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The effect of chemical pretreatment on the aerobic microbial degradation of PCB congeners in aqueous systems

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A series of experiments was conducted to examine the effects of chemical pretreatment on biodegradation of ^{14}C-labeled PCB congeners in aqueous systems. Fenton's reagent was used to generate hydroxyl radicals ($OH^{\cdot}$) which were successful in partially oxidizing/transforming otherwise recalcitrant molecules of tetrachlorinated PCB, but had little or no impact on the biodegradation of a monochlorinated congener. Application of Fenton's reagent (1% H_2O_2, 1 mM $FeSO_4$) followed by inoculation with pure cultures *Pseudomonas* sp, strain LB 400 and *Alcaligenes eutrophus*, strain H850 resulted in the removal of approximately 38% of 2-chlorobiphenyl and 51% of 2,2′,4,4′-tetrachlorobiphenyl in the form of $^{14}CO_2$. Comparison of the rate and extent of biodegradation of 2,2′,4,4′-tetrachlorobiphenyl after the application of Fenton's reagent with the dynamic and final level of radioactivity in the aqueous phase of experimental system suggests two possible means of microbial utilization of tetrachlorinated PCB congener altered by chemical oxidation: (a) consumption of the partially oxidized chemical dissolved in the aqueous phase, and (b) direct microbial attack on the transformed compound, which may still be adhered to the solid surface.

Keywords: polychlorinated biphenyls: Fenton's reagent; aerobic biodegradation; chemical-biological treatment

Introduction

Polychlorinated biphenyls (PCBs; trade names: Arochlor, Clophen, Phenoclor, Pyralene and Canechlor) have been used widely. Applications include production of pesticides, electric fluids, plasticizers, and cutting oils. Due to toxicity and hazardous effects on natural ecosystems, production and application of PCBs was stopped in the late 1970s. Nevertheless, PCBs continue to impact the environment. These compounds slowly leach from numerous contaminated sites into groundwater and seawater [13,23,38], and exhibit resistance to microbial and fungal degradation [1,11,37]. Low water solubility and strong sorptive capacity to the fine fractions of soils and sediments may also contribute to their recalcitrance [15].

Several studies have been published which deal with the aerobic degradation of PCBs by genetically engineered and enriched pure cultures [11,20,21], mixed consortia and enrichments [17,25] as well as indigenous soil and sediment populations [4]. Congeners with four or more chlorines, especially the industrial mixtures of highly chlorinated congeners, are more recalcitrant compared to mono-, di- and tri-chlorinated compounds [1,12]. This phenomenon is based on steric hindrance of 2,3-dioxygenation, due to chlorine moieties at either of these positions [1].

In order to overcome the recalcitrance of the highly chlorinated PCB congeners, several physical and chemical methods have been proposed for their partial oxidation [32,33,40]. Hydroxyl radicals generated from hydrogen peroxide by photo-decomposition or ferrous ion catalysis (Fenton's type reaction) were found to be effective oxidizers of aromatic rings due to nonspecificity of chemically induced oxidation [19,26,39]. The effectiveness of Fenton's reagent as a nonspecific oxidizer has been shown for various classes of organic pollutants, such as cyanides [8], PAHs [24], chlorobenzenes [31], PCBs [27,32], azo dyes [16, 35], and some pesticides [29,36]. The production of partially oxidized intermediates during the PCB degradation processes does not necessarily lead to their complete removal. This may result from increased toxicity of chemically produced intermediates compared to parent compounds, and the inability of PCB degraders to metabolize them [9,30,34].

Thus, the main obstacles preventing mineralization of PCBs in natural environments may be defined as: a) recalcitrance; b) toxicity of intermediates; and c) limited physical availability for microorganisms or chemical oxidants.

While some studies have indicated chemical oxidation of PCBs in aqueous solutions to different degrees [32], and others have concentrated on the microbial degradation of partially oxidized products of PCB transformations, including hydroxychlorobiphenyls and chlorobenzoates [22,34], there are no available reports on the direct effect of chemical pretreatment on the biodegradation of PCBs in aqueous systems. This study evaluates the effects of a combination of chemical and biological methods, in continuous experimental systems, on the rate and extent of degradation of various pure PCB congeners.

Materials and methods

Chemicals

All chemicals were reagent-grade and included toluene, hydrogen peroxide, and ferrous sulphate purchased from Aldrich Chemical Co (Milwaukee, WI, USA); HPLC-grade 2-chlorobiphenyl (2-CB), 2,2′,4,4′-tetrachlorobiphenyl (2,2′,4,4′-TCB) purchased from Ultra Scientific

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Co (North Kingstown, RI, USA); 2-chlorobiphenyl-UL-^{14}C (4.9 mCi mmol^{-1}, purity > 98%) and 2,2′4,4′-tetrachlorobiphenyl-UL-^{14}C (10.6 mCi mmol^{-1}, purity < 98%) purchased from Sigma Chemical Co (St Louis, MO, USA).

Microbial cultures

The organisms used in this experiment were *Alcaligenes eutrophus*, strain H850 and *Pseudomonas* sp, strain LB400, obtained from General Electric Co, Schenectady, NY, USA. Cultures were maintained as described elsewhere [11]. Cells were harvested by centrifugation near the midpoint of log phase of growth, washed twice with 0.05 M sodium phosphate buffer (pH 7), and resuspended in phosphate-buffered mineral salts medium to obtain a cell density of approximately 1×10^8 cells per ml. After mixing in equal proportions, cells were added to the experimental systems for a final cell density of 2.5×10^6 ml^{-1}.

PCB analysis

HPLC analyses of PCB congeners were accomplished with an instrument equipped with Gilson 305 and 306 series piston pumps (Gilson Medical Electronics, Inc, Middleton, WI, USA) and a Waters 991M Photodiode Array Detector (Millipore Corp, Milford, MA, USA). The samples were injected directly injected onto a Spherisorb ODS-2 (5 mm) reversed-phase column (250-mm long, 4-mm id) (Alltech Assoc, Inc, Deerfield, IL, USA) after filtration through a 0.22-mm syringe filter to remove solid particles and dissolved gases (Aronstein and Paterek, unpublished data). Elution was carried out by pumping acetonitrile and water (82:18 v/v) isocratically at a flow rate of 0.7 ml min^{-1}. The absorbance of PCB congeners was measured at a wavelength of 254 nm. The peak areas of the chromatograms were integrated using Millenium 2010 LC software package (version 1.10) (Millipore Corp, Milford, MA, USA). Standard solutions of the PCB congeners were prepared in a 1:1 (v/v) mixture of acetonitrile and water.

Measurement of PCB degradation

For PCB degradation studies, 160-ml serum bottles were modified [8]. To measure the rate of chemical transformation of PCBs by Fenton's reagent, unlabeled (0.25 mg) and labeled (100000 dpm) congeners, dissolved in toluene, were added to the bottom of the serum bottle apparatus. The toluene was allowed to evaporate, and acetate buffer and ferrous sulfate solutions were added. Acetate buffer was used to achieve pH 4.0. The serum bottles were crimp-sealed and 1 ml of 0.5 M NaOH was added to one 2-ml screw cap vial inserted in the bottle and a second vial received 1 ml of *n*-butanol. These vials allowed for trapping of CO_2 and volatile organics, respectively. Aliquots of hydrogen peroxide stock solution (30%) were injected into the bottles for a final concentration of 1%. The final volume in each bottle was 25 ml. The bottles were shaken on a rotary shaker (120 rpm) throughout the experiment at approximately 22° C. Periodically, the NaOH or *n*-butanol from the traps and 1.5 ml of liquid from the bottle were removed, and the NaOH or *n*-butanol was replaced. Samples from the liquid phase of the reaction mixture were passed through sterile nylon syringe filters (0.22-μm pore size; MSI, Westboro, MA, USA) to remove solid particles. The NaOH, *n*-butanol, and filtered liquid (1 ml), was mixed with 4 ml of Ultima Gold scintillation fluid (Packard Instrument Company, Inc, Meriden, CT, USA) in 7-ml scintillation vials, and the radioactivity was determined with a liquid scintillation analyzer (Tri-Carb 2000CA, Packard Instrument Co, Downers Grove, IL, USA). To assess the radioactivity associated with biomass and inorganic particles, the filters were dissolved in toluene, and the radioactivity was counted.

After completion of the chemical reaction, the pH in each of the experimental systems was adjusted to 8.0 with NaOH and microbial cells, along with phosphate-buffered mineral salts medium, were added to the bottles for a final volume of 40 ml. The pH was measured daily and adjusted to 8.0 thereafter. The evolution of CO_2 and organics, along with the dynamics of the specific radioactivity associated with solids and the liquid phase, were monitored as described above. After experiments were terminated, residues attached to the glass walls of the experimental vessels were extracted by adding 25 ml of toluene, shaking bottles for 24 h and counting toluene sub-samples.

Experiments on the biodegradation of PCBs without chemical pretreatment were carried out in phosphate buffer and maintained at pH 8.0 throughout the experiments. Duplicate bottles were used in all studies and mean values are presented. Data were analyzed statistically at the 95% confidence level.

Results

Experiments were conducted on the volatilization and mineralization of two test PCB congeners, and a mass-balance was generated. The total recovery of radioactivity added was greater than 84% in all experiments (Table 1). In the sterile system, sorption to the glass walls was the main factor affecting removal of both PCB congeners. Also, nearly 19% of 2-CB was removed from the system by volatilization, compared to only 5% for 2,2′4,4′-TCB. Only trace amounts of both PCB congeners were found in the liquid phase at the end of the experiment.

In 2-CB systems amended with microbial cultures, microbial degradation was the major factor affecting overall removal. For 2-CB, 45% of initially added compound was recovered in the form of CO_2, while only 8.5% of

Table 1 The final distribution of radioactivity between different parts of experimental systems, % of initial radioactivity added

PCBs	CO_2	Volatile	Liquid	Biomass	Glass	Total
		Control (no treatment)				
2-CB	1.0	18.5	0.9	–	70.2	90.6
TCB	0.1	5.2	0.1	–	82.5	87.9
		Biological treatment				
2-CB	45.0	8.7	3.4	15.3	22.9	96.4
TCB	8.5	1.5	2.8	7.1	75.1	95.0
		Chemical-biological treatment				
2-CB	37.5	1.8	3.5	25.4	22.6	90.8
TCB	51.2	0.6	3.3	22.1	20.2	84.8

2,2′,4,4′-TCB was mineralized. A portion of the added congeners was partially metabolized causing an increase in radioactivity in the liquid phase and its association with the cell biomass. The accumulation of water-soluble intermediates in the liquid phase and in the cells was more pronounced in the systems with 2-CB compared to those with 2,2′,4,4′-TCB. Microbial degradation appeared to compete with volatilization, and in controls with no microorganisms volatilization decreased more than two and three times in the bottles with 2-CB and 2,2′,4,4′-TCB, respectively (Table 1). Biodegradation of 2-CB also affected the amount of compound sorbed to the glass walls, with a 47.3% reduction, while the amount of 2,2′,4,4′-TCB sorbed to the glass walls in the presence of microbial cultures was reduced only 7.4% compared to sterile controls.

Application of chemical pretreatment by Fenton's reagent affected consequent biodegradation of mono- and tetrachlorinated PCB congeners differently (Table 1). While a slight decrease was observed for the extent of 2-CB mineralization, a six-fold increase was observed for 2,2′,4,4′-TCB, Chemical oxidation also decreased sorption of 2,2′,4,4′-TCB to the glass walls almost four-fold, while compared to the varieties with biological treatment only. At the same time, application of Fenton's reagent did not significantly affect the extent of accumulation of water soluble compounds in the liquid phase of the experimental system. On the contrary, at the end of the experiment more than 10% of the initially added radioactivity was found in the liquid phase of the bottles chemically treated but not inoculated with microorganisms. It is also noteworthy that chemical pretreatment resulted in increased radioactivity associated with biomass and particles of ferrous sulfate. Unfortunately, because of the inability to separate organic and inorganic material for consequent counting of radioactivity, it is impossible to assess the accumulation of radiolabeled intermediates associated with microbial cells at the end of chemical-biological treatment. The HPLC analyses of the liquid phase of experimental systems treated by Fenton's reagent showed no detectable amounts of the parent compounds tested during the course of the experiments.

Biodegradation kinetics in bottles with microorganisms and no chemical pretreatment approached first-order in the case of 2-CB, resulting in evolution of nearly 90% of all [14-C] CO_2 produced, in the first 125 h of the experiment (Figure 1a). Correlation analyses showed that kinetics in the case of 2,2′,4,4′-TCB were also close to first-order with a lag-phase of approximately 25 h following the addition of microbial cultures. Application of Fenton's reagent drastically changed the maximum rate of 2,2′,4,4′-TCB degradation from 0.14 $\mu g\ h^{-1}$ in the biological treatment alone to 0.92 $\mu g\ h^{-1}$ in the chemical-biological treatment, changing the shape of the degradation curve from first-order to a Monod-like curve, with a 75-h lag-phase. Chemical pretreatment of 2-CB resulted in a 15-fold decrease in the highest rate of 2-CB biodegradation, with an overall change in kinetics to a logistic curve (Figure 1b).

The analysis of radioactivity in the liquid phase of the experimental system amended with [14-C] 2-CB showed an increase from 5% to nearly 30% of initial radioactivity

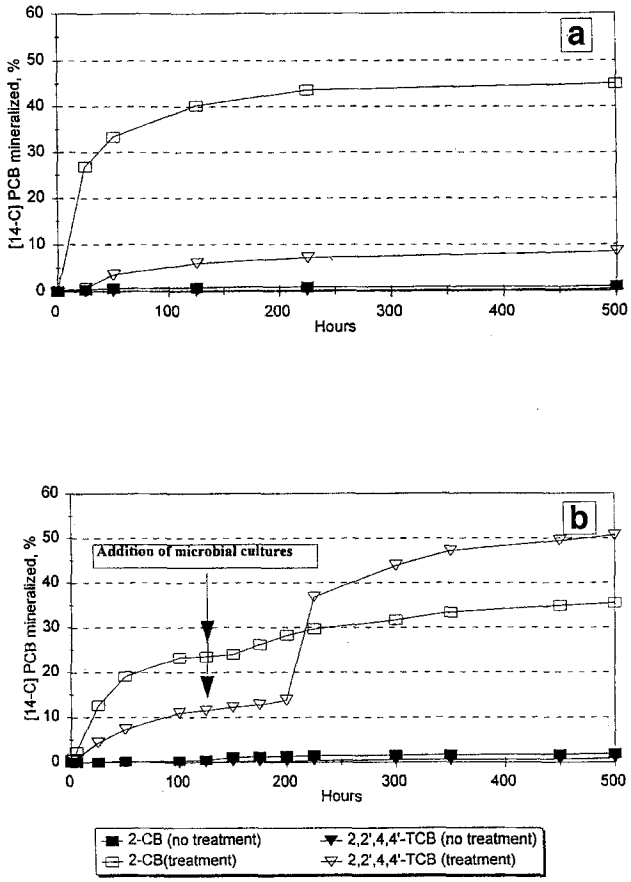

Figure 1 Mineralization of 2-chlorobiphenyl and 2,2′,4,4′-tetrachlorobiphenyl by biological (a) and sequential chemical-biological (b) treatments

added within the first hour after initiation of chemical treatment (insert in Figure 2), followed by a decrease to near the initial level at the end of the experiment (Figure 2). The shape of curve depicting the dynamics of radioactivity in the system amended with [14-C] 2,2′,4,4′-TCB was also biphasic, though in this case the beginning of the second

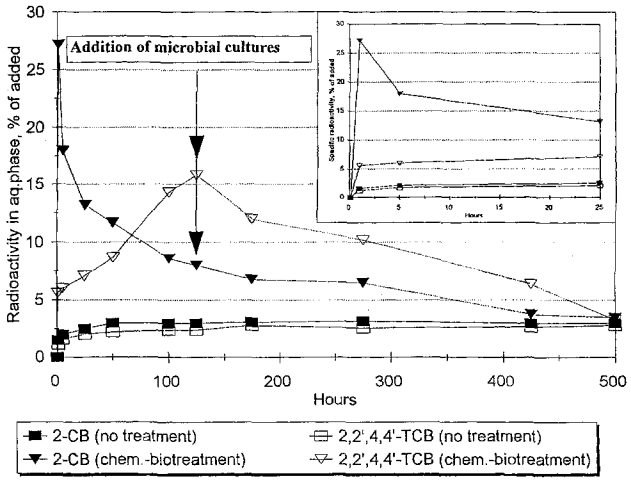

Figure 2 Dynamics of radioactivity in the liquid phase of the experimental system during the sequential chemical-biological treatment of 2-chlorobiphenyl and 2,2′,4,4′-tetrabiphenyl. The insert shows the detailed dynamics of radioactivity during the first 25 h of the experiment

phase was clearly associated with the addition of microbial cultures. During the first phase (chemical oxidation) the amount of radioactivity in the liquid phase increased to 16% of initial radioactivity added, and after inoculation decreased to 5% (Figure 2).

Discussion

Oxidation by Fenton's reagent followed by application of *Alcaligenes eutrophus*, strain H850 and *Pseudomonas*, strain LB 400 allowed greater than 50% removal of tetrachlorinated PCB congener. This result does not exceed the mineralization values for 2,2′,4,4′-TCB previously reported for microbial or chemical methods used separately [11,18,27,32]. It should be noted, however, that in the studies on biological degradation of PCBs, the limitations of mass-transfer of chemicals practically insoluble in water were avoided by direct addition of concentrated solutions of PCBs in acetone to the cell suspensions [11,18]. In chemical degradation experiments, the above limitations were overcome by either the addition of chemicals dissolved in non-aqueous phase liquids [14,27] or below their solubility limits [32]. In this study, substantial amounts of PCBs and their intermediates (up to 75% of added) were recovered from the glass walls at the end of experiments. Chemical and enzymatic reactions in the presence of sorbed and/or undissolved chemical are heterogeneous and thus environmentally realistic.

Application of Fenton's reagent dramatically increased the effectiveness of biodegradation of a highly chlorinated PCB congener. In contrast, the extent of combined chemical-biological degradation of a monochlorinated congener was slightly lower compared to that achieved by biological means alone. The lag-phase observed for biodegradation of partially oxidized products resulting from chemical treatment of PCB congeners indicates the possibility of the direct effect of these products on microbiological activity [2,3]. HPLC analyses of the liquid phase confirmed that all radioactivity existed in the form of unidentified water-soluble compounds. 5-Hydroxy-2-chlorobiphenyl is an intermediate in the chemical degradation pathway of 2-chlorobiphenyl [32]. Representatives of mono-hydroxybiphenyls have been found to act as antibiotic agents, and may inhibit microbial degradation [10]. Possible toxic intermediates generated by chemical oxidation of polychlorinated biphenyls include also polychlorinated dibenzofurans (PCDF) [28].

The amount of 2,2′,4,4′-TCB mineralized during the biological stage of combined chemical-biological treatment was nearly 40%. This was three times greater than the decrease of radioactivity in the aqueous phase of the experimental system after the application of microbial cultures. This suggests that microorganisms may have degraded the portion of parent compounds which had been chemically altered, but not desorbed. Marked enhancement of the rate and/or extent of biphenyl's biodegradation without its substantial desorption has been observed in soils and aquifer solids with application of two nonionic surfactants at low concentrations [5–7]. Although the surfactant-based and oxidant-based mechanisms of chemical alteration are different, they may both lead to the direct microbial consumption of some organic compounds adhered to the solid surfaces.

Thus, the evaluation of experimental data suggests two possible means of microbial utilization of tetrachlorinated PCB congeners altered by chemical oxidation: (a) consumption of the partially oxidized chemical dissolved in the aqueous phase, and (b) direct microbial attack on the altered compound, still adhered to the solid surface.

Additional experiments are required in order to optimize the combined chemical-biological treatment process for greater enhancement of PCB-degradation. Also, partially oxidized products of Fenton's degradation should be identified in order to isolate and apply microbial cultures capable of degrading these intermediates. Nevertheless, the data presented suggest the potential ability of proposed chemical pretreatment in increasing the availability of PCBs for microbial degradation.

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