Ground Water Monitoring&Remediation

Spatial Variation in MTBE Biodegradation Activity of Aquifer Solids Samples Collected in the Vicinity of a Flow-Through Aerobic Biobarrier

by Luis E. Lesser, Paul C. Johnson, Gerard E. Spinnler, Cristin L. Bruce, and Joseph P. Salanitro

Abstract

The spatial variation in methyl tert-butyl ether (MTBE) biodegradation activity of aquifer solids samples collected in the vicinity of a flow-through aerobic biobarrier was assessed through use of standard laboratory microcosms. These were prepared by collecting soil cores at a range of locations and depths along different flow paths through the biobarrier. Sections of core samples were placed in sealed bottles with MTBE-free groundwater from the site. The groundwater was filtered to remove microbes, and sparged with O_2. The initial MTBE concentration in the microcosms was adjusted to about 1 mg/L. Biodegradation activity was characterized by the magnitude of MTBE concentration reductions occurred over 4 weeks relative to control microcosms. Sampling locations and depths were selected to allow investigation of relationships between MTBE-degrading activity and dissolved oxygen (DO) concentration, MTBE, soil type, and initial microbial conditions (biostimulated vs. bioaugmented). The results suggest a relatively wide-spread presence of MTBE-degrading microbial consortia, with varying levels of MTBE-degrading activity. Significant changes in activity were observed over 0.3-m vertical distances in the same location; for example, cores from the most upgradient sampling locations contained sections with no discernible MTBE-biodegradation over 4 weeks, as well as sections that achieved order-of-magnitude MTBE concentration reductions within 2 weeks. None of those cores, however, achieved MTBE biodegradation to nondetect levels (<0.005 mg/L), as was observed in some cores from downgradient locations. Cores from the bioaugmented regions had the highest frequency of MTBE biodegradation to nondetect levels among their sections suggesting a direct effect of the inoculum and its distribution when it was implanted. Most cores with no activity were associated with the upgradient, low-DO, and high-MTBE concentration field environments, but low-DO field environments also yielded MTBE-degrading samples. There were no other clear correlations between MTBE-degrading activity in the microcosms and the local field environment conditions at the time of sampling.

Introduction

When biostimulation or bioaugmentation are chosen to treat a contaminated aquifer, the microbial community and its biodegradation performance characteristics (i.e., lag time, maximum utilization rate, effective first-order reaction rate, etc.) are often conceptualized as being uniform throughout the aquifer. This conceptualization then influences system design, the monitoring plan, and performance data interpretation. It is probably, however, that microbial communities and their ability to biodegrade target chemicals are more temporally and spatially variable than conceptualized, given the inherent heterogeneous nature of the physical and geochemical characteristics of most subsurface environments. When delivering microbial cultures to the subsurface, it is also difficult to achieve uniform distribution. The concept of a "bioactive zone" has been introduced in the literature; it can be defined as a region where a microbial community is sufficiently active to metabolize bioavailable substrates of interest (Yolcubal et al. 2003). The study of bioactive zones in laboratory experiments has shown heterogeneous biodegradation patterns and changes through time after inoculation (Thullner et al. 2002; Yarwood et al. 2002; Dorn et al. 2005). Some field studies have also demonstrated the spatial variability of microbial populations (Green-Blume et al. 2001; Sandrin et al. 2004).

Murphy et al. (1997) studied the effect of physical heterogeneities on the distribution of aerobic microbial biodegradation using a two-dimensional (100 × 20 × 10 cm) physical model. Individual biomass measurements were performed at 180 locations by colorimetric analysis of the

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polar lipid phosphates. Even though no units are reported for their data, a variability of at least 50% in biomass is suggested for samples spaced only a few centimeters apart. They found that low hydraulic conductivity inclusions created regions of slow transport and prolonged availability of dissolved oxygen (DO) which in turn enhanced microbial growth in these regions.

Yolcubal et al. (2003) studied the influence of DO and substrate availability on bioactive zones using 5-cm diameter × 10 cm-long columns equipped with five ports for data collection. Using fiberoptic/microbial luminescence techniques for localized in situ indirect measurements of microbial activity, they observed clearly delineated bioactive versus nonbioactive regions along the length of the column. They also found that the bioactive region location was influenced by DO and substrate concentrations.

Oates et al. (2005) developed a method to study reactive microbial transport using bioluminescence techniques, and applied this to a two-dimensional tank (31 × 6 × 2 cm) experiment. The data, which were collected with a digital camera, showed luminescence changes across a 3 to 4 cm distance, and a decrease in biodegradation activity as an oxygen-depletion zone developed.

In the field, Sandrin et al. (2004) examined the utility of a biotracer-based approach to study the spatial variability of microbial activity at two field sites. Their proof-of-concept test suggested that this was a promising approach, but the authors cautioned that further investigation was needed to better understand how to translate the observed variability in biotracer biodegradation to the desired knowledge of the variability in target chemical biodegradation. Naas et al. (2002) examined the spatial distribution of native aerobic methyl tert-butyl ether (MTBE) biodegradation activity at the MTBE plume in the Vandenberg Air Force Base, California, using 1-L aerobic microcosms with sediments from the site in triplicate. Upgradient of the source zone where no MTBE has historically been detected the initial MTBE concentration of the microcosms was 480 ug/L and was biodegraded within 20 d, including a lag period with no observed activity of 12 d. Within the source zone only one of the triplicates biodegraded MTBE within the 90 d of incubation. MTBE was biodegraded in mid-plume microcosms (from about 3 mg/L initial concentration) within 20 d with no apparent lag time. Distal-plume microcosms (0.45 mg/L initial concentration) biodegraded MTBE within 12 d after an 18-d lag period. All microcosms showed similar biodegradation characteristics with subsequent re-spiking of MTBE.

This study also focuses on the spatial variability in biodegradation activity. It differs from the studies discussed above in that the interest here is in assessing the spatial variability of MTBE biodegrading activity of aquifer solids samples collected in the vicinity of an operating and well-monitored aerobic flow-through MTBE biobarrier. The intent is not to determine the in situ rate of MTBE biodegradation at multiple points in the field, but instead the intent is to attempt to characterize the ability of each local microbial community to aerobically biodegrade MTBE under similar conditions. There are other related topics that are of a general scientific interest and arguably have important relevance to biodegradation activity (i.e., numbers of MTBE-degrading organisms and microbial community make-up), but those were not part of this study.

There has been discussion on methods for characterizing microbial activity (i.e., Sandrin et al. 2004), but there is also no clear resolution of this issue in the literature. In practice, enumeration techniques have been used for decades and with their use there is an inherent assumption that microbial biodegradation activity and degrader numbers are directly correlated; however, enumeration techniques are generally not contaminant and degrader-specific, and do not provide a direct measure of "activity," or how fast the microbial community can biodegrade a target chemical under a specific set of conditions. More recently, the development and use of phylogenetic probes has increased (i.e., Shi et al. 1999) to characterize populations and identify the presence and numbers of specific organisms, but again these do not provide measures of activity. Some have worked to develop more activity-related tools, including Yolcubal et al. (2003) and Oates et al. (2005) who proposed indirectly measuring microbial activity using luminescence methods rather than enumeration with soil cores.

This study is most similar to the Naas et al. (2002) study discussed above in that the conventional laboratory microcosm test is used as the tool to characterize the MTBE biodegrading activity of aquifer solids. It differs in that the interest here is assessing spatial variability in the vicinity of an operating and well-monitored engineered aerobic MTBE biobarrier, and that a consistent set of initial conditions was used for all microcosms.

Field Site

The dissolved MTBE plume at the Naval Base Ventura County, Port Hueneme, California, is attributed to a gasoline release that occurred in the mid-1980s at the Base service station. At the time of this study, the MTBE plume was about 1500-m long and about 150-m wide. Dissolved MTBE was present across the 3-m vertical extent of the surficial aquifer, which is encountered approximately 3-m below ground surface (bgs). The aquifer is bounded below by a clay aquitard and the saturated zone extends about 0.5 m into an upper silty fill layer. The majority of aquifer sediments between 3 to 6 m bgs are fine to medium sands, characterized by increasing coarseness with depth. Groundwater flows southwest, with hydraulic gradients ranging from 0.001 to 0.003 m/m, and the groundwater seepage velocity has been estimated from MTBE plume history and hydraulic property data to be about 0.3 m/d along the faster flow paths. The groundwater table fluctuates approximately 0.5 m throughout the year. The dissolved MTBE plume is anoxic under natural conditions, and although the MTBE plume is only weakly attenuated with distance, the dissolved benzene, toluene, ethylbenzene, and xylene cocontaminants are naturally attenuated within about 50 m of the downgradient edge of gasoline-containing source zone aquifer sediments. More site and MTBE plume details can be found in Johnson et al. (2002), Lesser et al. (2008), and Salanitro et al. (2000).

In August 2000, a full-scale aerobic flow-through biobarrier was installed immediately downgradient of the

source zone. The 150-m long biobarrier was oriented perpendicular to groundwater flow so that dissolved contaminants (MTBE, tertiary butyl alcohol [TBA], benzene, toluene, etc.) in groundwater would be aerobically biodegraded as they flowed through a well-oxygenated treatment zone. Oxygen was delivered in a pulsed mode by 144 injection wells and air by 108 injection wells, and the central section of the biobarrier was seeded with MTBE-degrading pure culture of a single organism (SC) and a mixed culture (MC). The study presented herein was performed over 2 years after the seeding occurred. More details on the cultures used for seeding and on the delivery methods can be found in Salanitro et al. (1994) and Salanitro et al. (2000). The different operating conditions and their position along the biobarrier are shown in Figure 1. MTBE treatment performance was assessed by sampling groundwater from about 400 monitoring wells and analyzing for dissolved oxygen (DO), MTBE, TBA, benzene, toluene, ethylbenzne, and xylene isomers. Within about 1 year, DO levels in groundwater increased from <1 mg/L to >5 mg/L in air injection zones and >20 mg/L in oxygen injection zones. Influent MTBE concentrations ranging from 0.01 to 10 mg/L across the width of the biobarrier were reduced to nondetect levels (<0.01 ug/L) as shown in Figure 2. The biobarrier was designed to ensure insignificant volatilization losses and to prevent groundwater flow from bypassing the treatment zone, and performance monitoring data verify that this was achieved. Thus, the MTBE attenuation along flow paths entering the biobarrier can confidently be attributed to aerobic biodegradation.

Study Design

In brief, this study involved the sampling of aquifer solids (referred to as "soil" below) and groundwater in the vicinity of the aerobic MTBE biobarrier, placing those soil and groundwater samples in microcosms, adjusting the initial conditions to consistent DO and MTBE concentrations, and then monitoring the decline in MTBE over 4 weeks. Below, the selection of sampling locations and sampling methods is discussed, and then followed with specific details of the microcosms.

Sampling locations were selected to provide data from four points along each of four different groundwater flow paths. As shown in Figure 1, the flow paths correspond to four different biobarrier operating conditions: biostimulation with air injection, biostimulation with O_2 injection, bioaugmentation using a pure culture of a SC with O_2 injection and bioaugmentation using an MC with O_2 injection. Along each flow path, one sample was collected upgradient in a higher MTBE/lower DO setting, a second was collected in the vicinity of significant MTBE groundwater concentration reductions with distance, the third was collected in the immediate vicinity of the gas injection wells and MTBE-degrading culture injection points (for two of the flow paths), and the fourth was located downgradient of the third

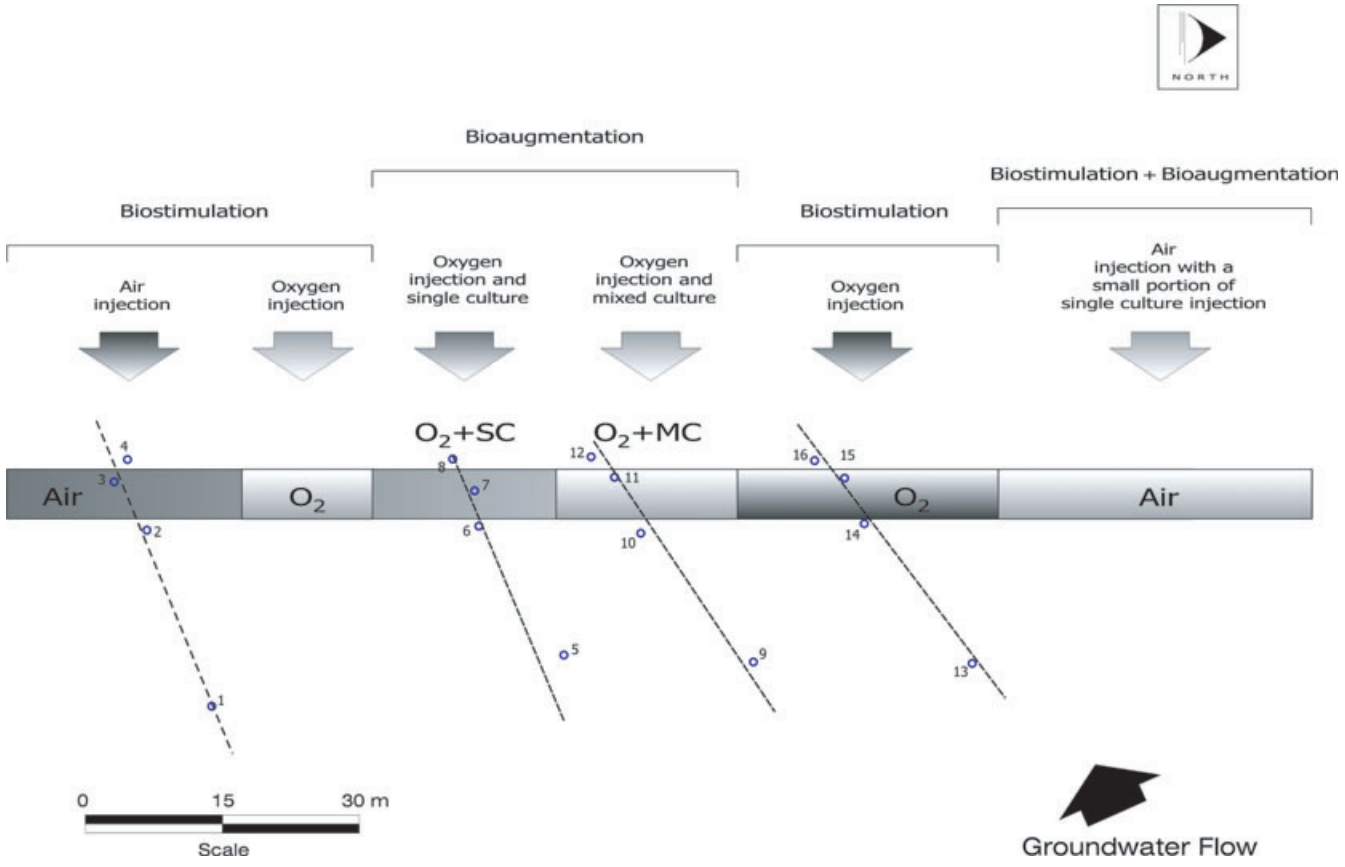

Figure 1. The different treatment zones in the biobarrier at Port Hueneme, California. Shown are the locations cored for at this study.

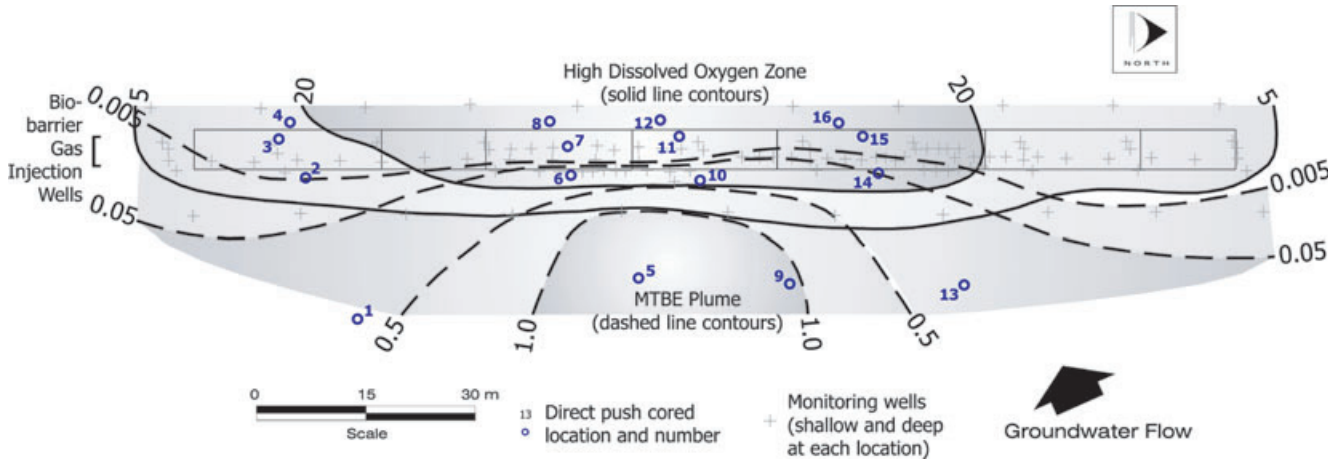

Figure 2. Dissolved MTBE plume and dissolved oxygen (DO) concentrations (mg/L) near the aerobic biobarrier. After 2 years of biobarrier performance, MTBE concentrations have dropped downstream to <0.02 mg/L and DO levels have increased to >20 mg/L. Data are from monitoring wells with screens from 3 to 4.6 m deep (modified from Johnson et al. 2002).

point, but generally still within the region of elevated DO concentrations.

Sample Collection

Soil cores and groundwater were collected during two sampling events spaced about 3 months apart (first locations 1 through 9 and later locations 10 through 16 shown in Figures 1 through 3). Samples were collected using Geoprobe® direct-push tools (Geoprobe Systems, Salina, Kansas). The core sampler was 1.2-m long × 3.8-cm ID (7.6-cm OD) and included an inner plastic liner and core sample retainer to minimize sample loss while pulling the core out of the ground. Inside and at the bottom of the core sampler is a drive point that is fixed until the desired sampling depth is reached, at which point it can be loosened so that it retracts through the core sampler as the sampler is advanced to a deeper depth and the soil core is collected. All cores were capped, labeled at the top and bottom with the depth interval, and then stored on ice in coolers until opened for use in the lab. Core sections were collected from depth intervals of 2.4 to 3.6 m bgs, 3.6 to 4.8 m bgs, and 4.8 to 6.0 m bgs at each location.

Groundwater samples were collected using a slotted 0.6-m long Geoprobe groundwater sampler (2.5-cm OD, 1.6-cm ID) and a peristaltic pump. At least 600 mL of groundwater were purged and the DO level, as measured in a flow-through cell, had stabilized prior to sample collection. Groundwater samples were collected in 3.8 L plastic cubitainers, which were stored in coolers and kept cold with ice until use in the lab. Groundwater samples were collected at shallow (2.5 to 3 m bgs) and deep (5.4 to 6 m bgs) depth intervals.

There was a maximum holding time of 5 d before the samples were processed in the lab.

Laboratory Microcosm Procedures

Each 1.2 m soil core was divided into two 0.6 m sections, so that there were six sections per location. First, the lithology of each was characterized according to the Wentworth classification (Davis, 1992). Then the aquifer solids from each section were manually homogenized in Ziplock® bags (SC Johnson & Son, Inc., Racine, Wisconsin). From each 0.6 m section one microcosm was prepared and labeled according to an alphabetical naming convention where the shallowest one (2.4 to 3.0 m bgs) was designated "a" and the deepest one (5.4 to 6.0 m bgs) was designated "f." The microcosms were prepared using 125 mL vials (20 mm neck) with crimp-seal caps. From each homogenized section, 80 g of soil was deposited in a vial. Duplicate microcosms were performed randomly on 10% of the 0.6 m soil core sections. Three respiratory inhibited controls were prepared by adding 2 g of solid sodium azide to those microcosms.

For each shallow soil sample collected from 2.4 to 4.2 m bgs, shallow groundwater from its location was added. For each deeper soil sample collected from 4.2 to 6.0 m bgs, deep groundwater from its location was added. All groundwater was filtered with 0.7 um filters (GF/F, Whatman filters) to avoid inoculating the microcosms with microorganisms present in the groundwater. The groundwater used to prepare the microcosms was also sparged with oxygen gas until the DO reached 30 mg/L. Using a plastic syringe, 60 mL of filtered groundwater was measured and deposited in each vial, leaving approximately 40 mL of headspace. The headspace was then briefly purged with oxygen gas. The vials were crimp-sealed with a Teflon septa and an aluminum cap, and then spiked with MTBE to an initial dissolved concentration of approximately 1 mg/L. The microcosms were kept in a constant temperature room at 23 °C and on a shaker table set at a speed as rapid as possible for continuous use.

Dissolved MTBE concentrations were analyzed weekly for 1 month by retrieving 0.5 mL of headspace for gas chromatography (GC) analyses. After sampling, each microcosm was refilled with 0.5 mL of oxygen. The GC (SRI Model 8610C) was equipped with a 0.53 mm ID × 60 m MXT-Vol glass capillary column with a 2.0 um df. The GC was also equipped with a flame ionization detector (FID—method detection limit of 0.01 mg/L) and a photo ionization detector (PID), with the latter being used only for the low (<0.01 mg/L) concentration range. At least three concentration standards spanning the concentration range of interest

were used (10, 1, and 0.01 mg/L). After every 10 samples, one of the standards was re-run to ensure instrument consistency (equal standards varied less than 10%).

Results and Discussion

With oxygen and MTBE addition to the microcosms, any microcosm containing even a few aerobic MTBE-degraders will eventually show MTBE loss. Thus, the loss of MTBE (relative to control microcosms) is indicative of the presence of aerobic MTBE-degraders, but it alone does not discriminate differences in "activity," or how fast the microbial community can biodegrade MTBE. Therefore, in our interpretation of the data from these microcosm tests, we focused on the extent of MTBE biodegradation achieved over fixed periods of 1, 2, 3, and 4 weeks. For each time frame, each microcosm was classified into one of the following three categories:

Nonactive (labeled "0" in Table 1): No MTBE concentration drop observed during the time period relative to the control microcosms.

Partially active (labeled "1" in Table 1): A clear decline in MTBE concentration during the time period, but less than a one-order-of-magnitude concentration decrease.

Active (labeled "2" in Table 1): A clear decline in MTBE concentrations during the time period, with at least a one-order-of-magnitude concentration decrease.

The results are presented in Table 1, which lists sample location, depth, biobarrier transect/operating condition, soil description, field-DO concentration, and the activity classification at 1, 2, 3, and 4 weeks.

Microcosm results from locations 6 and 13 are presented in Figure 3 to illustrate typical data, the range of behaviors observed, and their classification; included are the results for the six depths plus a duplicate of the 3.0 to 3.6 m bgs location 13 microcosm. All microcosms from location 6 were classified as active within 1 week, except for the "a" sample collected from 2.4 to 3.0 m bgs. That sample was classified as partially active after 1 week and active after 2 weeks. Location 13 microcosms exhibit the full range of behaviors. The two deepest samples (4.8 to 5.4 and 5.4 to 6.0 m bgs) were classified as nonactive over the 4-week period, the remaining samples were classified partially active over 1 week and active over 4 weeks, except for the 4.2 to 4.8 m bgs intermediate depth sample. For reference, control samples are also shown in Figure 3. All microcosm results are presented in the supplemental information Figures S1 through S4.

Upon review of the data and classifications presented in Table 1, it can be seen that:

- After 4 weeks, only 8 of the 96 soil core sections were classified as being nonactive; thus, the majority had sufficient degrader populations (either indigenous or a result

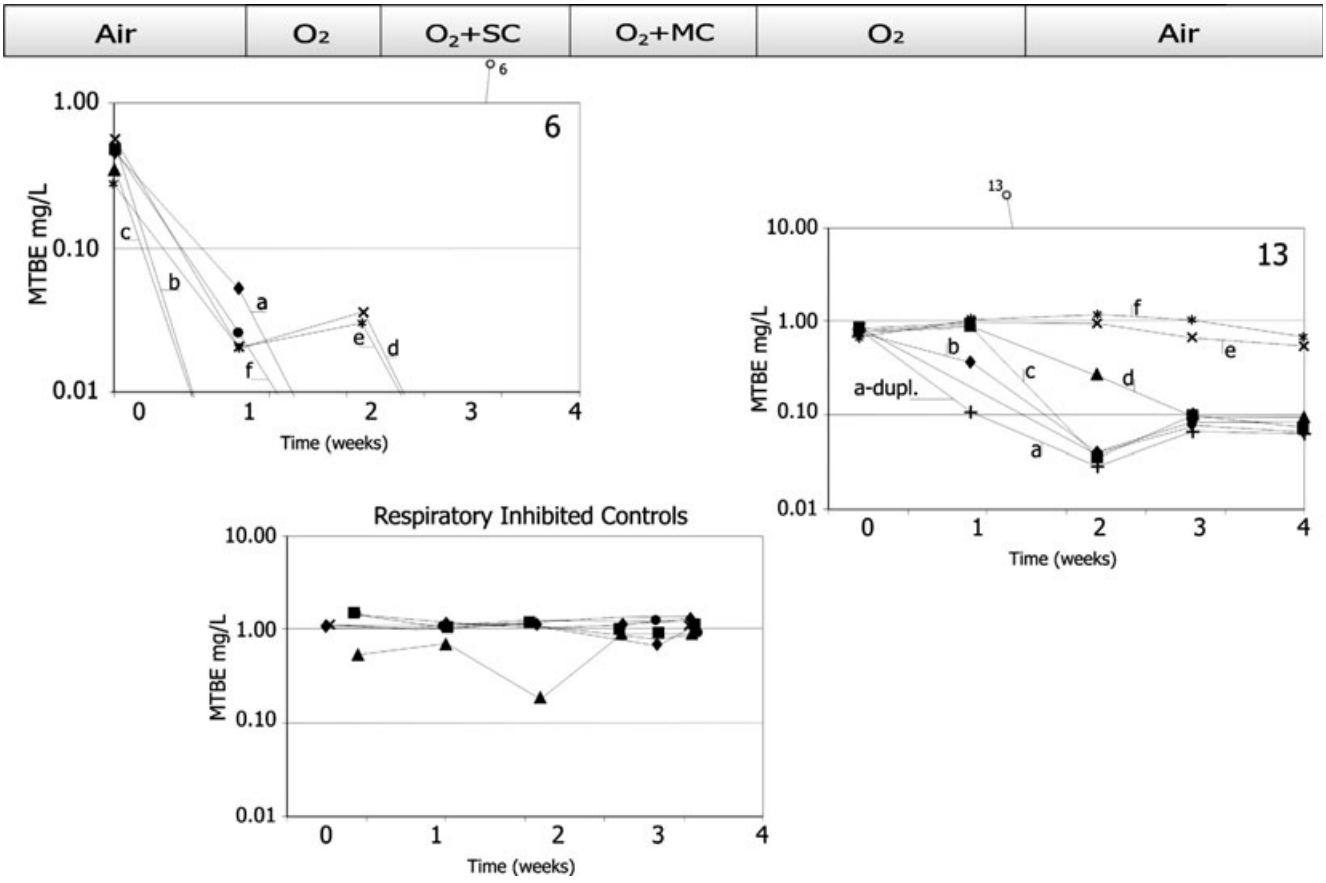

Figure 3. Results of the microcosms for sampling locations 6 and 13. Each graph contains the results for all the microcosms at the specified location. Subscripts *a, b, c, d, e,* and *f* correspond to depths 2.4 to 3.0, 3.0 to 3.6, 3.6 to 4.2, 4.2 to 4.8, 4.8 to 5.4, and 5.4 to 6.0 mbgs, respectively.

Table 1
Microcosms (Terminology Explained at the End of the Table)

Sampling Location	Location	Transect	Depth (m bgs)	MTBE Activity by Week				Biodegradation to Nondetect Levels? (Y/N)	Field-DO (mg/L)	Lithology
				First	Second	Third	Fourth			
1	S	Air	2.4–3.0	0	0	0	0	N	1.2	3
1	S	Air	3.0–3.6	0	1	2	2	N	1.2	3
1	S	Air	3.6–4.2	1	2	2	2	N	1.2	4
1	S	Air	4.2–4.8	0	1	1	2	N	<1	3
1	S	Air	4.8–5.4	0	0	0	1	N	<1	1
1	S	Air	5.4–6.0	0	0	0	0	N	<1	4
2	N	Air	2.4–3.0	0	0	0	1	N	3.4	2
2	N	Air	3.0–3.6	1	2	2	2	N	3.4	3
2	N	Air	3.6–4.2	1	2	2	2	N	3.4	4
2	N	Air	3.6–4.2*	2	2	2	2	N	3.4	4
2	N	Air	4.2–4.8	0	1	1	1	N	<1	4
2	N	Air	4.8–5.4	0	0	0	0	N	<1	1
2	N	Air	5.4–6.0	0	0	0	0	N	<1	4
3	B	Air	2.4–3.0	0	0	1	1	N	7.2	2
3	B	Air	3.0–3.6	1	2	2	2	Y	7.2	2
3	B	Air	3.6–4.2	0	0	1	1	N	7.2	3
3	B	Air	4.2–4.8	0	1	2	2	N	8.5	4
3	B	Air	4.8–5.4	0	1	2	2	N	8.5	3
3	B	Air	5.4–6.0	2	2	2	2	Y	8.5	4
3	B	Air	5.4–6.0*	2	2	2	2	Y	8.5	4
4	D	Air	2.4–3.0	0	1	1	1	N	5.3	3
4	D	Air	3.0–3.6	0	2	2	2	N	5.3	3
4	D	Air	3.6–4.2	0	1	1	1	N	5.3	3
4	D	Air	4.2–4.8	2	2	2	2	N	1.5	4
4	D	Air	4.8–5.4	1	1	2	2	N	1.5	3
4	D	Air	4.8–5.4*	0	1	1	2	N	1.5	3
4	D	Air	5.4–6.0	0	2	2	2	N	1.5	4
5	S	O_2 + SC	2.4–3.0	0	0	0	1	N	<1	3
5	S	O_2 + SC	3.0–3.6	0	0	0	0	N	<1	3
5	S	O_2 + SC	3.6–4.2	0	0	0	0	N	<1	3
5	S	O_2 + SC	4.2–4.8	0	2	2	2	N	<1	3
5	S	O_2 + SC	4.8–5.4	0	2	2	2	N	<1	4
5	S	O_2 + SC	5.4–6.0	0	0	2	2	N	<1	3
6	N	O_2 + SC	2.4–3.0	1	2	2	2	Y	>20	3
6	N	O_2 + SC	3.0–3.6	2	2	2	2	Y	>20	2
6	N	O_2 + SC	3.6–4.2	2	2	2	2	Y	>20	3
6	N	O_2 + SC	4.2–4.8	2	2	2	2	Y	4.6	3
6	N	O_2 + SC	4.8–5.4	2	2	2	2	Y	4.6	3
6	N	O_2 + SC	5.4–6.0	2	2	2	2	Y	4.6	4
7	B	O_2 + SC	2.4–3.0	2	2	2	2	Y	>20	3
7	B	O_2 + SC	3.0–3.6	1	2	2	2	N	>20	3
7	B	O_2 + SC	3.6–4.2	2	2	2	2	Y	>20	3

Table 1 Continued

7	B	O_2 + SC	3.6–4.2	2	2	2	2	Y	>20	3
7	B	O_2 + SC	4.2–4.8	2	2	2	2	Y	>20	3
7	B	O_2 + SC	4.8–5.4	2	2	2	2	Y	>20	3
7	B	O_2 + SC	5.4–6.0	2	2	2	2	Y	>20	4
8	D	O_2 + SC	2.4–3.0	0	2	2	2	Y	8.6	3
8	D	O_2 + SC	3.0–3.6	1	2	2	2	N	8.6	3
8	D	O_2 + SC	3.0–3.6*	2	2	2	2	N	8.6	3
8	D	O_2 + SC	3.6–4.2	0	2	2	2	N	8.6	3
8	D	O_2 + SC	4.2–4.8	1	2	2	2	Y	>20	3
8	D	O_2 + SC	4.8–5.4	2	2	2	2	Y	z0	3
8	D	O_2 + SC	5.4–6.0	2	2	2	2	Y	>20	3
9	S	O_2 + MC	2.4–3.0	0	1	1	1	N	<1	3
9	S	O_2 + MC	3.0–3.6	0	0	0	0	N	<1	3
9	S	O_2 + MC	3.0–3.6*	0	0	0	0	N	zz	3
9	S	O_2 + MC	3.6–4.2	0	0	1	1	N	<1	3
9	S	O_2 + MC	4.2–4.8	1	2	2	2	N	<1	4
9	S	O_2 + MC	4.8–5.4	1	2	2	2	N	<1	3
9	S	O_2 + MC	5.4–6.0	0	0	0	1	N	<1	3
10	N	O_2 + MC	2.4–3.0	2	2	2	2	N	<1	3
10	N	O_2 + MC	3.0–3.6	0	2	2	2	N	<1	2
10	N	O_2 + MC	3.6–4.2	0	2	2	2	N	<1	3
10	N	O_2 + MC	4.2–4.8	2	2	2	2	N	<1	3
10	N	O_2 + MC	4.8–5.4	2	2	2	2	N	<1	2
10	N	O_2 + MC	5.4–6.0	2	2	2	2	N	<1	4
11	B	O_2 + MC	2.4–3.0	2	2	2	2	N	>20	2
11	B	O_2 + MC	3.0–3.6	0	2	2	2	Y	>20	3
11	B	O_2 + MC	3.0–3.6*	0	2	2	2	Y	>20	3
11	B	O_2+MC	3.6–4.2	1	2	2	2	Y	>20	3
11	B	O_2 + MC	4.2–4.8	2	2	2	2	Y	16.3	4
11	B	O_2 + MC	4.8–5.4	1	2	2	2	N	16.3	3
11	B	O_2 + MC	5.4–6.0	1	2	2	2	Y	16.3	1
12	D	O_2 + MC	2.4–3.0	0	0	0	1	N	>20	2
12	D	O_2 + MC	3.0–3.6	1	2	2	2	N	>20	3
12	D	O_2 + MC	3.6–4.2	0	2	2	2	N	>20	3
12	D	O_2 + MC	4.2–4.8	1	2	2	2	N	>20	3
12	D	O_2 + MC	4.2–4.8*	1	2	2	2	N	>20	3
12	D	O_2 + MC	4.8–5.4	1	2	2	2	Y	>20	4
12	D	O_2 + MC	5.4–6.0	1	2	2	2	N	>20	4
13	S	O_2	2.4–3.0	1	2	2	2	N	<1	3
13	S	O_2	2.4–3.0*	1	2	2	2	N	<1	3
13	S	O_2	3.0–3.6	1	2	2	2	N	<1	3
13	S	O_2	3.6–4.2	0	2	2	2	N	<1	3
13	S	O_2	4.2–4.8	0	1	1	1	N	<1	3

Table 1 Continued

13	S	O_2	4.8–5.4	0	0	0	0	N	<1	4
13	S	O_2	5.4–6.0	0	0	0	0	N	<1	4
14	N	O_2	2.4–3.0	0	2	2	2	N	11.3	2
14	N	O_2	3.0–3.6	0	2	2	2	N	11.3	2
14	N	O_2	3.6–4.2	1	2	2	2	N	11.3	3
14	N	O_2	4.2–4.8	1	2	2	2	N	<1	3
14	N	O_2	4.2–4.8*	1	1	2	2	N	<1	3
14	N	O_2	4.8–5.4	2	2	2	2	N	<1	4
14	N	O_2	5.4–6.0	1	2	2	2	N	<1	4
15	B	O_2	2.4–3.0	0	2	2	2	N	>20	2
15	B	O_2	3.0–3.6	0	1	2	2	N	>20	3
15	B	O_2	3.6–4.2	0	0	1	2	N	>20	3
15	B	O_2	4.2–4.8	0	0	1	2	N	>20	2
15	B	O_2	4.8–5.4	0	0	1	2	N	>20	4
15	B	O_2	5.4–6.0	2	2	2	2	N	>20	4
16	D	O_2	2.4–3.0	1	2	2	2	Y	>20	3
16	D	O_2	3.0–3.6	1	2	2	2	Y	>20	3
16	D	O_2	3.6–4.2	1	2	2	2	Y	>20	3
16	D	O_2	4.2–4.8	0	1	2	2	N	>20	3
16	D	O_2	4.8–5.4	0	1	Broken	Broken	Broken	>20	1
16	D	O_2	5.4–6.0	1	2	2	2	N	>20	4

Notes:
Location:
S = upgradient (not affected by the biobarrier).
N = upgradient affected by the biobarrier.
B = biobarrier.
D = downgradient (not affected directly by the biobarrier).
Transect:
Air = Air injection (Biostimulation).
O_2 + SC = Oxygen and pure culture of a single organism injection (Bioaugmentation).
O_2 + MC = Oxygen and mixed culture injection (Bioaugmentation).
O_2 = Oxygen injection (Biostimulation).
*Duplicate sample.
mbgs = meters below ground surface.
MTBE activity:
0 = Nonactive microcosm.
1 = Partially active microcosm (MTBE concentration dropped less than one-order-of-magnitude during the analized period).
2 = Active microcosm (MTBE concentration dropped at least one-order-of-magnitude during the analized period).
Total biodegradation:
Y = MTBE concentration dropped to nondetect levels at the end of the microcosms experiment.
N = measurable MTBE concentration at the end of the microcosms experiment.
Lithology:
1 = clay, 2 = fine sand, 3 = medium sand, 4 = coarse sand and gravel.

of bioaugmentation in some areas) to effect some MTBE biodegradation in a 4-week period. The eight nonactive sections come from 5 of the 16 coring locations (locations 1, 2, 5, 9, and 13), and these included all of the most upgradient locations in the transects shown in Figure 1 (locations 1, 5, 9, and 13). All were also collected at locations/depths with low field-DO. At each of the five locations (locations 1, 2, 5, 9, and 13), core sections from other depths were classified as active or partially active, and sections immediately above and below the nonactive depths were classified as partially active except in one case.

- Of the 88 soil core sections that were classified as being active or partially active, 31 were collected from low field-DO environments (<2 mg/L); thus, many more low field-DO samples were classified as active or partially active than were classified as being nonactive.
- Only about one-fourth of the soil core sections (24 of 96) corresponded to active microcosms that achieved MTBE biodegradation to below detection levels (<0.005 mg/L) during the 4 weeks. All of those microcosms corresponded to locations and depths with elevated DO levels (all above 4.6 mg/L and many >20 mg/L). In their study,

Naas et al. (2002) showed that most of the microcosms biodegraded to nondetect levels within 30 d, except for a couple of microcosms from their source zone, which showed some biodegradation activity but failed to decline one-order-of-magnitude in the analyzed time period.

- Those locations where microcosms showed biodegradation to nondetect levels at four or more of the six sampling depth intervals (locations 6, 7, 8, and 11) were located in close proximity to bioaugmented regions of the biobarrier.

With respect to the transects shown in Figure 1:

- At their most upgradient locations (1, 5, 9, and 13 in Figure 1), all transects exhibited the full spectrum of activity (nonactive, partially active, active) across their depths. These locations correspond to low field-DO and the highest MTBE concentrations for each transect.
- At the next downgradient locations (2, 6, 10, and 14), almost all sections were classified as active within 2 weeks, except for those from location 2. These locations correspond to mixed conditions having lower to nondetectable MTBE (Figure 2) and low to elevated field-DO concentrations (Figure 2 and Table 1). Although the nonactive sections correspond to low field-DO, other low field-DO sections were classified as active.
- The two most downgradient locations in each transect (3, 4, 7, 8, 11, 12, 15, and 16) correspond to nondetect field MTBE concentrations and elevated field-DO concentrations, except for location 4 below about 4.2 m bgs where low field-DO concentrations were found. All sections from these locations were classified as active or partially active. The only distinguishing feature between them is the extent to which MTBE was biodegraded in the active sections. As discussed above, biodegradation to nondetect levels occurred across more sampling depths at the locations found within bioaugmented regions (7, 8, 11, and 12).

Although the data clearly show spatial variations in aerobic MTBE biodegradation activity, the data do not show any strong correlations between activity (as defined in this work) and factors defining the local environment where each sample was collected (i.e., field-DO and MTBE concentrations, soil type, depth, etc.). For example, although it is true that all nonactive samples correspond to low field-DO settings and all elevated field-DO sections were either classified as active or partially active, there were also many low field-DO sections classified as being active or partially active. MTBE concentration is also not a significant factor as sections collected at locations/depths with high- and low-field MTBE concentrations showed the full spectrum of activity. The same lack of correlation is true for the effect of soil type.

Thus, one could state the following for this data set:

Elevated DO environments, with or without MTBE present, always correspond to active or partially active microcosms. This suggests widely distributed indigenous aerobic MTBE-degraders, which is not too surprising given the performance of the biostimulated sections (air or O_2 injection only) of the biobarrier.

Microcosms that showed no activity were most likely to be constructed with soil from locations that were upgradient of the biobarrier where field concentrations of DO were low and concentrations of MTBE were elevated. However, those two conditions in themselves do not ensure no activity as partially active and active sections are also present in those regions.

The activity in microcosms from locations close to bioaugmentated regions of the biobarrier seems to be a direct effect of the inoculum and its distribution when it was implanted. In contrast, the slow or no complete degradation observed in microcosms (from either low- or high-field DO environments) represent fewer indigenous MTBE-degraders.

Other researchers have reported strong correlations between microbial activity and in situ dissolved oxygen concentrations (Murphy et al. 1997; Yolcubal et al. 2003; Oates et al. 2005); however, that was not the case in this work.

Conclusions

This study was conducted to examine the aerobic MTBE biodegrading activity of microorganism populations in the aquifer at various locations and depths around an operating and well-monitored engineered aerobic MTBE biobarrier. In the absence of well-accepted methodology for activity assessment, this work relied on conventional microcosms and used the extent of aerobic biodegradation achieved over 4 weeks to guide a three-level activity classification scheme (nonactive, partially active, active).

The results indicated a relatively widespread and uneven presence of MTBE-degrading activity, although the activity level was spatially variable and not clearly correlated with local field environmental conditions (MTBE concentration, DO concentration, soil type). Significant changes in activity level were observed over 0.3-m vertical distances in the same location. Microcosms from locations close to bioaugmentated regions show completeness of MTBE degradation. The highest degree of variability with depth (full spectrum of activity) was generally encountered at the most upgradient sampling locations and the least degree of variability with depth was generally encountered in bioaugmented portions of the biobarrier.

Supporting Information

The following supporting information is available for this article:

Fig. S1. Air Addition (Biostimulated Transect): Results of the microcosms from samples collected through the biobarrier. Each graph contains the results for all the microcosms at the specified location. Subscripts a, b, c, d, e, and f correspond to depths 2.4 to 3.0, 3.0 to 3.6, 3.6 to 4.2, 4.2 to 4.8, 4.8 to 5.4, and 5.4 to 6.0 m bgs, respectively.

Fig. S2. Oxygen and Pure Culture of a Single Organism Addition (Bioaumented Transect): Results of the microcosms from samples collected through the biobarrier. Each graph contains the results for all the microcosms at the specified location. Subscripts a, b, c, d, e, and f correspond to depths 2.4 to 3.0, 3.0 to 3.6, 3.6 to 4.2, 4.2 to 4.8, 4.8 to 5.4, and 5.4 to 6.0 m bgs, respectively.

Fig. S3. Oxygen and Mixed Culture Addition (Bioaumented Transect): Results of the microcosms from samples collected through the biobarrier. Each graph contains the results for all the microcosms at the specified location. Subscripts a, b, c, d, e, and f correspond to depths 2.4 to 3.0, 3.0 to 3.6, 3.6 to 4.2, 4.2 to 4.8, 4.8 to 5.4, and 5.4 to 6.0 m bgs, respectively.

Fig. S4. Oxygen Addition (Biostimulated Transect): Results of the microcosms from samples collected through the biobarrier. Each graph contains the results for all the microcosms at the specified location. Subscripts a, b, c, d, e, and f correspond to depths 2.4 to 3.0, 3.0 to 3.6, 3.6 to 4.2, 4.2 to 4.8, 4.8 to 5.4, and 5.4 to 6.0 m bgs, respectively.

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Biographical Sketches

***Luis E. Lesser**, corresponding author, is formerly a Ph.D. student at the Department of Civil, Environmental, and Sustainable Engineering, Arizona State University (ASU), Tempe, Arizona. Prior to receiving his Ph.D. from ASU he received a B.Sc. and M.Sc. in hydrogeology from the University of Waterloo, Waterloo, Ontario, Canada. He currently teaches at Universidad Autonoma de Queretaro (UAQ) in Queretaro, Mexico, and works at Lesser & Associates; (52-442) 223-1515; Rio Guadalquivir #3, Col. Pathé. Querétaro, Qro., C.P. 76020, México; luis_lesser@prodigy.net.mx.*

***Paul C. Johnson** is affiliated with Department of Civil, Environmental, and Sustainable Engineering, Arizona State University, Tempe, AZ 85287.*

***Gerard E. Spinnler** is affiliated with Shell Global Solutions (US) Inc., Houston, TX 77210.*

***Cristin L. Bruce** is affiliated with Shell Global Solutions (US) Inc., Houston, TX 77210.*

***Joseph P. Salanitro** is affiliated with Shell Global Solutions (US) Inc., Houston, TX 77210.*