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IN SITU MONITORING OF CHLORINATED HYDROCARBON REMEDIATION POTENTIAL USING BAITED BIO-SEP® TRAPS

Aaron D. Peacock (apeacock@utk.edu) (Microbial Insights, Inc., Rockford, TN and University of Tennessee, Knoxville, TN)
Kerry L. Sublette and Aditya Moralwar (University of Tulsa, Tulsa, OK)
Greg A. Davis (Microbial Insights, Inc., Rockford, TN)
Mark C. Harrison (S&ME Engineering, Louisville, TN)
David C. White (University of Tennessee, Knoxville, TN)

ABSTRACT: Bio-Sep®-bead traps, some baited with milk solids, molasses, or sodium acetate, were incubated in groundwater monitoring wells in a PCE-contaminated aquifer for 30 days. Collected biofilms were compared by extraction and analysis of phospholipids and PCR-amplified 16S rDNA. "Baiting" of Bio-Sep®-bead traps with molasses and milk solids resulted in increased viable biomass recovery and changes in community structure, including increased diversity and increased anaerobic character, when incubated in a PCE-contaminated aquifer. We propose that the microbial community collected in the non-baited beads is indicative of the untreated aquifer while the more diverse and more anaerobic community collected in the milk- and molasses-baited traps is predictive of a post-milk- or post-molasses-amended aquifer. Although no *Dehalococcoides* was detected these results do suggest that injection of milk solids and molasses in this aquifer will reduce the redox potential of the aquifer to favor growth of anaerobes and further reductive dechlorination activity. Recently completed microcosm studies confirm these conclusions. These data further suggest that the effects of potential remediation amendments can be readily evaluated by incorporating the amendments into Bio-Sep®-bead traps, incubating the traps in the aquifer to be treated, and evaluating the effects of the amendments on the microbial ecology of bead biofilms using biomarker analysis, all of which can be accomplished a greatly reduced costs relative to field injection of amendments.

INTRODUCTION

Implementation of a comprehensive groundwater-monitoring program to assess whether desired bioprocesses are occurring is critical to a defensible risk-based management approach for sites contaminated with chlorinated hydrocarbons. Geochemical parameters must typically be collected over an entire plume and in suitable control areas over an extended period of time. From this data the predominant bioprocesses are deduced. When active intervention is necessary remediation amendments must be evaluated for their effects on *in situ* microbial ecology to ensure that their introduction into a contaminated aquifer will have the desired effect of stimulating reductive dechlorination. These field tests are labor and analytically intensive.

The pre- or post-amendment microbial ecology of a contaminated aquifer is better represented by *in situ* biofilms than planktonic organisms in sampled groundwater. By conventional means subsurface biofilm sampling requires coring of aquifer sediments and extraction of viable microorganisms or biomarkers (lipids, DNA, etc.). However, the

efficiency of these extractions varies with the geochemistry of the sediments. We propose that biofilms characteristic of aquifer conditions can be rapidly and efficiently collected using a biofilm-sampling system based on Bio-Sep® technology. Bio-Sep® consists of 3-4 mm diameter spherical beads engineered from a composite of 25% aramid polymer (Nomex) and 75% powdered activated carbon (PAC) with a porosity of 75%. The median pore diameter is 1.9 microns, however, large macropores (> 20 microns) also exist inside the beads. Beads are surrounded by an ultrafiltration-like membrane with pores of 1-10 microns and the internal surface area is greater than 600 m^2/g. Bio-Sep® beads may be heated to 300°C for sterilization and to render the beads free of fossil biomarkers.

Biomarkers are more efficiently extracted from Bio-Sep® beads than aquifer sediments and provide measures of viable biomass, redox environment, microbial community composition, and nutritional status. Bio-Sep® beads are also more efficient collectors of biofilms than materials like glass wool. When like bulk volumes of Bio-Sep® beads and glass wool were incubated in a PCE-contaminated aquifer for 30 days the beads collected over seven times as much viable biomass (as represented by extracted phospholipids) as glass wool (Sublette et al., 2002). Bio-Sep® beads have also been shown to collect detectable biofilms in a drinking water distribution system in one day (White et al., 2003). The efficiency of biofilm formation in Bio-Sep® has been attributed to the high internal surface area, low-shear conditions in the bead, the concentration of limiting nutrients by the PAC, and the rapid formation of pre-conditioning films.

Potential remediation amendments may be incorporated into the Bio-Sep® beads during fabrication by entrapment or post-fabrication by adsorption onto the PAC component of the beads. The availability of nutrients, like Hydrogen Release Compound (HRC®, Regenesis), inside the bead have been shown to alter the community structure of biofilms formed in the bead. "Baiting" of Bio-Sep® beads with HRC® resulted in increased viable biomass recovery, increased metabolic activity, and changes in community structure, including reduced diversity, when incubated in a PCE-contaminated aquifer (Sublette et al., 2003). There was also evidence of stimulation of CVOC-degrading bacteria in HRC®-amended beads. These data suggested that the effects of potential remediation amendments can be readily evaluated by incorporating the amendments into Bio-Sep® beads, incubating the beads in the aquifer to be treated, and evaluating the effects of the amendments on the microbial ecology of bead biofilms using biomarker analysis, all of which can be accomplished a greatly reduced costs relative to field injection of amendments.

In this paper we describe the results of the incubation of Bio-Sep®-bead traps baited with sodium acetate, molasses, or milk solids and non-baited beads in groundwater monitoring wells in the same PCE-contaminated aquifer in which HRC®-baited beads were previously incubated. However, a new tube-in-tube trap design was utilized to provide a continuous low concentration of these very water soluble "baits" in the presence of Bio-Sep® beads where biofilms were collected. Collected biofilms were compared by extraction and analysis of phospholipids and PCR-amplified 16S rDNA.

Site Description. The source of the PCE plume at the test site was leakage from an above-ground storage tank (AST) of PCE at a dry cleaning business. The AST was in use from 1964-1991. Groundwater at the site is found in fracture systems and solution features in underlying bedrock (calcareous shale with embedded limestone) at 10-13 m.

Horizontal flow is very slow (about 19 cm/yr) and controlled by fractures and bedding planes. Groundwater monitoring wells (5-cm OD) have been installed at locations shown in Figure 1 and are screened over 3 m across the top of bedrock. Note that MW1 is upgradient of the PCE plume. The results of groundwater analysis from the site are summarized in Table 1. Daughter products of PCE dechlorination (TCE and *cis*-DCE) were found in the plume in addition to elevated chloride concentrations. Elevated concentrations of ethene (92 ng/L) were also found in MW2A associated with the highest concentrations of chlorinated hydrocarbons. These results indicate that the requisite organisms and environmental conditions necessary for reductive dechlorination exist at some locations in the plume despite the fact that the bulk dissolved oxygen concentration in the plume ranges from 2.5 – 5.5 mg/L.

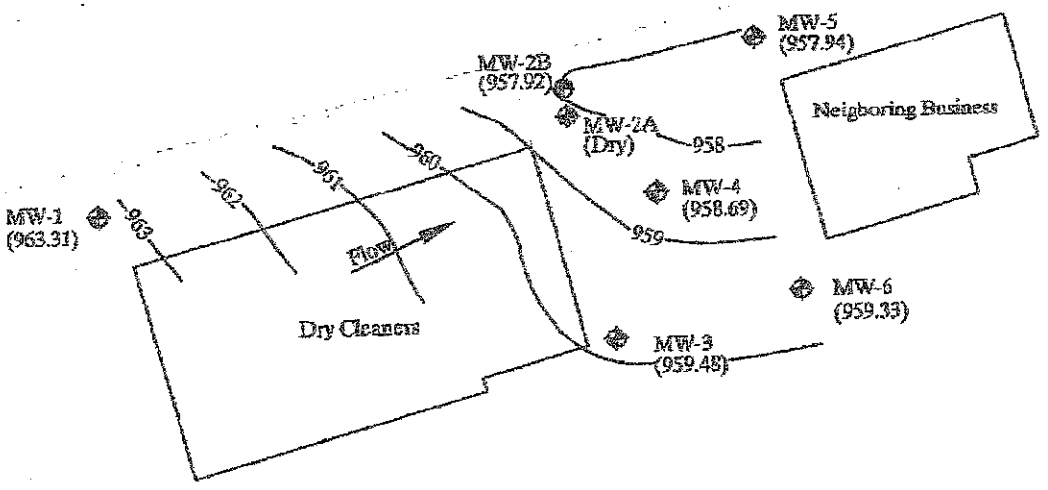

FIGURE 1. Dry cleaners site map showing monitoring well locations.

TABLE 1. Groundwater analysis at dry cleaners PCE site.

MW (Sampling Depth, m)	PCE (mg/L)	TCE (µg/L)	cis-DCE (µg/L)	Chloride (mg/L)
MW1 (10.7)	ND*	ND	ND	27
MW1 (13.7)	ND	ND	ND	27
MW2A (11.9)	5.9	50	42	
MW2B (13.4)	0.03	1.6	3.5	29
MW2B (16.5)	0.03	1.6	3.5	29
MW3 (9.4)	2.2	40	25	400
MW3 (12.5)	2.2	40	25	400
MW4 (8.5)	2.6	52	31	480
MW4 (11.6)	2.6	52	31	480

*not detected

MATERIALS AND METHODS

Preparation of Bio-Sep®-Bead Traps. Two types Bio-Sep®-bead traps were installed at the PCE site. One type was prepared using about 100 Bio-Sep® beads in 11.5-cm sections of PFA tubing (12.7 mm OD, 9.5 mm ID). The tubing was perforated with six rows of 2.3-mm holes spaced 0.6 cm apart to provide contact between the beads and the groundwater. Beads were held in place in the tubing with glass wool and nylon ties. The second type of trap consisted of two concentric tubes of PFA tubing separated by nylon spacers. The center 11.5-cm tube (12.7 mm OD, 9.5 mm ID) contained three adsorbents (PAC, neutral alumina, and silica gel) in a ratio of 1:1:1 by weight. Prior to packing the adsorbents into the center tube the mixtures were made into a paste with concentrated solutions of sodium acetate, milks solids or molasses. The wall of the center tube was cut with numerous thin slits. The function of this inner tube was to slow release the electron donor when the traps were exposed to groundwater. Surrounding the center tube in the annular space was a bed of about 450 Bio-Sep® beads. The outer tube (8.9 cm) was also PFA (25-mm ID, 28-mm OD) and was also perforated with 2.3-mm holes. The Bio-Sep® beads and glass wool were heat treated at 300°C for 4 hours to destroy fossil biomarkers and PFA tubing and nylon ties were washed with methanol prior to trap assembly. Pairs of traps, one tube-in-tube baited trap and one single-tube non-baited trap, were attached to braided nylon line with nylon ties about 30 cm from the end of the line to which was attached an epoxy-coated lead weight. These bug traps were lowered into each accessible monitoring well at the site and secured to place each pair of traps in the screened interval. All traps were retrieved after 30 days of incubation and bead biofilms characterized using PLFA and DNA analyses.

Biomarker Analysis. PLFA analysis was performed using previously reported important precautions to achieve quantitative results (Pinkart et al., 2002). The beads were extracted with the single-phase, chloroform-methanol-buffer system of Bligh and Dyer. The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography. The polar lipids were transesterified to the fatty acid methyl esters (FAMEs) by a mild alkaline methanolysis. The FAMEs from the phospholipids (PLFA) were analyzed by gas chromatography/mass spectroscopy using an Agilent 6890 series gas chromatograph interfaced to an Agilent 5973 mass selective detector with a 50 m non-polar column (0.2 mm I.D., 0.11 μm film thickness) utilizing the conditions described (Pinkart et al., 2002).

Nucleic acid was precipitated directly from the PLFA Bligh/Dyer aqueous phase with an equal volume of isopropanol in an ice bath for 30 min. DNA was pelleted by centrifugation at 13,000Xg at 4°C for 15 min, washed with 80% ethanol twice, air-dried, and re-dissolved in TE buffer (pH 8.0). The DNA extract was then purified and eluted in deionized water as described (Chang et al., 1999). PCR amplification of the 16S rDNA fragments prior to DGGE (denaturing gradient gel electrophoresis) was performed as described (Muyzer et al., 1993). The primers targeted eubacterial 16S regions corresponding to *E. coli* positions 341-534. DGGE was performed using a D-Code 16/16 cm gel system (BioRad, Hercules, CA). Prominent bands were excised and subjected to sequencing. Sequence identification was performed by use of the BLASTN facility of the National Center for Biotechnology Information and "Sequence Match" facility of the

Ribosomal Database Project (Madiak et al., 1999). Sequences were assembled using "SeqPup Version 0.6" (Gilbert, 1996). Phylogenetic algorithms (DNA-DIST, NEIGHBOR and SEQBOOT) were operated within the ARB software environment (Strunk and Ludwig, 1997).

RESULTS AND DISCUSSION

Table 2 gives the total biomass collected by all traps in terms of pmoles of prokaryote PLFA recovered. In each well the greatest biomass was collected in the milk- and molasses-baited traps. Figure 2 shows a hierarchical analysis of the fatty acid components of phospholipids extracted from the traps. This figure shows that the microbial community structures in the molasses and milk-baited traps were generally similar to each other but different from those collected in the non-baited and acetate-baited traps. Further the communities collected in the non-baited traps and acetate-baited traps were similar to each other.

TABLE 2. Biomass collected in Bio-Sep®-bead traps in terms of pmoles of prokaryote PLFA.

Monitoring Well	Control	Acetate	Milk	Molasses
MW1	1510	1300	12300	4500
MW2A	310	1540	6150	13900
MW2B	1100	220	3370	4380
MW3	780	1350	12950	20690
MW4	5090	540	13350	16600

When Shannon's diversity index was calculated for fatty acids in the PLFA it was seen that the greatest diversity was evident in the microbial communities from the molasses and milk-baited Bio-Sep®-bead traps ($H = 2.3 \pm 0.3$ in molasses- or milk-baited traps vs. $H = 1.6 \pm 0.3$ in non-baited or acetate-baited traps). This increase in diversity is in contrast to the decrease in diversity observed when HRC® was used as a bait in this aquifer (Sublette et al., 2003). The increase in diversity seen with the milk solids and molasses is likely due to the complexity of these materials in terms of the number of different electron donors available. Figure 3 shows the concentration of anaerobic indicators in each trap as represented by the sum of the mole fractions of the terminally branched saturated, branched monoenoic, and mid-chain branched saturated fatty acids. These results suggest that anaerobic environments are more likely to be found in the presence of milk components or molasses.

Figure 4 is a gel image from the DGGE analysis of 16S rDNA fragments amplified from DNA extracted from bug trap beads from the non-contaminated well (MW1) and one well in the plume (MW3). As seen in this figure the presence of all of the electron donors produced changes in the eubacterial community structure compared to the non-baited traps in both the plume and upgradient of the plume. However, no *Dehalococcoides* were detected through sequence identification or target gene analysis using specific primers directed to a conserved region of the 16S rRNA gene (He et al., 2003).

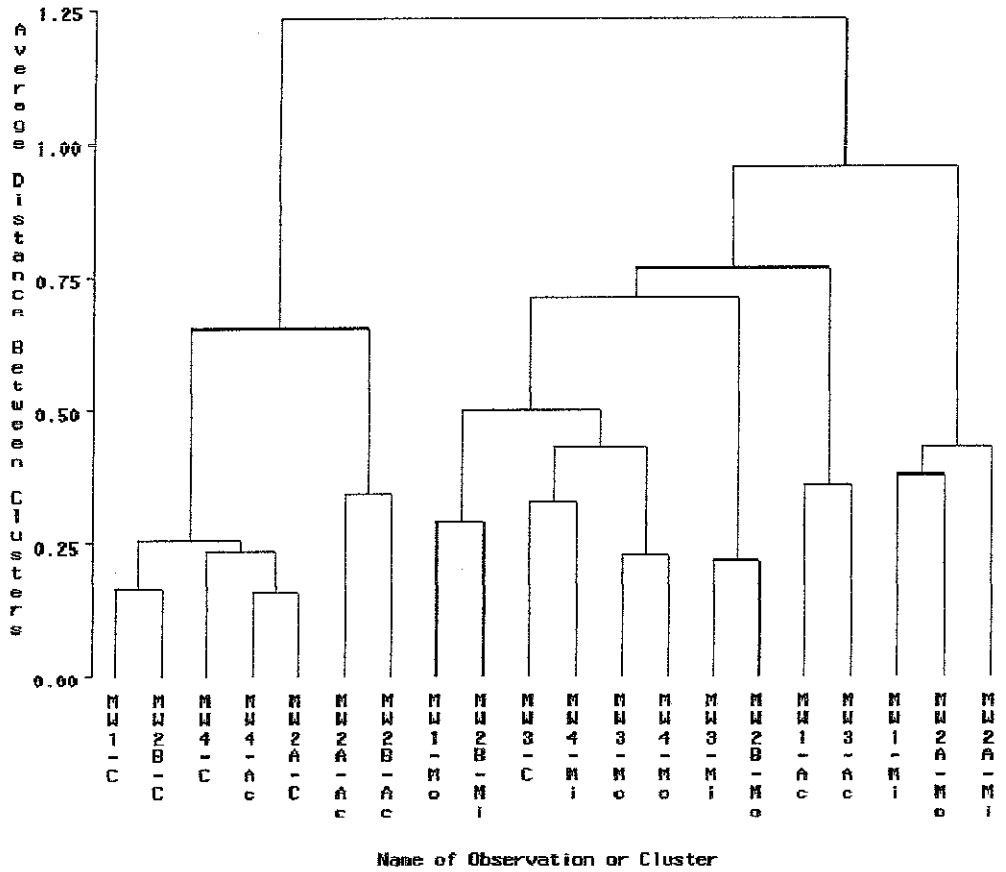

FIGURE 2. Hierarchical analysis of the fatty acid components of phospholipids extracted from the traps.
C = non-baited; Ac = acetate-baited; Mi = milk-baited; and Mo = molasses-baited

CONCLUSIONS

"Baiting" of Bio-Sep®-bead traps with molasses and milk solids resulted in increased viable biomass recovery and changes in community structure, including increased diversity and increased anaerobic character, when incubated in a PCE-contaminated aquifer. We propose that the microbial community collected in the non-baited beads is indicative of the untreated aquifer while the more diverse and more anaerobic community collected in the milk- and molasses-baited traps is predictive of a post-milk- or post-molasses-amended aquifer. These results suggest that the addition of molasses or milk solids to the aquifer in the proper quantities will create the requisite anaerobic conditions for reductive dechlorination activity. Since no *Dehalococcoides* were found in trap biofilms this site is a candidate for bioaugmentation to ensure full reductive dechlorination activity. An *ex situ* microcosm study, just completed, has confirmed these conclusions. Using groundwater and sediment from MW4, 100% conversion of PCE to ethene was observed in 33 days only with molasses or milk solids as electron donors (vs. lactate and HRC®) and bioaugmentation with *D. ethenogenes*. Further details of these microcosm studies will be published at a later date.

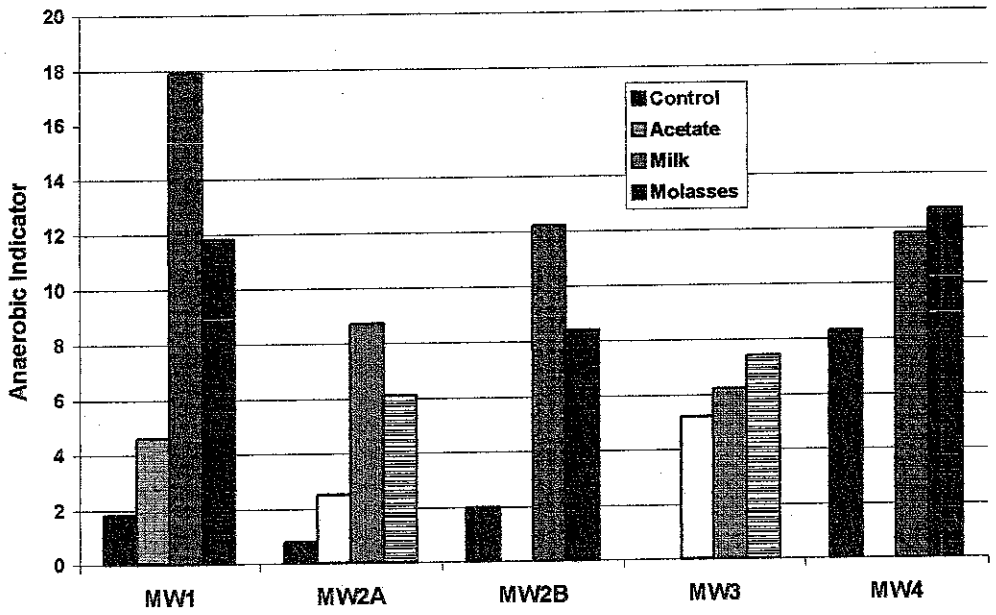

FIGURE 3. Anaerobic indicators from microbial communities extracted from all baited and non-baited traps. Control = non-baited; Anaerobic indicator is defined as the sum of the mole fractions of terminally branched saturated, branched monoenoic, and mid-chain branched saturated fatty acids.

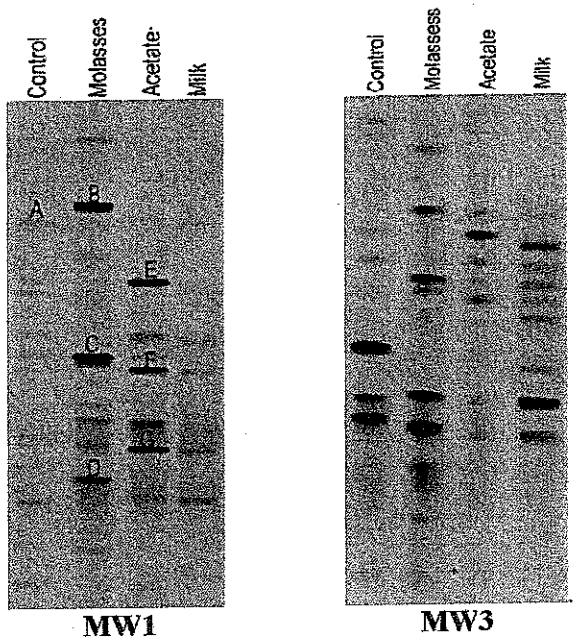

FIGURE 4. Gel images from the DGGE analysis of 16S rDNA fragments amplified from DNA extracted from the bug trap beads (C = non-baited).

Although more field tests are necessary these data support the hypothesis that the effects of potential remediation amendments can be readily evaluated by incorporating the amendments into Bio-Sep®-bead traps, incubating the beads in the aquifer to be treated, and evaluating the effects of the amendments on the microbial ecology of bead biofilms using biomarker analysis, all of which can be accomplished a greatly reduced cost relative to field injection of amendments.

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