be different from that observed in the 13 mM samples, although the extent of dechlorination in these two samples was similar. It is possible that the dechlorination in the high sulfate concentration samples is inhibited by the suppression of growth of methanogen, which have been shown to be *meta*-dechlorinating microorganisms.

Key words: Polychlorinated biphenyls, anaerobic dechlorination, sulfate concentration, dechlorinating microorganisms

Anaerobic dechlorination is the process which is primarily responsible for the degradation of haloaromatic compounds under anaerobic conditions, and is the only known degradative process of many highly halogenated pollutants (Mohn and Tiedje, 1992). The reductive dechlorination of polychlorinated biphenyls (PCBs) is important, because the dechlorinated products are more susceptible to aerobic degradation, including ring opening and mineralization, thereby reducing the toxicity associated with PCBs (Bedard *et al.*, 1986; Bedard and Quensen, 1995). It has been reported that lots of environmental parameters influence the activity and extent of dechlorination. These factors include redox level, temperature, pH, salinity, and available carbons (Rhee *et al.*, 1993a; Sokol *et al.*, 1994a; Bedard and Quensen, 1995). Reductive PCB dechlorination functions as a respiratory process (Lee *et al.*, 1998; Holliger *et al.*, 1999), and may therefore be influenced by the presence of alternative electron acceptors. In the presence of multiple electron acceptors, the most energetically favorable one may be the dominant, resulting in the preferential use of the respective electron acceptor (Rhee *et al.*, 1993c). A number of studies have determined that sulfates and other sulfur oxyanions either partially or completely inhibit aryl dehalogenation (Kuhn *et al.*, 1990; Allard *et al.*, 1992; Mohn and Kennedy, 1992; Häggblom *et al.*, 1993).

In marine and estuarine sediments, sulfate is found in abundance (Capone and Kiene, 1988), and sulfate reduction is probably the most important electron-accepting process influencing dehalogenation and degradation of haloaromatic compounds in these environments (Alder *et al.*, 1993; Monserrate and Häggblom, 1997).

PCB contamination of the Hudson River is the single most important threat to the ecosystem of the river (Cho *et al.*, 2002). Even if the "hot spots" are dredged, the PCB problems will still remain, albeit with less urgency. The remaining areas outside these spots, in particular the tidal estuary of the river, still retain substantial quantities of PCBs. In contrast to the extensive dechlorination of PCBs observed in the upper Hudson River, no clear evidence of dechlorination has been detected in the downstream of the river. One of important differences between the lower and upper Hudson River is the sulfate concentration; it is higher in lower Hudson River sediments than in upstream. In order to develop a sound restoration and management strategy, it is essential to obtain accurate data with regard to the transformation of PCBs related to the high sulfate concentrations of the tidal estuary of this river. Thus, in this study, we have attempted to characterize the effects of high sulfate concentrations on PCB dechlorination in estuarine sediments.

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Materials and Methods

Culture preparation and sampling

PCB-free sediments collected from the Grasse River (NY, USA) were air-dried and sifted through a 150 μm sieve prior to use. These sediments were spiked with Aroclor 1242 (AccuStandard, USA), a commercial mixture of PCBs, in hexane to yield a concentration of 300 μg of Aroclor 1242/g of sediment (ppm), and hexane was removed by evaporation. The PCB-spiked sediments were then made into slurries by adding reduced synthetic mineral medium (Balch *et al.*, 1979) in an anaerobic chamber (Coy, USA), with an $N_2:CO_2:H_2$ atmosphere (85:5:10). Resazurin (0.0001%, w/v) was added to the slurries as a redox indicator. Sample vials were prepared by the dispensing of 50 ml aliquots of sediment slurry into 100 ml serum vials. Final sulfate concentrations were adjusted to 3, 13, and 23 mM by adding sodium sulfate; the sulfate concentration in the reduced synthetic mineral medium is 3 mM. All vials were crimp-sealed with Teflon®-lined rubber septa and aluminum crimps. These vials were autoclaved for 40 min on three consecutive days.

In order to obtain an inoculum of the natural dechlorinating microbial population, 10 g of Hudson River sediment, collected near Moses Kill (NY, USA), was made into slurries in 100 ml of sterilized and reduced synthetic mineral medium, and then stirred for 20 min. The supernatant from this process was used as an inoculum. The inoculated sediment vials were incubated statically in the dark at room temperature. A 3 ml aliquot of the sediment slurries from triplicate samples was taken at 4 week intervals, for up to 24 weeks, in order to analyze the PCBs.

PCB extraction and analysis

For congener-specific analysis, Aroclor 1242 was extracted from the sediments using acetone and hexane, with ultrasonication, as described in an earlier report (Rhee *et al.*, 1993b). Elemental sulfur was removed by treating the extracts with tetrabutylammonium hydrogen sulfate and sodium sulfite (Jensen *et al.*, 1977). Sample cleanup was performed in a chromatographic column containing 10 g of 4% deactivated Florisil (60-100 mesh; Sigma, USA), topped with 1 g of anhydrous sodium sulfate. Congener-specific PCB analysis was carried out on a Hewlett Packard 5890 gas chromatograph, equipped with a ^{63}Ni electron-capture detector. PCBs were separated on a 60-m HP-5 capillary column. The gas chromatography conditions used to analyze PCBs were described elsewhere (Sokol *et al.*, 1994b). 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 of each in hexane). Peaks were identified and calibrated as described in a previous report (Rhee *et al.*, 1993b; Sokol *et al.*, 1994b). The calibration standards were run after every 6th sample for recalibration as part of quality assurance/quality control. Un-inoculated PCB-spiked sediment controls, set up at the beginning of the experiment and sampled at each time point, were used to monitor extraction efficiency.

Dechlorination pattern analysis

It is more difficult to determine a dechlorination pattern shift with Aroclor 1242 than with either Aroclor 1248 or Aroclor 1254, due primarily to the fact that the general extent of dechlorination associated with Aroclor 1242 is much lower (Rhee *et al.*, 1993c; Sokol *et al.*, 1998; Cho *et al.*,2001), and that the original congener profile of Aroclor 1242 is similar to the congener profile seen after dechlorination, due to the pre-existence of less-chlorinated congeners in Aroclor 1242, which are also dechlorination products (Rhee *et al.*, 1993c). Thus, we used Euclidean distance to compare the dechlorination patterns of Aroclor 1242 in the sediment samples of various sulfate concentrations, which can quantify the differences in dechlorination patterns (Cho *et al.*, 2001).

Euclidean distance, d_{ij}, can be calculated from the following formula (Eq. 1), in which m is the number of dimensions (the number of congeners), and x is the mole fraction values;

$$d_{ij} = \left[\sum_{a=1}^{m} (x_{ia} - x_{ja})^2 \right]^{\frac{1}{2}} \quad \text{(Eq. 1)}$$

The distance between the two samples indicates the differences between them, with longer distances indicating greater dissimilarities (Cho *et al.*, 2001).

Results

PCB dechlorination and sulfate concentration

The time course of dechlorination clearly demonstrated that dechlorination was inhibited at higher sulfate concentrations (Fig. 1). In the samples containing 3 mM sulfate, Aroclor 1242 was dechlorinated after an 8 week lag period. After 12 weeks of incubation, 4.4% of chlorines were removed, and by 20 weeks 12.2% was removed, when expressed as total chlorines/biphenyl (Cls/BP). There was no further dechlorination during the subsequent 4 weeks of incubation. Analysis after 24 weeks showed that total Cls/BP was reduced from 3.22 (±0.02) in the original Aroclor 1242 to 2.83 (±0.03) (Fig. 1). The lag period was longer in samples with over 3 mM sulfate; dechlorination started after 12 weeks of incubation. After 24 weeks of incubation, the extent of dechlorination in the 13 mM sulfate samples was not significantly different with that occurring in the 3 mM sulfate samples (t-test; $p<0.05$). In the samples with the higher sulfate concentrations, however, the extent of dechlorination was much less than that of the 3 mM or 13 mM sulfate samples. The total Cls/BP

in the 23 mM sulfate samples was 2.92 (± 0.02) (9.3% reduction). This result indicated that the dechlorination of PCBs was inhibited at high concentration of sulfate.

An analysis of 3 mM sulfate samples after 24 weeks of incubation showed a clear shift from highly-chlorinated congeners to lightly-chlorinated ones, with significant decreases in tetra- and tri-chlorinated biphenyls as well as concomitant increases in di-chlorinated ones (Fig. 2). In the sediment samples containing 23 mM sulfate, the amount of tetra-chlorinated biphenyls which were dechlorinated to di-chlorinated congeners was significantly less than the amounts in the 3 and 13 mM samples (*t*-test; $p < 0.02$).

A closer examination of dechlorinating characteristics disclosed that *meta*- as well as *para*-chlorines were dechlorinated in all of the samples. However, there was a quantitative as well as a qualitative difference in *meta* dechlorination between the 3 mM and 23 mM sulfate samples. In the 3 mM sulfate samples, the average number of *meta* Cls/BP decreased by 32.1%, from 0.90 (± 0.01) to 0.61 (± 0.02) *meta* Cls/BP. In the 23 mM samples, this number was reduced by 24.8% [0.90 (± 0.01) to 0.68 (±0.02) *meta* Cls/BP] after 24 weeks of incubation. The difference between the 3 mM and 23 mM samples with regard to the decrease in the average number of *para* Cls/BP was much less than that of *meta*-chlorines; 0.82 (± 0.02) and 0.84 (± 0.02) *para* Cls/BP in the 3 mM and 23 mM samples, respectively, after 24 weeks of incubation. These results indicated that *meta* dechlorination was inhibited in the samples with higher sulfate concentrations, relative to *para* dechlorination.

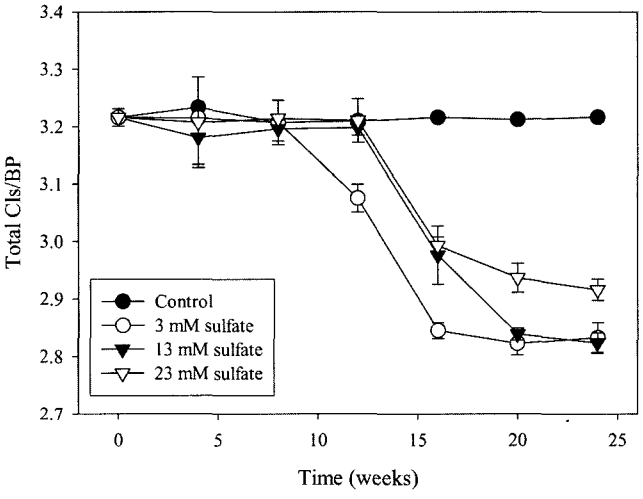

Fig. 1. Time course of dechlorination of Aroclor 1242 at various sulfate concentrations.

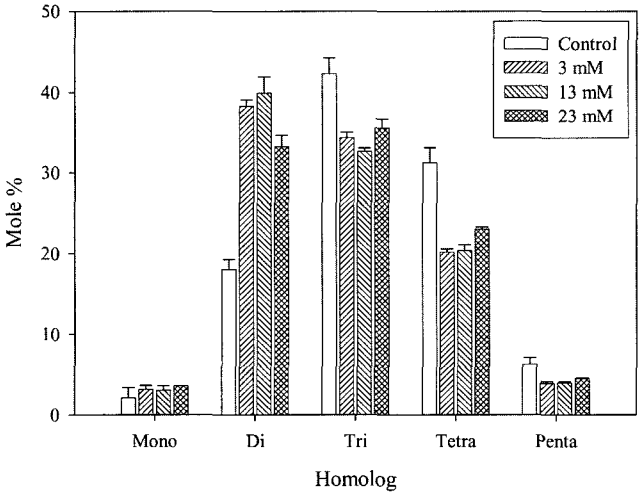

Fig. 2. The difference of relative concentrations of homologs (±SD) in sediments spiked with Aroclor 1242 after 24 weeks of incubation.

Dechlorination pattern and sulfate concentration

When we compared the congener patterns of the Aroclor 1242 control and the inoculated samples, a reduction in parent congeners 2,5,2'-, 2,3',4'-, 2,5,2',5'-, 2,3,2',5'-,

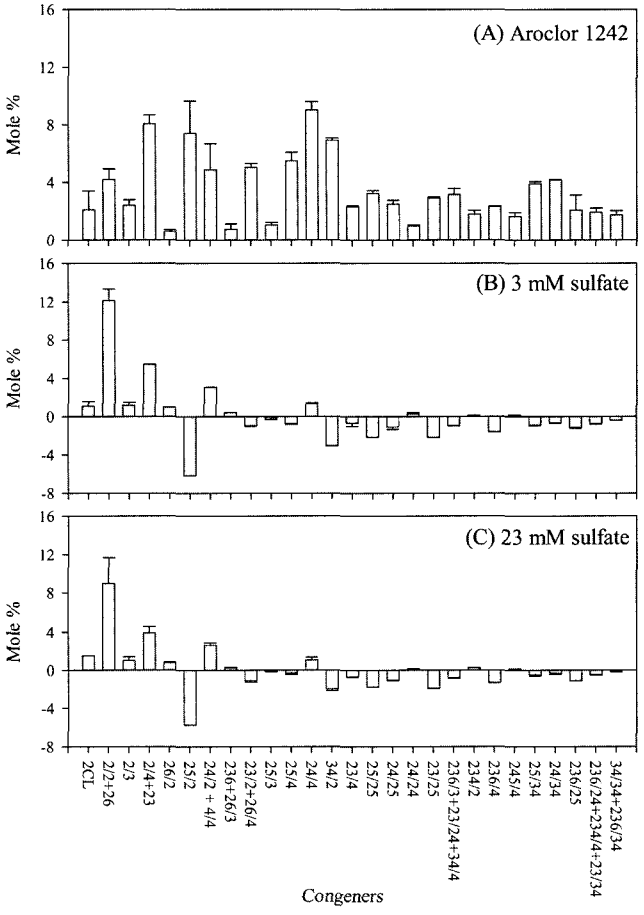

Fig. 3. Moles percent (±SD) of Aroclor 1242 congeners in the autoclaved control (a), and the differences of moles percent (±SD) between the Aroclor 1242 control and samples with 3 mM (b) and 23 mM (c) sulfate after 24 weeks of incubation. Congeners in which the moles percent were higher than 1% at any time point were selected for the graph.

2,4,2',5'-, and 2,3,6,4'-chlorobiphenyl (CBP), resulting in the accumulation of 2-, 2,2'- + 2,6-, 2,4'- + 2,3-, and 2,4,2'- + 4,4'-CBP, was noted (Fig. 3). The 3 mM sulfate samples indicated that dechlorination was not inhibited at this concentration of sulfate, because the dechlorination pattern observed in these samples appeared to be consistent with the patterns observed in the natural sediment from the Hudson River, Ft. Edward site (Cho *et al.*, 2001). The mole percent of 2,2' + 2,6-, 2,4'- + 2,3-, 2,6,2'-, and 2,4,2'- + 4,4'-CBP in the 23 mM sulfate samples, however, was much lower than that measured in the 3 mM sulfate ones (*t*-test; $p < 0.05$), indicating the inhibition of dechlorination at high sulfate concentrations.

The Euclidean distances in the same sulfate concentration samples correlated well with the extent of dechlorination; the values between 8 weeks and 12 weeks, and 12 weeks and 16 weeks in the 3 mM sulfate samples were high, at 0.089 (± 0.01) and 0.091 (± 0.01), respectively. The values between 16 weeks and 20 weeks in the 3 mM sulfate samples, and 20 weeks and 24 weeks in the 23 mM sulfate samples were relatively low, at 0.027 (± 0.003) and 0.025 (± 0.015), respectively (Fig. 4), because these samples reached the plateau level of dechlorination. It is interesting, however, to note that the Euclidean distance between the 3 mM sulfate samples at 20 weeks and the 13 mM samples at 24 weeks was as high as 0.073 (± 0.025), although the extent to which these two samples exhibited dechlorination was similar (see Fig. 1). These results indicate that the dechlorination pathway was influenced by the sulfate concentration, resulting in different congener patterns, although the final extents to which they were dechlorinated were similar.

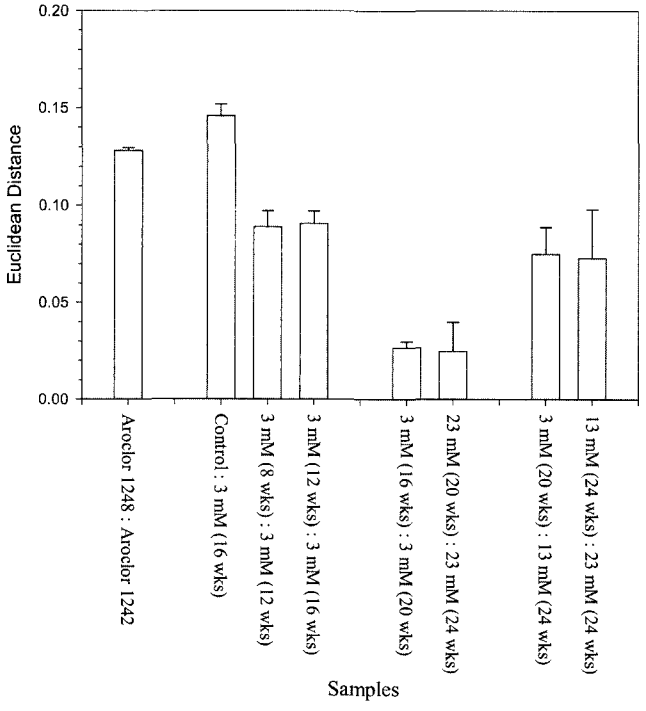

Fig. 4. The Euclidean distance (±SD) calculated from the mole fractions of each congener in two samples. The value determined between Aroclor 1248 and 1242 is indicated as a reference.

Discussion

Dechlorination of Aroclor 1242 was inhibited at high sulfate concentrations in this study. It is consistent with the results of other studies (Morris *et al.*, 1992; Rhee *et al.*, 1993c). The inhibition of *meta* dechlorination under sulfidogenic condition was also observed in earlier study. Under methanogenic condition, 12.4% of total chlorines were removed from Aroclor 1242, whereas 7.12% of chlorines were removed under sulfidogenic condition. This difference was caused by the differences in *meta* dechlorination; *meta* Cls/BP was reduced to 0.72 or 0.89 Cls/BP from 0.95 in the original Aroclor 1242 under methanogenic or sulfidogenic conditions, respectively (Rhee *et al.*, 1993c).

In contrast to the results presented here, one study has demonstrated that the addition of sulfate actually stimulates PCB dechlorination (Zwiernik *et al.*, 1998). In this study, $FeSO_4$ was used as the source of sulfate as well as iron. They concluded that sulfates stimulate the growth of sulfate reducers, which are responsible for *para* dechlorination activity, and that iron reduces the toxicity associated with sulfides by forming insoluble FeS. In our study, however, there was no change in *para* dechlorination activity in any of the sulfate concentration samples. It is possible that this difference originated from the different sources of used sulfate; we used sodium sulfate as a sulfate source. The sediment samples containing sodium sulfate, however, revealed a more enhanced dechlorination than un-amended control samples (Zwiernik *et al.*, 1998). We believe that this discrepancy comes from the different sources of inoculum. There are many examples to suggest that the microbial populations which were obtained from different locations reveal different dechlorination competence. The patterns of PCBs reveal differences in natural sediment samples which are located in the same river and contaminated by the same kinds of PCBs (Sokol *et al.*, 1994 b). This difference is caused by the differences in the populations of dechlorinating organisms (Cho *et al.*, 2000). The addition of the same compound enriches different dechlorinating microbial populations at different sites. The compound 2-bromoethanesulfonate, which is a growth inhibitor for methanogens, suppressed the dechlorination of *meta*-rich congeners when an extract from St. Lawrence River sediment was used as an inoculum (Kim and Rhee, 1999), but inhibited *para* dechlorination in another study, in which a Hudson River inoculum was used (Ye *et al.*, 1995).

In order to find evidence of the presence of dechlorinating microorganisms and to determine the dechlorinat-

ing potential, sediment samples were taken from the lower Hudson River [river miles (RM) 18.6 - 56.0], and the mixed microbial populations extracted from these sediments were then inoculated into sediments spiked with either 2,3,4- or 2,5,3',4'-CBP. No evidence of dechlorination was observed, except for the samples from RM 56.0 after 1.5 years of incubation (G-Y. Rhee, unpublished data). The extensive dechlorination, however, has been observed in upper Hudson River sediments (Brown *et al.*, 1987; Cho *et al.*, 2001). These results indicate that PCB dechlorination in the lower Hudson River has been restricted, and that the growth of PCB dechlorinating microorganisms has been inhibited by some environmental factors.

Our present results suggest that high sulfate concentrations in the lower Hudson River exert an inhibitory influence on PCB dechlorination. The less PCB dechlorination at higher sulfate concentrations appears to be caused primarily by the lower degree of dechlorination of *meta*-substituted congeners. According to the previous report, methanogens are a part of the dechlorinating microbial consortium, and are responsible for *meta* dechlorination (Kim and Rhee, 1999). Thus, it is possible that dechlorination in the high sulfate concentration samples can be inhibited by the suppression of the growth of methanogens, which compete with sulfate-reducing bacteria for hydrogen sources (Yang and McCarty, 1998).

However, this, by itself, is insufficient to explain the absence of dechlorination in the lower Hudson River sediment with high ambient sulfate concentrations, as dechlorination was shown to occur in the samples with high concentrations of sulfate even though these processes were inhibited relative to the samples with fewer sulfates. Another explanation for the absence of dechlorination in the lower Hudson River sediment lies in the PCB concentrations. The effects of PCB concentrations on dechlorination are well known (Sokol *et al.*, 1998; Chang *et al.*, 1999; Rhee *et al.*, 2000; Cho *et al.*, 2002). Recently, it has been reported that PCB dechlorination is closely linked to the growth of dechlorinating microorganisms (Kim and Rhee, 1997), and there is a threshold concentration level of dechlorination, below which no growth of dechlorinating microorganisms was observed in experiments conducted with the microorganisms from the St. Lawrence River (Sokol *et al.*, 1998; Rhee *et al.*, 2000). A similar relationship between the concentration of PCBs and dechlorination, and the presence of a threshold concentration of PCB dechlorination has also been observed with Hudson River sediment (Cho *et al.*, 2002). Studies focusing on the determination of the factors which influence PCB dechlorination in the lower Hudson River sediments may prove helpful in the development of strategies to remediate the contaminated areas in this river, as well as other estuary sediments with high sulfate concentrations.

Acknowledgement

This work was supported by the Kyung Hee University Research Fund in 2004 (No. 20040462).

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