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Dechlorination of 2,3,5,6-tetrachlorobiphenyl by a phototrophic enrichment culture

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1. SUMMARY

A phototrophic enrichment culture, using acetate as carbon source, reductively dechlorinated 2,3,5,6-tetrachlorobiphenyl, *ortho* chlorines were removed preferentially over *meta* chlorines. Tri- and dichlorobiphenyls were the major products. During 14 months incubation, chlorine was removed from 58% of the target molecules; 19% of the total chlorines were removed. Dechlorination did not occur in a control culture incubated in the dark.

2. INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent environmental toxins which tend to accumulate in sediments. PCBs are reductively dechlorinated in anaerobic sediments [1–3], but dechlorination is slow and the microorganisms responsible have not been identified or isolated. Reductive dechlorination leaves the biphenyl structure (and total PCBs concentration) intact, but tends to decrease the toxicity and render the partially dechlorinated PCBs more susceptible to complete degradation by aerobic microbes.

Various organic substrates were found to support reductive dechlorination of PCBs suggesting that the low rates in natural sediments could be due to limitations in availability of readily fermentable substrates [4]. We have speculated that reductive dechlorination of PCBs and some other compounds might be a fortuitous and non-specific reaction, i.e. that virtually any microorganism might be able to transfer electrons to these halogenated compounds, given a sufficient source of reducing power [5].

Anoxygenic phototrophic bacteria are able to use light-driven 'reverse electron transport' to regenerate low-potential electron donors at the expense of organic substrates [6]. Therefore, we speculated that phototrophic bacteria might be uniquely qualified to reductively dechlorinate

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PCBs. We report herein that a phototrophic enrichment culture, apparently dominated by purple non-sulfur bacteria, is able to reductively dechlorinate both a tetrachlorobiphenyl and its trichlorobiphenyl product.

Due to the low solubility of PCBs, a substantial portion of PCBs in the environment is associated with anaerobic sediments. Therefore, phototrophic microbes are not likely to be significant agents of PCBs dechlorination in the environment. However, this finding may aid in understanding the process of reductive dechlorination and may lead to processes for detoxifying other chlorinated xenobiotic compounds.

3. MATERIALS AND METHODS

3.1. Microorganisms and growth conditions

The medium contained the following (mg/l): NH_4Cl (100), KH_2PO_4 (1000), K_2HPO_4 (500), NaCl (10), MgCl (10), $CaCl_2$ (5), $NaHCO_3$ (500), $Na_2S \cdot 9H_2O$ (480). The following were also added (μg/l): $CuCl_2 \cdot 2H_2O$ (39), $FeCl_2 \cdot 4H_20$ (702), $AlCl_3$ (110), $MnCl_2 \cdot 4H_2O$ (280), $NaSO_4$ (140), $ZnCl_2$ (135), $NiCl_2$ (13), $CoCl_2 \cdot 6H_2O$ (38), H_3BO_3 (62), and $NaMoO_4 \cdot 2H_2O$ (25). Medium was prepared anaerobically for use in serum-stoppered bottles or tubes. Teflon-faced rubber septa (The West Co., Lancaster, PA) were used when PCBs or organic solvents were present; otherwise, butyl rubber stoppers were used.

A reddish film of growth developed on the inner surface of a glass column in which methanogenic bacteria were being enriched. The column was originally inoculated with anaerobic digestor sludge from the Jackson (MI) wastewater treatment plant and was fed sodium acetate (11 mM), sodium formate (15 mM), and methanol (21 mM); substantial methane production occurred. A phototrophic enrichment culture inoculated with the column effluent was fed acetate and incubated at room temperature (approx. 21–27°C), receiving solar, as well as room (fluorescent), light. When the column was covered with aluminum foil, the reddish growth disappeared.

3.2. PCB dechlorination assays and PCB analysis

50 ml of the enrichment culture was grown in medium containing 11 mM sodium acetate. After addition of 1 mg 2,3,5,6-tetrachlorobiphenyl (Ultra Scientific, North Kingstown, RI), dissolved in 100 μl acetone, the culture was incubated at room temperature by an east-facing window, where it recieved insolation in addition to fluorescent room lighting. An identical culture wrapped in aluminum foil served as dark control. 5-ml samples were taken periodically with a nitrogen-flushed syringe. Acetate was replenished thrice, but other nutrients may have limited growth.

Sample extraction and analytical methods were essentially as described previously [4]. Congeners were identified by gas-chromatographic (GC) retention times vs. those of known congeners. The results were verified by comparison to the relative retention times of Mullin et al. [7]. Identities of critical homologs were also verified by GC-mass spectrometry. Relative response factors [7] were used to quantify congeners for which standards were not available. Complete dechlorination or degradation of PCB was assumed not to occur. Therefore, the sum of concentrations of all congeners was assumed to remain constant (see INTRODUCTION) within each culture.

4. RESULTS AND DISCUSSION

The mixed phototrophic culture was tested for its ability to dechlorinate 2,3,5,6-tetrachlorobiphenyl while growing with variable solar and fluorescent light. Accumulation of 2,3,6- and 2,3,5-trichlorobiphenyls was detected after 15 days of incubation (Fig. 1). During 422 days of incubation, 58% of the PCB molecules had one or more chlorines removed (Table 1). Approximately one-third of the products were dichlorobiphenyls. As a first approximation, the rate of *ortho*-dechlorination of the tetrachlorobiphenyl was between two and three times higher than that of *meta*-dechlorination (Table 1). Monochlorobiphenyls were not detected. The homolog identities of di- and tri-chlorobiphenyls were confirmed by GC-mass spectrometry. This is the first report

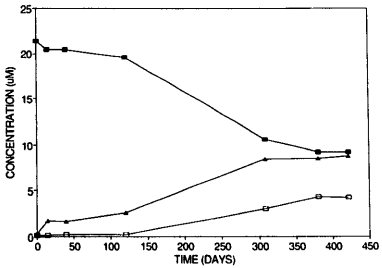

Fig. 1. Time course of conversion of 2,3,5,6-tetrachlorobiphenyl by phototrophic enrichment culture. Symbols: filled square, 2,3,5,6-tetrachlorobiphenyl in illuminated culture; triangle, trichlorobiphenyls; open square, dichlorobiphenyls.

of anaerobic phototrophic transformation of PCBs by bacteria.

The prominence of *ortho*-dechlorination contrasts with the low level occurring in sediments [2,8]. When 2,3,5,6-tetrachlorobiphenyl was dechlorinated by anaerobic sediment microbes, only a negligible amount of 2,3,5-trichlorobiphenyl was detected, and 2,5-dichlorobiphenyl was produced (probably from 2,3,6(= 2,5,6)-trichlorobiphenyl) at only one-third the level of 2,6-dichlorobiphenyl [8]. Even that low level of *ortho*-dechlorination (approx. 11% of chlorines removed) was considered to be unusually high for sediments. In contrast, 68% of the chlorines removed by the phototrophic culture were *ortho*-chlorines.

The lack of dechlorination in the dark control culture (Table 1) demonstrates that light was required. However, it could not be proved that dechlorination was carried out by the phototrophs rather than by other organisms. The latter could have occurred if non-phototrophic organisms were dependent upon the phototrophs to produce an unknown metabolite, or if the non-phototrophic organisms produced a metabolite which photocatalysed PCB dechlorination. A model for the latter is the photodecomposition of anilines and phenols by riboflavin (and degradation products thereof) [9]. Photochemical transformation of PCBs can occur, but is mediated by ultraviolet wavelengths which would be largely eliminated by the glass of the windows and culture containers in this experiment. We have observed only trace levels of dechlorination with abiotic controls exposed to far higher levels of fluorescent lighting than used here.

To determine whether the observed dechlorination was mediated by a phototrophic organism, strain PSM12D, provisionally identified as *Rhodopseudomonas palustris*, has been isolated from the mixed (enrichment) culture. Preliminary results (not shown) indicate that this organism photoreductively dechlorinates PCB in pure culture. The characteristics of this isolate will be described elsewhere.

These results might have led to a model system to increase our understanding of reductive PCB dechlorination occurring in the environment. However, the high level of *ortho*-dechlorination suggests that the mechanism differs from that dominant in sediments. If the dechlorinating abilities of the phototrophic enrichment culture were simply explained by the capacity to generate (non-specific) reducing power, the pattern of dechlorination should be similar to that found in sediments.

Table 1

Dechlorination of 2,3,5,6-tetrachlorobiphenyl by a phototrophic enrichment culture

PCB congener	Initial concentration (μM)	Final concentration (μM) [a]	
		Dark incubation	Illuminated
Dichlorobiphenyls			
2,3-	0	0	1.20
2,5-	0	0	1.25
2,6-	0	0	0.11
3,5-	0	0	1.48
Trichlorobiphenyls			
2,3,5-	0	0.06	5.95
2,3,6-	0	0.01	2.63
Tetrachlorobiphenyl			
2,3,5,6-	21.36	21.36	9.27

[a] After 422 days incubation; concentrations other than for 2,3,5,6-tetrachlorobiphenyl were corrected for contaminating levels in the PCB added.

Considerable research is directed at finding a practical process for dechlorination and degradation of PCBs in the environment. Although the findings reported here are quite interesting, a light-dependent process is unlikely be useful for treating a contaminant which is largely associated with sediments. However, these organisms might be capable of modifying xenobiotic compounds which are more soluble than PCBs.

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