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An ex situ evaluation of TBA- and MTBE-baited bio-traps

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ABSTRACT

Aquifer microbial communities can be investigated using Bio-traps® ("bio-traps"), passive samplers containing Bio-Sep® beads ("bio-beads") that are deployed in monitoring wells to be colonized by bacteria delivered via groundwater flow through the well. When bio-beads are "baited" with organic contaminants enriched in ^{13}C, stable isotope probing allows assessment of the composition and activity of the microbial community. This study used an *ex situ* system fed by groundwater continuously extracted from an adjacent monitoring well within an experimentally-created aerobic zone treating a *tert*-butyl alcohol (TBA) plume. The goal was to evaluate aspects of bio-trap performance that cannot be studied quantitatively *in situ*. The measured groundwater flow through a bio-trap housing suggests that such traps might typically "sample" about 1.8 L per month. The desorption of TBA or methyl *tert*-butyl ether (MTBE) bait from bio-traps during a typical deployment duration of 6 weeks was approximately 90% and 45%, respectively, of the total initial bait load, with initially high rate of mass loss that decreased markedly after a few days. The concentration of TBA in groundwater flowing by the TBA-baited bio-beads was estimated to be as high as 3400 mg/L during the first few days, which would be expected to inhibit growth of TBA-degrading microbes. Initial inhibition was also implied for the MTBE-baited bio-trap, but at lower concentrations and for a shorter time. After a few days, concentrations in groundwater flowing through the bio-traps dropped below inhibitory concentrations but remained 4–5 orders of magnitude higher than TBA or MTBE concentrations within the aquifer at the experimental site. Desorption from the bio-beads during *ex situ* deployment occurred at first as predicted by prior sorption analyses of bio-beads but with apparent hysteresis thereafter, possibly due to mass transfer limitations caused by colonizing microbes. These results suggest that TBA- or MTBE-baited bio-traps could be baited at lower initial total mass loading with no detriment to trapping ability. The bio-traps were able to collect detectable amounts of microbial DNA and thus allow some insight into the sparse microbial community present in the aquifer during remediation of the low concentration plume.

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1. Introduction

In situ bioremediation is frequently the most cost-effective method for remediation of a gasoline spill that has impacted- groundwater. However, demonstrating that a microbially-mediated degradation pathway is actively destroying contaminant mass can be challenging and time-consuming. To date, microbial community activity has been assessed by 1) microbial analysis of sediment and groundwater samples, 2) microcosm studies in the laboratory with site groundwater and/or sediments, or 3) *in situ* microcosms within wells or boreholes. A widely used, commercially available *in situ* microcosm is the Bio-trap ("trap"), which has been used in many configurations and applications, but is typically a narrow, slotted PVC pipe similar to a well screen, filled with Bio-Sep® beads ("bio-beads") (25% Nomex and 75% powder activated carbon [PAC]) (White et al., 2003). Bio-traps are typically "deployed," i.e., incubated, in a monitoring well or borehole for 4–6 weeks, similar to other *in situ* microcosms (Geyer et al., 2005; Peacock et al., 2004; Reardon et al., 2004; Stelzer et al., 2006). Groundwater is assumed to flow through the slots in the trap housing, contacting the bio-beads, and microbes in the groundwater are assumed to make their way in to colonize the internal porosity of the bio-beads. Since traps are sterilized prior to deployment, any microbes that accumulate on or within the bio-beads during deployment must be derived from the subsurface microbial community.

It has been reported that microbes accumulate in bead pore spaces, but not on external bead surfaces (White et al., 2003). The external bead surfaces are reportedly predominantly Nomex, which is thought to form an ultra-filtration-type membrane with 1–10 μm holes and tears, of an average 1.9 μm size (Busch-Harris et al., 2008; Sublette et al., 1996). Microbes are thought to move through these tears and colonize the internal pores of the beads, where they are protected from groundwater flow and also from larger, predatory microorganisms (Busch-Harris et al., 2008).

Traps can be "baited" prior to *in situ* deployment by exposing the bio-beads to the contaminant of concern, typically from a vapor phase (e.g., hydrocarbons, fuel oxygenates, etc.). Sorption is predominantly to the surfaces within the internal pore structure of the particles of PAC used to make the bio-beads. The bait can be artificially enriched in the stable carbon isotope, ^{13}C, in order to conduct "stable isotope probing," i.e. to directly connect metabolism of the enriched compound to microbial groups or specific microbes by their incorporation of the stable isotope into cellular components (Geyer et al., 2005). DNA, RNA, or phospholipid fatty acids (PLFAs) extracted from the biomass can be analyzed for enrichment in ^{13}C and provide insight into which microbes or groups of microbes are utilizing the ^{13}C-enriched substrate for cell growth (Busch-Harris et al., 2008; Geyer et al., 2005). Detection of ^{13}C-enriched carbon dioxide is evidence that some of the substrate or intermediate metabolites are oxidized for cellular energy (Busch-Harris et al., 2008).

Bait must desorb to some extent into the porewater within or immediately surrounding the beads in order for it to be available to microbes. If not all of the desorbed bait is degraded by microbes within the beads, some may escape the bio-bead and the bio-trap via diffusion or advection. The potential for loss of the bait from the bio-traps inversely correlates with the affinity of the bait for the activated carbon. For example, in the absence of biological activity, benzene loses are typically less that 5% over a 30-day period. This loss of desorbed bait would be most rapid immediately after trap deployment, i.e. before significant microbial colonization of the beads has occurred, since microbial growth could potentially create barriers to diffusion of bait from smaller pores to larger pores or ultimately to the porewater surrounding the bio-beads. This diffusion barrier has been noted for biofilms on granular activated carbon (GAC) (Ehrhardt and Rehm, 1989; Mason et al., 2000).

In this work, we studied two baits that do not sorb very strongly to activated carbon and thus, to bio-beads: *tert*-butyl alcohol (TBA) and methyl *tert*-butyl ether (MTBE) (Lal et al., 2008). Our motivation was triggered by the observation of significant increases in concentrations of both compounds in bio-trap deployment wells during field trials of TBA- and MTBE-baited bio-traps at Vandenberg AFB (VAFB), Site 60, between March 2006 and October 2008 (Mackay, unpublished results). The primary objectives of this work were to quantify: i) the rate of groundwater flow through a bio-trap housing and ii) the rate of elution of ^{13}C-enriched TBA and MTBE from the bio-beads within a bio-trap during a typical deployment duration. The rate of groundwater flow through a bio-trap housing was estimated using an approach similar to passive flux meters (Annable et al., 2005; Hatfield et al., 2004), i.e. based on loss of tracers presorbed to GAC loaded in the trap housing. We then estimated the rate of flow through a housing loaded with bio-beads based on differences between the bio-beads and the GAC, as described later. Since this experiment was not possible with an *in situ* deployment, we used an "*ex situ* system" designed to mimic, as closely as possible, the *in situ* environment within the TBA plume undergoing aerobic remediation at VAFB Site 60. In addition, we sought to determine whether desorption of the bait followed expectations based on prior sorption studies of TBA and MTBE on bio-beads (Lal et al., 2008). To our knowledge, this is the first attempt at quantification of flow through a bio-trap under the conditions encountered during deployment in a well.

2. Experimental site

Site 60, Vandenberg Air Force Base (VAFB) in Lompoc, California, is the location of a former service station and associated groundwater contamination. The area encompassing the original source and the impacted area 200 feet downgradient (Fig. 1) served as a research site for the *in situ* investigation of the bioremediation of gasoline oxygenates and the impacts of ethanol contamination in the subsurface from 1999 to 2009. The following is a brief release history and summary of hydrogeological characteristics of the site; more detail is provided by Mackay et al. (2006).

An estimated 572 gallon (2165 L) gasoline leak occurred in late 1994. The underground storage tanks and piping were removed in early 1995 and the excavation was backfilled with sand and gravel. Monitoring at the site has shown that sulfate

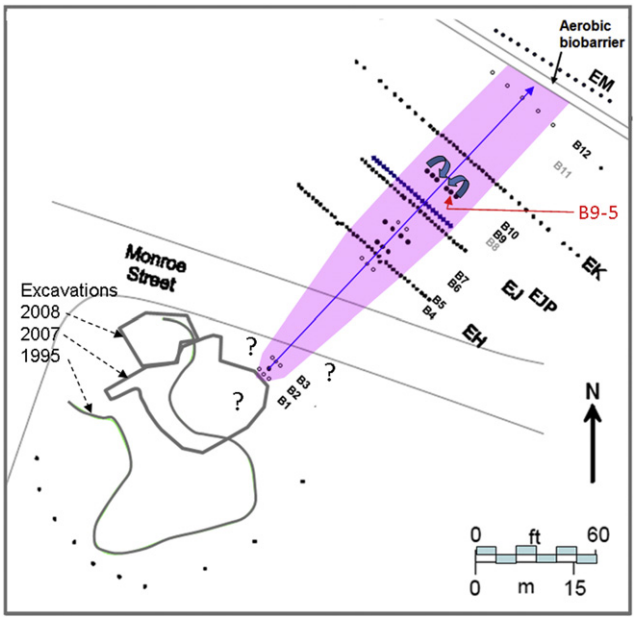

Fig. 1 – VAFB site 60 site map indicating the location of excavations, monitoring well transects, and the approximate extent and centerline of the TBA plume at the time of the *ex situ* experiment. The locations of monitoring wells used for the double well pair recirculation/oxygenation system are indicated with arrows in B9 transects. Monitoring well B9-5, located in between the injection and extraction wells on the eastern side of the system, was used to supply groundwater from the aerobic treatment zone to the *ex situ* system.

is the predominant dissolved electron acceptor in groundwater; dissolved oxygen has not been detected in the groundwater unless added experimentally or by an engineered system. The BTEX plume resulting from the spill was detected no further than 25 m from the source but the MTBE plume extended to at least 520 m from the source, as reported by consultants to VAFB. Within a decade after the gasoline release, BTEX concentrations in groundwater had diminished to below detectable levels. In contrast, a plume of MTBE and its transformation product TBA continued to emanate from the original source at concentrations above acceptable levels until 2010 (Shaw Environmental, Inc., 2010). TBA was the primary contaminant detected in groundwater at the experimental site during the *ex situ* evaluation of bio-traps; the TBA concentration maximum along the plume centerline was 193 μg/L and MTBE was non-detect (≤0.8 μg/L).

Previous research at VAFB Site 60 demonstrated the ability of the native microbial community to degrade TBA and MTBE *in situ* under experimentally-created aerobic conditions (Wilson et al., 2002). Hristova et al. (2003) identified a strain of bacteria with 99% similarity in 16S ribosomal DNA (rDNA) to *Methylibium petroleiphilum* strain PM1 within the experimentally-created aerobic zone. Strain PM1 is one of the few microbial strains isolated in pure culture that is capable of mineralizing MTBE and its metabolites, including TBA, when supplied with oxygen (Deeb et al., 2000; Hanson et al., 1999; Wilson et al., 2002). However, laboratory studies have shown that MTBE concentrations in excess of 1000 mg/L are inhibitory to growth of PM1 (Schmidt et al., 2008); a similar inhibitory limit has been observed for TBA (R. Schmidt, personal communication, 2011).

The *ex situ* experiment relied on extracting groundwater from an aerobic treatment zone created by a recirculation/oxygenation system utilizing two well pairs located in the B9 transect (Fig. 1) (North et al., in press). This two well pair system successfully promoted aerobic biodegradation of the TBA-dominated plume within and downgradient of the recirculation zones. The aerobic zone was established over 7 months before the beginning of the *ex situ* experiment and was continued until completion of the experiment.

3. Materials and methods

3.1. Ex situ *experimental system*

The *ex situ* experimental system continuously extracted a small flow of groundwater from monitoring well B9-5, located midway between the eastern extraction (B9-6) and injection (B9-4) wells of the recirculation/oxygenation system, which were 1.6 m apart (Fig. 1). Well B9-5 sampled the middle of the eastern half of the *in situ* aerobic treatment zone created by recirculation/oxygenation. At B9-5, the *in situ* dissolved oxygen concentrations ranged from 7 to 10 mg/L and TBA concentrations were non-detect (<3 μg/L).

The *ex situ* system (Fig. 2) was housed in an air-conditioned, insulated box located immediately adjacent to well B9-5. Groundwater was extracted from B9-5 and pumped into a 3-L HDPE reservoir using a peristaltic pump (Masterflex model HV-07591-00). Water was withdrawn from the bottom of the reservoir into a manifold with seven effluent tubes using a second multichannel peristaltic pump. A continuous length of pump tubing (Masterflex Norprene L/S 14) ran from the manifold and though the pump cartridges to the influent of each of seven chambers (discussed below). Effluent from each chamber was conveyed by tubing to 60 mL plastic syringe bodies; the flow rate could be quickly confirmed by counting drops from the tube into the syringe body during a known time interval. The effluent from the bottom of the syringe was directed to a waste container outside the cooled shed. The total rate of extraction of groundwater from well B9-5 was at all times greater than the total flow directed through the seven chambers; thus there was a constant overflow from the reservoir, which also drained to the waste container outside the shed. This overflow was maintained to ensure that water directed to the chambers was not exposed to the atmosphere.

The bio-trap housings used in this experiment were supplied by Microbial Insights, Inc. (MI); each was a 2.1-cm outer diameter (OD), 8.6-cm tall section of PVC with horizontal slots (2 mm, designed to retain Bio-Sep® beads of 3–4 mm diameter) that were 8 mm apart, similar to a well screen, and PVC end caps. The seven chambers each contained one bio-trap housing (Fig. 2c) filled with one of seven different materials, as specified in Fig. 2a. In this paper, we discuss only the results for chambers 5, 6, and 7. All bio-trap housing materials were sterilized in an autoclave prior to deployment. The tracer-laden GAC was silver-impregnated by

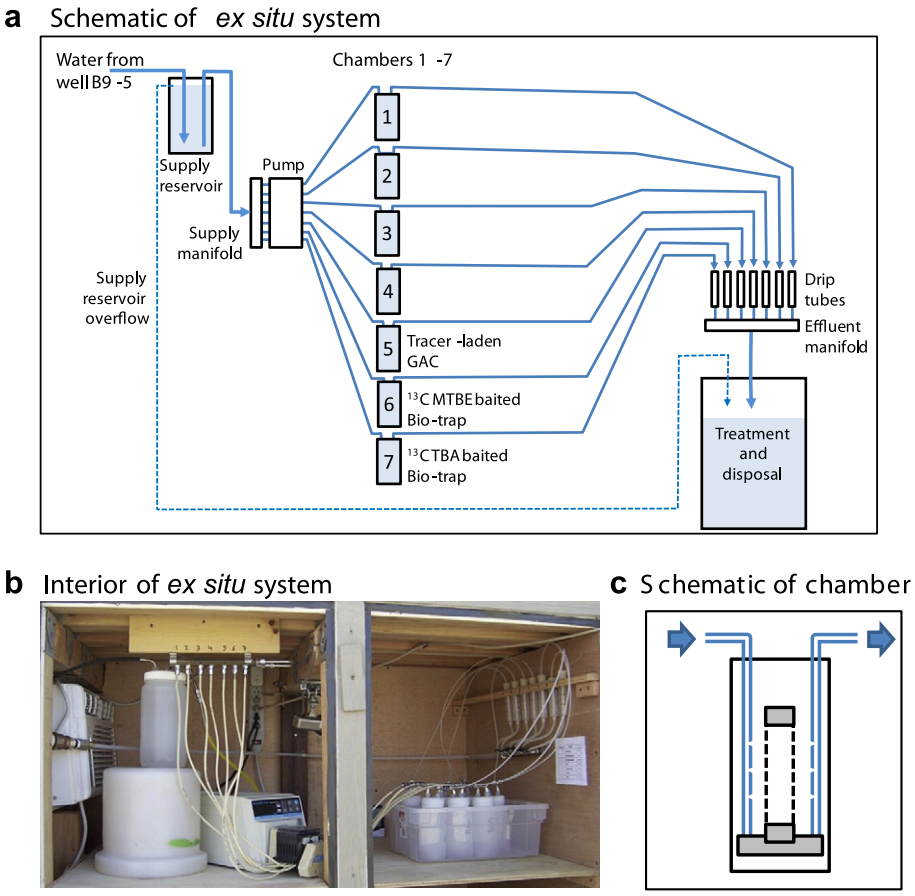

Fig. 2 – *Ex situ* system set-up: a) schematic of system, illustrating parallel flows through 7 chambers, each containing bio-trap housings with various contents, as listed, b) photo of the interior of the insulated box housing the system, c) schematic of chamber, showing influent and effluent stainless steel lines, each with small slots filed into the inner facing surfaces, and the bio-trap housing centered between influent and effluent lines by bottom fitting and top stainless steel wire (not depicted).

the manufacturer (Barnebey & Sutcliffe) to prevent microbial growth from interfering with tracer desorption; the bio-trap beads were sterilized in a muffle furnace at 400 °C by MI prior to shipment.

The chambers were 500 mL cylindrical HDPE bottles (Nalgene), 7.6 cm OD and 16.5 cm high. The chambers were therefore intermediate in diameter between that of a standard 2" (5.1 cm) or 4" (10.2 cm) PVC well screen. The groundwater captured by an unpumped well with a highly efficient screen in a permeable aquifer comes from a zone within the aquifer that is approximately twice the width of the well (Wilson et al., 1997). Thus the groundwater discharge through a hypothetical 3" (7.6 cm) diameter well, which our *ex situ* chambers were represented, was estimated at 2.7 mL/min, based on the estimated site groundwater velocity of 0.5 m/day, measured aquifer porosity of 0.34, and the height of the chamber.

The tracer-laden GAC (Chamber 5) was similar to that used in passive flux meters (Annable et al., 2005; Hatfield et al., 2004). The tracer-laden GAC particle size was 12 × 30 mesh (0.6–1.7 mm) and was impregnated by the manufacturer, Barnebey & Sutcliffe, with 0.026% silver to prevent microbial growth. Table 1 lists selected physical properties of the GAC. Nylon mesh fabric (Precision Woven Nylon Mesh 24.6 × 24.6,

Table 1 – Physical properties of Bio-Sep® beads and tracer-laden GAC.

Property	Units	Bio-beads	Tracer GAC
Particle diameter	mm	***3–4***	***0.6–1.7***
Specific surface area	m^2/g	***460***	***1103***
Total intraparticle porosity	%	***74***	66
Total interparticle porosity	%	***47***	30
Volume of pores with diameter < 0.3 μm	cm^3/g	***0.10***	***0.22***
Volume of pores with diameter > 0.3 μm	cm^3/g	1.54	0.81
Total intraparticle pore volume	cm^3/g	1.63	1.03
Total intraparticle pore volume	cm^3/bead	0.017	n/a

Measured values are in ***bold italic*** (source of information is either manufacturer or results of surface area and pore volume analysis by nitrogen adsorption by commercial laboratory). Other values are calculated from the measured values.

0.0295" opening, McMaster Carr) was used to line the bio-trap housing prior to filling it with tracer-laden GAC, which was too small to be retained by the 2 mm slots. Based on prior work, it is known the tracer mass loss is related to cumulative flow passing through the tracer-laden GAC (Annable et al., 2005; Hatfield et al., 2004). Five tracers were used with known and varying sorption strength (Hansch et al., 1995) (Table 2) so that at least one would optimally desorb at ~50% during deployment: methyl alcohol, ethanol, isopropyl alcohol, *tert*-butyl alcohol, and 2,4-dimethyl-3-pentanol. A bio-trap housing filled with tracer-laden GAC was deployed in a chamber. After 15 days, the estimated necessary deployment time, the housing was removed from the chamber. The GAC transferred to a clean glass jar with a Teflon-lined lid, which was then shipped overnight with ice packs to the University of Florida for analysis.

Baited bio-beads, emplaced in bio-trap housings, were provided by Microbial Insights, Inc. (MI). Bio-traps were stored at 4 °C and handled sparingly with nitrile gloves, as recommended by MI protocols. Table 1 lists selected physical properties of the bio-beads. Bio-beads are 3–4 mm in diameter and weigh approximately 10.7 mg each. The bio-beads were loaded by vapor-phase adsorption by MI with 100% ^{13}C MTBE or TBA (Busch-Harris et al., 2008); the initial mass loading is listed in Table 3. Loading was determined by MI as described by Busch-Harris et al. (2008); measurement error was estimated to be ±5%. The baited bio-traps were sealed in a plastic bag and delivered overnight with ice packs. After deployment in the *ex situ* system for 6 weeks (42 days), the bio-traps were removed from the chambers, sealed in plastic bags, and couriered back to MI with ice packs. MI analyzed a subset of the bio-beads for remaining bait by the previously-referenced method. The total number of beads in the baited bio-traps used in this experiment was not counted but instead estimated from counting beads in five bio-traps obtained from MI six months later (range 225–266 beads, with an average of 247).

3.2. *Sampling and analysis*

Dissolved oxygen concentrations were collected weekly from the influent and effluent of the *ex situ* experimental system. Samples were analyzed using a test kit with drop count titration (HACH Model OX-2P), per manufacturer instructions.

Water samples for TBA and MTBE analysis were collected from the effluent lines of chambers containing the TBA- and MTBE-baited traps. Samples were collected in 22 mL glass hypovials containing 0.5 g of powdered trisodium phosphate dodecahydrate. Subsequent handling, storage transport and analysis as described by Mackay et al. (2006). The detection limits for this method were 3 μg/L for TBA and 0.8 μg/L for MTBE.

DNA was extracted from approximately 30 bio-beads by Microbial Insights, Inc., using the Bio101 FastDNA SPIN Kit and according to manufacturer specifications. DNA was analyzed for universal bacteria 16S rRNA and *M. petroleiphilum* strain PM1 16S rRNA by quantitative PCR (qPCR) by MI. The primers and probes for universal bacteria and PM1 are detailed in Harms et al. (2003) and Hristova et al. (2001), respectively. Total uncertainty of the PM1 16S rRNA cell estimates for environmental samples is quantified in Hristova et al. (2001).

Table 2 – Sorption characteristics of the five tracers applied to the tracer-laden GAC.

Compound	Log K_{ow}	R_f	Reference
Methyl alcohol	−0.77	5	Hansch et al., 1995
Ethanol	−0.31	27	Hansch et al., 1995
Isopropyl alcohol	0.05	120	Hansch et al., 1995
Tert-butyl alcohol	0.35	1.003	Schmidt et al., 2001; Mackay et al., 2006
2,4-Dimethyl-3-pentanol	2.09	25,000	Hansch et al., 1995

4. Results

4.1. *System operation*

Water was extracted from the *in situ* aerobic treatment zone by well B9-5 and then supplied by the *ex situ* system to each chamber at a constant flow of 2.3 mL/min for the entire duration of the experiment (measured weekly). The extracted water was aerobic and remained so during flow through the chambers; dissolved oxygen ranged between 5 and 8 mg/L in both the influent and effluent for the duration of the experiment.

4.2. *Flow through a bio-trap housing*

Analysis of the tracer-laden GAC indicated that 50.3% of the isopropyl alcohol mass was lost during the 15-day deployment. Based on this loss, the known sorption affinity of the tracer for the GAC, the deployment duration, and the internal volume of the trap, we calculated that the average flow rate through the trap was 0.028 mL/min. This was approximately 1.2% of the total flow through the chamber, which is reasonable since the internal void volume of the bio-trap housing was approximately 3.4% of the total chamber volume. It was expected that water would preferentially flow around the bio-trap rather than through it since the screen and tracer-laden GAC present slight obstructions to flow. If we assume flow was horizontal within the chambers, only a portion of that flow would be able to pass through the bio-trap, i.e. the portion moving through the vertical interval of the chamber corresponding to the bio-trap screen. Compared to flow within the screened vertical interval, we estimate that approximately 2.3% of the total flow passed through the GAC-filled bio-trap.

The GAC served as a proxy for bio-beads in the bio-trap housing. A simple laboratory experiment, analogous to constant-head permeametry, indicated that, for similar conditions, the flow through a bio-bead-filled bio-trap is twice the flow through a GAC-filled bio-trap. Thus we calculate that approximately 4.6% of flow through the chamber would have traveled through the comercially-available, bio-bead-filled bio-trap.

Both tracer-laden GAC and bio-beads, when packed into housings, have large interparticle porosities (Table 1) and are designed to allow high flow through them, i.e., both have high hydraulic conductivities. If we assume that the hydraulic conductivities of the tracer-laden GAC and bio-beads are sufficiently similar, we can estimate the volume of

Table 3 – Estimates of TBA and MTBE bait lost from Bio-Sep® beads during the 6-week *ex situ* deployment.

Bait	Mass loss of bait based on analysis of beads before and after deployment; assumes 247 beads per trap				Mass loss of bait based on analysis of effluent from experimental chambers
	Pre (mg/bead)	Post (mg/bead)	Lost (mg/bead)	Total mass lost (mg/trap)	Total mass lost (mg/trap)
TBA	1.36 ± 0.14	0.06 ± 0.003	1.30 ± 0.14	321 ± 35	338 ± 34
MTBE	1.27 ± 0.07	0.15 ± 0.004	1.12 ± 0.07	277 ± 18	149 ± 15

groundwater that would typically be sampled during an *in situ* deployment of a standard bio-bead-filled bio-trap in a standard diameter monitoring well. Assuming a groundwater velocity of 0.5 m/d, as measured at VAFB, and horizontal flow in the aquifer through a standard 2" (5.1 cm) deployment well, the total flow through the appropriate vertical interval of the well (the height of the slotted section of the trap) would be 0.95 mL/min. Approximately 4.6% of that flow (i.e., 0.044 mL/min) would be expected to go through the bio-bead-filled bio-trap. Therefore, in a typical deployment duration of 6 weeks, about 2.7 L of groundwater could be expected to flow through and thus be "sampled" by a bio-trap. In effect, that volume of water "seeds" the bio-beads, and it is expected, especially for baited bio-beads, that there would be growth of microbes within the beads.

4.3. *Elution of bait from bio-beads*

While groundwater flow through the bio-trap has the advantage of allowing beads to be colonized by native microbes over the duration of deployment, the flow can also remove bait with low carbon affinity presorbed to the bio-beads. This is clear from Fig. 3, which presents the results of sampling effluent from chambers containing the TBA- and MTBE- baited bio-traps. Effluents from the chambers show an initial spike in bait concentration (TBA and MTBE maxima are 155 mg/L and 12.7 mg/L, respectively, then a rapid decline followed by more constant concentrations (approximately 1.3 mg/L and 0.4 mg/L, respectively) for the duration of the experiment.

The total mass of bait lost from the bio-traps (Table 3) was estimated by 1) analysis of bait concentration on bio-beads before and after deployment by MI and 2) analysis of the bait concentration in effluent from the chambers containing the baited bio-traps. On the basis of bio-bead analyses by MI, approximately 321 ± 35 mg of TBA and 277 ± 18 mg of MTBE were lost from the baited bio-traps during the *ex situ* deployment. This measurement results in percent losses for TBA and MTBE of 96% and 88%, respectively. Some losses may have occurred during shipment of the bio-traps to the site and back to MI, volatilization of the baits between the time the bio-traps are removed from the plastic bags and deployed, elution by groundwater flowing through the bio-traps, and degradation of the baits by microbes colonizing the bio-beads.

To evaluate the mass of bait lost to elution by groundwater flow through the bio-traps, we integrated the effluent concentration data in Fig. 3, assuming constant flow through the chambers of 2.3 mL/min. The total mass eluted is listed in Table 3. Over the 6-week experiment, approximately 338 g of TBA was estimated to have eluted from the TBA-baited bio-trap, whereas approximately 149 mg of MTBE was estimated to have eluted from the MTBE-baited bio-trap, i.e., approximately 90% and 45%, respectively. Approximately 93% of the TBA elution and 65% of MTBE elution occurred within the first week.

The total TBA mass lost from the bio-beads, based on bio-bead analysis, is in good agreement with the total mass eluted from the bio-trap based on effluent analysis. In contrast, the total MTBE mass loss based on bio-bead analysis was considerably higher than what was estimated to have eluted from the bio-beads by water flow through the bio-trap. The large discrepancy between the MTBE mass loss estimates may have resulted from losses of MTBE during transport to/from the site and handling at the site. Busch-Harris et al. (2008) included trip blanks in their work and found that two sets of MTBE-baited beads lost 13% and 31% of their mass during transport and handling during deployment. In this experiment, the difference in percent loss is 43%, but we believe this is expected considering that more time-consuming handling was required to insert the bio-traps in the *ex situ* system chambers than would be the case for deploying them in wells. The apparent difference in mass loss estimates could also be due to estimation methods; the bio-bead analysis is based on a sub-set of beads and an unknown, though constrained, number of total beads per trap.

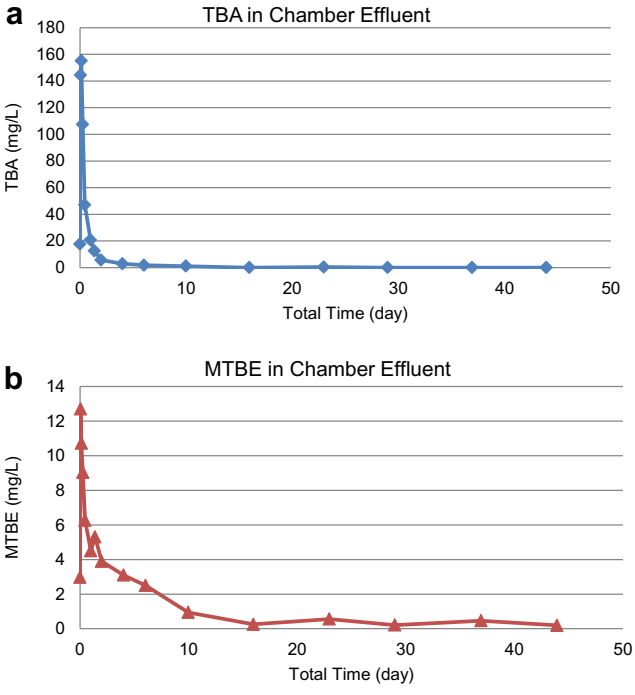

Fig. 3 – Concentration of TBA (a) and MTBE (b) In chamber effluents over the 42-day deployment. Note different y-axis scale for each plot.

The total mass eluted estimate is based on an integration of concentrations over time (after Levenspiel, 1979); the effluent measurements could have missed a higher MTBE concentration in the first several hours.

4.4. *Microbial communities collected on bio-traps*

Table 4 summarizes the average measured population densities of total universal bacteria 16S and PM1 16S as well as the proportion of PM1 and the estimated total number of PM1 cells in each trap. The TBA-baited trap collected more total bacteria and a higher proportion of PM1 than the MTBE-baited trap. In general, the results indicate there were low populations of fuel-oxygenate degraders present at the site during the experiment.

5. Discussion

5.1. *Elution of bio-trap bait into deployment wells*

The peak concentrations of TBA and MTBE in effluent from the chambers were 155 mg/L and 12.7 mg/L, respectively, both very high compared to concentrations in the plume at VAFB Site 60 at the time of this research, and quite high compared to concentrations in many TBA and MTBE plumes (Wilson et al., 2005). Thus, our results suggest that sampling of deployment wells during TBA- or MTBE-baited bio-trap deployment at any site may yield bait concentrations considerably higher than the ambient value in the aquifer at the time of sampling. In addition, because the baited compounds are enriched with ^{13}C, the release of bait into monitoring wells has the potential to interfere with compound-specific isotope analysis (CSIA). Some of the desorbed bait may also migrate downgradient of the deployment well within the aquifer, but this leads to very localized increases in TBA or MTBE concentrations (Mackay, unpublished results).

5.2. *Bait concentration in porewater within the bio-traps*

Because approximately 4.6% of flow through each *ex situ* chamber entered and exited the bio-bead-filled bio-trap, the porewater within the bio-trap had bait concentrations approximately 22 times higher than the chamber effluent at any given time. Fig. 4 presents calculated inter-bead porewater concentrations of TBA and MTBE within the baited bio-traps over the experiment duration. TBA and MTBE concentrations in porewater moving between the TBA- or MTBE-baited bio-beads reached approximately 3,400 mg/L and 280 mg/L, respectively. These concentrations are much higher than concentrations ever detected in ambient groundwater at VAFB Site 60 or, especially for TBA, generally observed in TBA/MTBE plumes at other sites. Wilson et al. (2005) reviewed monitoring data from various MTBE plumes in the US and found that the maximum MTBE concentration near the spill source was on the order of 500 mg/L Wilson and Adair (2007) reported the geometric mean concentrations of TBA and MTBE across three sets of U.S. fuel tank spill sites; the highest TBA mean was 1.73 mg/L and the highest MTBE mean was 1.72 mg/L (not from the same set). After the initial spike, the estimated TBA and MTBE concentrations declined to approximately 30 and 10 mg/L, respectively.

That the TBA and MTBE concentrations within the bio-traps were significantly higher than typical ambient values has the potential advantage of making the bio-beads an attractive location for planktonic microbes to colonize, as indeed this and past studies have suggested. However, concentrations of either TBA or MTBE above 1000 mg/L may be inhibitory to growth of many organisms. Laboratory strains of PM1, one known MTBE- and TBA-degrading bacterium detected at Site 60, are inhibited at these concentrations (Schmidt et al., 2008; R. Schmidt, personal communication, 2011); Busch-Harris et al. (2006) suggested TBA concentrations above 200–300 mg/L may have inhibited microbial colonization of bio-traps in their field applications. Thus, for a short period after deployment, the high concentrations of TBA in porewater surrounding the baited bio-beads investigated in this research likely would have inhibited growth, and possibly attachment, of the microbes they were meant to sample. In this work, the TBA-baited bio-trap may have been inhibitory for at least 5 h. The actual period of inhibition may have been greater since the concentrations of the desorbing baits necessarily were higher within the bio-bead pore structure than they were in the inter-bead porewater. However, the durations of the potential inhibition period were short compared to a typical 6-week deployments; thus, the baited bio-beads in this work quickly became hospitable to colonizing microbes and remained as such for the deployment duration.

5.3. *Desorption and elution of baits*

Lal et al. (2008) studied adsorption and desorption of TBA and MTBE by bio-beads in batch laboratory tests. They found that adsorption for single solute experiments was well described by a Freundlich isotherm, and furthermore, that there was little evidence of hysteresis during desorption of MTBE but clearer evidence during desorption of TBA. However, their studies were limited to equilibrium TBA or MTBE concentrations under 100 mg/L, which is one order of magnitude lower than estimated in this study for TBA or MTBE within bio-traps.

The observed elution of TBA and MTBE baits from bio-beads during deployment in the *ex situ* system is consistent in general

Table 4 – Average PM1 and total bacterial copy numbers from the TBA- and MTBE- baited traps, in cells/g bead.

Sample/Trap	UNIVERSAL 16S	PM1 16S	Percentage of Universal 16S that is PM1	Total PM1 (cells/trap) based on 247 beads/trap
TBA-baited Trap	1.7E+07	5.1E+04	0.30%	1.2E+07
MTBE-baited Trap	3.6E+06	1.6E+03	0.05%	4.0E+05

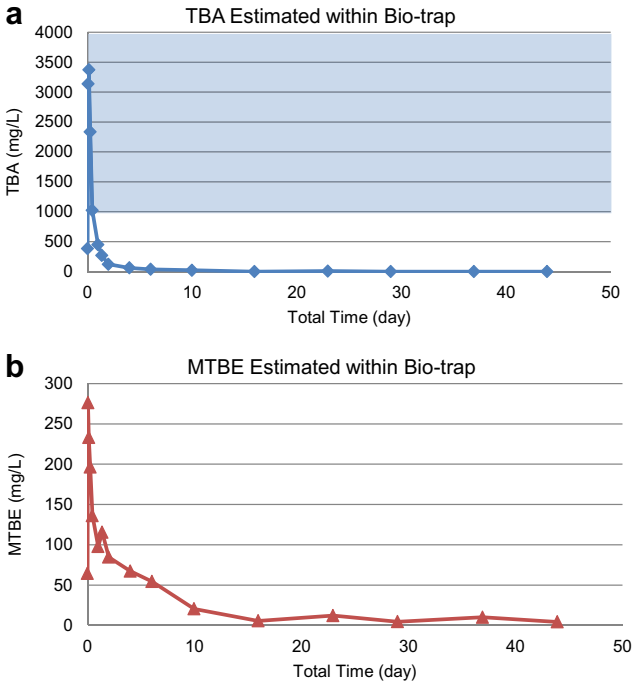

Fig. 4 – Estimated concentrations of bait in porewater within the bio-traps: (a) TBA-baited bio-trap, (b) MTBE-baited bio-trap. Note that concentrations above 1000 mg/L may be inhibitory to growth of aerobic MTBE/TBA degraders (shaded area in TBA plot).

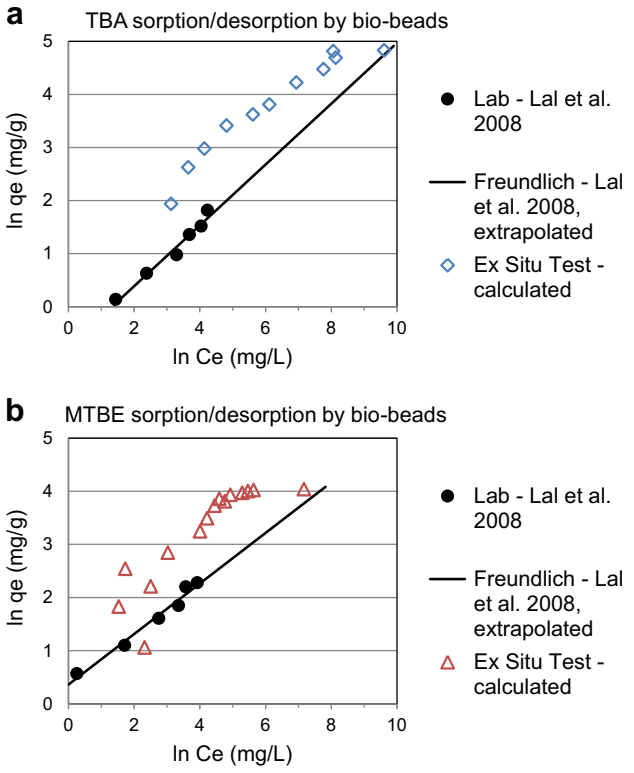

Fig. 5 – Plots of sorbed concentration of bait (qe) versus solution concentration of bait (Ce) for bio-beads in a lab study and this *ex situ* experiment: (a) TBA, (b) MTBE.

terms with expectations of desorption following a Freundlich isotherm, i.e., an initial spike and a rate of concentration decrease which lessens with time. As a quantitative evaluation of bait desorption from the bio-beads, we estimated sorbed concentrations of bait for each time we sampled effluent, from which we could estimate concentrations within the bio-traps. The sorbed concentration over time was calculated as the initial bait loading minus the bait mass estimated to have eluted in the chamber effluent up to that time, adjusted to the units required for a Freundlich plot (mg of bait per g of sorbent, i.e., bio-bead). Initial bait loading was calculated from the pre-deployment concentrations measurements made by MI. The mass of bait eluted was estimated by integrating the area under concentration versus elution volume plots (after Levenspiel, 1979). Elution volume was estimated as the product of elution time and the constant effluent flow rate. Thus, we generated a set of estimates of bait mass sorbed to bio-beads (mg/g) and concentration of bait in contact with the bio-beads. Assuming desorption was at equilibrium, we created a set of desorption isotherm data, which are plotted for TBA and MTBE in Fig. 5. Also plotted in Fig. 5 are the sorption data of Lal et al. (2008) as well as a line extrapolating their Freundlich isotherm to concentrations beyond their experimental data, which had a maximum concentration of 100 mg.

Fig. 5 shows that the Freundlich isotherms determined by Lal et al. (2008) are applicable to concentrations far beyond those they studied. The highest concentration data point calculated for TBA or MTBE in this study is in effect a part of an extended sorption isotherm, since the bio-beads are loaded with bait sorption prior to deployment. Although the data indicate physical nonequilibrium to desorption, the points in fact fit the extrapolated isotherm quite well, especially given the variability in the sorption data upon which the isotherm was based. This finding is quite useful, since it allows confident prediction of initial bait concentrations in porewater internal to bio-traps with bio-beads loaded to known TBA or MTBE sorbed concentrations. Thus, it is straightforward to determine the initial bait loading that would ensure initial aqueous bait concentrations were below inhibitory levels. Lower bait loading is advisable since higher bait loading may only lead to elution of the bait mass until the solution concentrations drop below inhibitory levels, i.e., loss of bait with no advantage for trapping targeted microbes.

Because bio-beads are specifically designed to promote microbial growth, desorption of bait from bio-beads into groundwater moving through the bio-trap would be expected to eventually be limited by microbial attachment and growth within the bead pores. Not only could this result in utilization of some of the bait but, given sufficient growth, could also create a barrier to diffusion of un-metabolized bait out of the bio-bead. Apparent hysteresis, such as that seen in Fig. 5, leads to solution concentrations that are lower than expected based on equilibrium assumptions for given sorbed concentrations (i.e., desorption may not have been at equilibrium under the *ex situ* experimental conditions). The impact of bait metabolism is less clear: if the microbes created diffusion barriers and also degraded a significant portion of the dissolved bait diffusing

out of the pores, then the solution concentrations of the bait outside the beads would be even lower than from diffusive limitations alone. The disappearance of bait due to microbial metabolism or desorption and elution from the *ex situ* chambers cannot be differentiated by this experiment.

Since bacteria are too large to fit into the micropores of the bio-beads, which have diameters less than 0.3 microns (Table 1), colonizing bacteria would be limited to the larger pores. We estimate that microbial biomass would have occupied only 0.03% of the bead macropore volume (>0.3 μm) in the TBA-baited trap, which had the highest biomass; this calculation assumed a typical bacterium has a volume of 3.0×10^{-13} cm^3 (Sylvia et al., 2004). Past work suggests that the dominant mechanism for sorption of TBA and MTBE solutes onto synthetic carbonaceous sorbents is micropore filling (Davis and Powers, 2000; Bi et al., 2005) i.e., initially "sorption" is more like condensation in the micropores rather than coverage of the bead surfaces. Thus, microbes attracted to the bait would presumably attach around openings of micropores and their colonization would eventually lead to bait degradation and/or limit bait diffusion into surrounding water. However, since the total TBA mass eluted from the TBA-baited bio-beads was very close to the total initial loading, it seems likely that the degradation of TBA was minimal in this *ex situ* study. If so, then the primary limitation to desorption must have been factors leading to physical nonequilibrium, i.e. narrow pore throats causing limitations to diffusion out of pores and/or pore blockage caused by microbial colonization.

5.4. *Enumeration of microbial populations*

Although microbes that attach or grow on beads can detach, in general, the quantitative PCR data indicate the presence of small populations of fuel-oxygenate degraders at the site during the experiment. Biomass was higher in the TBA-baited bio-trap than the MTBE-baited bio-trap, suggesting that the microbial community was more acclimated for TBA degradation and thus better able to colonize the TBA-baited trap at the time of this test. Indeed, MTBE had largely been undetectable at the site for approximately three years prior to the experiment. Laboratory strains of PM1 are capable of degrading both MTBE and TBA (Schmidt et al., 2008); however, in the field, populations of PM1-like organisms may have adapted more to preferentially degrading TBA rather than MTBE due to selective pressures associated with the presence of only TBA.

Two liter groundwater samples, filtered and extracted for DNA immediately prior to the *ex situ* experiment, did not yield measureable DNA. The fact that we could extract, however, measurable DNA from the bio-beads (Table 4) demonstrates the utility of the bio-trap approach, even in such a low concentration plume with low microbial activity. For comparison, measurements made in groundwater samples from the Site 60 TBA plume, during oxygenation two years prior when TBA concentrations were approximately four times higher than in this study (Kayne, 2008), were 1.2E + 05 and 6.6E + 02 copies/mL for total bacteria and PM1, respectively. The microbial community collected by bio-traps may not be identical to those in the planktonic or attached communities in the aquifer. Nevertheless, as described in detail by Busch-Harris et al. (2008), stable isotope probing enabled by the ^{13}C-enriched compounds sorbed to bio-beads allows bio-traps to detect the *in situ* capacity for biodegradation of specific compounds, even if the biodegradation within the aquifer is undetectable at the time of bio-trap deployment using traditional monitoring of groundwater.

6. Conclusions

An *ex situ* system was used to quantitatively evaluate the performance of bio-traps. The flow of water through baited bio-traps eluted sorbed bait from the traps as expected based on the sorption properties determined by others. The majority of the TBA and MTBE baits were desorbed from the Bio-Sep® beads and eluted from the traps in the first few days of deployment. The concentration of bait calculated at the bead surface during the first several hours of deployment of the TBA-baited trap would be expected to temporarily inhibit growth of the microbes able to metabolize the baits at lower concentrations. Once the elution lowered bait concentrations, microbes would have presumably have found the conditions within the bio-beads attractive and conducive to growth. Based on these results, it is possible to predict what maximum TBA or MTBE baiting levels to use to avoid inhibition in future bio-trap applications and minimize bait loss to the well and surrounding aquifer. Nevertheless, in this work, few microbes were collected by either the TBA- or MTBE-baited bio-traps, suggesting the native populations are active but low in this low-concentration TBA plume, even within the experimentally established *in situ* aerobic treatment zone. On the other hand, the bio-traps were more successful in collecting sufficient biomass for analysis, which was not the case for filtration of 2 L groundwater samples. Finally, when provided with high concentrations of the two fuel oxygenates, greater microbial biomass accumulated on the bio-beads baited with TBA, the contaminant the community was biodegrading *in situ* at the time.

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