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Enrichment of anaerobic polychlorinated biphenyl dechlorinators from sediment with iron as a hydrogen source

Jason P. Rysavy, Tao Yan, Paige J. Novak*

The Department of Civil Engineering, University of Minnesota, 122 Civil Engineering Building, 500 Pillsbury Drive S.E., Minneapolis, MN 55455-0220, USA

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Abstract

Little is known about anaerobic polychlorinated biphenyl (PCB) dechlorination, although it is believed that some microorganisms are capable of respiring PCBs, gaining energy for growth from PCB dechlorination. If this is the case, the amendment of appropriate electron donors to contaminated sediment should stimulate dechlorination. The effect of elemental iron (Fe^0) addition, an easily amended electron donor, on the microbial dechlorination of the PCB congeners 3,4,5-trichlorobiphenyl (3,4,5-CB) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (2,2′,3,4,4′,5,5′-CB) was investigated in microcosms containing estuarine sediment from Baltimore Harbor. Results showed that the addition of 0.1 g Fe^0/g sediment reduced the lag time for removal of doubly flanked *para* chlorines by approximately 100 days. Because Fe^0 is a source of cathodic hydrogen (H_2), the effect of direct H_2 addition to sediment microcosms was also tested. The addition of 0.001 atm H_2 in the headspace generated the same dechlorination activity and reduction in lag time as the addition of 0.1 g Fe^0/g. Higher concentrations of Fe^0 or H_2 increased the lag prior to dechlorination. Additional results showed that an alkaline pH ($\geqslant$7.5), high [Fe^{2+}] (3.3 g/L), or HS^- ($\geqslant$0.1 mg/L total sulfide) inhibited dechlorination. Elevated concentrations of Fe^{2+}, OH^-, and HS^- are products of Fe^0 oxidation or increased microbial activity (methanogenesis, homoacetogenesis, and sulfate reduction), both of which would result from the amendment of large quantities of Fe^0 or H_2 to sediment. This research shows that not only can PCB dechlorination be stimulated through the addition of electron donor, but implies that the dechlorinators are enriched by the continuous addition of low concentrations of H_2, similar to other known dechlorinators, such as the dehalorespirer *Dehalococcoides ethenogenes*. These results suggest that the direct addition of controlled amounts of Fe^0 to sediments may be an effective remediation tool to reduce the lag period prior to dechlorination at PCB-impacted sites. They also suggest that PCB dechlorinators may be enriched using techniques similar to those used with known dehalorespirers.

Keywords: Hydrogen; PCBs; Elemental iron; Bioremediation; Sediment; Anaerobic dechlorination

1. Introduction

Polychlorinated biphenyls (PCBs) were produced in the United States from 1929 to 1977 for many applications, including electrical insulators, coolants in capacitors and transformers, hydraulic fluids, and carbonless copy paper (Abramowicz and Olson, 1995).

*Corresponding author. Tel.: +1 612 626 9846; fax: +612 626 7750.

E-mail address: novak010@tc.umn.edu (P.J. Novak).

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In the mid-1960s, it was discovered that PCBs were accumulating in the environment as a result of their chemical stability and resistance to biological degradation; this led to their eventual ban in the United States in 1979. It has been estimated that 2.1×10^8 kg of PCBs, one-third of the total quantity produced, have been released into the environment as contaminants in soil and river, lake, and ocean sediments (Hutzinger and Verrkamp, 1981). This is of great concern because these compounds act as endocrine disrupters, teratogenic agents, and carcinogens and are distributed throughout the biological food chain via bioaccumulation (Cogliano, 1998).

PCB treatment options are few in number and often prohibitively expensive, leading researchers to investigate the potential for biological dechlorination to serve as an in situ remediation option. Anaerobic dechlorination can attack more highly chlorinated PCBs and has the potential to reduce their toxicity, particularly if *meta* and *para* chlorines are removed (Mohn and Tiedje, 1992). Such dechlorination has been observed at PCB-contaminated sites and also in microcosms containing sediment from non-impacted sites (Wiegel and Wu, 2000). Nevertheless, our current understanding of anaerobic biological PCB dechlorination is limited. Methanogens (Ye et al., 1995) and sulfate reducers (Zwiernik et al., 1998) have been implicated in PCB dechlorination, although no anaerobic PCB-dechlorinating organisms have been isolated. Two uncultured anaerobic PCB dechlorinators (DF-1 and *o*-17) that are phylogenetically related to known dehalorespiring microorganisms have been recently identified using molecular techniques (Cutter et al., 2001; Wu et al., 2002). Little is known, however, about the physiology of PCB dechlorinators in general. Moreover, the varying dechlorination patterns observed in complex PCB mixtures have been attributed to different microbial populations (Wiegel and Wu, 2000), suggesting that different techniques may be required to enrich for varying dechlorination patterns.

Long lag periods before PCB dechlorination begins, ranging from months to years (Wiegel and Wu, 2000), are often observed. This is a significant obstacle to the use of anaerobic bioremediation as sediment clean-up technology. Because of our lack of understanding regarding the identity and physiology of PCB dechlorinators, we are limited in our ability to understand and overcome these long lag periods. The creation of particular environmental conditions, or niches, for the PCB dechlorinators, the low bioavailability of PCBs (i.e., Bedard et al., 1998), and/or competition for electron donor could each contribute to long lag periods.

Hydrogen (H_2) has been reported to be an electron donor for the dechlorination of 2,3,4-trichlorobiphenyl (2,3,4-CB) in Hudson River sediment microcosms (Sokol et al., 1994). Nevertheless, in additional experiments H_2 was observed to be inhibitory to dechlorination (Wu, 1996). More recently, H_2 was shown to serve as an electron donor for a specific PCB-dechlorinator enriched from Charleston Harbor sediment, bacterium DF-1, which removes doubly flanked chlorines from PCB congeners (Wu et al., 2000, 2002). The general role of H_2 in a complex community that includes PCB-dechlorinators is currently unknown, but needs to be clarified if this process is to be understood and then manipulated for sediment remediation. Because of this, the overall goal of this study was to further investigate the apparent physiology of PCB-dechlorinators. Specifically, it was hypothesized that the addition of H_2 generated by the corrosion of elemental iron (Fe^0) would stimulate the microbial reductive dechlorination of PCBs in sediment microcosms if added at the appropriate rate. H_2, which is generated in anaerobic environments as a fermentation product, is maintained at low steady-state concentrations as a result of the catabolic activity of sulfate reducing bacteria and methanogenic archaea. Competition for H_2 may thus contribute to the long lag periods prior to PCB dechlorination observed in the environment and laboratory. The oxidation of Fe^0 to H_2 has an oxidation–reduction potential of 0.44 V, making this chemical reaction (shown below) thermodynamically favorable:

$$Fe^0 + 2H_2O = Fe^2 + 2OH^- + H_2.$$

In addition to providing H_2 in situ to stimulate microbial activity, the production of Fe^{2+} will sequester potentially inhibitory sulfide, an end product of biological sulfate reduction.

Herein we report that Fe^0 reduced the lag period prior to microbial reductive dechlorination of 3,4,5-CB and 2,2′,3,4,4′,5,5′-CB in sediment microcosms. We show that the enrichment of dechlorinators with the ability to remove doubly flanked chlorines resulted from the generation of H_2 via Fe^0 corrosion. The results of these microcosm studies suggest that treatment of PCB-impacted sediments with Fe^0 could serve as part of a tractable and inexpensive biological remediation technology to promote in situ microbial reductive dechlorination. These results also shed light on the physiology of PCB-dechlorinators, which appear to have a high affinity for H_2, much like known dehalorespirers.

2. Materials and methods

2.1. Chemicals

The PCB congeners, 3,4,5-CB and 2,2′,3,4,4′,5,5′-CB (AccuStandard) were dissolved in acetone (HPLC-UV grade) to concentrations of 1–10 mg/mL. Single PCB congeners (99% purity, AccuStandard) used to prepare

calibration standards were obtained dissolved in iso-octane at concentrations of 35 or 100 μg/mL. Hexane (GC Resolv) and Fe^0 (unwashed, 99% pure, 100 mesh) were from Fisher Scientific.

2.2. Sediment collection and storage

Baltimore Harbor (BH) sediment, which has been shown to contain PCB dechlorinators, was obtained as described previously (Berkaw et al., 1996, Cutter et al., 1998, Wu et al., 1998). Upon arrival at the laboratory, samples were stored at room temperature in an anaerobic glove bag (Coy Laboratory Products) to minimize loss of dechlorinating activity over time (Wu, 1996).

2.3. Experimental setup

2.3.1. Effect of Fe^0 addition on dechlorination

Microcosms were prepared in 125-mL crimp-top serum bottles. All bottles were prepared and maintained under anaerobic conditions. Each 125-mL microcosm contained 100 mL anaerobic mineral media and 20 g wet BH sediment (5 g dry sediment equivalent) under an N_2 headspace. The anaerobic mineral media was similar to that used previously with BH sediment (Berkaw et al., 1996) and contained (per liter of deionized (DI) water): 0.001 g resazurin (a color indicator of redox potential), 0.1 g $MgCl_2 \cdot 6H_2O$, 0.1 g $CaCl_2 \cdot 6H_2O$, 3.0 g Na_2CO_3, 0.5 g NH_4Cl, 0.6 g Na_2HPO_4, 10 mL vitamin solution (Wolin et al., 1963), and 10 mL mineral solution (Wolin et al., 1963). The medium was boiled and allowed to cool under a stream of N_2 (industrial grade) before addition to the microcosms. The following treatments were prepared, both with and without the addition of 2.7 g sulfate/L: no Fe^0 control, 0.002 g Fe^0/g dry sediment (0.002 g Fe^0/g), 0.1 g Fe^0/g dry sediment (0.1 g Fe^0/g), and 2.0 g Fe^0/g dry sediment (2.0 g Fe^0/g). These quantities of Fe^0 were chosen to generate a relatively wide range of H_2 production rates in the microcosms, and represent 0.007%, 0.36%, and 7% Fe^0 on a volumetric basis in the wet sediment. Treatments to which no sulfate was added contained approximately 1.0 g/L sulfate from the sediment itself. Sterile controls were also prepared (both with and without 2.7 g sulfate/L added) containing 2.0 g Fe^0/g or no added Fe^0 to determine whether Fe^0 could serve as a direct electron donor for abiotic PCB dechlorination. Sterile controls were prepared by autoclaving sealed microcosms 5 times for 40 min at 120 °C. Sodium azide (50 mM) was added to these microcosms if CH_4 production occurred after autoclaving. All treatments were prepared in triplicate.

The PCB congeners 3,4,5-CB and/or 2,2′,3,4,4′,5,5′-CB (2.6 μmol/g dry sediment) were added to each microcosm using a 7.21 mg/mL acetone stock, after which the bottle was sealed with a Teflon-lined stopper and shaken on a wrist-action shaker for 1 h. The microcosms were incubated on a shaking incubator (120 rpm) in the dark over the course of the experiment. Microcosms were sampled (approximately 3 mL) for pH, sulfate, methane and PCBs approximately once a month over the course of 10 months. After sampling, the pH of each microcosm was adjusted to approximately 7.0 with the addition of a 50% (by volume) HCl solution. Samples (2 mL) were withdrawn from the microcosms for Fe^{2+} concentration determination after 8 months of incubation.

Additional microcosms were prepared as described above without the addition of Fe^0 and 2.7 g sulfate/L. The microcosms were flushed with either 100% H_2 or 100% N_2. Additional H_2 was added to some of the N_2-flushed bottles using a syringe, resulting in treatments with the following H_2 partial pressure in the headspace (prepared in triplicate): no H_2 control (1.0 atm N_2), 0.001 atm H_2, 0.01 atm H_2, and 1.0 atm H_2. The headspace of each microcosm was flushed once a week and after sampling. Each septum was replaced after sampling for PCBs and prior to flushing the bottles. The PCB congener 3,4,5-CB (2.6 μmol/g dry sediment) was added to each bottle as described above to begin the experiment.

2.3.2. Effect of Fe^0 oxidation products or microbial sulfate reduction products on dechlorination

Additional microcosm experiments were performed to understand the impact of pH, Fe^{2+}, and a high H_2 partial pressure on 3,4,5-CB dechlorination once a dechlorinating culture (*para* chlorine removal) had been enriched. Microcosms were prepared in 37-mL crimp-top serum bottles. The bottles contained an N_2 headspace unless otherwise specified. The contents of the 125-mL sediment microcosms to which 0.1 g Fe^0/g and 3,4,5-CB were added (both with and without added sulfate) were combined and homogenized. Active PCB dechlorination was occurring in these microcosms. Twenty milliliters of this homogenized mixture was then added to each of the 37-mL bottles. Treatments (prepared in triplicate) included a positive control (with no additions made to the 20 mL mixture), an Fe^{2+}-added treatment (3.3 g/L), an Fe^0-added treatment (2.0 g/g dry sediment), a high pH treatment (pH of 8.5), and a high H_2 treatment (1.0 atm H_2 in the headspace).

An experiment was also performed to understand the impact of sulfide on 3,4,5-CB dechlorination. Microcosms (in triplicate) were prepared in 37-mL crimp-top serum bottles. The bottles contained an N_2 headspace. Twenty mL of a nearly sediment-free culture enriched from BH sediment that was actively dechlorinating 3,4,5-CB (*para* chlorine removal) was added to each

bottle. Na_2S was added to each bottle via a pH-adjusted (7.0) stock solution to reach final concentrations of 0.3, 0.1, and 0.03 mg/L total sulfide. This quantity of sulfide corresponds to the reduction and release into solution of approximately 0.03%, 0.01%, and 0.003% of the original 1.0 g/L sulfate present in the sediment microcosms. An additional experiment was performed with this culture to more precisely determine the effect of pH on dechlorination. Microcosms were prepared (in triplicate) as described above, except sulfide was not added to any of the microcosms. The pH of the culture was adjusted through the addition of HCl or NaOH to prepare the following treatments: pH 7.5, pH 8.0, and pH 8.5. A positive control (with no sulfide addition to the 20 mL mixture and at a pH of 7.0) was also prepared.

To begin these experiments, 3,4,5-CB was added to each bottle (2.6 μmol/g dry sediment), which was sealed with a Teflon-lined stopper and shaken on a wrist-action shaker for 1 h. The microcosms were incubated on a shaker table (120 rpm) in the dark over the course of the experiments and were sampled every 2–30 days for pH and PCBs. If necessary, microcosm pH was adjusted after sampling using a 50% (by volume) HCl solution or a 1 N NaOH solution.

2.4. Analytical methods

2.4.1. PCB analysis

Methods used for sample preparation and extraction were similar to those described elsewhere (Quensen et al., 1990). Microcosms were shaken for 2 min, the septum was removed, and approximately 2 mL of the sediment slurry was withdrawn with a filed-off Pasteur pipette. After the sample was weighed, PCBs were extracted using sequential liquid extraction. Acetone (10 mL) was added to the 2 mL sample, the mixture was shaken for 2 min, and the acetone layer was transferred to a 125-mL bottle. This was followed by the addition of 10 mL of hexane-acetone (1:1) to the 2 mL sample. The mixture was shaken for 2 min and the solvent layer was transferred into the 125-mL bottle. This step was repeated, giving a final solvent volume in the 125-mL bottle of 30 mL. Ten milliliters of DI water containing 2% (by volume) NaCl was added to the 125-mL bottle containing the pooled solvents from the previous step. The mixture was shaken for 2 min and the hexane layer was removed and placed in a 37-mL bottle containing 4 mL 30% (by volume) sulfuric acid. This mixture was shaken for 2 min and the hexane layer was removed and placed into a 37-mL bottle containing 10 mL DI water containing 2% (by volume) NaCl. This mixture was shaken for 2 min and the hexane layer was again removed, dried with Na_2SO_4, and filtered through a florisil-copper (25% copper by weight) column. The final hexane volume was adjusted to 25 mL before analysis. PCBs were analyzed using a gas chromatograph (GC) (Hewlett Packard (HP) 5890 series) equipped with an electron capture detector (ECD). An HP-1 capillary column (25 m × 0.200 mm × 0.11 μm film thickness) was used for congener separation. Samples (2.0 μL) were injected using an autosampler (HP 6890 Series). The carrier gas flow (He, UHP zero grade) was 3 mL/min. The oven temperature was held at 100 °C for 2 min, ramped to 160 °C at 15 °C/min, ramped to 235 °C at 5 °C/min, held at 235 °C for 3 min, ramped to 270 °C, and held at 270 °C for 2 min. Extraction efficiencies were approximately 80%. The method detection limit for the congeners 3,4,5-CB and 2,2′,3,4,4′5,5′-CB was 1.2×10^{-5} and 7.6×10^{-6} μmol/mL, respectively, as determined by Standard Method 1030C (APHA, 1995).

External calibration standards for specific PCB congeners diluted in isooctane were purchased and diluted further with hexane to produce a range of concentrations. Neither peak retention times nor peak areas changed when isooctane was used as the solvent instead of hexane. These standards were analyzed by GC-ECD as described above.

Results are plotted as number of chlorines per biphenyl. This was calculated as follows:

$$\frac{(\sum C_{dt}N_d) + C_{pt}N_p}{\sum C_{dt} + C_{pt}}.$$

Here C_{dt} is the concentration of a given daughter product at the sampling time, t, N_d is the number of chlorine atoms per biphenyl in that daughter product, C_{pt} is the concentration of the parent PCB congener at a given sampling time, t, and N_p is the number of chlorine atoms per biphenyl in the parent congener.

2.4.2. Methane and H_2 analysis

Prior to opening the microcosms for PCB sample withdrawal, volumes (100 μL) for headspace methane and H_2 analysis were removed with a gas-tight locking syringe and analyzed using a GC (HP 6890 series) equipped with a thermal conductivity detector (TCD). An HP molecular sieve column was used for sample separation. The carrier gas flow (He, high purity) was 20 mL/min. The method detection limits for H_2 and methane were 1.03×10^{-5} and 8.02×10^{-6} atm, respectively.

External calibration standards for methane and H_2 were prepared using 37-mL serum bottles to which measured quantities of methane or H_2 were added. These standards were analyzed by GC-TCD as described above.

2.4.3. Fe^{2+} analysis

Fe^{2+} concentrations were measured using the Ferrozine method (Viollier et al., 2000). A 100-μL liquid

sample was removed from each microcosm under anaerobic conditions and transferred to a 100-mL volumetric flask. Four mL of a 4.9×10^{-3} M Ferrozine iron reagent solution ($C_{20}H_{13}N_4S_2O_6Na \cdot H_2O$) along with 4 mL of a 160 g/L ammonium acetate buffer solution (CH_3COONH_4) were added to the flask. The volume was adjusted to 100 mL using DI water. Samples were analyzed using a Beckman DU 530 Life Science UV/Vis Spectrometer at a wavelength of 562 nm. The method detection limit for Fe^{2+} was 0.0002 g/L.

External calibration standards for Fe^{2+} were prepared under anaerobic conditions using a 0.073 g/L Fe^{2+} stock solution. The stock solution was freshly prepared with $FeCl_2$ in an anaerobic glovebag prior to use and was diluted to produce a range of concentrations. The standards were analyzed as described above.

2.4.4. Sulfate analysis

A Dionex DX-100 ion chromatograph (IC) with an IonPac AS4A 4 mm (10–32) column was used to measure the sulfate concentration in the microcosms. Approximately 2 mL of supernatant was withdrawn from each microcosm and filtered (0.45 μm Ion Chrom Acrodisc, Gelman Sciences) into a vial. The vials were sampled by an autosampler (Dionex Autosampler Module). Eluent (1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$) was pumped through the column at a rate of 2 mL/min. The regenerant consisted of a 0.02 N H_2SO_4 solution. The method detection limit for sulfate was 0.027 mg/L.

External sulfate calibration standards were prepared from a 3 g/L stock solution prepared gravimetrically with K_2SO_4. These standards were analyzed by IC as described above.

3. Results

3.1. Effect of Fe^0 addition on dechlorination

The effect of Fe^0 on the microbial reductive dechlorination of PCBs was tested with congeners 3,4,5-CB and 2,2′,3,4,4′,5,5′-CB. The congener 3,4,5-CB was examined because it has been shown to be *para*-dechlorinated rapidly to 3,5-CB in sediment microcosms (Sokol et al., 1995, Wu et al., 2000, Wu et al., 2002); 2,2′,3,4,4′,5,5′-CB is transformed more slowly, but it is reported to be both *meta*- and *para*-dechlorinated (Bedard et al., 1998) and could therefore enrich for organisms with varying dechlorination specificity. Fig. 1 shows the effect of Fe^0 on 3,4,5-CB dechlorination in BH sediment microcosms. In microcosms amended with 0.1 g Fe^0/g, dechlorination began between days 27 and 76, effectively decreasing the lag time by 100 days compared with untreated microcosms. The addition of 2,2′,3,4,4′,5,5′-CB or excess

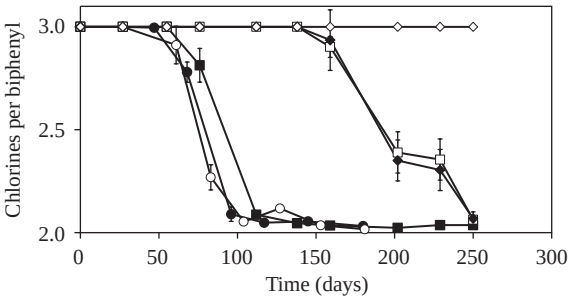

Fig. 1. The effect of Fe^0 addition on 3,4,5-CB dechlorination in microcosms containing Baltimore Harbor sediment with varying amounts of Fe^0. Symbols are as follows: ● 0.1 g Fe^0/g dry sediment, ○ 0.1 g Fe^0/g dry sediment + sulfate, ■ 0.1 g Fe^0/g dry sediment + 2,2′,3,4,4′,5,5′-CB, ◆ no Fe^0, □ 0.002 g Fe^0/g dry sediment, and ◇ all other treatments (2.0 g Fe^0/g dry sediment, no Fe^0 sterile control, and 2.0 g Fe^0/g dry sediment sterile control). Error bars represent the standard deviation between triplicate samples. Note: all dechlorination resulted in the stoichiometric formation of 3,5-CB.

sulfate did not affect the lag time nor extent of 3,4,5-CB dechlorination (Fig. 1). Greater than 0.4 g/L sulfate remained in the microcosms treated with excess sulfate when dechlorination began. In contrast, Zwiernik et al. (1998) and Ye et al. (1999) found that sulfate at a concentration above 0.096 g/L inhibited the dechlorination of Aroclor 1242 in Hudson River sediment microcosms. This indicates that in the BH microcosms sulfate reducers were not directly responsible for PCB dechlorination. Biological and/or abiotic dechlorination of 3,4,5-CB was never observed in microcosms that contained 2.0 g Fe^0/g or in autoclaved controls. This clearly demonstrates that Fe^0 itself could not serve as an electron donor for abiotic PCB dechlorination. In all of the microcosms where dechlorination occurred, the doubly flanked *para* chlorine of 3,4,5-CB was removed, resulting in the stoichiometric formation of 3,5-CB. Further dechlorination of 3,5-CB was not observed in any of the treatments. This process is similar to that described by Wu et al. (2000, 2002) in which only doubly flanked chlorines in the *meta* or *para* positions were removed from a number of PCB congeners. The average H_2 level in the headspace of the BH microcosms was 0.001, 0.001, and 0.005 atm for the 2.0 g Fe^0/g, no Fe^0 autoclaved, and 2.0 g Fe^0/g autoclaved treatments, respectively. H_2 was below the detection limit (1.03×10^{-5} atm) in the biologically active microcosms to which no Fe^0, 0.002 g Fe^0/g, and 0.01 g Fe^0/g was added. The results indicate that H_2 was generated by the Fe^0 and was generally consumed to below the detection limit in the biologically active microcosms, as would be expected (Rajagopal and LeGall, 1989).

Fig. 2 shows the dechlorination of 2,2′,3,4,4′,5,5′-CB. As observed with microcosms containing 3,4,5-CB, the

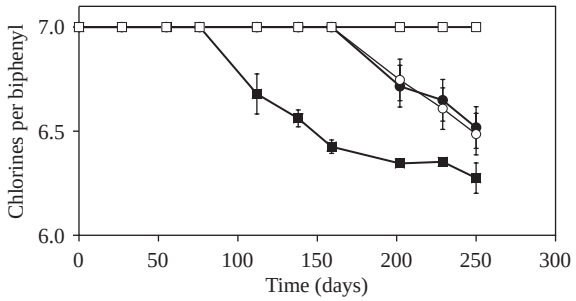

Fig. 2. The effect of Fe^0 addition on 2,2′,3,4,4′,5,5′-CB dechlorination in microcosms containing Baltimore Harbor sediment with varying amounts of Fe^0. Symbols are as follows: ● no Fe^0, ○ 0.002 g Fe^0/g dry sediment, ■ 0.1 g Fe^0/g dry sediment, and □ all other treatments (2.0 g Fe^0/g dry sediment, no Fe^0 sterile control, and 2.0 g Fe^0/g dry sediment sterile control). Error bars represent the standard deviation between triplicate samples. Note: all dechlorination resulted in the stoichiometric formation of 2,2′,3,4′,5,5′-CB.

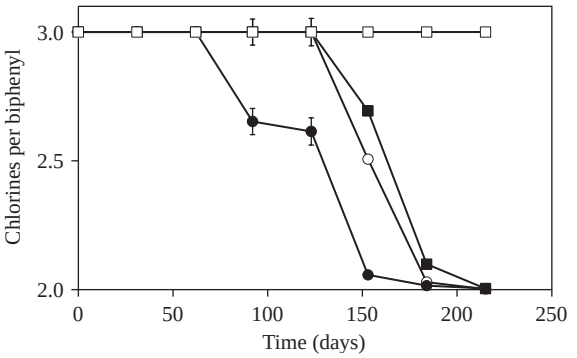

Fig. 3. The effect of H_2 addition on 3,4,5-CB dechlorination in microcosms containing Baltimore Harbor sediment with varying amounts of H_2 in the microcosm headspace. Symbols are as follows: ● 0.001 atm H_2, ○ 0.01 atm H_2, ■ 1.0 atm N_2, and □ 1.0 atm H_2. Error bars represent the standard deviation between triplicate samples. Note: all dechlorination resulted in the stoichiometric formation of 3,5-CB.

addition of 0.1 g Fe^0/g decreased the lag time prior to dechlorination of 2,2′,3,4,4′,5,5′-CB by approximately 100 days. Dechlorination of 2,2′,3,4,4′,5,5′-CB in microcosms with 0.1 g Fe^0/g and 3,4,5-CB began between days 76 and 112. Biological and/or abiotic dechlorination of 2,2′,3,4,4′,5,5′-CB was not observed in treatments that contained 2.0 g Fe^0/g nor in sterile microcosms. Again, this shows that abiotic Fe^0-mediated PCB dechlorination did not occur. Dechlorination was also not observed in any of the 2,2′,3,4,4′,5,5′-CB microcosms that were not amended with 3,4,5-CB (data not shown). Once again, dechlorinating activity removed the doubly flanked *para* chlorine of 2,2′,3,4,4′,5,5′-CB, resulting in the formation of 2,2′,3,4′,5,5′-CB. Further dechlorination of 2,2′,3,4′,5,5′-CB was not observed. The H_2 partial pressure in the microcosms was 0.0001, 0.001, and 0.005 atm H_2 for the 2.0 g Fe^0/g, no Fe^0 autoclaved, and 2.0 g Fe^0/g autoclaved treatments, respectively. H_2 was below the detection limit in the biologically active microcosms to which no Fe^0, 0.002 g Fe^0/g, and 0.01 g Fe^0/g were added. These results demonstrate that H_2 was generated by the added Fe^0 and was again generally consumed in the biologically active treatments. They also indicate that enrichment on 3,4,5-CB was required for 2,2′,3,4,4′,5,5′-CB to be dechlorinated.

The effect of H_2 itself on the dechlorination of 3,4,5-CB was investigated (Fig. 3). The addition of 1.0 atm H_2 to the headspace completely inhibited dechlorination, whereas the addition of 0.001 atm H_2 decreased the lag period prior to dechlorination by about 100 days (Fig. 3). Interestingly, the addition of 0.01 atm H_2 did not impact dechlorination compared to the microcosms incubated under an N_2 headspace. If the system was at equilibrium, the microcosms with 1.0 and 0.001 atm H_2 in the headspace should have had 785 μM and 785 nM H_2 dissolved in the liquid, respectively.

As expected, the addition of Fe^0 increased the microcosm pH. The pH rose to between 7.3 and 8.4 (7.9 ± 0.3) in the microcosms to which 2.0 g Fe^0/g was added. The pH was between 6.9 and 7.6 (7.3 ± 0.3) in the microcosms to which 0.1 g Fe^0/g was added. These values were measured over time when samples were removed for PCB analysis (approximately once every 3 weeks). The Fe^{2+} concentrations also increased in the microcosms to which Fe^0 was added, rising to 2.8 ± 0.06 g/L in systems to which 2.0 g Fe^0/g was added.

3.2. *Effect of Fe^0 oxidation products or microbial sulfate reduction products on dechlorination*

Fig. 4 shows the effect of Fe^0 oxidation products on PCB dechlorination in sediment cultures pre-enriched for PCB dechlorinating activity. A high pH (pH 8.5) and excess Fe^{2+} (3.3 g/L) inhibited the dechlorination of 3,4,5-CB. In contrast to the initial microcosms, once enriched for PCB dechlorinating activity, there was no lag time prior to dechlorination in the positive control. The addition of 1.0 atm H_2 to the microcosm headspace or 2.0 g Fe^0/g did not inhibit dechlorination over the short 21-day experiment, in contrast to results in the microcosms that had not been pre-enriched for dechlorinators. Again, only dechlorination of the doubly flanked *para* chlorine was observed. Further dechlorination of 3,5-CB was not observed in any of the treatments.

The production of sulfide from the anaerobic respiration of sulfate reducing bacteria is a potential inhibitor of PCB dechlorinating activity. A sulfate mass balance

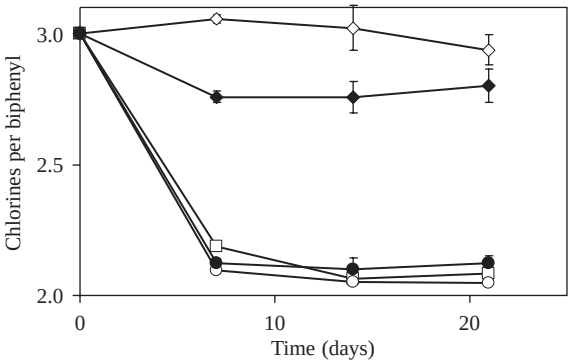

Fig. 4. The effect of Fe^0, 1.0 atm H_2, Fe^{2+}, and a pH of 8.5 on 3,4,5-CB dechlorination in microcosms containing a homogenized mixture from the 0.1 g Fe^0/g dry sediment microcosms. Symbols are as follows: ● Fe^0, ○ 100% H_2, □ positive control (no additions), ◆ Fe^{2+}, and ◇ pH of 8.5. Error bars represent the standard deviation between triplicate samples. Note: all dechlorination resulted in the stoichiometric formation of 3,5-CB.

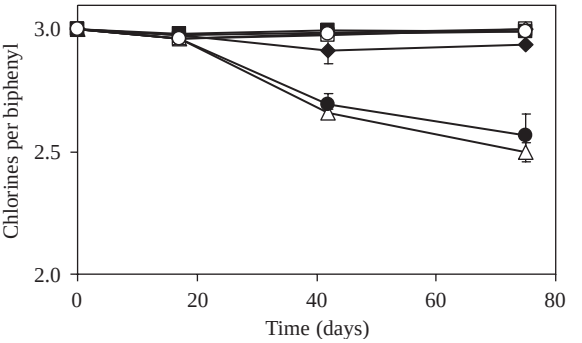

Fig. 5. The effect of sulfide concentration and pH on 3,4,5-CB dechlorination in microcosms containing a dechlorinating culture enriched from Baltimore Harbor sediment. Symbols are as follows: △ no added sulfide at a pH of 7.0 (positive control), ● 0.03 mg/L total sulfide (pH 7.0), ◆ 0.1 mg/L total sulfide (pH 7.0), ■ 0.3 mg/L total sulfide (pH 7.0), ○ pH 7.5 (no added sulfide), ◇ pH 8.0 (no added sulfide), and □ pH 8.5 (no added sulfide). Error bars represent the standard deviation between triplicate samples. Note: all dechlorination resulted in the stoichiometric formation of 3,5-CB.

was not performed on the sediment microcosms, as the sulfide produced during sulfate reduction was expected to rapidly precipitate in the sediment in the form of amorphous iron sulfide minerals (Swider and Mackin, 1989; Rickard, 1995) or organic sulfide complexes (Swider and Mackin, 1989). Nevertheless, when experiments were performed in a well-defined system with no sediment and controlled quantities of sulfide, sulfide inhibition was observed. The addition of 0.3 mg/L total sulfide to a nearly sediment-free culture enriched for PCB dechlorinators from BH sediment resulted in the cessation of dechlorination activity (Fig. 5). The addition of 0.1 mg/L total sulfide also inhibited dechlorination in this culture (Fig. 5). The addition of 0.03 mg/L total sulfide resulted in no inhibition of dechlorination (Fig. 5). The average pH in these microcosms throughout the experiment was 7.14 ± 0.05. This dechlorinating culture was also extremely pH sensitive. As the pH increased to 7.5 (averaging 7.47 ± 0.04 throughout the experiment) from 7.0 (7.14 ± 0.06), dechlorination ceased (Fig. 5). The complete inhibition of dechlorination was also observed in the microcosms adjusted to a pH of 8.0 (8.00 ± 0.05) and 8.5 (8.44 ± 0.04).

4. Discussion

The corrosion of Fe^0 generates H_2, which can serve as an electron donor for microbial growth and PCB dechlorination (Sokol et al., 1994; Wu et al., 2002). Methanogens (Daniels et al., 1987; Belay and Daniels, 1990), sulfate reducers (Rajagopal and LeGall, 1989), and homoacetogenic bacteria (Rajagopal and LeGall, 1989) have been reported to grow chemolithotrophically with cathodic H_2 as their sole electron source. Cathodically produced H_2 has also been shown to be an effective electron donor for the dechlorination of reduced contaminants such as carbon tetrachloride, chloroform, and trichloroethene (Weathers et al., 1997; Novak et al., 1998; Lampron et al., 2001). In this study the addition of Fe^0 to BH sediment at a level of 0.1 g Fe^0/g enriched for 3,4,5-CB and 2,2′,3,4,4′,5,5′-CB dechlorinators. The role of cathodic H_2 in enriching for PCB dechlorinators was supported by two lines of evidence: (1) detection of H_2 in microcosms amended with Fe^0 and (2) reduction of the lag time equivalent to that observed with Fe^0 by direct addition of 0.001 atm H_2 in the microcosm headspace.

It was initially assumed that the microcosms receiving the highest level of Fe^0 (2.0 g Fe^0/g) and H_2 (1.0 atm) would show the shortest lag period with respect to the dechlorination of 3,4,5-CB and/or 2,2′,3,4,4′,5,5′-CB as a result of the large quantities of electron donor added. The higher levels of Fe^0 and H_2, however, were inhibitory. We believe that this inhibition was a result of (1) an increase in pH and [Fe^{2+}] as the Fe^0 oxidized (Figs. 4 and 5), and (2) the stimulation of H_2-utilizing methanogens, homoacetogens, and sulfate reducers. An increase in the supply of electron donor (H_2) would result in an increase in the growth rate of these organisms. This increase in growth rate would then increase the rate at which HS^- was produced, trace limiting nutrients were consumed, and acidity was consumed in the surrounding pore water. This rate increase could therefore result in high transient HS^-

concentrations prior to amorphous iron sulfide mineral precipitation or high transient pH values in the pore water, both of which were observed to be inhibitory (Figs. 4 and 5).

When Fe^0 oxidizes it also produces Fe^{2+}, which can have a complex effect on PCB dechlorination. Fe^{2+} reacts with sulfide and rapidly precipitates as iron sulfide minerals (Rickard, 1995), which may effectively reduce inhibition by sequestering free sulfide. Zwiernik et al. (1998) found that the addition of $FeSO_4$ to microcosms containing sediment and Aroclor 1242 reduced the soluble concentration of inhibitory sulfide through the formation of insoluble iron sulfide minerals. The decrease in soluble sulfide in turn increased the rate of PCB dechlorination (Zwiernik et al., 1998). This could have occurred in the microcosms to which 0.1 g Fe^0/g was added, resulting in a shorter lag time prior to PCB dechlorination. Large quantities of soluble Fe^{2+}, however, have been reported to be inhibitory or toxic to microorganisms (Braun, 1997, Dunning et al., 1998). This was also observed in the pre-enriched sediment microcosms to which 3.3 g/L Fe^{2+} was added and may have been the case with the addition of 2.0 g Fe^0/g to BH sediment, which generated 2.8 ± 0.1 g/L Fe^{2+} in the water overlying the sediment.

Finally, we believe that the concentration of H_2 and the rate of cathodic H_2 production in the microcosms played a critical role in the enrichment of a dechlorinating population and in the subsequent dechlorination of PCBs. Wu (1996) found that at a headspace H_2 concentration of 0.1 atm, the dechlorination rate of 2,3,4,6-CB was lower than when H_2 was added at 0.01 atm. In our work, we saw that H_2 addition at 1.0 atm completely inhibited PCB dechlorination and when added at a concentration of 0.01 atm H_2, no effect on PCB dechlorination was observed (Fig. 3). The addition of 0.001 atm H_2, however, enriched for PCB dechlorinators, decreasing the lag period prior to dechlorination (Fig. 3). Several identified dehalorespirers have low H_2 half velocity coefficients (Smatlak et al., 1996; Ballapragada et al., 1997), which provides them with a competitive advantage over other H_2-utilizers at low H_2 concentrations (<100 nM). We hypothesize that the PCB dechlorinators in BH sediment also have a low H_2 half velocity coefficient, enabling them to compete most effectively with H_2-utilizing methanogens and sulfate reducers at low H_2 partial pressures, such as 0.001 atm.

The addition of 1.0 atm H_2 to the pre-enriched dechlorinating sediment microcosms, however, did not inhibit dechlorination (Fig. 4). This observation shows that high concentrations of H_2 do not directly inhibit dechlorination in BH sediment, particularly when the dechlorinators have been enriched. Nevertheless, when dechlorinators are only a small part of a consortium that includes H_2-utilizing sulfate reducers, methanogens, and homoacetogens, the addition of high concentrations of H_2 is not an effective way to stimulate their growth and activity because other populations are likely to grow more rapidly at high electron donor concentrations, and change the local environment. It is possible that if high H_2 concentrations did stimulate the growth of homoacetogenic bacteria, the concomitant production of acetate might stimulate the growth of different, acetate-utilizing PCB respirers, such as *o*-17 (Cutter et al., 2001).

5. Conclusions and engineering signficance

The addition of low concentrations of Fe^0 or H_2 enriched for a population of dechlorinators specifically capable of doubly flanked chlorine removal. Our observations also suggest that these PCB dechlorinators shared a physiological trait with known dehalorespirers, a high affinity for H_2, in that they were effectively enriched at low H_2 concentrations. In addition, although the amendment of Fe^0 to BH sediment only enriched for one particular population (capable of removing only one chlorine per biphenyl), the fact that it was able to effectively create a niche (via H_2 production) for this dechlorinating population is exciting and suggests that Fe^0 may be useful for creating similar niches in other sediments. It does indicate, however, that some environments, such as Baltimore Harbor, may lack sufficient diversity in their native dechlorinating populations to provide effective dechlorination of the variety of congeners present in contaminated sediment.

Dechlorination eventually began in the sediment microcosms to which no Fe^0 or H_2 was added (Figs. 1–3). This could have been a result of the addition of bioavailable PCB congeners or the enhanced release of organic matter in the sediment as a result of handling and homogenization (Ma et al., 2003; McCarty, 1997). In contaminated sediment, the amendment of solid Fe^0 offers a way to enrich for H_2-utilizing dechlorinators that should be easier to place and handle in sediment than other common H_2 sources, such as fermentable organic compounds, and should provide a longer-lasting source of electron donor than the labile portion of sediment organic matter. In addition, the Fe^{2+} that is released as the Fe^0 corrodes can be effective in precipitating inhibitory sulfide and may be generally stimulatory at low concentrations (Martin, 1992). The amendment of likely critical bioavailable PCB congeners, however, is not possible outside of the laboratory. Bioaugmentation of exogenously enriched PCB dechlorinators combined with a long-term biostimulation approach, such as adding low levels of Fe^0 to sediment, may therefore be necessary in practice if a sediment does not possess either adequate labile PCBs or a sufficient diversity of PCB dechlorinators.

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