Published in final edited form as:

Bioremediat J. 2006 ; 10(4): 143–151.

The Impact of Sediment Characteristics on PCB-dechlorinating Cultures: Implications for Bioaugmentation

Tao Yan, Timothy M. LaPara, and Paige J. Novak

University of Minnesota, Department of Civil Engineering, 500 Pillsbury Drive S.E., Minneapolis, MN 55455-0116

Abstract

PCB-dechlorinating cultures with complimentary activities, previously derived from estuarine Baltimore Harbor (B), marine Palos Verdes (P) and riverine Hudson River (H) sediments, were mixed and then inoculated into sterile sediments from the same sources. In the treatments containing sterile B sediment, the different inocula had limited impact on the bacterial community development and on dechlorination patterns, all of which were similar. In treatments containing sterile P or H sediment, however, different inocula resulted in significantly different PCB dechlorination patterns and bacterial communities. The B sediment appeared to support not only the most extensive and rapid dechlorination of the three sediments, but also supported a more diverse bacterial community. This was thought to be a result of nutritional richness, as it was high in organic carbon and micronutrients such as zinc and cobalt. Although mixing three PCB-dechlorinating cultures was able to produce a culture capable of enhanced PCB-dechlorinating activity as compared to single cultures, some activities were lost upon culture transfer. This indicates that care must be taken to establish robust PCB-dechlorinating cultures capable of extensive dechlorination prior to pursuing bioaugmentation. In addition, our results indicate that the concentration and availability of macro- and micro-nutrients could have a significant impact on the microbial community structure, and thus a thorough characterization of the sediment at contaminated sites is essential for implementing bioaugmentation for PCB bioremediation.

Keywords

Dechlorination; Polychlorinated biphenyls; Bioaugmentation; Anaerobic; Microbial community

Introduction

Contamination of soil and sediment by polychlorinated biphenyls (PCBs) is a worldwide environmental problem (Hutzinger and Veerkamp, 1981). PCBs are chemically stable and extremely lipophilic, which enable them to persist in the environment and bioaccumulate through the food chain (Safe et al., 1987). Growing evidence indicates that PCBs pose significant threats to both the ecosystem and human health (Cogliano, 1998; Tan et al., 2003). Therefore, remediation of PCB-contaminated sites is important for the protection of the environment and public health.

Correspondence to: Paige J. Novak.

Inquiries to: P. J. Novak, University of Minnesota, Minneapolis, MN, 55455-0116, Telephone: (612) 626-9846. Fax: (612) 626-7750. E-mail: novak010@umn.edu

Tao Yan, University of Minnesota, Minneapolis, MN, 55455-0116, Telephone: (612) 625-6725. Fax: (612) 625-2208. E-mail: yanx0046@umn.edu

Timothy M. LaPara, University of Minnesota, Minneapolis, MN, 55455-0116, Telephone: (612) 624-6028. Fax: (612) 626-7750. E-mail: lapar001@umn.edu

Due to the differences in initial introduction and subsequent transportation and redistribution, PCB-contaminated sites may differ in terms of scale and PCB concentration. Physical methods have been applied to remediate soils and sediments that were contaminated with high concentrations of PCBs and/or present in a confined locale (Bergen et al., 2005; Praeger et al., 1996). This approach, however, entails subsequent treatment of excavated soil/sediment and thus is costly for large sites (Richards et al., 2005). Bioremediation, therefore, has been extensively investigated as a cost-effective method for treating PCB-contaminated sediment (Bedard and Quensen, 1995). PCBs can be anaerobically dechlorinated to less-chlorinated congeners (Brown et al., 1987; Quensen et al., 1988) that are generally more amenable to further aerobic biodegradation (Bedard et al., 1987). Recent evidence also suggests that they may be completely dechlorinated to biphenyl (Rhee et al., 1993; Williams, 1994; Natarajan et al., 1996), thus eliminating the need for additional treatment.

Nevertheless, detailed knowledge about the microbiology of anaerobic PCB dechlorination is still lacking. Recent studies have begun to identify the microorganisms involved in PCB dechlorination by the 16S rRNA approach (Cutter et al., 2001; Wu et al., 2002; Yan et al., 2006). The increasing availability of phylogenetic information about PCB-dechlorinating bacteria permits detailed analysis of indigenous microbial communities and monitoring of their development during the PCB remediation process. Therefore, the absence of known PCB-dechlorinating bacteria at a particular contaminated site, or the failure to enrich indigenous dechlorinating bacteria *in situ*, can be observed and mitigated by introducing pre-enriched PCB-dechlorinating bacteria at a high population density. This bioremediation strategy is termed bioaugmentation (Vogel and Walter, 2001), and its application in bioremediation has received increasing attention, mainly for the treatment of recalcitrant organic compounds, such as petroleum products (da Silva and Alvarez, 2004), halogenated organic solvents (Ellis et al., 2000), and herbicides (Silva et al., 2004). Despite numerous successful cases of bioaugmentation, it has at times yielded inconsistent results (Vogel and Walter, 2001). One issue critical to successful bioaugmentation is the survival and growth of the augmented bacteria in the target environment (Bouchez et al., 2000). For remediation, the chemical properties of the soil or sediment, including total organic carbon (TOC), ionic composition, and the availability of critical trace metals, may play a significant role in such survival and growth. No studies, however, have been conducted to investigate how variations in the chemical properties of a soil or sediment impact the ecology and the functional performance of augmented bacteria.

The objective of this study was to investigate the effect of different sediments on augmented PCB-dechlorinating cultures, in terms of PCB-dechlorinating activity and microbial community structure. PCB-dechlorinating cultures, previously enriched from three different sediment sources in our laboratory (Rysavy et al., 2005; Yan et al., 2006), were investigated in combination by inoculating them into microcosms amended with different sediments. The results of this study are important for determining whether the sediment characteristics at a contaminated site could significantly affect the success of bioaugmentation.

Material and Methods

Source cultures and sediments

The enrichment of PCB-dechlorinating cultures from Baltimore Harbor estuarine sediment (B culture), Palos Verdes marine sediment (P culture), or Hudson River riverine sediment (H culture) were described previously (Yan et al., 2006). Subsamples of the sediments used to develop the enrichment cultures were air dried and then autoclaved (121°C; 15 psig) for 40 min on two consecutive days. These sterile subsamples of the sediments were then stored in an anoxic glovebag (COY labs, MI) until needed.

Sediment characterization was performed by the Research Analytical Laboratory at the University of Minnesota. The total N was measured according to the Dumas method using a LECO FP-528 Nitrogen Analyzer (Yeomans and Bremner, 1991), with a method detection limit (MDL) of 0.01%. Sulfate was measured using a Klett colorimeter following extraction by $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ and precipitation with $\text{BaCl}_2 \cdot \text{H}_2\text{O}$ (MAES, 1998); this method had an MDL of 1 part per million (ppm). Phosphate was extracted with NaHCO_3 , reduced by ascorbic acid, and then analyzed using a Brinkmann PC 600D probe colorimeter (MAES, 1998); the MDL was 1 ppm. Nitrate was extracted with CaSO_4 and quantified colorimetrically with an Alpchem Rapid Flow Analyzer (Willis and Gentry, 1987), with an MDL of 0.2 ppm. The total organic carbon (TOC) and inorganic carbon were first converted by dry combustion and phosphoric acid, respectively, to CO_2 , which was subsequently measured by IR spectrum absorption using a Skalar Primacs carbon furnace (MAES, 1998). The MDLs for TOC and total inorganic carbon were 0.21% and 0.15%, respectively. Elemental analysis was accomplished by extracting the sediments with HNO_3 in a perfluoralkoxy (PFA) vessel using microwave heating, followed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (USEPA, 1992). The MDLs for the analyzed elements were 1.44 (Fe), 0.32 (Mn), 0.64 (Zn), 2.16 (Cu), 6.8 (Pb), 1.84 (Ni), 0.56 (Cd), 1.2 (Cr), 1.04 (Co), 14.4 (Al), 1.92 (B), 3.36 (Ca), and 15.28 ppm (Mg). For the sediment analysis, duplicate blank controls were included for each and every analysis. Triplicates were also analyzed for the Baltimore Harbor and Hudson River sediment and duplicates were analyzed for the Palos Verdes sediment.

Experimental set-up

Reduced anaerobic mineral medium (RAMM) was prepared as described by Shelton and Tiedje (1984), except that 2 mM L-cysteine hydrochloride was used as a reducing agent instead of Na_2S , as sulfide ion was previously reported as inhibitory to PCB dechlorination (Rysavy *et al.*, 2005). The pH of the media was adjusted to 7.0 with H_3PO_4 or NaOH. The media was sterilized by autoclaving and was stored in an anaerobic glove bag until use.

Microcosms were set up in 160-mL serum bottles. Each bottle was first amended with 5 g of sterile dry sediment; 2,3,4,5-chlorinated biphenyl (2,3,4,5-CB, 10 μmoles in 0.3 mL hexane) was added directly onto the sediment. The hexane was allowed to fully evaporate (verified by observation) in the glovebag for approximately 1 hour. RAMM medium (100 mL) and 1 mL of an anoxic fatty acid solution (acetate, butyrate, and propionate) were then added to each bottle to reach a final concentration of 20 mM for each acid. The pH of the medium was then adjusted to 7.0 using NaOH or H_3PO_4 .

Four culture mixtures (i.e. BP, PH, BH, and BHP) were obtained by mixing together either two or three of the PCB-dechlorinating source cultures (the B, P, or H cultures). These were used to inoculate the microcosms (Table 2). For example, 2 mL of each of the supernatants of cultures B and P were withdrawn and mixed to make culture mixture BP; this mixture was then used to inoculate microcosms containing sterile and dry sediment B and microcosms containing sterile and dry sediment P. As a quality assurance measure, the source cultures themselves (e.g., the B culture) were also inoculated back into the sterilized sediments of the same origin (e.g., sediment B) to verify the cultures' activities. The bottles were then capped with Teflon-lined rubber stoppers and sealed with aluminum crimps. The bottles were shaken on a wrist-action shaker for 2 hours to homogenize the added 2,3,4,5-CB, after which they were incubated on a rotating shaker (120 rpm) at room temperature for the duration of the experiment. All treatments were prepared in triplicate. The treatments prepared are summarized in Table 2.

PCB extraction and analysis

The sample extraction method for PCB analysis was based on the method of Quensen *et al.* (1988) and has been described in detail previously (Rysavy *et al.*, 2005; Yan *et al.*, 2006).

Briefly, sediment slurry samples were withdrawn from thoroughly mixed microcosms in an anaerobic chamber. Samples were weighed and then extracted, first with acetone (10 mL), followed by two hexane-acetone (1:1) (10 mL) extractions. The pooled extracts (30 mL) were sequentially purified with a 2% (v/v) NaCl solution (10 mL), a 30% (v/v) sulfuric acid solution (4 mL), and again with a 2% (v/v) NaCl solution (10 mL). The purified extracts were then dried with anhydrous Na₂SO₄, filtered through a florisil-copper (25% copper w/w) column, and normalized to a total volume of 25 mL hexane for subsequent analysis. The extracted PCBs were analyzed on a gas chromatograph (GC, Hewlett Packard, HP 5890) 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. Identification of 2,3,4,5-CB and its dechlorination products was primarily based on their respective retention times in reference to those of authentic standards (AccuStandard). Product congeners were further verified using a GC (HP 5890, Series II) equipped with an HP 5972 mass selective detector (MS). External 7-point calibration curves were established for the quantification of individual congeners; the MDL for 2,3,4,5-CB was 0.01 μM.

Microbial community analysis

Samples for community analysis were taken from the microcosms in the same way as the samples for PCB analysis. The samples were centrifuged at 10,000 × g for 10 minutes; after removing supernatant, the sediment pellets were frozen at -70 °C until needed. Cell lysis was accomplished by a combination of detergent and bead-beating (Miller et al., 1999). Briefly, 450 μL phosphate buffer (100 mM, pH=8) and 450 μL of lysis buffer (100 mM NaCl, 500 mM Tris [pH=8], 10% [w/v] sodium dodecyl sulfonate) were added to the sediment pellets, and incubated at 70°C for 90 min. Genomic DNA extraction by bead-beating and subsequent purification were accomplished using a FastDNA kit for soil (Q-BIOgene; Vista, CA), according to the manufacture's procedure. Partial 16S rRNA genes were amplified from the extracted genomic DNA by polymerase chain reaction (PCR) using bacterial universal primers PRBA338F and PRUN518R, with a GC-clamp attached to the forward primer (Muyzer et al., 1993). The final 50 μL reaction mixture contained: 1× PCR buffer (Promega; Madison, WI), 175 μmol MgCl₂, 4 nmol deoxynucleoside triphosphates, 2% bovine serum albumin, 25 pmol forward and reverse primers, 1.25 units of *Taq* polymerase (Promega) and ~1 ng of template DNA. The PCR protocol included a 5 min initial denaturation at 94°C, thirty cycles of 92°C for 30 s, 55°C for 30 s, and 72°C for 30s, and a final extension for 10 min at 72°C.

PCR products were loaded onto 8% (w/v) polyacrylamide gels with the denaturing gradient ranging from 30% to 55% (100% denaturant contains 7M urea and 40% formamide in 0.5X Tris-acetate-EDTA (TAE) buffer). Electrophoresis was performed on a D-Code apparatus (BioRad; Hercules, CA) in 0.5X TAE buffer at 60oC, initially at 20 V for 20 min followed by 200 V for 270 min. Gels were stained with SYBR Green I (Molecular Probes; diluted 1:5000 in 0.5X TAE), visualized on a UV transilluminator, and photographed with a digital CCD camera (BioChemi System; UVP, Inc; Upland, CA).

Statistical Analysis

The differences in dechlorination extent among treatments were analyzed by the Student's t-test to determine statistical significance ($P \leq 0.05$). Principal component analysis was used to group cultures based on their bacterial community structures as illustrated by PCR-DGGE. Bands were automatically identified by the BioChemi system, and horizontally co-migrating bands in different gel lanes were considered to represent the same bacterial population. A binary matrix was established based on the presence or absence of identified bands in each gel lane. The extraction of principal components was performed using Microsoft Excel with statistiXL version 1.4 (Kalamunda, Australia), and the first two major principal components were plotted using SigmaPlot version 8.02 (SPSS, Chicago, Illinois).

Results

Sediment characteristics

The three sediments used in this study were substantially different (Table 1). As expected, the estuarine sediment B and marine sediment P had a much higher sulfate content than the riverine sediment H. After dilution, about 7.05 and 6.15 mg/L sulfate was present in the B and P sediment-containing microcosms, respectively. Generally, sediment B had the highest concentrations of macro- and micronutrients. Taking into consideration the 18 mmole TOC introduced by the medium itself, the TOC in the microcosms containing B sediment was approximately 1.8 and 1.9 times that in the microcosms containing H and P sediment, respectively. The sediment from B also contained the highest concentration of total N.

The trace element analyses of the sediments showed that B sediment was also much richer in trace nutrients than the other two sediments (Table 1). Among the three sediments, the H sediment contained the lowest concentrations of all of the trace elements. The B sediment contained approximately 4, 5, 8, and 2 times more zinc, copper, lead, and cobalt than the P sediment, respectively, and approximately 12, 23, 7, and 6 times more zinc, copper, lead, and cobalt than the H sediment, respectively.

Sediment effects on the dechlorination of 2,3,4,5-CB

Identical mixtures of the cultures exhibited distinctively different 2,3,4,5-CB dechlorination patterns depending upon which sediment was present (Fig. 1). When culture mixture BP was inoculated into microcosms containing sediment B (BP-B), microbial dechlorination started before Day 62, whereas significant dechlorination was observed only after 120 days of incubation when the BP culture mixture was inoculated into sediment P (BP-P) (Fig. 1A). Dechlorination commenced at approximately the same time in microcosms inoculated with the BH culture; nevertheless, by the end of the experiment, more extensive dechlorination was observed in the presence of sediment B (BH-B) as compared to cultures incubated in the presence of sediment H (BH-H) (Fig. 1B). Differences, although less distinctive, were also discernable in the dechlorination of 2,3,4,5-CB by culture mixture PH in the presence of sediments H or P; dechlorination began slightly earlier in the presence of sediment H, while slightly more dechlorination occurred in the presence of sediment P (Fig. 1C).

The effect of sediment type on dechlorination by the mixture of all three cultures (BPH) was complex (Fig. 1D); nevertheless, careful examination reveals the same general patterns as observed with mixtures containing only 2 of the cultures. Dechlorination in microcosms containing sediment B occurred with the shortest lag period and was the most extensive, while dechlorination in microcosms containing sediment P occurred after the longest lag period and that in microcosms containing sediment H was the least extensive.

The molar distribution of 2,3,4,5-CB and its dechlorination products, (2,3,5-CB, 2,4,5-CB, and 2,4/2,5-CB) at the end of the experimental period were also consistent with the observations made over time (Fig. 2). All treatments containing sediment B behaved similarly, with 2,3,4,5-CB almost gone and extensive dechlorination observed after 189 days of incubation (columns 1~4, Fig. 2). This uniformity indicates that the characteristics of sediment B exerted a significant impact on the microbial dechlorination of 2,3,4,5-CB.

In contrast, variations were obvious in treatments containing sediments P or H (columns 5–12, Fig. 2), demonstrating that these two sediments had a less significant overall effect on dechlorination than sediment B. In fact, in these microcosms a synergistic effect from mixing the three cultures together could be observed. In the microcosms containing sediment P, the final dechlorination products varied, with 2,4/2,5-CB only observed in treatments PH-P and BHP-P, and dechlorination to only 2,3,5-CB and 2,4,5-CB in treatments BP-P, PH-P, and P-

P. Treatment BHP-P exhibited almost complete loss of the parent congener 2,3,4,5-CB, with a dechlorination extent comparable to that observed in microcosms containing sediment B. Similar observations can also be made with microcosms containing sediment H. For example, treatment BHP-H exhibited more extensive dechlorination than treatments PH-H, BH-H, and H-H. This synergistic effect in the BHP cultures was not obvious in microcosms containing sediment B, likely because sediment B exerted a stronger influence on the community development, and therefore, dechlorination pattern.

Bacterial community analysis

Bacterial community structures were profiled to further illustrate the impact of sediment characteristics on the development of 2,3,4,5-CB-dechlorinating communities. At the end of the experiment (Day 189), the bacterial communities in one replicate of each of the treatments were analyzed by PCR-DGGE (Fig. 3A). Principal component analysis (PCA) of the bacterial community fingerprints identified two principal components (PCA1 and PCA2) accounting for 38.6% of the variability (Fig. 3B). The bacterial communities in microcosms containing sediment B grouped closely, which is in agreement with their similar dechlorination activities (Figs. 1 and 2). The similarity in both the community structure and dechlorination activity of the cultures incubated with sediment B suggests that this sediment was able to strongly affect the enrichment and survival of the bacterial community, including those members capable of dechlorination. Sediments P and H also appeared to affect the bacterial community development, although less markedly. This is evident in treatments PH-P and PH-H where different bacterial community structures developed (Fig. 3A) despite identical inocula.

Discussion

The three source cultures used for bioaugmentation in this study initially exhibited different PCB-dechlorinating activities that were to some degree complementary to each other (Yan *et al.*, 2006). For example, microorganisms enriched from sediment P dechlorinated 2,3,4,5-CB to approximately equal quantities of 2,4,5-CB and 2,3,5-CB, the latter of which persisted as a dead-end product while 2,4,5-CB was further dechlorinated (Yan *et al.*, 2006). The cultures enriched from sediments B and H, however, dechlorinated 2,3,4,5-CB mainly to 2,3,5-CB, which was further dechlorinated (Yan *et al.*, 2006). Nevertheless, these varied dechlorination activities were not maintained upon mixing the cultures with each other. This is further compounded by the observation that in some cases even subculturing lead to a loss of PCB-dechlorinating activity; for example, microcosms P-P and H-H showed less extensive dechlorination than their respective source cultures (Yan *et al.*, 2006). This observation is in agreement with previous studies that reported difficulty in maintaining and transferring PCB-dechlorinating activity (Bedard and Quensen, 1995) and suggests that environmental factors or microbial interactions may significantly affect the function of a particular community, and its consequent application in bioaugmentation.

In this study, we demonstrated that the incubation of initially identical cultures in the presence of different sediments affected the microbial dechlorination of 2,3,4,5-CB. This was thought to be a result of the sediment stimulating the selective enrichment of certain populations over time, as observed by differences in the PCR-DGGE patterns after 189 days of incubation. It was an intriguing observation that sediment B imposed a stronger influence on the bacterial community structure and dechlorination pattern, regardless of the source of the inoculum, than sediments P and H did. One potential explanation is that sediment B was rich in nutrients, as demonstrated by sediment characterization, and thus was able to provide more nutrients to the dechlorinating bacteria, thereby impacting the community structure and dechlorination activity more strongly.

Among the differences in the sediments, particularly interesting were the relatively high trace metal concentrations in sediment B. The availability of trace metals, particularly cobalt, may be of particular significance as the dehalogenases of previously isolated dehalorespirers, such as *Dehalococcoides ethenogenes* strain 195 (Maymó-Gatell et al., 1997) and *Dehalobacter restrictus* (Holliger et al., 1998), required cobalt for the synthesis of corrinoids (Schumacher et al., 1997; Magnuson et al., 1998). The stimulatory effect of cobalt on *Dehalococcoides ethenogenes* strain 195 was previously demonstrated, as cobalamin significantly enhanced dechlorination of trichloroethene (Maymó-Gatell et al., 1997). In experiments with the methanogen *Methanosarcina barkeri*, it was found that the availability of cobalt, nickel, and iron strongly influenced the synthesis of corrinoids, coenzyme F₄₃₀, and cytochromes (Lin et al., 1989). Therefore, the enhanced availability of such trace metals in sediment might be critical for the expression and/or production of dehalogenases, or may provide niches for synergistic organisms, such as methanogens (Adamson and Parkin, 2001), that can provide these cofactors to the dechlorinating bacteria. This warrants further investigation to improve the applicability and practicality of bioaugmentation as a remediation technology (Vogel and Walter, 2001). The strong influence of sediment B may provide useful insights with respect to important factors in the survival of PCB-dechlorinating bacteria.

In conclusion, we found in this study that the sediment origin and chemistry significantly affected the activity and community structure of bioaugmented PCB-dechlorinating microorganisms. These effects were most likely a result of the survival and enrichment of particular microbial populations as a result of macro- and micronutrient concentrations and availability. The impact that different sediments impose on augmented microorganisms suggests that thorough biological and chemical characterization of a contaminated site may be essential for successful bioaugmentation.

Acknowledgements

Funding for this work was provided by the Office of Naval Research (Grant N00014-99-1-0923), the National Institute of Environmental Health Sciences (Grant ES12810-01), and the Hudson River Foundation (Graduate Fellowship). T.Y. was financially supported by a Sommerfeld fellowship from the University of Minnesota and by a Hudson River Foundation Graduate Fellowship (Grant GF/03/02).

References

- Adamson DT, Parkin GF. Dependence of a high-rate PCE-dechlorinating enrichment culture on methanogenic activity. *Biorem J* 2001;5:51–62.
- Bedard DL, Haberl ML, May RJ, Brennan MJ. Evidence for novel mechanisms of polychlorinated biphenyl metabolism in *Alcaligenes eutrophus* H850. *Appl Environ Microbiol* 1987;53(5):1103–1112. [PubMed: 3111366]
- Bedard, DL.; Quensen, JF. Microbial reductive dechlorination of polychlorinated biphenyls. In: Young, LY.; Cerniglia, CE., editors. *Microbial transformation and degradation of toxic organic chemicals*. John Wiley & Sons; New York: 1995. p. 127–216.
- Bergen BJ, Nelson WG, Mackay J, Dickerson D, Jayaraman S. Environmental monitoring of remedial dredging at the New Bedford Harbor, Ma, superfund site. *Environ Monit Assess* 2005;111(1–3):257–275. [PubMed: 16311831]
- Bouchez T, Patureau D, Dabert P, Juretschko S, Dore J, Delgenes P, Moletta R, Wagner M. Ecological study of a bioaugmentation failure. *Environ Microbiol* 2000;2(2):179–190. [PubMed: 11220304]
- Brown JF Jr, Bedard DL, Brennan MJ, Carnahan JC, Feng H, Wagner RE. Polychlorinated biphenyl dechlorination in aquatic sediments. *Science* 1987;236(4802):709–712. [PubMed: 17748310]
- Cogliano VJ. Assessing the cancer risk from environmental PCBs. *Environ Health Perspect* 1998;106(6):317–323. [PubMed: 9618347]
- Cutter LA, Watts JEM, Sowers KR, May HD. Identification of a microorganism that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl. *Environ Microbiol* 2001;3(11):699–709. [PubMed: 11846760]

- da Silva MLB, Alvarez PJJ. Enhanced anaerobic biodegradation of benzene-toluene-ethylbenzene-xylene-ethanol mixtures in bioaugmented aquifer columns. *Appl Environ Microbiol* 2004;70(8):4720–4726. [PubMed: 15294807]
- Ellis DE, Lutz EJ, Odom JM, Buchanan RJ Jr, Bartlett CL, Lee MD, Harkness MR, DeWeerd KA. Bioaugmentation for accelerated in situ anaerobic bioremediation. *Environ Sci Technol* 2000;34(11):2254–2260.
- Holliger C, Hahn D, Harmsen H, Ludwig W, Schumacher W, Tindall B, Vazquez F, Weiss N, Zehnder AJ. *Dehalobacter restrictus* gen. nov and sp nov., a strictly anaerobic bacterium that reductively dechlorinates tetra- and trichloroethene in an anaerobic respiration. *Arch Microbiol* 1998;169(4):313–321. [PubMed: 9531632]
- Hutzinger, O.; Veerkamp, W. Microbial degradation of xenobiotics and recalcitrant compounds. Academic Press; New York: 1981. p. 3–45.
- Lin DG, Nishio N, Mazumder TK, Nagai S. Influence of cobalt, nickel, and iron on the production of tetrapyrroles by *Methanosarcina barkeri*. *Appl Microbiol Biotechnol* 1989;30(2):196–200.
- MAES. Recommended chemical soil test procedures for the north central region, north central regional research publication No. 221 (Revised). Missouri Agricultural Experiment Station. 1998
- Magnuson JK, Stern RV, Gossett JM, Zinder SH, Burris DR. Reductive dechlorination of tetrachloroethene to ethene by a two-component enzyme pathway. *Appl Environ Microbiol* 1998;64(4):1270–1275. [PubMed: 10671186]
- Maymó-Gatell X, Chien Y-t, Gossett JM, Zinder SH. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* 1997;276(5318):1568–1571. [PubMed: 9171062]
- Miller DN, Bryant JE, Madsen EL, Ghiorse WC. Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Appl Environ Microbiol* 1999;65(11):4715–4724. [PubMed: 10543776]
- Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 1993;59(3):695–700. [PubMed: 7683183]
- Natarajan MR, Wu WM, Nye J, Wang H, Bhatnagar L, Jain MK. Dechlorination of polychlorinated biphenyl congeners by an anaerobic microbial consortium. *Appl Microbiol Biotechnol* 1996;46(56):673–677.
- Praeger TH, Messur SD, DiFiore RP. Remediation of PCB-containing sediments using surface water diversion “dry excavation”: A case study. *Water Sci Technol* 1996;33(6):239–245.
- Quensen JF III, Tiedje JM, Boyd SA. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. *Science* 1988;242(4879):752–754. [PubMed: 17751997]
- Rhee GY, Sokol RC, Bethoney CM, Bush B. A long-term study of anaerobic dechlorination of PCB congeners by sediment microorganisms: pathways and mass balance. *Environ Toxicol Chem* 1993;12(10):1829–1834.
- Richards MK, Partymiller KG, Dauchy JW, Brown KW. SITE demonstration of minergy glass furnace treatment of PCBs, PCDDs/Fs, and metals in river sediment. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* 2005;9(3):158–166.
- Rysavy JP, Yan T, Novak PJ. Enrichment of anaerobic polychlorinated biphenyl dechlorinators from sediment with iron as a hydrogen source. *Water Res* 2005;39(4):569–578. [PubMed: 15707629]
- Safe, S.; Safe, L.; Mullin, M. Polychlorinated biphenyls: environmental occurrence and analysis. In: Safe, S.; Hutzinger, O., editors. *Environmental Toxin Series*. Springer-Verlag; Berlin: 1987. p. 1–13.
- Schumacher W, Holliger C, Zehnder AJ, Hagen WR. Redox chemistry of cobalamin and iron-sulfur cofactors in the tetrachloroethene reductase of *Dehalobacter restrictus*. *FEBS Lett* 1997;409(3):421–425. [PubMed: 9224702]
- Silva E, Fialho AM, Sa-Correia I, Burns RG, Shaw LJ. Combined bioaugmentation and biostimulation to cleanup soil contaminated with high concentrations of Atrazine. *Environ Sci Technol* 2004;38(2):632–637. [PubMed: 14750741]
- Tan Y, Li D, Song R, Lawrence D, Carpenter DO. Ortho-substituted PCBs kill thymocytes. *Toxicol Sci* 2003;76(2):328–337. [PubMed: 14514967]

- USEPA. Test Methods of Evaluating Solid Waste, Physical/Chemical Methods, SW-846. 3. Environmental Monitoring and Support Laboratory, Office for Research and Development; Cincinnati: 1992. Microwave assisted acid digestion of sediments, sludges, soils, and oils.
- Vogel, TM.; Walter, MV. Bioaugmentation. In: Hurst, CJ.; Crawford, RL.; Knudsen, GR.; McInerney, MJ.; Stetzenback, LD., editors. Manual of Environmental Microbiology. American Society of Microbiology Press; Washington, DC: 2001. p. 952-959.
- Williams WA. Microbial reductive dechlorination of trichlorobiphenyls in anaerobic sediment slurries. *Environ Sci Technol* 1994;28(4):630–635.
- Willis RB, Gentry CE. Automated method for determining nitrate and nitrite in water and soil extracts. *Communications in Soil Science and Plant Analysis* 1987;18(6):625–636.
- Wu Q, Watts JEM, Sowers KR, May HD. Identification of a bacterium that specifically catalyzes the reductive dechlorination of polychlorinated biphenyls with doubly flanked chlorines. *Appl Environ Microbiol* 2002;68(2):807–812. [PubMed: 11823222]
- Yan T, LaPara TM, Novak PJ. The reductive dechlorination of 2,3,4,5-chlorobiphenyl in three different sediment cultures: Evidence for the involvement of phylogenetically similar *Dehalococcoides*-like bacterial populations. *FEMS Microbiol Ecol* 2006;55(2):248–261. [PubMed: 16420633]
- Yeomans JC, Bremner JM. Carbon and nitrogen analysis of soils by automated combustion techniques. *Communications in Soil Science and Plant Analysis* 1991;22(9–10):843–850.

Figure 1. Differentiated dechlorination of 2,3,4,5-CB by culture mixtures BP (A), BH (B), PH (C), and BPH (D) due to the presence of different sediments. Dechlorination in the controls, B-B, P-P and H-H, are shown in compartment E. Error bars represent the standard deviation of triplicates.

Figure 2.

Molar distribution of 2,3,4,5-CB (■) and its dechlorination products, 2,3,5-CB (▨), 2,4,5-CB (▩), and 2,4/2,5-CB (□), in each treatment on Day 189. Mean values of triplicates are presented.

Figure 3.

Bacterial community structures revealed by PCR-DGGE (A) and community similarity analysis based on gel patterns using principal component analysis (B). In compartment B, axes were the first two major principal components (PCA1 and PCA2) and the values in parenthesis stand for the percentages of the variability the principal components represent.

Table 1
Characteristics of sediments used in the 2,3,4,5-CB dechlorination experiments^a

Parameters	Baltimore Harbor	Hudson River	Palos Verdes	Parameters	Baltimore Harbor	Hudson River	Palos Verdes
Total N (%)	0.500	0.075	0.155	Cu (ppm)	3.53×10^2	15.6	68.6
Water pH	7.7	7.1	8.0	Pb (ppm)	2.87×10^2	41.6	37.4
Sulfate (ppm)	141	11	123	Ni (ppm)	51.5	5.27	29.73
Phosphate (ppm)	40.0	10.0	39.5	Cd (ppm)	2.47	0.53	4.50
Nitrate (ppm)	1.7	1.6	1.6	Cr (ppm)	229.36	22.59	126.36
TOC (%)	6.80	2.12	1.60	Co (ppm)	21.3	3.85	9.65
Inorganic carbon (%)	0.615	≤ 0.15	1.16	Al (ppm)	2.28×10^4	4.36×10^3	1.80×10^4
Fe (ppm)	3.16×10^4	8.15×10^3	2.55×10^4	B (ppm)	12.2	1.95	21.9
Mn (ppm)	3.18×10^2	1.44×10^2	3.05×10^2	Ca (ppm)	2.00×10^4	2.33×10^3	2.73×10^4
Zn (ppm)	5.89×10^2	4.88×10^1	1.54×10^2	Mg (ppm)	1.18×10^4	1.49×10^3	1.91×10^4

^a Mean values of triplicates are presented for sediments from Baltimore Harbor and Hudson River, and mean values for duplicates are presented for sediment from Palos Verdes

Table 2

Experimental treatments and designations

	Sediment B (Baltimore Harbor)	Sediment P (Palos Verdes)	Sediment H (Hudson River)
B + P cultures	BP-B	BP-P	-
P + H cultures	-	PH-P	PH-H
B + H cultures	BH-B	-	BH-H
B + H + P cultures	BHP-B	BHP-P	BHP-H
Controls	B-B	P-P	H-H