DECHLORINATION OF PCBs BY ANAEROBIC MICROBIAL GRANULES

M. R. Natarajan, W. Wu, R. Rajan, J. Nye, H. Wang, and M. K. Jain
Michigan Biotechnology Institute, P.O. Box 27609, Lansing, MI 48909, U.S.A.

1. Introduction

Widespread contamination of PCBs in lake and river ecosystems, and their indirect involvement in the human food chain pose serious threats to public health and wildlife[1]. Hundreds of PCB-contaminated sites are reported in the U.S. alone. Many laboratory studies have shown that PCBs dechlorinated partially under anaerobic conditions can be subsequently oxidized aerobically[2]. Attempts made to isolate anaerobic dechlorinating microorganisms from sediments are not yet successful. Most of the reductive dechlorination processes of PCBs catalyzed by anaerobic microorganisms were through losses of *meta* and *para* chlorines, resulting in the accumulation of *ortho* substituted mono- or di-chlorinated congeners[3,4]. An alternative and environmentally benign approach is the development of an *in situ* process that involves use of externally added PCB-dechlorinating microbial consortia at contaminated sites. Some of the advantages include: (1) substantial enhancement of rate and extent of dechlorination, (2) maximization of desired and selective metabolic performances, (3), no dredging and incineration, (4) environmentally benign process and, (5) acceptable to the public. We have developed anaerobic microbial consortia in the form of granules that are capable of dechlorinating lower as well as highly chlorinated PCBs for an *in situ* bioremediation of sediment in the contaminated areas. These self-immobilized granules were developed in our laboratory and found to extensively dechlorinate PCBs under methanogenic conditions[5].

In this investigation, we describe dechlorination of PCB mixtures such as Aroclor 1242 and 1254 by the anaerobic microbial consortia including the impact of surfactant, Triton X-100 to enhance the bioavailability of PCBs in sediments for these microbial granules. The overall objective of this study was to evaluate the dechlorinating performance of these granules under laboratory conditions.

2. Methodology

Experiments were conducted in serum bottles under strict anaerobic conditions at 30°C. Dechlorination experiments were performed using phosphate-buffered basal (PBB) medium[6] with glucose and methanol as carbon sources. PCBs were spiked in 158 ml serum vials containing PBB medium just before inoculation. The

bioavailability studies using Triton X-100 were conducted in serum vials containing Lake sediments spiked with Aroclor 1254. The experimental bottles were sealed with Teflon-coated rubber stoppers and incubated without shaking. At appropriate time intervals, samples were withdrawn for extraction and analysis of PCBs and dechlorination products. PCBs were extracted from samples as described by Quensen *et al.* (1990). Uniformly suspended samples were withdrawn from the experimental bottles at different time periods and subjected to liquid phase extraction. Octachloronapthalene (1.6 ppm) was used as an internal standard in extraction solvents. PCB samples were analyzed using a Varian 3400 Gas Chromatography equipped with an electron captured detector and DB-5 capillary column (30 m x 0.25 m I.D.).

3. Results and Discussion

In order to understand the extent of dechlorination of PCBs, two highly chlorinated PCB mixtures such as Aroclor 1242 and Aroclor 1254 were treated with MBI's PCB-dechlorinating granules. These Aroclor mixtures are predominant PCBs in most of the contaminated sites. Figure 1 illustrates the fate of Aroclor 1242 when incubated with the anaerobic granules over a period of time. Extensive dechlorination of Aroclor 1242 was observed in the presence of the anaerobic granules. Important to note is the predominant dechlorination of highly chlorinated compounds (i.e., more than 5 Cl / biphenyl) of Aroclor 1242. However, no accumulation or appearance of mono and di-chlorinated bi-phenyl compounds was observed throughout these experiments. Results based on the homolog distribution analysis revealed that Aroclor 1242 was substantially dechlorinated at 30°C, almost 50% in 24 weeks.

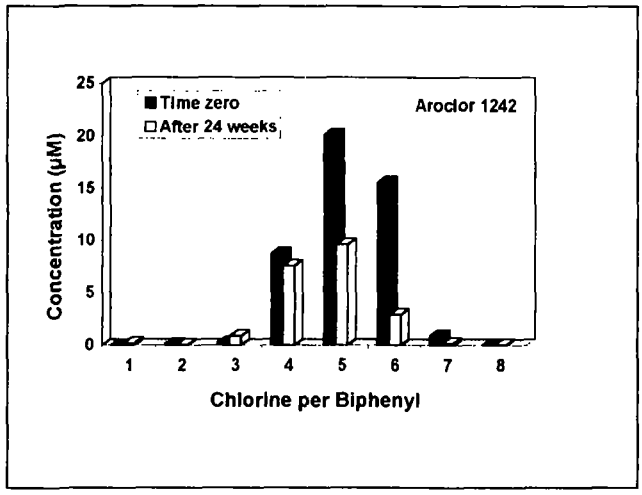

Figure 1. Dechlorination of Aroclor 1242 by the anaerobic microbial granules.

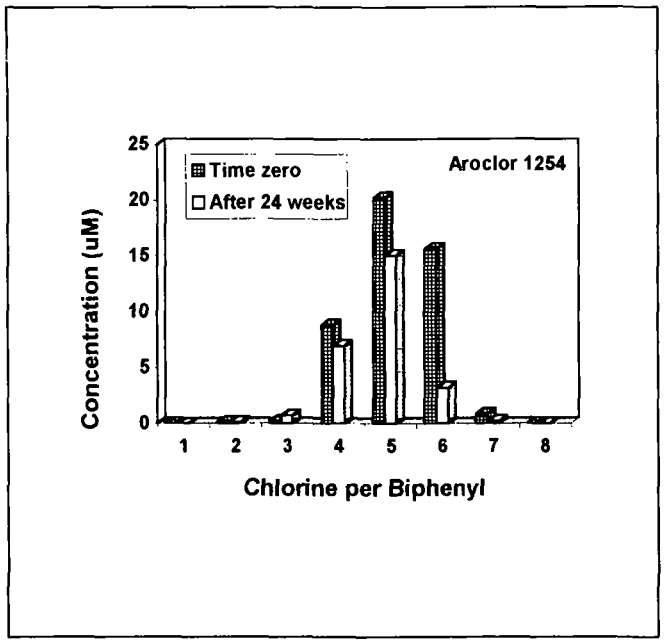

Figure 2. Dechlorination of Aroclor 1254 by the anaerobic microbial granules.

Similarly, the extent of dechlorination of Aroclor 1254 was also studied with anaerobic granules. Figure 2 illustrates the homolog distribution of Aroclor 1254 at time zero and after 24 weeks of incubation with these granules in PBB medium. Significant shift in distribution due to dechlorination and reduction in relative amounts of PCBs was observed. Aroclor 1254 predominantly contains penta and hexachlorobiphenyl congeners. A notable observation was the occurrence of dechlorination, predominantly of higher chlorinated compounds (i.e., more than 5 Cl / biphenyl) and relative changes on tri and tetra chlorinated congeners. These changes clearly indicate that the progressive dechlorination of PCBs occurred due to the biological activity of the anaerobic microbial granules. Approximately, 50% of Aroclor 1254 was dechlorinated in 24 weeks. The pattern and extent of dechlorination was similar to Aroclor 1242. In both cases, absence of accumulation of lower chlorinated products indicates further dechlorination of these products.

Bioavailability of PCBs is an important issue for *in situ* bioremediation since the PCBs are hydrophobic and strongly bind to sediments and soil particles. In many studies, addition of surfactants enhanced the solubility of the hydrophobic hydrocarbons and eventually increased biodegradation. Therefore, surfactant Triton X-100 was selected in our studies to enhance the availability of PCBs in the Lake sediments for the dechlorination. In parallel, control bottles were maintained under the same conditions without Triton X-100. Figure 3 shows homolog distribution of congeners of Aroclor 1254 after incubation with the anaerobic granules in the presence and absence (control) of Triton X-100.

In the absence of Triton X-100, but presence of microbial granules extensive dechlorination of PCBs was observed. After 48 weeks, considerable dechlorination was observed even in the vials supplemented with Triton X-100. These results suggest that at the concentrations used Triton X-100 was initially toxic and inhibited the dechlorinating activity. However, after a prolonged incubation period, these granules became tolerant to Triton X-100 and recovered their metabolic activity. Approximately 70% of dechlorination was noticed in the absence of Triton X-100 in 48 weeks. After 48 weeks of incubation except for residual tri, tetra and penta, no accumulation of other chlorinated compounds was observed in the absence of Triton X-100. These data indicate that availability may not be an issue with the microbial granules.

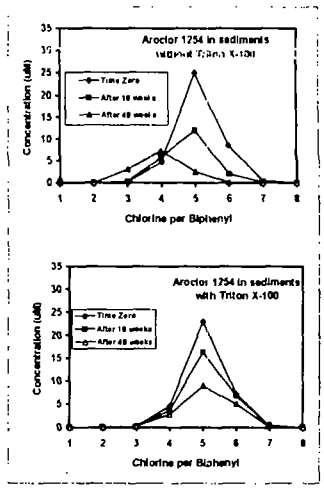

Figure 3. Dechlorination of Aroclor 1254 in the lake sediments with and without Triton X-100 by the anaerobic microbial granules.

4. References

1. Mes, J., Davies, D. J., Doucet, J., Weber, D. and Mullen, E. D. 1993. Environmental Technology, 14, 555-565.

2. Abramowicz, D. A. 1990. Critical review in biotechnology. 10, 241-249.

3. Brown, J. F. Bedard, D. L., Brennan, M. J., Carnahan, J. C., Feng, H., Wagner, R. E. 1987. Science 26, 709-712.

4. Quensen, J. F., Boyd, S. A., Tiedje, J. M. 1990. Appl. Environ. Microbiol. 56, 2360-2369.

5. Natarajan, M. R., Wang, H., Hickey, R., and Bhatnagar, L. 1995. Appl. Microbiol. Biotechnol. (in press).

6. Jain, M. K., Zeikus, J. G. and L. Bhatnagar. 1990. In: Anaerobic microbiology, A practical approach, P.N. Levett (ed) Oxford University press, Oxford, A review chapter 12; 223-245.

Acknowledgment

This study was supported by funds from the Great Lakes Protection Fund and the Kellogg Foundation.