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Enumeration of aromatic oxygenase genes to evaluate biodegradation during multi-phase extraction at a gasoline-contaminated site

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ABSTRACT

Multi-phase extraction (MPE) is commonly used at petroleum-contaminated sites to volatilize and recover hydrocarbons from the vadose and saturated zones in contaminant source areas. Although primarily a physical treatment technology, the induced subsurface air flow can potentially increase oxygen supply and promote aerobic biodegradation of benzene, toluene, ethylbenzene, and xylenes (BTEX), the contaminants of concern at gasoline-contaminated sites. In this study, real-time PCR enumeration of aromatic oxygenase genes and PCR-DGGE profiles were used to elucidate the impact of MPE operation on the aquifer microbial community structure and function at a gasoline-contaminated site. Prior to system activation, ring-hydroxylating toluene monooxygenase (RMO) and naphthalene dioxygenase (NAH) gene copies were on the order of 10^6 to 10^{10} copies L^{-1} in groundwater samples obtained from BTEX-impacted wells. Aromatic oxygenase genes were not detected in groundwater samples obtained during continuous MPE indicating decreased populations of BTEX-utilizing bacteria. During periods of pulsed MPE, total aromatic oxygenase gene copies were not significantly different than prior to system activation, however, shifts in aromatic catabolic genotypes were noted. The consistent detection of RMO, NAH, and phenol hydroxylase (PHE), which catabolizes further oxidation of hydroxylated BTEX metabolites indicated the potential for aerobic biodegradation of dissolved BTEX during pulsed MPE.

1. Introduction

Multi-phase extraction (MPE) is becoming an increasingly common treatment technology at petroleum-contaminated sites because it is effective for source removal even in low permeability soils [1], treats both vadose and saturated zones, can recover non-aqueous phase liquids, provides hydraulic plume control, and may accelerate aerobic biodegradation [2,3]. In the simplest configuration, a liquid-ring pump applies a vacuum to drop tubes within recovery wells with screens extending above the water table into the vadose zone. The applied vacuum results in volatilization and advective transport of volatile contaminants for subsequent recovery and above-ground treatment. In addition, the applied vacuum enhances the hydraulic gradient and provides groundwater recovery not only removing dissolved contaminants but also limiting potential off-site plume migration.

MPE is related to a group of ventilation-based technologies including soil vapor extraction/air sparging (SVE/AS) and bioventing/biosparging. The primary difference between these approaches is the air flow rate and thus the contaminant removal mechanism. Typically, MPE and SVE/AS are employed as physical treatment technologies for contaminant source remediation in which the main removal mechanism is through contaminant volatilization and recovery. With bioventing/biosparging, induced air flow rates are typically an order of magnitude lower (5–100 cfm) to maximize aeration to enhance aerobic biodegradation while minimizing contaminant volatilization [4]. Despite the potential for ventilation-based technologies to promote aerobic biodegradation, measurement of in situ respiration rates is often the only estimate of the effect of system operation on microbial activity [5]. While providing valuable information, respiration rates do not necessarily relate to the specific contaminant of interest and may be complicated by the presence of reduced species such as sulfides [6]. The use of nucleic acid-based techniques, however, would provide more direct assessment of the impact of remediation technologies on microbial community functions

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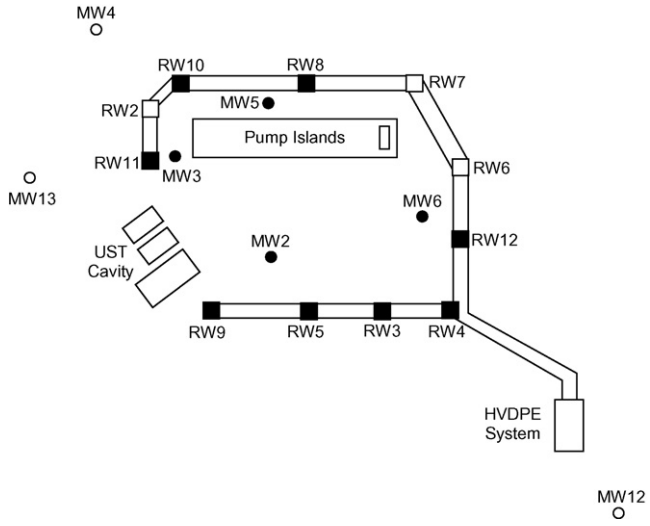

Fig. 1. Multi-phase extraction (MPE) study site. Gasoline underground storage tanks (USTs) are depicted as rectangles. Solid squares indicate MPE system recovery wells that were active for the duration of the study (January 2003 through July 2005). Recovery wells RW2, RW7, and RW6 (open squares) were deactivated in September 2004. Monitoring wells with BTEX concentrations exceeding maximum contaminant levels (solid circles) are based on the January 2003 groundwater sampling event prior to MPE system activation. Open circles indicate monitoring wells in which BTEX concentrations were below laboratory detection limits at the start of the study.

including evaluation of the biodegradation potential of specific pollutants.

Although not the primary objective, MPE can increase oxygen supply, which in turn can potentially accelerate bioremediation. Furthermore, MPE is usually a first step in a tiered approach to site remediation. To meet groundwater maximum contaminant levels (MCLs) for benzene, toluene, ethylbenzene, and xylenes (BTEX) at petroleum-contaminated sites, MPE is often followed by monitored natural attenuation or enhanced bioremediation. Thus the impact of MPE on the microbial community will ultimately play a critical role in site remediation. The purpose of the current study was to determine the effect of MPE on the bacterial community structure and function in the saturated zone at a gasoline-contaminated site. Groundwater samples were obtained over a 2.5-year period from BTEX-impacted and downgradient monitoring wells for chemical analysis and microbial characterization prior to and during MPE operation. PCR-DGGE profiles were used to assess overall changes in bacterial community structure resulting from MPE. A suite of real-time PCR assays targeting aromatic oxygenase genes [7] were used to evaluate the impact of MPE on bioremediation of BTEX, the contaminants of principal concern at petroleum-contaminated sites.

2. Materials and methods

2.1. Field site history

The site is an operating gasoline fueling station located in southeastern Indiana, US. Monitoring wells MW2 and MW3 were installed in 1987 adjacent to three gasoline underground storage tanks (USTs; Fig. 1). During UST upgrade activities, BTEX compounds were detected in a groundwater sample taken from the base of the excavation cavity and a confirmed release was reported. The site is underlain by a silty clay to depths of 2.1–3.8 m below land surface (BLS). Below the silty clay, a sandy, silty clay unit extends to depths between 2.7 and 4.6 m BLS. This water-bearing unit is underlain by a hard, red clay. Monitoring well screens extend from 1.5 to 4.6 m BLS. Historically, groundwater flow was toward the east/southeast. During operation of the MPE system, a groundwater depression centered on MW3 was created, which encompasses the pump island and recovery well network.

2.2. High vacuum dual phase extraction system

The MPE system and an accompanying network of 12 recovery wells (Fig. 1; RW) were installed at the site in January 2003. Essentially, the MPE system simultaneously couples soil vapor extraction and groundwater pump and treat by applying a vacuum on a series of sealed recovery wells to volatilize and recover subsurface contaminants. The MPE system at the site consisted of a 10-hp liquid-ring pump piped to 1.9 cm PVC drop tubes installed in each recovery well. Recovered vapor and groundwater were passed through an air–water separator (knockout tank). Extracted groundwater was treated by air stripping followed by adsorption on granular activated carbon (GAC) prior to discharge. Due to the relatively low vapor phase BTEX concentrations, recovered subsurface vapors were exhausted to the atmosphere without additional treatment. Emission rate was calculated based on field measurements of the exhaust concentrations (flame ionization detector), vapor flow rate, and assuming an average molecular weight of 102 g mole^{-1}. Based on groundwater drawdown and vacuum measurements during a pilot study, the estimated radius of influence of each recovery well was 3.7 m. Groundwater drawdowns at MW2, MW3, and MW5 were on the order of 1 m during system operation indicating study wells MW2 and MW5 were under direct influence of MPE.

2.3. Groundwater sample collection

Groundwater samples were obtained from monitoring wells (Fig. 1; MW) using sterile, disposable, PVC bailers. Monitoring wells were purged of approximately 3-well volumes by manual bailing prior to sampling to obtain groundwater samples representative of the aquifer. Three 40 mL groundwater samples were sent on ice to a certified laboratory for benzene, toluene, ethylbenzene, xylene analysis by established methods (US EPA Method 8021B). One liter groundwater samples were collected in sterile glass bottles and shipped overnight on ice to Purdue University for molecular genetic analysis. One set of groundwater samples from monitoring wells MW2, MW5, and MW12 was collected in January 2003 ($t = 0$), less than 1 week before the activation of the MPE system. Additional samples were collected over a period of 2.5 years in August 2003 ($t = 1$), July 2004 ($t = 2$), January 2005 ($t = 3$), and July 2005 ($t = 4$).

2.4. Sample preparation and DNA extraction

Suspended solids in groundwater samples were collected by centrifugation at 4 °C immediately upon receipt then stored at −80 °C until processed further. Following the manufacturer's instructions the FastDNA® Spin Kit for soil (QBIOgene, Carlsbad, CA, USA) was used to extract total DNA from approximately 0.5 g of the groundwater solids. Agarose gel (0.7%) electrophoresis was used to check DNA quality and quantified by fluorometry (model 8000-003; Turner Design, Sunnyville, CA, USA) calibrated with calf thymus DNA. DNA was stored at −20 °C when not in use. DNA extractions from control strains were performed as previously described [7].

2.5. Multiplex and real-time PCR

Samples were screened for aromatic oxygenase genes using the PCR primers and multiplex PCR protocols described previously [7]. Briefly, 10-fold dilutions of each sample were screened by multiplex

PCR assays for naphthalene dioxygenase (NAH), ring-hydroxylating toluene monooxygenase (RMO), phenol hydroxylase (PHE), and three subfamilies of biphenyl dioxygenase (BPH) genes. Presence of multiplex PCR products were determined by visualization on agarose gels (Bio-Rad, Richmond, CA, USA). The two DNA dilutions that produced the brightest bands for each positive sample were used for real-time PCR enumeration of aromatic oxygenase genes. Real-time PCR was performed on an ABI 7700 Sequence Detector (version 1.7 software; Applied Biosystems, Foster City, CA, USA). All real-time PCR conditions were the same as described previously [7]. All PCR experiments included negative controls containing no template and reactions with DNA extracts from appropriate positive control strains: NAH-*Pseudomonas putida* G7, RMO-*Pseudomonas aeruginosa* JI104, PHE-*Pseudomonas* sp. CF600, BPH1-*Comanonas testosteroni* B-356, BPH2-*Pseudomonas pseuodoalcaligenes* KF707, and BPH4-*Rhodococcus* sp. RHA1.

2.6. Denaturing gradient gel electrophoresis (DGGE) and analysis

The V3 region (primers PRBA338f [8] and PRUN518r [9] of the 16S rRNA gene was amplified by PCR [9–11] from the DNA extracted from groundwater solids. The 16S rRNA gene products were then loaded on 8% polyacrylamide gels with a linear gradient of 35–65% denaturant in 0.5% TAE buffer and electrophoresis at 60 °C at 200 V for 5.5 h. Gels stained with SYBR Green I (Molecular Probes, Eugene, OR, USA) were viewed on a UV transilluminator and images were captured digitally (UVP Imaging). Similarities between PCR-DGGE fingerprints based on the presence and absence of bands were calculated using Bionumerics software (Applied Maths, Kortrijk, Belgium). Similarity was calculated using the Dice coefficient and unweighted pair-group method using arithmetic averages (UPGMA) was used for cluster analysis [12].

3. Results

3.1. MPE system performance

Overall the MPE system performed effectively with an estimated 119 kg of petroleum hydrocarbons recovered from the subsurface during the study (January 2003–July 2005). During consistent operation, vapor flow rates were typically near 850 L min^{-1} but ranged from 510 to 1133 L min^{-1}. A total of 1.4 ML of petroleum-impacted groundwater were recovered during MPE system operation resulting in a depression of the local groundwater table centered near MW3 during consistent operation. The average groundwater drawdowns at MW2, MW3, and MW5 were between 0.9 and 1.5 m indicating the influence of the MPE system on the dissolved contaminant plume on the western portion of the site. Despite its proximity to RW12, the average drawdown at MW6 was less than 0.15 m suggesting MW6 was not dramatically influenced by MPE. Overall, the system operational data indicate that study wells MW2 and MW5 were under direct influence of MPE and MW6 may also have been affected to a more limited degree by system operation.

The MPE system is an unmanned mobile remediation unit designed to operate continuously, however, safety considerations dictate that any process faults deactivate the system. Since they likely impacted the microbial community composition and sampling results, scheduled and unscheduled system deactivations must be considered during data interpretation. The January 2003 (t = 0) groundwater samples were obtained under monitored natural attenuation (MNA) conditions 1 week prior to system startup. From September 2003 to July 2004, the system was nearly 100% operational and the July 2004 sampling event (t = 2) represents optimal MPE performance. During all other groundwater sampling events (t = 1, 3, and 4), the system was less than 50% operational due to system deactivations of various durations. Although unscheduled, these system shutdowns afforded the opportunity to evaluate the effect of pulsed MPE operation on the microbial community.

3.2. BTEX concentrations

Prior to MPE system activation, dissolved BTEX concentrations ranged from 2110 μg L^{-1} near the pump islands (Fig. 1; MW5) to below laboratory detection limits (<21 μg L^{-1}) in upgradient (MW4 and MW13) and downgradient wells (MW12). BTEX compounds were also detected in pre-startup groundwater samples obtained downgradient from the UST cavity (MW2), adjacent to the pump islands (MW3), and immediately downgradient of the pump islands (MW6). Although BTEX compounds were not detected in MW4, MW12, and MW13 within the study period, methyl *tert*-butyl ether (MTBE) was detected. In environmental releases of MTBE-blended gasoline, MTBE is typically the first compound detected in downgradient wells [13] and commonly dominates the leading edge of the gasoline-impacted groundwater plume [14]. Thus MW4, MW12 and MW13 at the site are also considered gasoline-impacted and likely represent the edge of the dissolved gasoline plume. As is typical of system activation, BTEX concentrations initially increased as the system depressed the groundwater elevation and captured the contaminant plume (Fig. 2A). Following the initial increase, BTEX concentrations generally declined in monitoring wells within the system radius of influence.

During system startup, troubleshooting, and pulsed operation, petroleum hydrocarbon recovery rates were relatively low (Fig. 2B). Hydrocarbon recovery rates increased substantially during continuous operation (September 2003 through July 2004). Following this period of rapid recovery, ventilation-based technologies are prone to "tailing" in which air–water mass transfer [15] or desorption [16] become rate limiting. When hydrocarbon recovery becomes mass transfer limited, MNA, enhanced bioremediation or pulsed venting

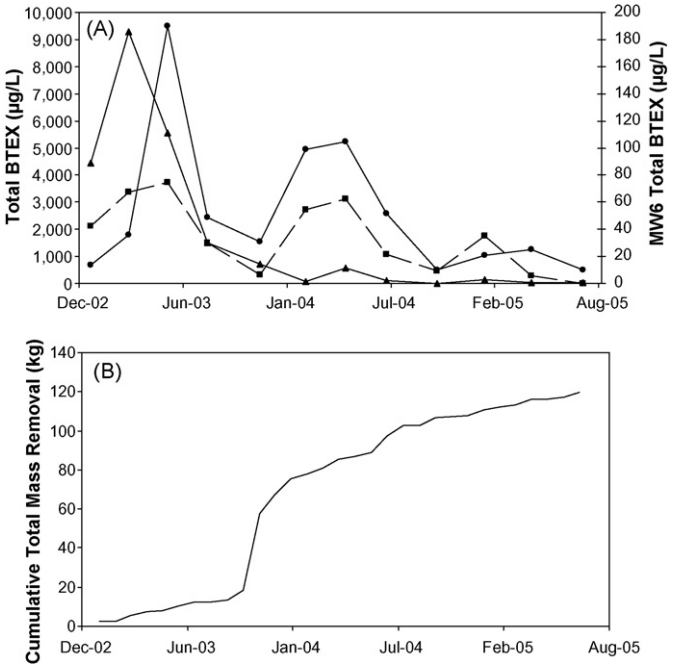

Fig. 2. (A) Changes in total BTEX concentrations over the course of the study in monitoring wells MW2 (•), MW5 (■), and MW6 (▲). Initial total BTEX concentrations are based on the January 2003 groundwater sampling event less than one week prior to MPE system activation. (B) Cumulative petroleum hydrocarbon removal.

are common supplemental measures to reach remediation goals. By the July 2005 groundwater sampling event, dissolved benzene concentrations were at or below drinking water MCLs in all monitoring wells except MW2, hydrocarbon concentrations in recovered soil vapors had decreased, and hydrocarbon recovery approached an asymptotic value (Fig. 2B). While MPE particularly in the vicinity of MW2 may still have been required, long-term MPE may not have been cost effective and a transition to a bioremediation approach may have been warranted.

3.3. Community profiling by DGGE

Visual inspection of the PCR-DGGE gels indicated a smear of bands in the background of each profile with 3 to ~20 bands of greater intensity. The well samples that were most impacted by BTEX (MW2 and MW5) had the most intense bands and were more similar to each other than to the communities in MW12. MW5 at $t = 4$ was the only sample that yielded insufficient DNA for use in analyses. By $t = 4$, BTEX levels in MW5 had decreased to near detection limits so there may have been insufficient nutrients to support a community at this time. MW12 is on the downgradient edge of the contaminant plume and BTEX was not detected in these samples therefore it was not surprising that differential selection of specialized populations within this community would be limited and it would differ from the other wells. We have seen similar community profile changes under controlled conditions using microcosms and addition of hydrocarbons [11].

Cluster analysis of community profiles in wells over time reveals there are temporal changes in community fingerprints (Fig. 3). With the exception of MW2, there was more similarity in communities within a well than between wells. Pairwise comparison of similarity values ranged from 70 to 90% in samples collected over time in profiles from MW5 or MW12. Whereas the temporal differences

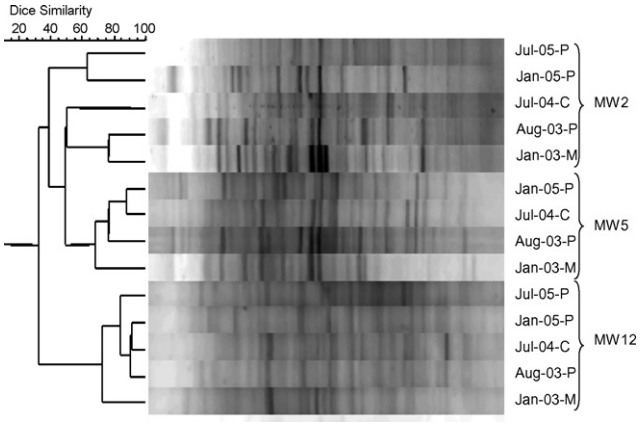

Fig. 3. DGGE fingerprint of small-subunit ribosomal DNA PCR products (positions 338–518, *Escherichia coli* rRNA sequence numbering). Lanes are designated as monitoring well (MW)-date of sample collection-system operation mode. System operation modes: M = monitored natural attenuation conditions prior to system activation; P = Pulsed; C = Continuous. The Dice similarity coefficient is indicated at the top left of the figure.

in community structure were far greater in the MW2 with only about 40% similarity between the January 2005/July 2005 and the January 2003/August 2003/July 2004 branches. Whereas this latter branch had 50% similarity to the MW5 profiles. This suggests that there may be some common populations in these wells when BTEX concentrations are high and disappear with the substrate.

3.4. Detection and quantification of aromatic oxygenase genes

Overall, aromatic oxygenase gene quantification corresponded to BTEX concentrations and system operating conditions with few

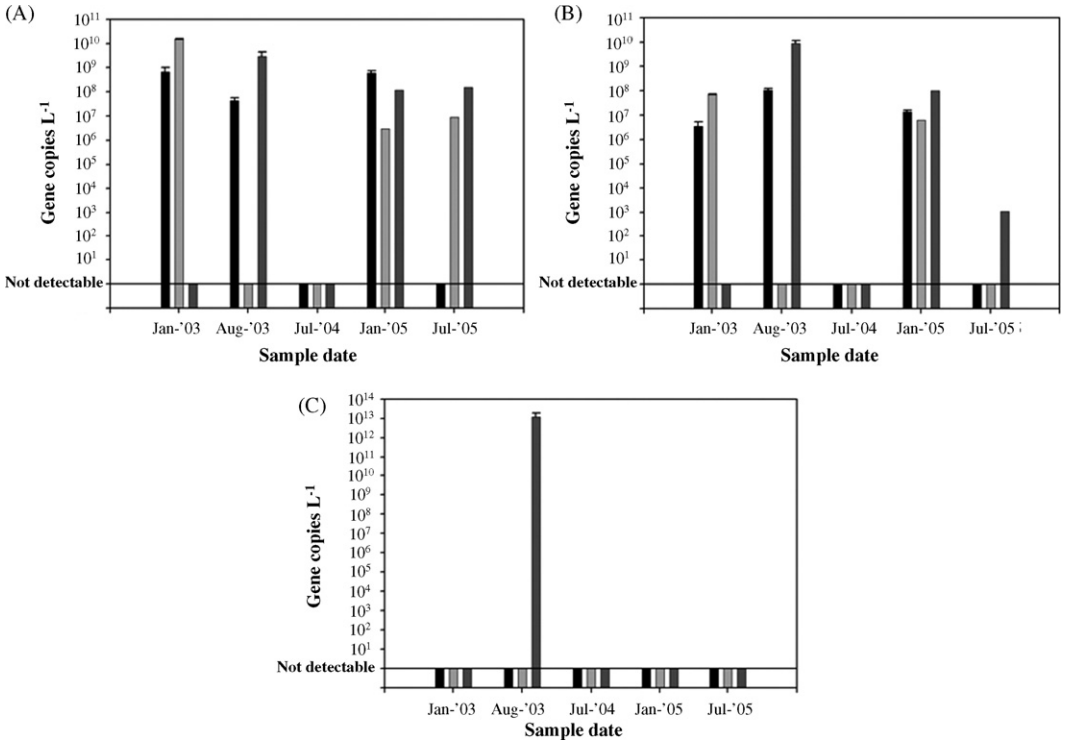

Fig. 4. Changes in aromatic oxygenase gene copy number as indicated by real-time PCR over the course of the MPE study in monitoring wells MW2 (A), MW5 (B), and MW12 (C). The MPE system was activated less than one week after the January 2003 samples were collected. Error bars are indicated for samples where PCR products were detected and replicates reactions were performed. Black bars, ring-hydroxylating toluene monooxygenase (RMO) genes; light gray, naphthalene dioxygenase (NAH) genes; dark gray, phenol hydroxylase (PHE) genes.

Table 1
Summary of dissolved BTEX concentrations and aromatic oxygenase gene copy numbers detected in groundwater samples obtained throughout the study

Well	Date	MPE operation	Total BTEX ($\mu g\,L^{-1}$)	Log (oxygenase gene copies L^{-1})			
				RMO	NAH	PHE	Total
MW2	January 2003	Pre-startup	1170	8.8	10.2	–	10.2
	August 2003	Pulsed	2437	7.6	–	9.5	9.5
	July 2004	Continuous	2592	–	–	–	–
	January 2005	Pulsed	1023	8.8	6.5	8.1	8.9
	July 2005	Pulsed	498	–	7.0	8.2	8.2
MW4	January 2003	NA	ND	NS	NS	NS	NS
	August 2003	NA	ND	6.5	–	11.9	11.9
	July 2004	NA	ND	–	–	–	–
	January 2005	NA	ND	–	–	–	–
	July 2005	NA	2	–	–	–	–
MW5	January 2003	Pre-startup	2110	6.5	7.8	–	7.9
	Aug 2003	Pulsed	1506	8.0	–	9.9	10.0
	July 2004	Continuous	1076	–	–	–	–
	January 2005	Pulsed	1740	7.1	6.8	8.0	8.1
	July 2005	Pulsed	3	–	–	3.0	3.0
MW6	January 2003	Pre-startup	89	NS	NS	NS	NS
	August 2003	Pulsed	30	8.7	–	9.7	9.8
	July 2004	Continuous	2.1	7.1	–	5.7	7.1
	January 2005	Pulsed	2.8	–	–	–	–
	July 2005	Pulsed	1	–	–	7.0	7.0
MW12	January 2003	NA	ND	–	–	–	–
	August 2003	NA	ND	–	–	13.1	13.1
	July 2004	NA	ND	–	–	–	–
	January 2005	NA	ND	–	–	–	–
	July 2005	NA	ND	–	–	–	–

January 2003 ($t = 0$) samples were collected less than 1 week prior to MPE system activation. ND indicates BTEX were not detected and NS denotes that the well was not sampled. Dash (–) denotes samples in which aromatic oxygenase genes were not detected. Monitoring wells MW4 and MW12 are outside of the MPE capture zone and system operation mode is not applicable (NA).

exceptions. Prior to system activation, RMO and NAH genes were detected in impacted wells MW2 and MW5 (Fig. 4) but not in downgradient well MW12 (Table 1). Following system startup, NAH was no longer detected, but RMO and PHE were detected in copy numbers on the order of 10^6 to 10^9 copies L^{-1} in BTEX-impacted wells MW2, MW5, and MW6 as well as MW4, MW12, and MW13 on the edge of the contaminant plume (Fig. 4 and Table 1). Between August 2003 and July 2004, the MPE system operated nearly continuously. In the July 2004 groundwater samples ($t = 2$), aromatic oxygenase genes (RMO and PHE) were only detected in BTEX-impacted well MW6 and upgradient well MW13. As noted previously, MW6 did not appear to be dramatically influenced by MPE and MW13 is outside the groundwater capture zone. The lack of detectable aromatic oxygenase genes in July 2004 groundwater samples from impacted wells MW2 and MW5 following consistent MPE operation suggested that vapor extraction and dewatering dramatically decreased aromatic hydrocarbon degrader populations in the saturated zone. Total bacterial biomass may have also decreased, however, the presence of distinct bands in MW2 and MW5 DGGE profiles generated for these samples (Fig. 3: lanes marked Jul-04-C) indicated the lack of detectable aromatic oxygenase genes was not due to sampling or sample handling issues. After pulsed MPE operation in December 2004, RMO, NAH, and PHE were again detected in January 2005 samples ($t = 3$) from MW2 and MW5 but not from MW6 likely due to low BTEX concentrations ($<5\ \mu g\,L^{-1}$). As with the January 2005 samples, aromatic oxygenase genes were detected in the July 2005 samples ($t = 4$) from MW2 and MW5 following pulsed operation. PHE gene copy numbers in $t = 4$ samples from MW5 were relatively low, however, total BTEX concentrations had decreased to 2.61 $\mu g\,L^{-1}$ by July 2005.

4. Discussion

Corrective action plans for petroleum-contaminated sites with high BTEX concentrations in a defined source area often follow a tiered approach in which a physical remediation method for rapid contaminant removal is followed by a bioremediation approach to meet groundwater MCLs. The aggressive source removal technology is implemented to reduce contaminant mass flux, reduce overall time to closure, reduce risk of exposure, and ideally would promote biodegradation during both the source removal and post-treatment phases [17–20]. MPE is one of a group of ventilation-based technologies that can accomplish source reduction goals and could potentially accelerate aerobic bioremediation in the vadose and saturated zones through increased oxygen supply and induced groundwater mixing [1]. Following continuous MPE, aromatic oxygenase genes were no longer detected in BTEX-impacted wells MW2 and MW5 suggesting a decrease in aerobic BTEX-utilizing populations and PCR-DGGE fingerprints seemed to indicate a general decrease in the aquifer bacterial community. In principle, MPE is a physical process resulting not only in volatilization but also advective transport. Experimental results have shown that under advective flow the diffusion of substrate to cells attached to particulate surfaces can limit the biodegradation rate [21,22]. Although local groundwater fluxes were not measured, advective–diffusive mass transfer could have limited substrate or nutrient bioavailability. When substrate flux decreased below cellular maintenance requirements, cell abundance would have also decreased [23]. Regardless of the mechanism, the qPCR results clearly show that consistent MPE decreased the abundance of BTEX-utilizing bacteria in groundwater samples and suggest biodegradation of dissolved BTEX compounds was negligible during full-time operation. How-

ever, MPE was extremely effective for contaminant source removal and any stimulation of aerobic biodegradation of the dissolved contaminant plume during the initial period of rapid recovery would have been an additional but secondary benefit.

Following initial rapid recovery, ventilation-based technologies are prone to "tailing" in which air–water mass transfer [15] or desorption [16] become rate limiting. When recovery becomes mass transfer limited, pulsed operation is relatively common [24], may aid contaminant recovery [25], and could potentially promote aerobic biodegradation during the post-recovery phase. In the current study, aromatic oxygenase genes were detected in BTEX-impacted wells in high copy numbers (10^7 to 10^{10} copies L^{-1}) prior to system activation but were not detected following continuous MPE. During periods of pulsed operation, total oxygenase genes were again detected on the order of 10^7 to 10^9 copies L^{-1} in BTEX-impacted wells under direct influence of the system. While total oxygenase gene copy numbers during pulsed operation were not greater than in pre-startup samples, shifts in aromatic catabolic genotypes were noted. RMO copy numbers in MW5 samples increased significantly (t-test; $\alpha = 0.05$) following system startup in the pulsed mode ($t = 1$). RMO and NAH were not detected during continuous operation ($t = 2$) but were detected in MW2 and MW5 samples at $t = 3$ following pulsed operation. Furthermore, PHE, which catalyzes the further oxidation of hydroxylated intermediates of monoaromatic hydrocarbon catabolism [26], was only detected during pulsed MPE. Although the presence of a specific catabolic genotype does not necessarily indicate the corresponding catabolic activity, the increase in aromatic oxygenase gene copy numbers particularly from below detection limits at $t = 2$ to greater than 10^7 copies L^{-1} at $t = 3$ implies growth of bacteria harboring that catabolic ability. The apparent recovery of RMO- and NAH-harboring organisms and the enrichment of the PHE genotype during pulsed MPE have important implications for overall corrective actions at the site. First, since pulsed operation is a common approach to combat tailing, the detection of aromatic oxygenase genes during this operation mode suggests the potential for biodegradation of dissolved BTEX during system deactivations. Second, the qPCR and PCR-DGGE results indicate recovery of the microbial community structure and BTEX-degrading populations suggesting MPE can be successfully utilized in tandem with bioremediation serving as a polishing step to achieve groundwater MCLs.

To date, relatively few studies have examined the impact of ventilation-based technologies including bioventing on the subsurface microbial community structure and function. Most rely on O_2 consumption, CO_2 production, or dehydrogenase activity [5,27–29] as an indicator of microbial activity. While providing valuable information, these general approaches do not necessarily relate to biodegradation of the contaminant of concern. The use of cultivation-independent, molecular methods would complement these classical measurements and provide quantitative results for the evaluation of the biodegradation potential of specific pollutants. For example, Chandler and Brockman [30] quantified naphthalene dioxygenase (*nah*Ac) and catechol-2,3-dioxygenase (*xyl*E) genes at a bioventing site to conclude that poor bioremediation performance as indicated by in situ respirometry was not due to a lack of genetic potential for aerobic biodegradation but more likely related to physical conditions. Zucchi et al. [31] found increases in catechol-2,3-dioxygenase gene abundance corresponded to increases in respiratory activity showing not only increased microbial activity but also the catabolic ability to degrade aromatic hydrocarbons in air sparging column studies. In the present study, quantification of aromatic oxygenase genes conclusively demonstrated the potential for aerobic BTEX biodegradation of dissolved BTEX during pulsed MPE and showed that MPE and bioremediation are compatible technologies to achieve remediation goals.

5. Conclusions

As a source removal technology, MPE was extremely effective with approximately 119 kg of petroleum hydrocarbons recovered during the 2.5 years of operation. In addition to physical contaminant recovery, MPE is also believed to enhance aerobic biodegradation of petroleum hydrocarbons. While the impact of MPE on the vadose zone microbial community was not investigated, the lack of detectable aromatic oxygenase genes and the loss of intense bands in PCR-DGGE profiles in BTEX-impacted wells indicated that continuous MPE decreased the abundance of BTEX-utilizing bacteria in the saturated zone of the source area and suggested biodegradation of dissolved BTEX was negligible during full-time operation. Conversely, during pulsed MPE operation, RMO, NAH, and PHE were routinely detected on the order of 10^7 to 10^9 copies L^{-1} in groundwater samples. Although the presence of a catabolic gene does not indicate activity, the apparent growth of BTEX-utilizing bacteria and the recovery of the aquifer microbial community structure suggest aerobic BTEX biodegradation could have been a component of dissolved BTEX removal during pulsed operation.

Acknowledgement

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Appendix A

MPE continued for approximately 1 year following the conclusion of this study. When the system was deactivated in November 2006, the dissolved BTEX concentrations were below 5 $\mu g\,L^{-1}$ in all monitoring wells except MW2 (32 $\mu g\,L^{-1}$). In January 2007, the site was granted "No Further Action" status.

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