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Anaerobic Dechlorination of Polychlorinated Biphenyls (PCBs) and PCB-Dechlorinating Microorganisms

Young-Cheol Cho
Department of Environmental Science and Engineering, College of Environmental Applied Chemistry, Kyung Hee University

Introduction

Polychlorinated biphenyls (PCBs) are a class of chlorinated hydrocarbons with a biphenyl nucleus on which hydrogens are substituted by chlorines. With 10 substitutable positions, 209 different congeners are possible. PCBs are thermodynamically stable. Therefore, they were widely used as dielectric fluids in capacitors and transformers, hydraulic fluids, lubricants, plasticizers, and additives in pesticides, paints, and copying papers. Their manufacturing in the United States ended in 1977, because PCBs have caused a variety of potential health effects such as reproductive toxicity, growth inhibition, immuno-, hepato-, neuro- and endocrine toxicity, and carcinogenicity (Safe, 1994).

PCBs are biodegraded by microorganisms under aerobic conditions. A large number of PCB-degrading microorganisms have been isolated from many environments (Commandeur *et al.*, 1996). However, PCB degradation of these isolate is generally limited to lightly chlorinated congeners with a few chlorines. Although some isolates were capable of breaking down congeners containing as many as six chlorines in laboratory assays (Bedard *et al.*, 1987), there is no evidence that such biodegradation is occurring in natural environments.

The evidence for PCB dechlorination is wide-spread in natural environments such as freshwater sediments (Sokol *et al.*, 1994) as well as estuarine and coastal sediments (Wu *et al.*, 1998). PCB dechlorination involves preferential removal of chlorines from *meta* and *para* positions with the replacement by hydrogens. Since the final products of PCB dechlorination are congeners with fewer chlorine substitutions, dechlorination may serve as a crucial initial step which removes the barrier to aerobic breakdown especially in case of highly chlorinated PCB congeners. Therefore, a sequential treatment of PCBs by anaerobic followed by aerobic degradation offers a promising conceptual framework upon which new and cost-effective bioremediation technologies can be developed.

Dechlorinating microorganisms

Although evidence for microbial PCB dechlorination has been found in most contaminated sediment, dechlorinating microorganisms are still poorly understood because of the failure so far to

isolate them. Therefore, all studies have been carried out using mixed microbial populations eluted from PCB-contaminated sediments as the inoculum.

Judging from the dechlorination pattern of Aroclors, which are the commercial products of PCBs made by Monsanto Co., and individual congeners, it appears that there are many different kinds of dechlorinating microorganisms. When mixed sediment microbial populations from the St. Lawrence River were treated with the metabolic inhibitor, 2-bromoethanesulfonate, two distinct dechlorination patterns were found (Kim and Rhee, 1999). When methanogenesis was inhibited, *meta*-substituted congeners such as 2,5,2',5'-, 2,3,2',5'- and 2,5,2'-chlorobiphenyls were not dechlorinated, although the total number of dechlorinating microorganisms, as determined by the most probable number (MPN) technique, was not significantly different from the number in untreated sediments. These results indicate that there are at least two different dechlorinating populations: one of which requires the presence of methanogens for dechlorination and the other which can dechlorinate independently. Different dechlorination patterns were also found at low PCB concentrations (Sokol *et al.*, 1998).

When sediment microbial populations in St. Lawrence River sediments were fractionated by serial dilution and estimated by the MPN technique, two distinct dechlorination patterns emerged, indicating different population types (Cho *et al.*, 2000). In serially diluted sediment slurries, the extent of dechlorination in high dilutions was significantly less than in low dilutions. This was mainly because major *meta*-substituted congeners were absent. These results indicate that *meta*-dechlorinating microorganisms were present in low numbers. When the population size was estimated based on the frequency of dechlorination patterns in MPN vials, the dechlorinators of the *meta*-rich congeners were two orders of magnitude less than those for other types of dechlorination. Despite the lower numbers, however, they were responsible for nearly a half of the total dechlorination.

Recently, we investigated the effects of non-PCB haloaromatic compounds (HACs) on PCB dechlorination and PCB-dechlorinating microorganisms (Cho *et al.*, 2002a). When the natural microbial populations eluted from St. Lawrence River sediments were enriched with 15 different kinds of chlorobenzoates, chlorophenols, and chlorobenzenes in PCB-free sediments, PCB-dechlorinating microorganisms were found in all but pentachlorophenol-amended sediments, when determined by the MPN method using PCB-amended sediments. In sediments spiked with Aroclor 1248, all HACs, except pentachlorophenol and pentachlorobenzene, enhanced the overall extent of PCB dechlorination. It also appears that different HACs enrich PCB dechlorinators of different capabilities (Cho *et al.*, 2002a). Of the eleven effective HACs, six (2,3-, 2,4-, and 2,4,6-chlorobenzoates; 3,4- and 2,3,6-chlorophenols; and 1,2,3-chlorobenzene) enhanced only *meta*-dechlorination. The remaining five (3-, 2,5- and 2,3,5-chlorobenzoates; 2,3- and 2,5-chlorophenols; and 1,2- and 1,2,4-chlorobenzenes) significantly increased both *meta*- and *para*-dechlorination. These results indicate that selective enrichment of specific dechlorinating microorganisms may be possible by using appropriate HACs.

The rate and the maximum level of overall dechlorination of Aroclor 1248 are concentration-dependent (Sokol *et al.*, 1998; Rhee *et al.*, 2001). No dechlorination was found at concentrations below 40 ppm (μg Aroclor 1248/g of sediment), indicating a threshold level. Dechlorination reached a maximum level at concentrations above 80 ppm. At 60 ppm, dechlorination was less than the maximum. This reduced level was not due to a lower level of dechlorination involving all congeners; rather, it stemmed from the fact that a group of certain congeners was not dechlorinated at all this concentration. The similar linear relationship between PCB concentration and the maximum extent of dechlorination was also observed in the study with Aroclor 1242 and microorganisms eluted from the Hudson River sediments (Cho *et al.*, 2002b).

To investigate the threshold concentration of individual congeners in Aroclor 1248, the initial concentration of each congener was plotted against the dechlorination rate of that congener (Cho *et al.*, 2003). The relationship was linear. Therefore, the concentration intercept of this linear function was considered as the threshold. These kinetic characteristics indicate that there are three different groups of congeners in Aroclor 1248: Group A, which consists of congeners with the threshold value not significantly different from their levels at the threshold concentration of Aroclor 1248 (40 ppm); Group B, which is comprised of congeners whose threshold concentration are higher their levels in Aroclor 1248 at its threshold concentration; and Group C, the final products of dechlorination, whose concentrations increased over time. Detailed analyses of dechlorination showed that Group B members were not dechlorinated at a sub-saturation level of 60 ppm of Aroclor 1248 but accumulated. Therefore, the different dechlorination patterns of Aroclor 1248 between high and low PCB concentrations are caused by the absence of dechlorination of Group B congeners at the slurry samples with lower concentrations of PCBs.

Conclusions

There are diverse PCB-dechlorinating microorganisms with different capabilities. Their capabilities appear to be specific to the pattern of chlorine substitution on the PCB congeners. The extent of dechlorination in mixtures of various congeners thus depends on the kinds of dechlorinators present. Therefore, if one can artificially enrich dechlorinating microorganisms tailored to the congeners present, it will be possible to maximize dechlorination. Enrichment studies of non-PCB HACs indicate that targeted enrichment may be possible. Artificial enrichment is difficult to use in most open systems. However, these techniques are well suited for a confined disposal facility. Either the direct on-site enrichment of indigenous microbial populations or an application of microorganisms enriched in mass culture may be used.

References

Bedard DL, Haberl ML, May RJ, and Brennan MJ. 1987. Evidence for novel mechanisms of PCB metabolism in *Alcaligenes eutrophus* H850. *Appl. Environ. Microbiol.* 53:1103-1112.

Cho YC, Kim J, Sokol RC, and Rhee GY. 2000. Biotransformation of polychlorinated biphenyls in St. Lawrence River sediments: reductive dechlorination and dechlorinating microbial populations. *Can. J. Fish. Aquat. Sci.* 57:95-100.

Cho YC, Ostrofsky EB, Sokol RC, Frohnhoefer RC, and Rhee GY. 2002a. Enhancement of microbial PCB dechlorination by chlorobenzoates, chlorophenols, and chlorobenzenes. *FEMS Microbiol. Ecol.* 42:51-58.

Cho YC, Sokol RC, and Rhee GY. 2002b. Kinetics of polychlorinated biphenyl dechlorination by Hudson River, New York, USA, sediment microorganisms. *Environ. Toxicol. Chem.* 21:715-719.

Cho YC, Sokol RC, Frohnhoefer RC, and Rhee GY. 2003. Reductive dechlorination of polychlorinated biphenyls: threshold concentration and dechlorination kinetics of individual congeners in Aroclor 1248. *Environ. Sci. Technol.* 37:5651-5656.

Commandeur LCM, May RJ, Mokross H, Bedard DL, Reineke W, Govers HAJ, and Parsons JR. 996. Aerobic degradation of polychlorinated biphenyls by *Alcaligenes* sp. JB1: metabolites and enzymes. *Biodegradation* 7:435-443.

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

Rhee GY, Sokol RC, Bethoney CM, Cho YC, Frohnhoefer RC, and Erkkila T. 2001. Kinetics of polychlorinated biphenyl dechlorination and growth of dechlorinating microorganisms. *Environ. Toxicol. Chem.* 20:721-726.

Safe SH. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biological and toxic responses and implications for risk assessment. *Crit. Rev. Toxicol.* 24:87-149.

Sokol RC, Kwon OS, Bethoney CM, and Rhee GY. 1994. Reductive dechlorination of polychlorinated biphenyls (PCBs) in St. Lawrence River sediments and variations in dechlorination characteristics. *Environ. Sci. Technol.* 28: 2054-2064.

Sokol RC, Bethoney CM, and Rhee GY. 1998. Effect of Aroclor 1248 concentration on the rate and extent of PCB dechlorination. *Environ. Toxicol. Chem.* 17:1922-1926.

Wu QZ, Sowers KR, and May HD. 1998. Microbial reductive dechlorination of Aroclor 1260 in anaerobic slurries of estuarine sediments. *Appl. Environ. Microbiol.* 64:1052-1058.