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CRITICAL OXYGEN CONCENTRATIONS FOR BIODEGRADATION OF PCBs

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Introduction

An understanding of PCB biotransformation and biodegradation has developed during 25 years of study[1-3]; however, complex interactions among different populations of microorganisms and critical system parameters are not well understood. Complete mineralization of lightly chlorinated PCBs through oxidation can be achieved by many aerobic organisms[2,4-6]. More highly chlorinated PCBs can be dechlorinated in an anaerobic reductive process that results in more lightly chlorinated mono-, di- and tri-chlorobiphenyls or completely dechlorinated biphenyl[7-9]. Many researchers have suggested that complete removal of PCBs from contaminated sediments will rely on the sequential activities of anaerobic and aerobic populations of microorganisms[4,10]; accordingly, oxygen concentration will be a critical controlling parameter. The premise is that the more highly chlorinated PCB congeners will be reductively dechlorinated in anoxic sediments. Under these conditions, microorganisms generate energy for growth by coupling oxidation of sediment organic matter to reductive dechlorination of PCB congeners as the electron acceptor. (This is a form of anaerobic respiration and gives chloride ion and a less chlorinated PCB congener as reaction products.) PCB dechlorination requires a low redox potential; the presence of more energetically favorable electron acceptors (*e.g.*, O_2, NO_3^-, SO_4^{2-}) has been observed to inhibit dechlorination in some systems [11]. Following dechlorination to more lightly chlorinated congeners, aerobic growth conditions will allow for complete mineralization with the PCBs used as carbon source and electron donor. In this reaction, oxygen plays two roles. One function involves molecular oxygen as a substrate for the enzyme (dioxygenase) that attacks the PCB aromatic rings, forming a *cis* diol structure (adjacent hydroxyl groups) that effectively makes the molecule more amenable to further oxidative

mineralization. The second function is oxygen as the terminal electron acceptor for aerobic respiration. The dependence of these two processes on the concentration of dissolved oxygen is typically quite different, with the dioxygenases requiring a much higher threshold concentration of oxygen than the respiratory enzymes.

Thus, a combination of aerobic and anaerobic processes, carried out by different but complementary groups of bacteria, has the potential for complete destruction of PCBs. The major limitation to these processes being carried out concurrently in time and space is that one process requires substantial oxygen while the other is inhibited by the presence of oxygen. There are at least two naturally occurring situations in which aerobic and anaerobic processes occur within close physical proximity of each other. One is the oxic-anoxic transition zone in near-surface sediments. Here, the relevant physical dimensions are probably on the order of millimeters to centimeters depending on sediment conditions. The other occurs within microbial biofilms where the dissolved oxygen concentration can drop from saturation (approximately 8 mg/L D.O.) to zero over a film thickness of as little as 100 μm. Through a combination of aerobic and anaerobic processes, these microenvironments could make it possible for a consortium of microorganisms to achieve complete PCB destruction that couldn't happen solely in either type of environment.

The goal of the present research was to evaluate the concentration of oxygen necessary for aerobic PCB mineralization and the concentration of oxygen that inhibits anaerobic PCB reductive dechlorination. The potential for these reactions to happen in close proximity would presumably be enhanced by the existence of nearly overlapping concentrations such that the aerobic process could take place significantly below saturation while the anaerobic process could tolerate some low level of oxygen without significant inhibition.

Materials and Methods

We investigated the effect of dissolved oxygen concentration on PCB biodegradation by establishing replicate microcosms that have a graded series of controlled dissolved oxygen concentrations (0 to 8 ppm). The experiments were carried out in a commercial hypoxic chamber (Coy Laboratories) fitted with an oxygen sensor-controller. The dissolved oxygen concentration was maintained by adjusting the oxygen percentage in the headspace gas. To avoid significant volatilization while still maintaining equilibrium between the gas and dissolved phase oxygen, intermittent shaking was used (30 minutes of shaking at 12-hour intervals).

Microcosms were prepared in 150 mL serum bottles using a sediment sample collected from a region of the Grasse River in Massena, NY, where there is a low (< 5 ppm) concentration of sediment PCBs. Each bottle contained 50 g of a 15% sediment-water slurry (sediment dry weight basis). The sediment portion consisted of 80% wet sediment as viable inoculum and 20% that had been dried and then spiked with defined PCB congeners consisting of a mixture of BZ4 (2,2'-dichlorobiphenyl), BZ29 (2,4,5-trichlorobiphenyl) and BZ70 (2,3',4',5-tetrachlorobiphenyl) as indicator PCBs (final concentration of 12 ppm each in the microcosm). These congeners were chosen based

on our prior experience in using them in sediment microcosms and the fact that BZ4 and BZ29 can be aerobically biodegraded, whereas BZ70 cannot, and the fact that BZ29 and BZ70 can be anaerobically dechlorinated, whereas BZ4 cannot. The structures of these PCB congeners and the predominant pathways for dechlorination of BZ29 and BZ70 are presented in Figure 1.

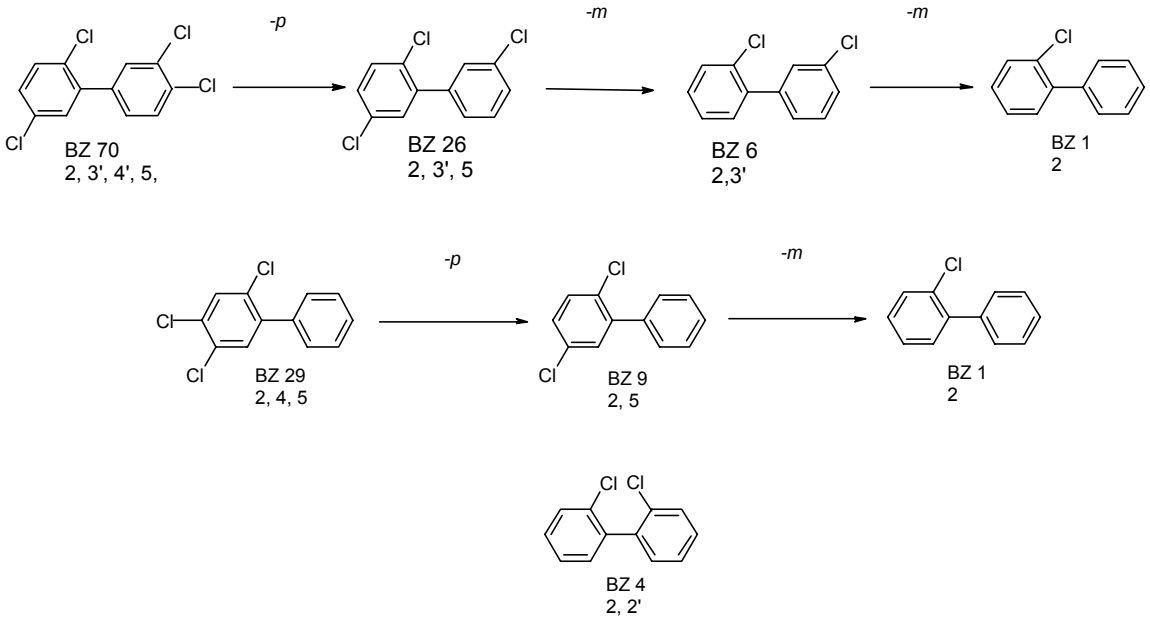

Figure 1. Predominant pathways for dechlorination of BZ70, BZ29, BZ4 and their daughter products in Grasse River sediment anaerobic microcosms.

The fully anaerobic microcosms were crimp sealed with Teflon®-lined butyl rubber stoppers and then purged with nitrogen. They were incubated with continuous shaking. Headspace gas analysis of the anaerobic microcosms by gas chromatography with a thermal conductivity detector confirmed the absence of oxygen and the appearance of carbon dioxide and methane over time. The fully aerobic microcosms had uncrimped rubber stoppers that were removed periodically to permit re-equilibration with atmospheric oxygen. Finally, the microcosms with intermediate dissolved oxygen concentrations were incubated without caps in the hypoxic chamber. Multiple replicates of microcosms for each condition tested were incubated at ambient laboratory temperature for the duration of the experiment. PCB analyses were carried out by extracting wet, centrifuged sediment with hexane-acetone (1:1), followed by florisil and sulfur clean-up. Quantitative PCB deteminations were made on a Perkin-Elmer Autosystem gas chromatograph with a DB-5 capillary column and ECD detector. A positive result for anaerobic PCB dechlorination was inferred from a decrease in the levels of BZ70 and BZ29, the appearance of their dechlorination products (see Figure 1), and no change in the amount of BZ4. A positive result for aerobic degradation was inferred from a decrease in the levels of BZ4 and BZ29 and no change in the amount of BZ70. Because there were small losses of BZ4 and BZ29 due to volatilization from the uncapped microcosms that were incubated in the hypoxic chamber, all changes in PCB

levels were assessed relative to sterile control microcosms. Micocosms were analyzed for aerobic biodegradation of PCBs after 1, 2 and 4 weeks of incubation and for dechlorination after 12 or 24 weeks of incubation.

Results and Discussion

Under fully aerobic conditions (8.1 ppm D.O.) the spiked BZ4 and BZ29 were both substantially biodegraded within a 4-week period whereas there was no BZ70 removal after either 12 or 24 weeks of incubation (see Table 1). In the corresponding fully anaerobic microcosms (0 ppm D.O.), after an initial lag period of approximately 6 weeks, the spiked BZ29 and BZ70 were both substantially dechlorinated to daughter congeners (see Figure 1) within a 12-week period whereas there was no dechlorination of BZ4 after either 12 or 24 weeks of incubation.

Table 1. Oxidation and reduction of PCBs at various D.O. levels

D.O., ppm	**PCB Degradation** (t = 4 weeks)	**PCB Dechlorination** (t = 12 weeks)
8.1	+	-
4	+	NT
2.1	-	NT
0.5	-	-
0	-	+

NT = not tested

Following these initial studies at the two extremes of dissolved oxygen concentration, we proceeded to identify the critical D.O. concentration below which aerobic PCB biodegradation shuts off and the concentration below which anaerobic dechlorination is no longer repressed/inhibited. Because of the substantial difference in rate between aerobic biodegradation and anaerobic dechlorination, we first identified the D.O. concentration below which aerobic PCB biodegradation is shut off, using a 4-week test incubation period. As shown in Table 1, biodegradation of the PCB congeners BZ4 and BZ29 was observed at 4 ppm D.O. (10% oxygen in the headspace gas of the hypoxic chamber) but not at 2.1 ppm D.O. (5% headspace oxygen) or lower. (Actual D.O. levels in the microcosms were determined using a D.O. meter.) Having thus determined the cut-off point for aerobic PCB biodegradation of the BZ4 and BZ29 congeners (between 2 and 4 ppm D.O.), we began longer (12-week) incubations to determine the D.O. concentration below which anaerobic dechlorination of BZ29 and BZ70 commences. As shown in Table 1, anaerobic dechlorination did not take place even at the lowest D.O. level that we could maintain with the hypoxic chamber (*i.e.*, 0.5 ppm D.O. at 1.25% headspace oxygen).

Conclusions

The dependence of aerobic PCB biodegradation on dissolved oxygen concentration ≥ 4 ppm required) is similar to that for other oxygenase-mediated processes. This suggests that our knowledge about other coupled systems (such as elimination of ammonia by oxidation to nitrate followed by nitrate-dependent respiration) may be applicable to

PCBs. The relatively high dissolved oxygen concentration required for significant aerobic PCB biotransformation activity implies that aerobic processes will be important only in the very-near surface region (*e.g.*, top 1 cm) of river sediments.

The inhibition of reductive dechlorination of PCBs at relatively low dissolved oxygen concentration (> 0.5 ppm) suggests that this process will take place only in sediment systems that are fully anaerobic.

The combination of these two observations indicates that there is an interval of dissolved oxygen concentration over which neither aerobic nor anaerobic PCB transformation processes can occur. *In situ* bioremediation of PCBs through a step-wise reductive dechlorination of highly chlorinated congeners followed by aerobic mineralization of the more lightly chlorinated congeners will be complicated by the need for strictly anaerobic conditions followed by strongly aerobic conditions.

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