

## **Final Report**

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Title: Identifying the Signature of the Natural Attenuation in the Microbial Ecology of Hydrocarbon Contaminated Groundwater Using Molecular Methods and 'Bug Traps'

Investigators: Kerry L. Sublette, University of Tulsa, Tulsa, OK (kerry-sublette@utulsa.edu)

Laura Ford, University of Tulsa, Tulsa, OK

David White, University of Tennessee, Knoxville, TN

Aaron Peacock, University of Tennessee, Knoxville, TN

Institution: University of Tulsa, Tulsa, OK

EPA Project Officer: Bala Krishnan

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## **Abstract**

Monitored natural attenuation can be a successful management strategy for groundwater contamination if requisite microbial and geochemical conditions are present. Subsurface microbial ecology of a contaminated aquifer is often investigated by sampling groundwater from the plume. However, the subsurface microbial ecology of a contaminated aquifer is better represented by *in situ* biofilms than planktonic organisms. Conventional sampling to collect the sessile subsurface microbial community requires coring of the aquifer sediments. However, the efficiency of extracting viable microorganisms and biomarkers from these sediments varies with site geochemistry. Therefore, we have developed a rapid and efficient microbial-sampling system based on Bio-Sep<sup>®</sup> technology. Bio-Sep<sup>®</sup> consists of 3-4 mm diameter spherical porous beads engineered from a composite of 25% aramid polymer and 75% powdered activated carbon. These beads encourage microbial growth by providing high internal surface area with low-shear conditions, and by concentrating limiting nutrients within the groundwater environment. Biomarkers can be efficiently extracted from Bio-Sep<sup>®</sup> to provide measures of viable biomass, redox environment, and microbial community composition. This paper presents a review of the Bio-Sep<sup>®</sup> technology and presents two case studies of its use for documenting subsurface microbial ecology and bioprocesses in contaminated aquifers.

Key words: bio-trap, benzene, chlorinated hydrocarbons, microbial ecology, groundwater

## **Introduction**

Releases of hydrocarbons and chlorinated solvents into the environment have resulted in the accumulation of toxic compounds in the subsurface which can potentially represent significant risks to human health. Past technology-based cleanup standards for impacted groundwater often resulted in the expenditure of financial resources on remediation that did little to actually protect society. This recognition has led to the development of risk-based site management strategies which allow resources to be devoted to sites with the greatest risk to human health. Natural attenuation, particularly intrinsic bioremediation, of contaminants is typically a central feature of a risk-based management strategy for impacted groundwater. When clear proof of natural attenuation exists and environmental receptors will not be threatened during the life of the plume, a risk-based management approach can be both cost-effective and protective of human health and ecological receptors (Small, 1998).

Documenting intrinsic bioremediation has frequently required a comprehensive groundwater monitoring program (National Research Council, 1993; ASTM, 1998). Geochemical parameters (electron acceptors, products of reduction of electron acceptors, DO, redox potential, hydrogen, etc.) must typically be collected over an entire plume and in suitable control areas over an extended period of time (Wiedemeier et al., 1995). From these data the predominant bioprocesses are deduced. However, it is often difficult and/or costly to

demonstrate that the desired bioprocesses are occurring with this approach. More direct evidence of intrinsic bioremediation and the operative mechanisms can be obtained from *ex situ* microcosms using groundwater and/or sediments from the site as source material (Krumholz et al., 1996 and references therein). However, *ex situ* microcosms require lengthy incubations and significant analytical costs. Furthermore, such studies suffer from the criticism that the *in situ* environment is not represented by the microcosms.

We propose that a definitive signature of intrinsic bioremediation can be obtained by analyzing the composition and nutritional/physiological status of the active component of the extant microbial community. Subsurface microbial ecology in a contaminated aquifer is often investigated by sampling groundwater from the plume. However, the microbial ecology of a contaminated aquifer is better represented by *in situ* biofilms than planktonic organisms in sampled groundwater. Traditional subsurface biofilm sampling requires the 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 (Zhou et al., 1995). Further, such extractions yield biomarkers from both the active and the inactive members of the community.

It is not uncommon that desired microorganisms and bioprocesses exist in an impacted aquifer but are limited by unfavorable redox conditions or limited amounts of electron acceptors. In these cases active intervention in the form of stimulation of subsurface bioprocesses with amendments can be effective. However, when active intervention is necessary remediation amendments must be evaluated for their effects on *in situ* microbial ecology to ensure that the introduction of amendments into a contaminated aquifer will have the effect of stimulating the desired biochemical processes. These field tests are labor and analytically intensive.

We propose that biofilms characteristic of aquifer conditions (pre- or post-amendment) can be rapidly and efficiently collected using a biofilm-sampling system or bio-trap based on Bio-Sep<sup>®</sup> technology. The biofilms can be analyzed for lipid and DNA biomarkers that define the metabolic capabilities and activity at the cellular level. These bio-traps can also be used to predict the effects of a potential remediation amendment on surface microbial ecology and/or monitor the effects of a remediation amendment *in situ* during injection. Lastly, bio-traps “baited” with <sup>13</sup>C-labeled contaminants can irrefutably demonstrate the *in situ* biodegradation potential of a contaminant by indigenous microorganisms under actual aquifer conditions. In this paper we review Bio-Sep<sup>®</sup> bio-trap technology and provide two case studies of the use of bio-traps to document subsurface microbial ecology.

### **Bio-Sep<sup>®</sup> Bio-trap Technology**

As noted above, the microbial ecology of a contaminated aquifer is better represented by *in situ* biofilms than planktonic organisms in sampled groundwater. Further, the relatively high spatial separation of microbes in groundwater can limit the detection and analysis of these microbial populations. We have demonstrated that biofilms characteristic of aquifer conditions can be rapidly and efficiently collected using *in situ* microcosms or “bio-traps” containing Bio-Sep<sup>®</sup> beads (The University of Tulsa) (Sublette et al., 2006 and references therein). Bio-Sep<sup>®</sup> beads are 3-4 mm in diameter and composed of 25% aramid polymer and 75% powdered activated carbon (PAC). They have a porous, sponge-like structure with 74% porosity and 600 m<sup>2</sup>/g of surface area surrounded by an ultrafiltration-like membrane that contains holes and tears of 1-10 microns in size (Figure 1). This size range allows for bacteria to enter into the beads while minimizing invasion by fungi and other larger organisms including predators. Bio-Sep<sup>®</sup> beads are heated to 280 °C for sterilization and to render the beads free of fossil biomarkers.

Much of the bead PAC surface is accessible and available to interact with soluble material that diffuses into the beads. The large internal surface area coupled with the adsorptive properties of the surface very likely explains the rapid formation of biofilms in these beads when exposed to a variety of aqueous environments. In some environments the PAC may also serve to concentrate limiting nutrients inside the bead or aid in the rapid formation of pre-conditioning films that are a prerequisite of biofilm formation.

A standard bio-trap generally consists of perforated PFA tubing packed with >100 clean and sterile Bio-Sep® beads. When bio-traps are deployed in groundwater indigenous microbes enter through the outside membrane and migrate into the porous internal matrix. The microbial biomass then attaches to this internal structure and reproduces to form biofilms. In this manner active microorganisms can be concentrated for analysis despite the relatively low density of microbes in the sampled groundwater. It is important to note that microorganisms must grow and reproduce within the bead to be detected. Thus the beads will collect only those organisms which are active under the specific subsurface conditions. Those microorganisms that are not capable of growth under *in situ* conditions due to a nutrient limitation, unfavorable redox potential, or adverse environmental conditions will not be collected in the beads at detectable levels.



Figure 1. Scanning electron micrograph of cross section of a Bio-Sep® bead

Research has shown that the material composition of Bio-Sep® beads can have a profound effect on the microbial composition of biofilms that form in the beads. Differences in the material composition can result in changes within the internal bead environment in terms of the availability of carbon and energy sources, electron acceptors, or other nutrients. The internal material composition may also affect pH within the bead. Therefore, changes in the composition of the internal matrix of the Bio-Sep® beads can be tailored to favor the growth of specific groups of microorganisms. These changes can be affected in two ways. First, carbon-adsorbable organics can be adsorbed onto the bead PAC post-fabrication. These adsorbed species are available as nutrients through a reversal of the adsorption equilibrium. The rate of desorption depends on the affinity of the compound for the PAC, the rate at which it is consumed by the microbes, the concentration of the compound in the bulk aqueous phase, etc. The second method of producing changes in Bio-Sep® beads composition is to incorporate new materials during the fabrication process in place of, or in addition to, PAC. Our experience has been that many different solids as fine powders can substitute in whole or in part for PAC while producing beads with very similar structural properties. Further, these new materials are accessible to the internal aqueous phase and to the microorganisms.

Another strategy for changing the internal environment of the beads is to slowly release water-soluble chemicals in close vicinity to the beads. This release can also occur within, or adjacent to, a bed of beads. This strategy is especially useful for chemicals that are highly water soluble and non-adsorbable on carbon. Released slowly these chemicals exhibit their highest concentrations within the bead bed and diffuse into the bead interiors as well. This sampling device uses an inner tube containing a “bait”, such as a potential remediation amendment, surrounded by a perforated, larger outer tube. The annular space between the tubes is packed with Bio-Sep® beads. As the bait or amendment is slowly released from the inner tube, it must diffuse through the bead bed and into the beads. This provides a means of customizing the localized environment for studying microbial ecology in groundwater.

Bio-traps are typically deployed in existing groundwater monitoring wells within the plume and as well as in control regions upgradient of the plume. Comparing the plume incubated bio-traps to the controls allows the effects of the aquifer contaminants on subsurface microbial ecology to be clearly discerned. Within the well itself the positioning of the bio-traps within the well is typically dependent on the type of contaminant. For heavy contaminants such as chlorinated hydrocarbons bio-traps are positioned in the middle of the screened interval. In contrast, the highest concentrations of light contaminants, such as fuel hydrocarbons, are near the water table. Thus, it is assumed that this is also the location of the most intense microbial activity associated with this contaminant. Therefore, bio-traps in these of aquifers are typically positioned 25-75 cm below the water table. If the water level is expected to rise or fall during the period of incubation the bio-traps can be suspended from a float. A typical incubation period is 30-60 days.

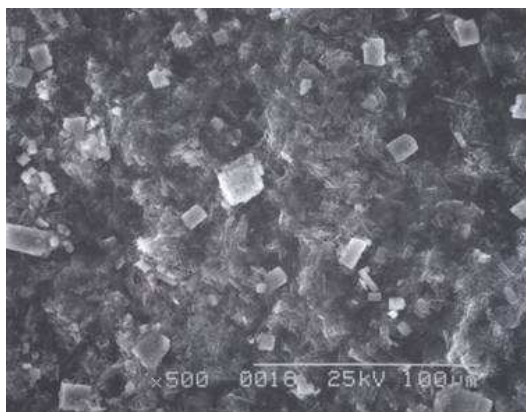


Figure 3. Scanning electron micrograph of interior of baited Bio-Sep<sup>®</sup> showing entrapped crystals of sodium sulfate

Following incubation, biofilms collected within Bio-Sep<sup>®</sup> beads can be analyzed through a variety of methods including extraction and analysis of biomarkers such as phospholipids fatty acids (PLFA), nucleic acids (DNA and RNA) and respiratory quinones. Biomarkers are more efficiently extracted from Bio-Sep<sup>®</sup> beads than aquifer sediments and provide measures of viable biomass, redox environment, microbial community composition, and nutritional (White and Ringelberg, 1998 and references therein). Phospholipid fatty acid (PLFA) analysis is based on the extraction and separation of lipid classes, followed by a quantitative analysis of derived fatty acid methyl esters (FAMES) using gas chromatography/mass spectrometry (GC/MS). Lipids are essential components of the cellular membranes and play a role as storage materials. A signature lipid biomarker analysis can provide quantitative insight into three important attributes of microbial communities; viable biomass, community structure and nutritional/physiological status. One type of deoxyribonucleic acid (DNA) analysis is based on structural differences in certain genes that all organisms contain. For prokaryotes (bacteria) this is the 16S rDNA gene (Sambrook et al., 1989). In this analysis 16S rDNA is extracted from the bacteria within an environmental sample and then amplified by an enzyme-catalyzed reaction called the polymerase chain reaction (PCR). The gene copies from different bacteria are then separated and visualized by a technique called denaturing gradient gel electrophoresis (DGGE). In this technique the 16S rDNA genes produce profiles visible as bands (or lines) in a gel. The banding patterns and relative intensities of the bands can provide a measure of differences among the communities. Gel bands from dominant species, which constitute at least 1-2% of the total bacterial community, can be excised and then sequenced. Sequence analysis of individual bands is used to infer the identity of the source organism based on database searches and phylogenetic methods. Phylogenetic affiliations are determined by comparing the DNA sequences retrieved from samples to DNA sequences of known bacterial sources in national databases such as the Ribosomal Database Project (<http://rdp.cme.msu.edu>) sponsored by the National Science Foundation, the National Institutes of Health and the U.S. Department of Energy Office of Biological and Environmental Research. In addition to the taxonomic identification of members of a microbial community, nucleic acid expression may be qualitatively monitored in order to determine the dynamics within the microbial community in changing microenvironments. As with PLFA analysis, numerous

studies have used PCR amplification of 16S rDNA genes to characterize microbial populations in a number of different environments. These studies have demonstrated that the dominant microorganisms isolated by traditional culture methods frequently do not match those identified by molecular techniques. The most recent developments in DNA analysis techniques are in the area of quantitative analysis. Specifically, quantitative PCR (qPCR) counts specific genes, either functional genes (Chang et al., 2001; Ivanova et al., 2000) or specific 16S rDNA sequences, that have been extracted from an environmental sample. This provides non-culture based counts of specific groups of microorganisms (such as sulfate reducing bacteria, methanogens, or hydrocarbon degraders), specific organisms (*Dehalococcoides*, for example), or total eubacteria.

### **Applications of Bio-Sep® Bio-traps**

Two case studies are presented here: 1) predicting the effect of a potential remediation amendment on subsurface microbial ecology and 2) demonstrating the *in situ* biodegradation potential of benzene by indigenous microorganisms under actual aquifer conditions.

#### Predicting the effect of a potential remediation amendment on subsurface microbial ecology

Bio-Sep® bio-traps, baited with either milk solids, molasses, or sodium acetate, were incubated in groundwater monitoring wells in a tetrachloroethylene (PCE)-contaminated aquifer for 30 days (Peacock et al., 2003). Groundwater monitoring wells were installed at several locations within the plume as well as upgradient of the source. The results of the groundwater analysis from the site are summarized in Table 1. Daughter products of PCE dechlorination [trichloroethylene (TCE) and *cis*-1,2-dichloroethylene (*cis*-DCE)] were found in the plume in addition to elevated chloride concentrations. Elevated concentrations of ethene (92 ng/L) were also associated with the highest concentrations of chlorinated hydrocarbons.

Collected biofilms were compared by extraction and analysis of phospholipids and PCR-amplified 16S rDNA. “Baiting” of bio-traps traps with molasses or milk solids resulted in increased viable biomass recovery and changes in community structure in comparison to non-baited and acetate-baited bio-traps. In each monitoring well tested the greatest biomass was collected in the milk- and molasses-baited traps. A hierarchical analysis of the extracted PLFA fatty acids showed 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. Figure 3 shows the concentration of anaerobic indicators in each trap as represented by the sum of the mole fractions of branched fatty acids (White et al., 1997). The microbial community collected in the non-baited beads is indicative of the untreated aquifer. Therefore, these results suggest that anaerobic environments are more likely to be stimulated in the presence of milk components or molasses. The results also suggest that 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 in these samples by qPCR these results do suggest that injection of milk solids and molasses into this aquifer would reduce the redox potential of the aquifer to favor the growth of anaerobes and further reductive dechlorination activity.

These data demonstrate 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. This entire process can be accomplished at a greatly reduced cost relative to the cost of the field injection of amendments. Since no *Dehalococcoides* were found in the bio-trap biofilms, this site was determined to be a candidate for bioaugmentation (inoculation with dechlorinating bacteria) to ensure full reductive dechlorination activity. An *ex situ* microcosm study (Table 2) has confirmed these conclusions (Mehta et al., 2004). Using groundwater and sediment from the site, conversion of PCE to ethene was observed only after bioaugmentation

with *D. ethenogenes*. A more rapid conversion was observed with molasses or milk whey as electron donors vs. lactate or Hydrogen Release Compound (HRC, Regenesis, San Clemente, CA).

Table

MW (Sampling Depth, m) Chloride	PCE (mg/L)	TCE (µg/L)	cis-DCE (µg/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

1.

Groundwater analysis at a dry cleaners PCE site where Bio-Sep® bio-traps were deployed

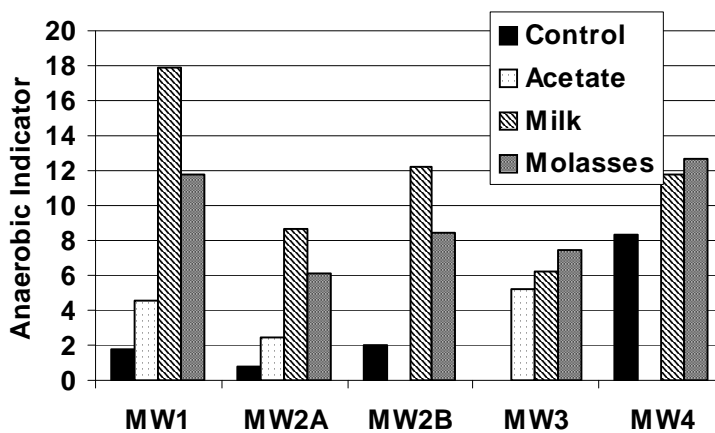


Figure 3. Anaerobic indicators from microbial communities extracted from all baited and non-baited traps deployed in the dry cleaners PCE site . Control = non-baited;

Table 2. PCE and daughter product concentrations in *ex situ* microcosms using groundwater and sediments from the dry cleaners PCE site

<b>Killed Control</b>						
Day	<b>μM</b>					Methane
	PCE	TCE	cDCE	VC	Ethene	
1	39	0.4	0.8	< 0.1	< 0.05	< 1
18	31	0.3	0.8	< 0.1	< 0.05	< 1
33	35	0.5	0.9	< 0.1	< 0.05	0.5
63	--	--	--	--	--	--
<b>Lactate</b>						
Day	PCE	TCE	cDCE	VC	Ethene	Methane
1	40	0.5	1	< 0.1	< 0.05	--
18	< 0.3	< 0.3	46	< 0.1	< 0.05	< 1
33	< 0.3	< 0.3	54	< 0.1	< 0.05	0.2
63	< 0.3	< 0.3	55	< 0.1	< 0.05	0.3
<b>231</b>	<b>&lt; 0.3</b>	<b>&lt; 0.3</b>	<b>41</b>	<b>&lt; 0.1</b>	<b>&lt; 0.05</b>	<b>936</b>
<b>Lactate-Bioaugmented</b>						
Day	PCE	TCE	cDCE	VC	Ethene	Methane
1	39	0.5	1	< 0.1	< 0.05	< 1
18	< 0.3	< 0.3	43	< 0.1	< 0.05	< 1
33	< 0.3	< 0.3	21	15	8.4	0.7
63	< 0.3	< 0.3	< 0.4	< 0.1	38	2.7
<b>HRC-Bioaugmented</b>						
Day	PCE	TCE	cDCE	VC	Ethene	Methane
1	40	0.5	1	< 0.1	< 0.05	--
18	< 0.3	< 0.3	40	< 0.1	< 0.05	< 1
33	< 0.3	< 0.3	3.4	7.2	24	0.6
63	< 0.3	< 0.3	< 0.4	< 0.1	33	4.8
<b>Whey-Bioaugmented</b>						
Day	PCE	TCE	cDCE	VC	Ethene	Methane
1	46	0.6	1	< 0.1	< 0.05	< 1
18	< 0.3	< 0.3	44	< 0.1	< 0.05	< 1
20	< 0.3	< 0.3	44	0.7	< 0.05	1
33	< 0.3	< 0.3	< 0.4	< 0.1	35	49
<b>Molasses-Bioaugmented</b>						
Day	PCE	TCE	cDCE	VC	Ethene	Methane
1	40	0.5	1	< 0.1	< 0.05	--
18	< 0.3	< 0.3	50	< 0.1	< 0.05	< 1
33	< 0.3	< 0.3	< 0.4	< 0.1	40	6
63	--	--	--	--	--	--

Demonstrating the *in situ* biodegradation potential of benzene by indigenous microorganisms under actual aquifer condition - Stable isotope probing.

Bio-Sep<sup>®</sup> beads can also be “baited” with a variety of organic compounds by vapor phase adsorption onto the PAC component of the beads post-fabrication. We have loaded benzene onto Bio-Sep<sup>®</sup> beads in this way at concentrations of up to 1.5 mg/bead. Benzene-baited beads show less than 5% loss of the hydrocarbon in an aqueous phase over a 30-day period in the absence of biological activity (data not shown). However, the adsorbed hydrocarbon has been shown to be bioavailable to bacteria that form biofilms in the beads during incubation in a contaminated aquifer. If the benzene, or other organic compound, is labeled with <sup>13</sup>C, polar lipids may be extracted from bead biofilms and derived FAMES analyzed for <sup>13</sup>C incorporation using GC-IRMS

(Boschker et al., 1998). Since the beads are clean of biomarkers and sterile when deployed, incorporation of  $^{13}\text{C}$  in phospholipids provides proof of *in situ* biodegradation of a target compound by indigenous microorganisms under actual aquifer conditions. This has the potential of significantly reducing the cost of site characterization while providing more precise evidence of biodegradation potential (Geyer et al., 2005). Further, identification of the specific lipids that become labeled can provide insight into what specific bacteria are involved in the degradation of the target compound (White et al., 1997).

The following case study illustrates the utility of this method. The site used in this experiment was an aquifer contaminated by a leaking underground storage tank at a retail gasoline station. Bio-traps containing 450 Bio-Sep<sup>®</sup> beads which had been loaded with a mixture of 92% natural benzene and 8%  $^{13}\text{C}$ -benzene were deployed in triplicate in a monitoring well 113 m downgradient from the source. The benzene concentration in the groundwater at the time of deployment of the bio-traps was 6  $\mu\text{g/L}$ . A Styrofoam float was to maintain the bio-traps 30 cm below the water level in the well. All traps were retrieved after 45 days of incubation. Aliquots of beads were extracted with methylene chloride (2 mL/bead) and analyzed for residual  $^{12}\text{C}$ -benzene and  $^{13}\text{C}$ -benzene by GC-MS. Polar lipids were extracted from the remaining beads and transesterified into FAMES that were then analyzed by GC-IRMS to determine  $^{13}\text{C}$  content.

Before deployment the baited Bio-Sep<sup>®</sup> contained  $1.51 \pm 0.30$  mg/bead total benzene (mean  $\pm$  std. dev., N=6). As noted above Bio-Sep<sup>®</sup> beads loaded with benzene have been shown to leach very little of the compound (<5%) during incubation in sterile water. However, a comparison of the benzene concentrations within pre- and post-incubation beads showed a 73% loss of benzene after deployment. There was also a significant increase in the percent  $^{13}\text{C}$ -labeled benzene compared to the pre-incubation composition (from  $7.6 \pm 0.97$ , N=6;  $9.4 \pm 0.75$ , N=3 pre- and post-incubation, respectively). This difference strongly suggests that the observed depletion of benzene in the bio-traps during incubation was due to biodegradation since it is well known that bacteria show a preference for  $^{12}\text{C}$ -labeled substrates. Therefore, the pool of benzene left in the beads would be expected to be enriched for  $^{13}\text{C}$  over time since  $^{12}\text{C}$ -benzene would be degraded at a faster rate (Baedecker et al., 1993).

When phospholipid fatty acids were extracted from the recovered bio-traps and analyzed for  $^{13}\text{C}$  several of the dominant fatty acids were shown to have been significantly enriched with  $^{13}\text{C}$  (Table 3). For comparison the  $\delta^{13}\text{C}$  of natural benzene is about -26 ‰ (Stehmeier et al., 1999). The only way for the phospholipid fatty acids to have become enriched in  $^{13}\text{C}$  is for the bacteria in the bio-traps to have metabolized the  $^{13}\text{C}$ -benzene and incorporated  $^{13}\text{C}$  into biomass components. Since the bio-traps were sterile and clean of biomarkers when they were introduced into the aquifer it was clear that bacteria indigenous to this aquifer were capable of benzene biodegradation under aquifer conditions.

Bio-traps with stable isotope probing have also been successfully used to document the *in situ* biodegradation potential of toluene, chlorobenzene, p-xylene, methyl-t-butyl ether (MTBE) and t-butyl alcohol (TBA).

## Conclusions

Documenting the geochemical and geomicrobial parameters necessary to support a natural attenuation management strategy for impacted groundwater can be a costly undertaking. The actual extent of the site characterization required to support a risk-based management strategy in the U.S. varies from state to state. However, the goals remain the same: to deduce the prevalent bioprocesses in the subsurface and to determine whether natural attenuation will prevent exposure of environmental receptors to the hydrocarbon plume. With respect to the prevalent bioprocesses the key word is to “deduce” – that is, these data amount to circumstantial evidence of intrinsic bioremediation. What is needed is a definitive signature as direct evidence of biodegradation potential. We propose that this signature lies in the *in situ* microbial ecology represented by subsurface biofilms. This signature can be obtained more definitively and at a reduced cost using the Bio-Sep<sup>®</sup> bio-traps in comparison to the more conventional site investigations methods. Bio-trap biofilms provide

interpretable and relevant data regarding subsurface microbial ecology that can be used to discern operative *in situ* bioprocesses. Bio-traps can predict the effect of a potential remediation amendment on subsurface microbial ecology and be used to evaluate the impact of the field injection of a remediation amendment. Lastly, bio-traps can be used to demonstrate the *in situ* biodegradation potential of specific pollutants by indigenous microorganisms under actual aquifer condition.

Table 3.  $\delta^{13}\text{C}$  Values of individual fatty acids derived from phospholipids from the  $^{13}\text{C}$ -benzene baited bio-traps

Phospholipid fatty acid	$\delta^{13}\text{C}$ (‰)*
16:1 $\omega$ 7c	+5699 $\pm$ 161**
16:0	+5342 $\pm$ 240
18:1 $\omega$ 7c	+3514 $\pm$ 756
18:1 $\omega$ 9c	+754 (n=1)***
cy19:0	+1055 (n=1)

\*The carbon isotope ratio of fatty acids is reported in  $\delta$ -notation (per mill) relative to Vienna Pee Dee Belemnite standard (PDB) according to

$$\delta_t^{13}\text{C} [\text{‰}] = \left( \frac{(^{13}\text{C}/^{12}\text{C})_{\text{Sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{Standard}}} - 1 \right) \cdot 1000$$

The  $\delta^{13}\text{C}$  of natural benzene is about -26 ‰. Values more positive reflect increasing enrichment with  $^{13}\text{C}$ .

\*\* Mean  $\pm$  std. dev. (N=3)

\*\*\*One observation

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