J. Microbiol. Biotechnol. (2008), **18**(9), 1564–1571

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Reductive Dechlorination of Low Concentration Polychlorinated Biphenyls as Affected by a Rhamnolipid Biosurfactant

Kim, Jongseol[1]*, Robert C. Frohnhoefer[2], Young-Cheol Cho[3], Du Wan Cho[1], and G-Yull Rhee[2,4]

[1]*Department of Biological Science, University of Ulsan, Ulsan 680-749, Korea*
[2]*Wadsworth Center, New York State Department of Health, Albany, New York 12201, U.S.A.*
[3]*Department of Environmental Engineering, Chungbuk National University, Cheongju 361-763, Korea*
[4]*School of Public Health, State University of New York at Albany, Albany, New York 12201, U.S.A.*

Received: January 21, 2008 / Accepted: May 10, 2008

We investigated whether the threshold concentration for polychlorinated biphenyl (PCB) dechlorination may be lower in biosurfactant-amended sediments compared with biosurfactant-free samples. At PCB concentrations of 40, 60, and 120 ppm, the surfactant amendment enhanced the PCB dechlorination rate at all concentrations and the rate was also faster at higher concentrations. On a congener group basis, dechlorination proceeded largely with group A (congeners with low threshold) in both surfactant-free and -amended sediments, accumulating mainly group C (residual products of dechlorination) congeners, and surfactant enhanced the dechlorination rate of group A congeners. Since the PCB threshold concentration for the inoculum in the experiment was lower than 40 ppm, we carried out another experiment using sediments with lower PCB concentrations, 10, 20, and 30 ppm. Sediments with 100 ppm were also performed to measure dechlorination at a PCB saturation concentration. Comparison between the plateaus exhibited that the extent of dechlorination below 40 ppm PCBs was much lower than that at a saturation concentration of 100 ppm. There was no significant difference in the extent of dechlorination between surfactant-free and -amended sediments. Moreover, surfactant did not change the congener specificity or broaden the congener spectrum for dechlorination at PCB concentrations below 40 ppm. Taken together, it seems that at a given PCB concentration, dechlorination characteristics of dechlorinating populations may be determined by not only the congener specificity of the microorganisms but also the affinity of dechlorinating enzyme(s) to individual PCB congeners.

Keywords: Dechlorination, polychlorinated biphenyl, biosurfactant, threshold, concentration

**Corresponding author*
Phone: 82-52-259-2387; Fax: 82-52-259-1694;
E-mail: jkim@ulsan.ac.kr

Environmental concerns over organic pollutants at low chemical concentrations have fostered interest in the biodegradation processes affecting trace concentrations of organic chemicals. As the interest grew, it became apparent that there exists a threshold, or more specifically, a concentration of a nutrient source below which microorganisms cannot grow [1]. A significant factor contributing to apparent threshold concentrations in soils, aquifer materials, and sediments may be the mass transfer of the compound to the organisms.

Earlier studies of dechlorination kinetics of polychlorinated biphenyls (PCBs) have also indicated that there is a threshold concentration below which no dechlorination takes place [5, 15, 16]. In the dechlorination of Aroclor 1248 by sediment microorganisms from the St. Lawrence River, which was contaminated by this Aroclor, the lowest concentration at which dechlorination occurred was 45 μg of Aroclor 1248/g sediment (ppm), and no dechlorination was observed below 35 ppm, indicating that the PCB threshold concentration existed between these two concentrations [5, 16].

The microbial degradation of hydrophobic compounds is often limited by the transfer of such compounds from the environment surrounding the microorganisms to the cell surface. Because of their amphiphatic nature, surfactants increase the apparent solubility and bioavailability of hydrophobic compounds including PCBs. Some microorganisms excrete products that facilitate pseudosolubilization by converting hydrophobic compounds into droplets with sizes smaller than 1 μm, and these droplets are then assimilated by the microorganisms [1, 11, 14]. For environmental applications, chemical surfactants are generally toxic to microorganisms at the effective concentration for solubilization [7, 13]. Microbial surfactants, on the other hand, are generally less toxic and more biodegradable than synthetic ones, and some of them are beneficial at concentrations even below the critical micelle concentration [2, 7, 9–11, 14].

The previous investigation showed that addition of the rhamnolipid biosurfactant to PCB-contaminated sediments increased the rate of dechlorination, but had little effect on overall dechlorination. However, the PCB concentration in the study was 300 ppm, a saturation level for dechlorination [4]. Therefore, it remains to be answered whether the surfactant promotes dechlorination at subsaturation concentrations where the bioavailability of PCBs is likely a limiting factor. In the present study, we investigated whether the PCB threshold concentration for dechlorination [5, 16] may be lower in biosurfactant-amended sediments compared with biosurfactant-free sediments.

MATERIALS AND METHODS

Sediment Slurry Preparation

All experiments were carried out using stringent anaerobic techniques. For sediment slurry preparation, clean sediments collected from the Grasse River, a tributary of the St. Lawrence River (NY, U.S.A.), were air-dried, sifted through a sieve with a 150-μm opening, and analyzed to confirm the absence of PCBs contamination. Two separate experimental set-ups were performed with different PCBs concentration ranges. Individual batches of the sediments were spiked with Aroclor 1248 in hexane to yield 40, 60, and 120 μg/g sediment on a dry weight basis for one experimental set-up, and 10, 20, 30, and 100 μg/g sediment for the other. The sediments were then made into slurries by adding reduced synthetic mineral medium [3]. The sediment slurries contained 2.5% (w/v) sediment on a dry weight basis. Aliquots (10 ml) of each slurry were dispensed into 20-ml serum vials in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, U.S.A.) with an atmosphere of N_2:H_2:CO_2 (85:10:5). The vials were then capped with Teflon-coated rubber stoppers and aluminum crimp seals and autoclaved for 40 min on three successive days. Half of the sediment vials were amended with filter-sterilized biosurfactant using a sterile syringe to yield a final concentration of 50 μg biosurfactant/g sediment. The rhamnolipid biosurfactant used in this study was prepared from cultures of *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 9027 according to the procedures described previously [4]. The vials were then inoculated with microorganisms eluted from the St. Lawrence River sediment, which was contaminated mostly by Aroclor 1248 [17]. The inocula were separately prepared for the two experimental set-ups by mixing an aliquot of the St. Lawrence River sediments with reduced mineral medium. After inoculation, the vials were incubated statically at room temperature up to 36 weeks for the first and 50 weeks for the second experimental set-ups. Autoclaved sediments with or without biosurfactant served as the control. Duplicate samples were taken regularly for PCB analysis.

PCB Extraction and Analysis

Sediment extraction and congener-specific analysis of PCBs were performed as described previously [15, 17]. Briefly, sediments were extracted using an Accelerated Solvent Extractor system (Dionex, Sunnyvale, CA, U.S.A.) according to US Environmental Protection Agency Method 3545. After removal of sulfur by treating with a tetrabutylammoniumhydrogen sulfite reagent, the hexane extracts were cleaned up on a Florisil column. Congener-specific analysis was carried out on a Hewlett-Packard 5890 II gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a ^{63}Ni electron capture detector, an autosampler, a splitless injector, an Rtx-5 capillary column (Restek, Bellefonte, PA, U.S.A.), and a computerized data acquisition system (ChromPerfect, Justice Innovations, Mountain View, CA, U.S.A.). The gas chromatography conditions were as previously described [15]. The PCB congeners were identified and quantitated 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 according to response factors [8, 15]. The number of chlorine molecules per biphenyl and the mol% of PCB congeners were calculated based on the concentration of each congener. Co-eluting congeners were assumed to be present in equal proportions for the calculations [17].

Paired *t* test was used to determine differences in dechlorination rates between surfactant-free and surfactant-amended sediments and between sediments with various Aroclor 1248 concentrations for the first experimental set-up. Paired *t* test was also used to determine differences in dechlorination at plateau levels of 4 sampling time points between surfactant-free and surfactant-amended sediments and between sediments with various Aroclor 1248 concentrations for the second experimental set-up. The PCB congener numbering system in the text uses a slash to represent the separation of rings to permit an easier visualization of the chlorination substitution pattern (*e.g.*, 2,4,5,4'-chlorobiphenyl (CBP) will appear as 245/4-CBP), and co-eluting congeners were connected with "+".

RESULTS

Effect of Biosurfactant on Dechlorination Rate

In the first experiment, we investigated the effect of the rhamnolipid biosurfactant at PCB concentrations of 40, 60, and 120 ppm. Sediments spiked with Aroclor 1248 at these concentrations were inoculated with microorganisms eluted from PCB-contaminated St. Lawrence River sediments with or without surfactant amendment. No dechlorination was evident in autoclaved controls up to 36 weeks of incubations, either with or without biosurfactant (Fig. 1). The extent of dechlorination, expressed as the total number of chlorine molecules per biphenyl, was examined with the time course of incubations. We found that the surfactant amendment enhanced the rate of dechlorination at all concentrations and the rate was also faster at higher concentrations (Fig. 1). Analysis after 36 weeks showed that the number of chlorines per biphenyl was not different between surfactant-free and surfactant-amended at 120 ppm sediments, reduced from 3.95 to 3.24 and 3.22 (18.0% and 18.6%), respectively. At 60 ppm, dechlorination was less in the surfactant-free sediments than in the surfactant-amended, removing 16.3% in contrast to 18.5% under surfactant amendment. Similarly, dechlorination was also less in surfactant-free sediments at 40 ppm (13.1% removal *vs.* 16.3%). However, the time course of dechlorination at the two lower concentrations (Fig. 1) indicates that dechlorination may not have reached a plateau. Therefore, it is not clear

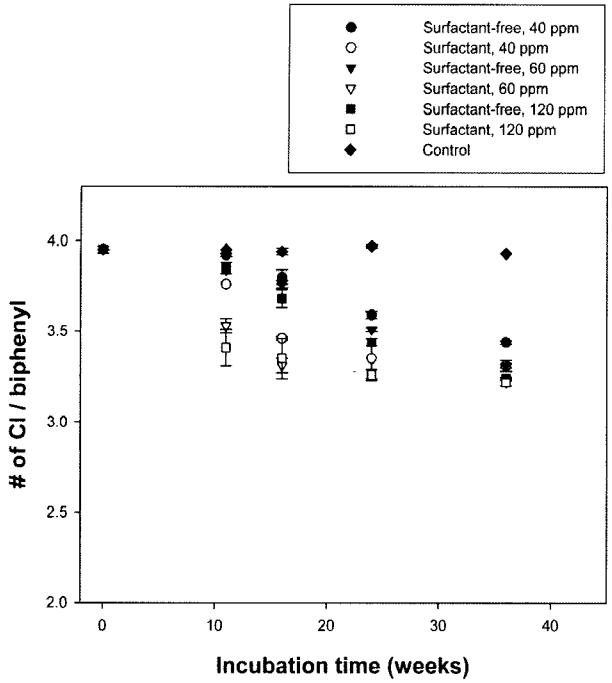

Fig. 1. Overall dechlorination of Aroclor 1248, expressed as total chlorine molecules per biphenyl, at 40, 60, and 120 ppm with and without biosurfactant amendment.

whether the differences between surfactant-free and -amended conditions represent an overall increase in dechlorination with the amendment.

In earlier studies, the congeners of Aroclor 1248 were divided into three groups based on the threshold level of dechlorination [5]. The first group (group A) consisted of congeners with low threshold concentrations for dechlorination to occur and the second group (group B) comprised those requiring high threshold concentrations. The third group (group C) consisted of the residual products of dechlorination. When the time course of Aroclor 1248 dechlorination was examined on a congener group basis, it proceeded primarily with group A in both surfactant-free and -amended sediments, accumulating mainly group C congeners (Fig. 2). By 36 weeks, group A congeners in 120 ppm sediments were reduced to 9.5 mol% in surfactant-free and 8.9 mol% in surfactant-amended sediments from the initial concentration of 46.3 mol%, as a reduction of 79.5% and 80.7%, respectively (Fig. 2A). At 60 ppm, group A concentrations were reduced by 73.1% without and 77.2% with surfactant. In 40 ppm sediments, dechlorination decreased group A by 58.5% without and 67.9% with surfactant on a mol% basis.

In contrast to group A, the concentration of group B exhibited a net increase (Fig. 2B). Initially, surfactant-amended sediments showed greater increase. At the end of 36 weeks incubations, however, the accumulation was greater in surfactant-free sediments with the increases ranging from 28.5 to 30.7 mol% compared with 25.9 to 28.5 mol% increases in surfactant-amended sediments (Fig. 2B).

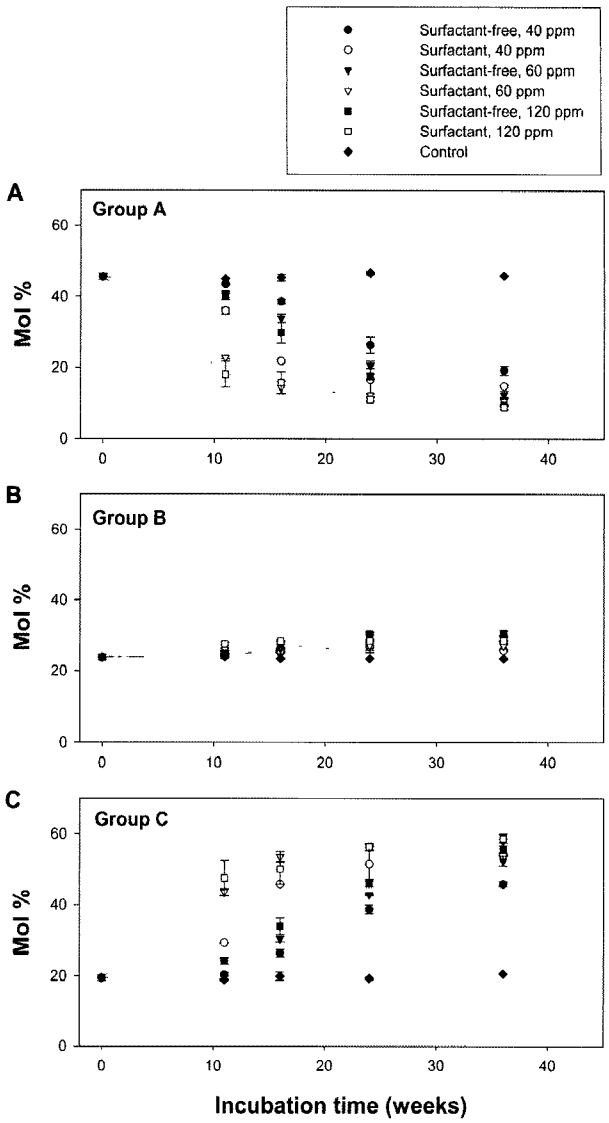

Fig. 2. Dechlorination of Aroclor 1248 congener group A (**A**), and concentrations of group B (**B**) and group C (**C**) at 40, 60, and 120 ppm with and without biosurfactant amendment.

The concentration of group C increased in a reversed pattern of the decrease in group A in both surfactant-free and -amended sediments. After 36 weeks, the concentration was the highest at 58.7 mol% (an increase of 209.2%) in surfactant-amended 120 ppm sediments and the lowest at 45.9 mol% (an increase of 141.8%) in surfactant-free 40 ppm (Fig. 2C).

Congeners, which were not included in groups A, B, or C, accounted for approximately 11 mol% in autoclaved controls. Although they were also dechlorinated, it was difficult to accurately quantify their changes because of their low concentrations.

Biosurfactant Effects on Individual Congeners

Regardless of PCB concentrations, surfactant enhanced the dechlorination rate in most of the group A congeners

(Figs. 3A and 3B). Surfactant-enhanced rate of dechlorination was observed in congeners such as 23/4- (chromatographic peak #23), 234/2-+236/4- (#34), 245/4- (#38), 25/34-+245/26- (#39), 24/34-+245/26- (#40), 23/34-+234/4-+235/25- (#42), 245/25-+235/24-+234/26- (#44), 245/24- (#45), 234/26- (#52), 245/34- (#57), and 234/34-CBPs (#64) (Figs. 3A and 3B). In contrast, concentrations of 236/24- (#41) and 236/23-CBPs (#43) did not change in all sediments.

Unlike group A, certain congeners in group B revealed a net increase in concentrations rather than a decrease; a significant accumulation was found in 25/2- (#11), 25/25- (#26), and 24/25-+235/2-CBPs (#27) in all sediments (Figs. 3C and 3D). Dechlorination in this group was observed in a limited number of peaks, 23/3-+25/26-+2/34- (#21), 23/25- (#31), and 236/3-+23/24-+34/4-CBPs (#32). These congeners were decreased with or without surfactant amendment, and surfactant-enhanced dechlorination was also found only in these peaks.

Regardless of PCB concentrations, group C congeners such as 2/2-+26- (#5), 2/3- (#7), 2/4-+23- (#8), 24/2-+4/4- (#12), 23/2-+26/4- (#14), 25/3- (#17), 24/3- (#18), 25/4- (#19), 24/4- (#20), and 24/24-+246/4-CBPs (#28) accumulated faster in sediments with surfactant than without surfactant (Figs. 3E and 3F). By 36 weeks, the accumulation of *para*-substituted congeners (#12, #20, and #28) was especially pronounced in the presence of surfactant. On the other hand, the concentration of peak #17 was higher in surfactant-free than surfactant-amended sediments after 36 weeks. In the cases of other group C congeners such as 24-+25- (#6) and 24/26-CBPs (#22), their concentrations were not increased above the control level in all sediments.

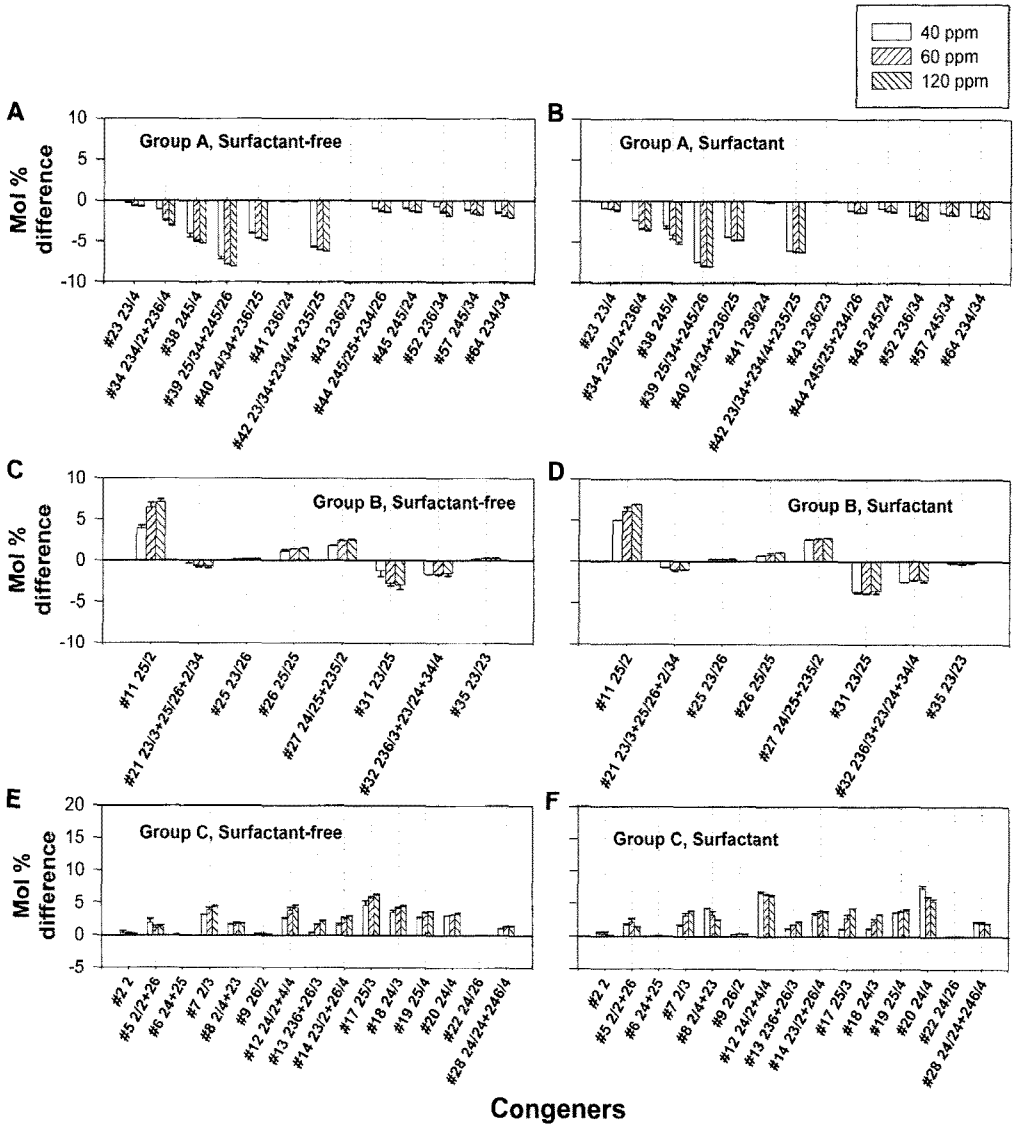

Fig. 3. Mole percent changes of group A (**A, B**), group B (**C, D**), and group C (**E, F**) congeners after 36 weeks of incubation at 40, 60, and 120 ppm with and without biosurfactant amendment.

Possible Pattern of Dechlorination

To reveal the dechlorination pattern by the inoculum, we traced possible parent congeners for major group C peaks (Fig. 3). The potential sources of 24/2-+4/4-CBPs (#12) accumulation include 234/2- (in #34), 245/25- (in #44), 245/24- (in #45), and 23/24-CBPs (in #32) for 24/2-CBP, and 34/4-CBPs (in #32) for 4/4-CBP. The *para* dechlorination of 24/24-CBP (in #28) to 24/2- and 2/2-CBPs was excluded since the congener was also accumulated in all sediments. The peak #19 (25/4-CBP) could be produced from the dechlorination of #38 (245/4-CBP) at the *para* position on the first ring, 25/34-CBP (in #39) at the *meta* position on the second ring, and #57 (245/34-CBP) at the *para* and the *meta* positions on the first and second rings, respectively. These congeners showed a surfactant-enhanced rate of dechlorination as well. The potential sources of #20 accumulation include 245/4-(in #38), 24/34- (in #40), 234/4- (in #42), and 234/34-CBPs (in #64), since *meta* dechlorination may produce the 24/4-CBP. These data indicate that the inoculum primarily has dechlorination specificity to PCB congeners with *meta*-substituted flanked chlorines, and their dechlorination is faster and more complete in the presence of surfactant.

Overall Dechlorination at Concentrations Below 40 ppm

It appears that the threshold concentration for the inoculum in the above experiment was lower than 40 ppm, the level found in our previous investigations [16], because dechlorination was still evident at 40 ppm regardless of the treatment. Therefore, we carried out another experiment using sediments with lower PCB concentrations, 10, 20, and 30 ppm. Sediments with 100 ppm were also set up to measure dechlorination at a saturation concentration. These sediments were inoculated with microorganisms eluted from the St. Lawrence River sediments.

The first sampling took place after 20 weeks of incubation. The congener pattern at this point showed that dechlorination apparently reached a plateau in all sediments, because no further changes were observed up to 50 weeks (data not shown). For data analysis, therefore, the values of the two time points (one single and three duplicate samples) were averaged as plateau levels of dechlorination.

At 100 ppm, the number of chlorines per biphenyl was reduced by 37.0% and 32.4% in surfactant-free and -amended sediments, respectively (Fig. 4). Dechlorination at the three concentrations below 40 ppm was much lower than that at a saturation concentration of 100 ppm; the sediment microorganisms removed 14.4% to 18.6% of chlorines in surfactant-free and 17.3% to 19.7% of chlorines in surfactant-amended sediments at subsaturation concentrations (Fig. 4). The difference between with and without surfactant was not statistically significant in

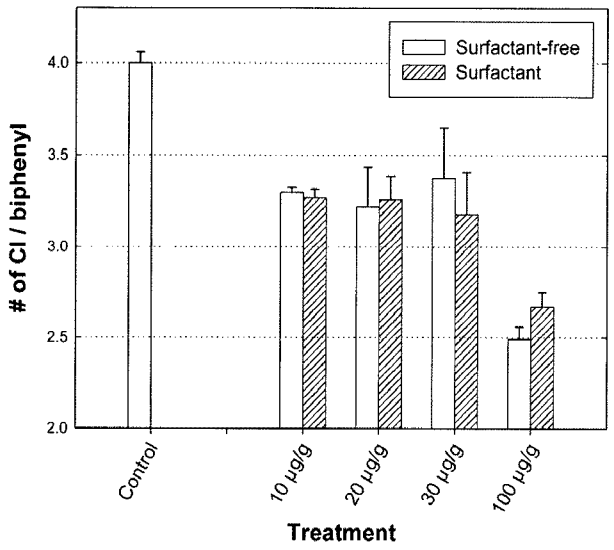

Fig. 4. The extent of Aroclor 1248 dechlorination, expressed as total chlorine molecules per biphenyl, at the plateau in sediments below 40 ppm with and without biosurfactant amendment.

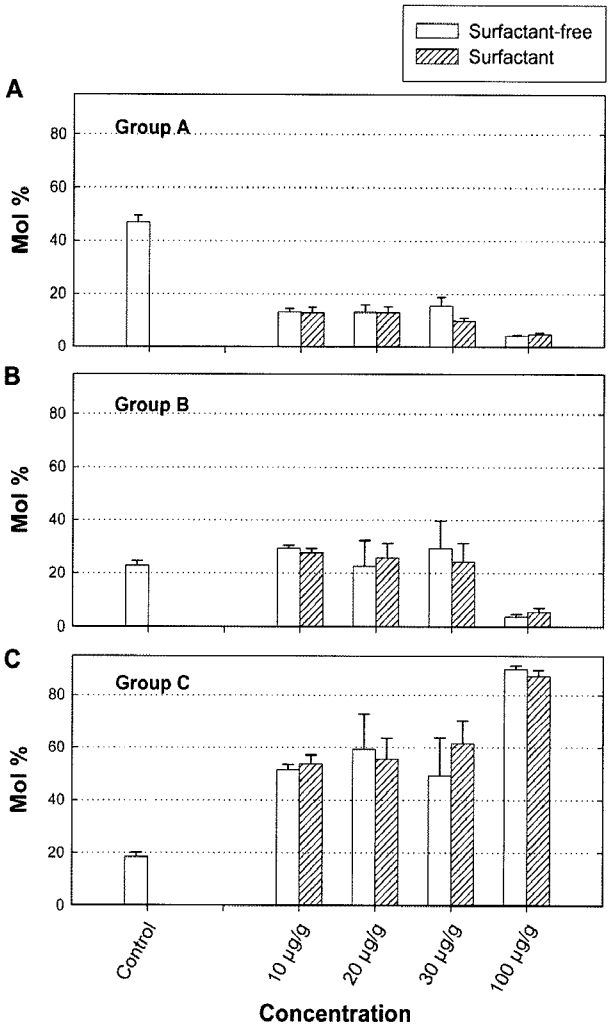

Fig. 5. The plateau level concentrations of congener group A (**A**), group B (**B**), and group C (**C**) at concentrations below 40 ppm with and without biosurfactant amendment.

sediments below 40 ppm. On a congener group basis, the effect of surfactant showed no difference between surfactant-free and -amended sediments (Fig. 5). Accordingly, surfactant amendment did not enhance overall dechlorination at concentrations below 40 ppm.

Dechlorination of Individual Congeners at Concentrations Below 40 ppm

Regardless of PCB concentrations, biosurfactant had no effect on the pattern of group A dechlorination (Figs. 6A and 6B). Whereas all group A congeners were dechlorinated at 100 ppm Aroclor 1248, dechlorination of 236/24- (#41) and 236/23-CBPs (#43) was not evident in sediments of 10, 20, and 30 ppm Aroclor 1248.

Unlike the pattern observed in group A, the dechlorination activity of group B was different depending on PCB concentrations (Figs. 6C and 6D). At 100 ppm, all group B congeners were dechlorinated, with little difference between surfactant-free and surfactant-amended sediments. On the other hand, congeners such as 25/2- (#11), 25/25- (#26), and 24/25-+235/2-CBPs (#27) were not dechlorinated but accumulated in 10 ppm sediments. At 20 and 30 ppm, the average plateau levels of individual group B congeners, especially 25/2- (#11), 25/25- (#26), and 23/25-CBPs (#31), showed larger standard deviations compared with those in 10 and 100 ppm sediments.

When the plateau levels of individual group C congeners were compared between 10 and 100 ppm sediments, concentrations of 2- (#2), 2/2-+26- (#5), 2/4-+23- (#8), 26/2- (#9), 24/2-+4/4- (#12), and 236-+26/3-CBPs (#13) were higher at 100 ppm, whereas congeners such as 25/3- (#17), 24/3- (#18), 25/4- (#19), 24/4- (#20), and 24/24-

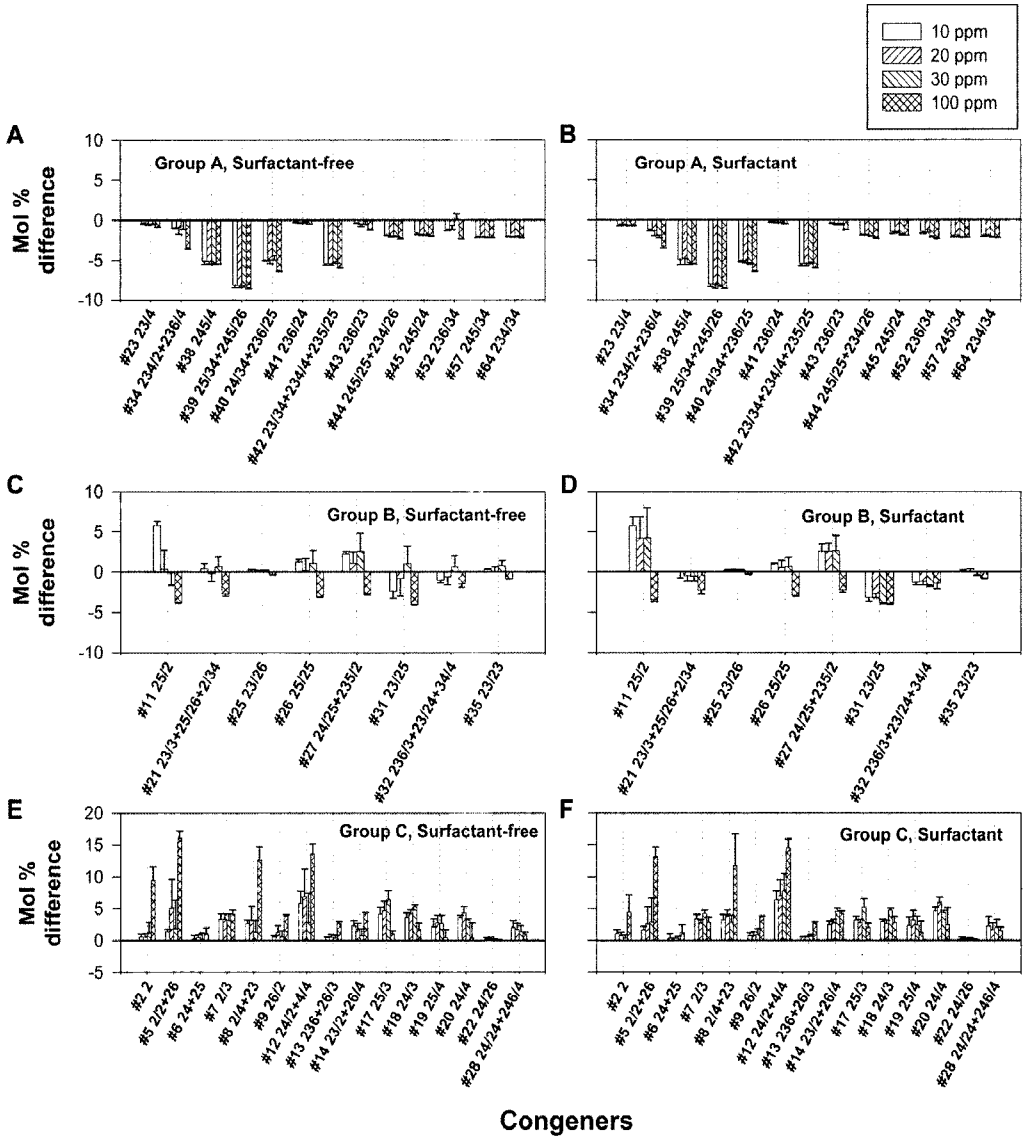

Fig. 6. Mole percent changes of group A (**A**, **B**), group B (**C**, **D**), and group C (**E**, **F**) congeners at the plateau in sediments below 40 ppm with and without biosurfactant amendment.

+246/4-CBPs (#28) showed higher concentrations at 10 ppm (Figs. 6E and 6F). At 20 and 30 ppm, the plateau level concentrations of 2/2-+26- (#5) and 24/2-+4/4- (#12) had larger standard deviations compared with those of other group C congeners.

Inoculum and Concentration Effects on Dechlorination

Although the same procedure was applied for inoculum preparation, the two inocula of the first and second experiments showed differences in their dechlorination characteristics at saturation Aroclor 1248 concentrations (Figs. 2 and 5). The concentrations of group A congeners were reduced to 4.0 and 4.6 mol% in surfactant-free and -amended sediments, respectively, from the initial concentration of 46.3 mol%, at 100 ppm in the second experiment (Fig. 5A). Group B congeners were also reduced from 23.3 mol% to 3.7 mol% without surfactant and 5.4 mol% with surfactant (Fig. 5B). On the other hand, the sediment microorganisms in the first experiment did not decrease, but increased the concentrations of group B (Fig. 2).

In the second experiment, the overall dechlorination was qualitatively different between the saturation and subsaturation Aroclor 1248 concentrations (Fig. 5). At 100 ppm, congeners in both groups A and B were dechlorinated and reduced in concentration, whereas those in group C increased. On the other hand, at concentrations below 40 ppm, no apparent decreases in group B congeners were found; indeed the congeners accumulated above the control level (Fig. 5). To determine whether the absence of group B dechlorination at these three low concentrations was caused by the inability of certain dechlorinating microorganisms to grow below the threshold value, the sediment microorganisms at each concentration were transferred into 300 ppm Aroclor 1248 sediment vials after 50 weeks. When the sediments were analyzed for dechlorination after 30 weeks, the inocula from the 100 ppm vials were able to dechlorinate both group A and group B congeners. In contrast, the inocula from the three low concentrations failed to dechlorinate group B congeners even at this high saturation concentration. In this regard, the specific dechlorinating microorganisms for group B congeners may die out or be inactivated at the low PCB concentrations, and the biosurfactant did not lower the threshold levels enough for the specific dechlorinators to maintain growth at the low PCB concentrations.

Discussion

Although the same procedure was applied for inoculum preparation, the inocula used in the two experimental set-ups showed differential dechlorination activities. In one experiment with 40, 60, and 120 ppm Aroclor 1248, the sediment microorganisms dechlorinated most group A congeners, and dechlorination of these congeners was faster in the presence of biosurfactant. The surfactant might stimulate the growth of dechlorinating populations by enhancing the bioavailability of these congeners through dissolution or pseudosolubilization. However, the enhanced rate of dechlorination may not be related to the reduced threshold levels of the congeners, since the group A congeners have a relatively lower threshold compared with group B. Surfactant did not change the congener specificity or broaden the congener spectrum for dechlorination of the inoculum. At a given PCB concentration, the dechlorination characteristics of dechlorinating populations may be determined by not only the congener specificity of the microorganisms but also the affinity of dechlorinating enzyme(s) to individual congeners.

In the second experimental set-up of 10, 20, 30, and 100 ppm Aroclor 1248, the maximum level of dechlorination was not increased by the surfactant amendment, as in the results of a previous investigation at 300 ppm [4]. The extent of dechlorination depended on the Aroclor 1248 concentrations, showing two different plateau levels. However, the differences between these two plateau levels were not overcome through the surfactant amendment. In this context, enhancing the bioavailability alone may not be enough to support the growth of certain dechlorinating populations at PCB concentrations below the threshold levels. For a dechlorinating population, the threshold levels may be mainly associated with the affinity (K_m) of dechlorinating enzyme(s) to individual congeners. Since the K_m value itself is constant regardless of bioavailability, surfactant, which affects the equilibrium sorption reaction between sediment particles and pore water, may have limited effect on lowering the threshold concentrations for the dechlorination. If the effective congener concentrations increased by surfactant amendment were still below the threshold levels, those congeners would not be dechlorinated or support the growth of dechlorinating populations. Thus, PCB concentrations may act as a selection force for dechlorinating populations, and those with relatively high affinity for certain congeners would be selected at low PCB concentrations.

As previous studies indicated [16], the extent of dechlorination depended on the initial PCB concentration. The results of this study also indicate that there exist at least two different PCB dechlorinating populations in the microbial community of the St. Lawrence River sediments. Whereas one population was excluded at low concentrations (below 40 ppm) of Aroclor 1248, another population could still maintain their activity at the low concentrations. The latter population has specificity to most group A congeners, but cannot dechlorinate such group B congeners as 25/2-, 25/25-, and 24/25-+235/2-CBPs, even at 300 ppm of Aroclor 1248. The addition of biosurfactant enhanced the dechlorination rate, but did not

broaden the congener spectrum for dechlorination by this latter population. The former population, which was inactivated at PCB concentrations below 40 ppm, appears to require higher concentrations for its growth and metabolism, and addition of surfactant cannot overcome the concentration barrier. This former population has dechlorination specificity to most group B congeners (*e.g.*, 25/2-, 25/25-, and 24/25-+235/2-CBPs), and may have dechlorination activity to group A congeners. Previous investigations with the St. Lawrence River sediments suggested that microorganisms dechlorinating group A congeners were different from those involved in the dechlorination of group B congeners [6, 12]. It is unclear regarding the interactions of the two populations on dechlorination characteristics at above threshold concentrations.

The previous study has demonstrated the presence of threshold concentration for dechlorination in Aroclor 1248 by sediment microorganisms from the St. Lawrence River; a clear threshold concentration was found between 35 and 45 ppm [5, 16]. In the present study, dechlorination of Aroclor 1248 revealed a limited dechlorination activity at concentrations below 40 ppm, which was previously indicated as the threshold level. It is suggested that a threshold can be lowered if degrading bacteria have certain alternative nutrient sources available to them [1]. Nonetheless, it seems that this discrepancy may have stemmed from the differential experimental conditions; in the present study, the sediment cultures were not disturbed throughout incubation because we sampled the whole sediment slurry of 20-ml serum vials, whereas in the previous study, an aliquot of sediment slurry was removed for analysis when 100-ml serum vials were opened in the anaerobic chamber at each sampling time. Alternatively, it is possible that the different sediment to liquid medium ratio (2.5% *vs* 20%, w/v) may influence the bioavailability of PCB congeners in pore water or particle surface, affecting the threshold level.

Acknowledgment

This work was supported by grants from US Environmental Protection Agency (R825449), the National Institute of Environmental Health Sciences Superfund Basic Research Program (ES04913), and the Hudson River Fondation (005/97A) to GYR. J.K. received financial support from the University of Ulsan Research Fund.

REFERENCES

1. Alexander, M. 1999. *Biodegradation and Bioremediation*, pp. 105–116. 2nd Ed. Academic Press, San Diego, California.
2. Baek, K.-H., B.-D. Yoon, B.-H. Kim, D.-H. Cho, I.-S. Lee, H.-M. Oh, and H.-S. Kim. 2007. Monitoring of microbial diversity and activity during bioremediation of crude oil-contaminated soil with different treatments. *J. Microbiol. Biotechnol.* **17:** 67–73.
3. Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: Reevaluation of a unique biological group. *Microbiol. Rev.* **43:** 260–296.
4. Cho, Y.-C., E. B. Ostrofsky, and G-Y. Rhee. 2004. Effects of a rhamnolipid biosurfactant on the reductive dechlorination of polychlorinated biphenyls by St. Lawrence River (North America) microorganisms. *Environ. Toxicol. Chem.* **23:** 1425–1430.
5. Cho, Y.-C., R. C. Sokol, R. C. Frohnhoefer, and G-Y. Rhee. 2003. Reductive dechlorination of polychlorinated biphenyls: Threshold concentration and dechlorination kinetics of individual congeners in Aroclor 1248. *Environ. Sci. Technol.* **37:** 5651–5656.
6. Cho, Y.-C., J. Kim, R. C. Sokol, and G-Y. Rhee. 2000. Biotransformations of polychlorinated biphenyls in St. Lawrence River sediments: Reductive dechlorination and dechlorinating microbial populations. *Can. J. Fish Aquat. Sci.* **57:** 95–100.
7. Desai, J. D. and I. M. Banat. 1997. Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol. Rev.* **61:** 47–64.
8. Frame, G. M., J. W. Cochran, and S. S. Bøwadt. 1996. Complete PCB congener distributions for 17 Aroclor mixtures determined by 3 HRGC systems optimized for comprehensive, quantitative, congener-specific analysis. *J. High Resolut. Chromatogr.* **19:** 657–668.
9. Georgiou, G., S. C. Lin, and M. Sharma. 1992. Surface active compounds from microorganisms. *Biotechnology* **10:** 60–65.
10. Herman, D. C., Y. M. Zhang, and R. M. Miller. 1997. Rhamnolipid (biosurfactant) effects on cell aggregation and biodegradation of residual hexadecane under saturated flow conditions. *Appl. Environ. Microbiol.* **63:** 3622–3627.
11. Joshi, S., S. Yadav, A. Nerurkar, and A. J. Desai. 2007. Statistical optimization of medium components for the production of biosurfactant by *Bacillus licheniformis* K51. *J. Microbiol. Biotechnol.* **17:** 313–319.
12. Kim, J. and G-Y. Rhee. 1999. Reductive dechlorination of polychlorinated biphenyls: Interactions of dechlorinating microorganisms with methanogens and sulfate reducers. *Environ. Toxicol. Chem.* **18:** 2696–2702.
13. Laha, S. and R. G. Luthy. 1991. Inhibition of phenanthrene mineralization by nonionic surfactants in soil-water systems. *Environ. Sci. Technol.* **25:** 1920–1930.
14. Lee, S.-C., J.-S. Yoo, S.-H. Kim, S.-Y. Chung, C.-W. Hwang, W.-H. Joo, and Y.-L. Choi. 2006. Production and characterization of lipopeptide biosurfactant from *Bacillus subtilis* A8-8. *J. Microbiol. Biotechnol.* **16:** 716–723.
15. Rhee, G-Y., R. C. Sokol, C. M. Bethoney, Y.-C. Cho, R. C. Frohnhoefer, and T. Erkkila. 2001. Kinetics of polychlorinated biphenyl dechlorination and growth of dechlorinating microorganisms. *Environ. Toxicol. Chem.* **20:** 721–726.
16. Sokol, R. C., C. M. Bethoney, and G-Y. Rhee. 1998. Effect of Aroclor 1248 concentration on the rate and extent of polychlorinated biphenyl dechlorination. *Environ. Toxicol. Chem.* **17:** 1922–1926.
17. Sokol, R. C., O.-S. Kwon, C. M. Bethoney, and G-Y. Rhee. 1994. Reductive dechlorination of polychlorinated biphenyls (PCBs) in St. Lawrence River sediments and variations in dechlorination characteristics. *Environ. Sci. Technol.* **28:** 2054–2064.