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A COMPARISON OF IN SITU BAITED BIO-SEP® BEAD TRAPS AND EX SITU MICROCOSMS IN THE EVALUATION OF POTENTIAL REMEDIATION AMENDMENTS IN A PCE-CONTAMINATED AQUIFER

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ABSTRACT: Bio-Sep® beads consist of 3-4 mm diameter spherical beads engineered from a composite of 25% aramid polymer (Nomex) and 75% powdered activated carbon (PAC). The bulk density is about 0.16 g/cm^3 with a porosity of 74%. Beads are surrounded by an ultrafiltration-like membrane with pores of 1-10 microns. Bio-Sep® beads have been shown to concentrate extant microbes in internal biofilms providing biomarkers for viable biomass, redox environment, microbial community composition, and nutritional status. Bio-Sep® beads may also be "baited" with potential remediation amendments during fabrication or amendments may be loaded onto PAC post-fabrication.

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 was indicative of the untreated aquifer while the more diverse and more anaerobic community collected in the milk- and molasses-baited traps was 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 has confirmed these conclusions. Using groundwater and sediment from the site, conversion of PCE to ethene was observed only after bioaugmentation with *D. ethenogenes* and more rapid conversion was observed with molasses or milk whey as electron donors (vs. lactate and HRC).

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.

We have also described 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 (Peacock et al., 2003). 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. In this paper we compare the results of the incubation of these "*in situ* microcosms" with *ex situ* microcosms which used groundwater and groundwater sediments from the same PCE-contaminated site as source material and molasses, whey, lactate, or HRC as electron donors for the reductive dechlorination of PCE.

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.

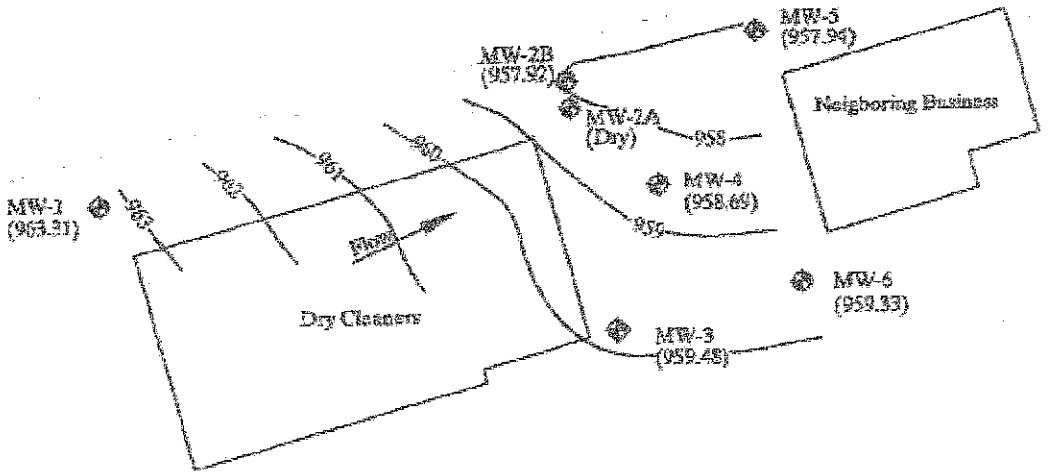

FIGURE 1. Dry cleaners site map showing monitoring well locations.

MATERIALS AND METHODS

Preparation and Deployment of Bio-Sep® Bead Traps. Construction of Bio-Sep® bead traps baited with molasses, milk solids, and sodium acetate has been described in detail elsewhere (Peacock et al., 2003). 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

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.

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. Details of biomarker analysis in Bio-Sep® beads have also been described previously (Peacock et al., 2003).

Microcosms. Microcosm tests were conducted as described by Findley and Fogel (2000). Groundwater and sediment from MW-4 were used as source material in the microcosms. Microcosms were constructed on June 13, 2003 using 160-mL microcosm bottles flushed with argon. Each microcosm contained 100 mL of the MW-4 groundwater sample. The microcosms were sealed and flushed to replace the argon in the headspace with 30% CO_2 in nitrogen. Bottles were then pressurized, placed upside down in a dark room at 22°C, and shaken three times per week. The six conditions constructed included: 1) killed control; 2) lactate-amended; 3) HRC-amended, bioaugmented; 4) whey-amended, bioaugmented; 5) molasses-amended, bioaugmented; and 6) lactate-amended, bioaugmented.

The killed control was prepared by adding enough 6 N HCl to lower the pH below 2. Microcosms 2-6 were initially amended with 30 mg/L phosphate, and then with 40 mg/L NH_3-N after NO_3^- was reduced. Sub-samples was removed from each microcosm on day 1, day 18, day 33, and day 63 of the test. These sub-samples were analyzed for PCE, daughter products (TCE, cDCE, VC, ethane, methane), nitrate and sulfate. Microcosms 3-6 were inoculated with a dechlorinating culture of *D. ethenogenes* (NJ-14) on day 19 of the test, after SO_4^{-2} had been reduced, in order to evaluate the potential for successful bioaugmentation at the site.

RESULTS AND DISCUSSION

***In Situ* Microcosms (Bio-Sep® Bead Traps).** 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. A hierarchical analysis of the fatty acid components of phospholipids extracted from the traps showed that the

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

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 seen to be similar to each other (for details see Peacock et al., 2003).

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 2 shows the concentration of anaerobic indicators in each trap as represented by the sum of the mole fractions of the terminally branched

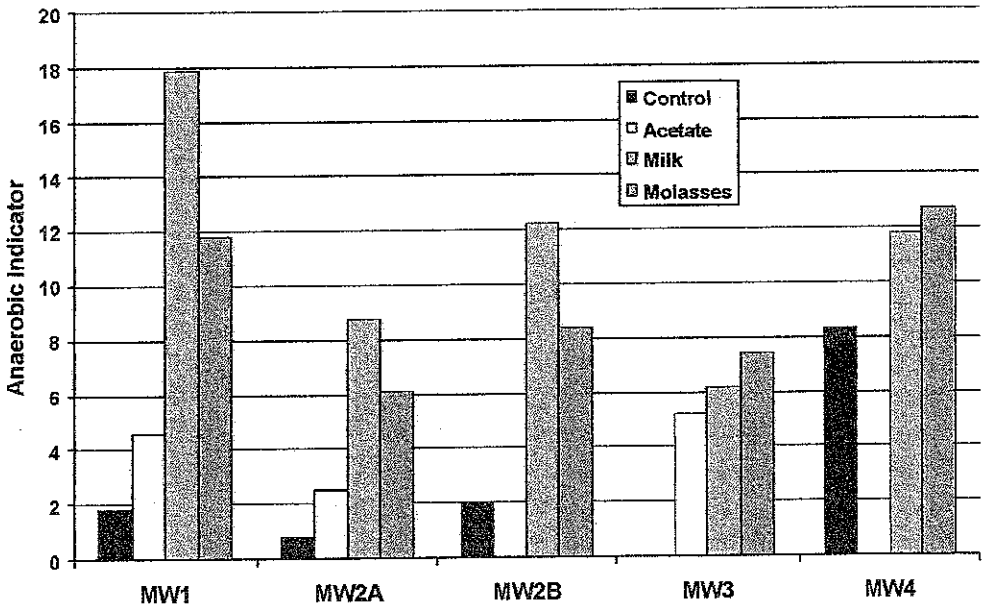

FIGURE 2. Anaerobic indicators from microbial communities extracted from all baited and non-baited traps. Control = non-baited; anaerobic indicator is defined as the sum of the mole fractions of terminally branched saturated, branched monoenoic, and mid-chain branched saturated fatty acids.

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.

DGGE analysis of 16S rDNA fragments amplified from DNA extracted from trap beads showed that 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* was 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).

***Ex Situ* Microcosms.** Of the six microcosms, four completely dechlorinated PCE to ethane (Table 3). The killed control maintained a 35 ± 4 µM PCE concentration, a 0.4 ±0.1 µM TCE concentration, and a 0.8 ±0.1 µM cDCE concentration throughout the course of the study. No VC or ethene was detected during the study, and only a minimal amount of methane (0.5 µM) was detected at day 33. The sulfate concentration exhibited a slight increase between Days 0 and 33 (from 25 to 31 mg/L). No nitrate was detected on Day 33.

The lactate-amended microcosm without bioaugmentation reduced PCE to cis-cDCE, but failed to reduce cDCE to VC or ethene by day 63, at which point the monitoring of most of the microcosms was concluded. However, this microcosm was analyzed again at 231 days and still showed no VC or ethane strongly suggesting the absence of active *Dehalococcoides*. Again, minimal amounts of methane were detected by day 63 (0.3 µM). Sulfate was completely reduced (originally 25 mg/L) by Day 13. No nitrate was detected throughout the test.

The lactate-amended microcosm with bioaugmentation was able to completely reduce PCE to ethene in 63 days. As the PCE was reduced, an accumulation of cDCE was noted, and as the cDCE was reduced, an accumulation of VC was noted. Ethene concentrations slowly increased as the cDCE and VC was reduced. At Day 63, no cDCE or VC was detected. Methane was detected in increasing concentrations from Day 33 reaching a concentration of 2.7 µM at day 63. The production of methane loosely mirrored that of ethene. Nitrate was completely reduced (originally 5 mg/L) by Day 4 of the test, and sulfate (originally 22 mg/L) was completely reduced by Day 18.

The HRC-amended microcosm with bioaugmentation was also able to completely reduce PCE to ethene in 63 days. The VOC constituent concentration patterns for dechlorination in this microcosm were very similar to that of the lactate-amended microcosm with bioaugmentation, which is not surprising because HRC is a form of lactate. The difference is that HRC was able to reduce more of the cDCE to ethene by Day 33 with a lower concentration of VC (7.2 µM as compared to 15 µM in the lactate-amended microcosm). More methane was also produced in this microcosm than the lactate-amended microcosm, attaining a concentration of 4.7 µM at Day 63, but again, the methane was not detected until the PCE was completely reduced, and its production loosely mirrored that of ethene. Nitrate was completely reduced (originally 5 mg/L) at Day 4, and sulfate (originally 23 mg/L) at Day 18.

The whey microcosm with bioaugmentation was able to completely reduce PCE to ethene in only 33 days with only a minimal accumulation of VC. Methane production in this microcosm was greater than any of the other five, attaining a maximum concentration

TABLE 3. *Ex situ* microcosm VOC data

Killed Control				uM			
Day	Date	PCE	TCE	cDCE	VC	Ethene	Methane
1	06/20/03	39	0.4	0.8	< 0.1	< 0.05	< 1
18	07/07/03	31	0.3	0.8	< 0.1	< 0.05	< 1
33	07/22/03	35	0.5	0.9	< 0.1	< 0.05	0.5
63	08/21/03	–	–	–	–	–	–
Lactate							
Day	Date	PCE	TCE	cDCE	VC	Ethene	Methane
1	06/20/03	40	0.5	1	< 0.1	< 0.05	–
18	07/07/03	< 0.3	< 0.3	46	< 0.1	< 0.05	<1
33	07/22/03	< 0.3	< 0.3	54	< 0.1	< 0.05	0.2
63	08/21/03	< 0.3	< 0.3	55	< 0.1	< 0.05	0.3
231	**02/05/04**	**< 0.3**	**<0.3**	**41**	**< 0.1**	**< 0.05**	**936**
Lactate-Bioaugmented							
Day	Date	PCE	TCE	cDCE	VC	Ethene	Methane
1	06/20/03	39	0.5	1	< 0.1	< 0.05	< 1
18	07/07/03	< 0.3	< 0.3	43	< 0.1	< 0.05	< 1
33	07/22/03	< 0.3	< 0.3	21	15	8.4	0.7
63	08/21/03	< 0.3	< 0.3	< 0.4	< 0.1	38	2.7
HRC-Bioaugmented							
Day	Date	PCE	TCE	cDCE	VC	Ethene	Methane
1	06/20/03	40	0.5	1	< 0.1	< 0.05	–
18	07/07/03	< 0.3	< 0.3	40	< 0.1	< 0.05	< 1
33	07/22/03	< 0.3	< 0.3	3.4	7.2	24	0.6
63	08/21/03	< 0.3	< 0.3	< 0.4	< 0.1	33	4.8
Whey-Bioaugmented							
Day	Date	PCE	TCE	cDCE	VC	Ethene	Methane
1	06/20/03	46	0.6	1	< 0.1	<0.05	< 1
18	07/07/03	< 0.3	< 0.3	44	< 0.1	<0.05	< 1
20	07/22/03	< 0.3	< 0.3	44	0.7	< 0.05	1
33	08/21/03	< 0.3	< 0.3	< 0.4	< 0.1	35	49
Molasses-Bioaugmented							
Day	Date	PCE	TCE	cDCE	VC	Ethene	Methane
1	06/20/03	40	0.5	1	< 0.1	<0.05	–
18	07/07/03	< 0.3	< 0.3	50	< 0.1	< 0.05	< 1
33	07/22/03	< 0.3	< 0.3	< 0.4	< 0.1	40	6
63	08/21/03	–	–	–	–	–	–

of 49 μM at Day 63. As with microcosm 3 and 4, the methane did not appear until after PCE reduction was complete. Nitrate was completely reduced (originally 6 mg/L) at Day 4 and sulfate (originally 25 mg/L) at Day 13.

The molasses microcosm with bioaugmentation was also able to completely reduce PCE to ethene in 33 days with no accumulation of VC. Methane was produced, first detected after the reduction of PCE and increasing as the ethene concentration increased, attaining a final concentration of 6 μM at Day 33. Nitrate was completely reduced (originally 6 mg/L) at Day 4 and sulfate (originally 28 mg/L) at Day 13.

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 these *in situ* microcosm results suggest that this site is a candidate for bioaugmentation to ensure full reductive dechlorination activity. The *ex situ* microcosm study confirmed these conclusions.

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 reduced cost relative to field injection of amendments or *ex situ* microcosms.

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