Ground Water Monitoring&Remediation

In Situ Biotreatment of TBA with Recirculation/Oxygenation

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Abstract

The potential for in situ biodegradation of *tert*-butyl alcohol (TBA) by creation of aerobic conditions in the subsurface with recirculating well pairs was investigated in two field studies conducted at Vandenberg Air Force Base. In the first experiment, a single recirculating well pair with bromide tracer and oxygen amendment successfully delivered oxygen to the subsurface for 42 d. TBA concentrations were reduced from approximately 500 µg/L to below the detection limit within the treatment zone and the treated water was detected in a monitoring transect several meters downgradient. In the second experiment, a site-calibrated model was used to design a double recirculating well pair with oxygen amendment, which successfully delivered oxygen to the subsurface for 291 d and also decreased TBA concentrations to below the detection limit. *Methylibium petroleiphilum* strain PM1, a known TBA-degrading bacterium, was detectable at the study site but addition of oxygen had little impact on the already low baseline population densities, suggesting that there was not enough carbon within the groundwater plume to support significant new growth in the PM1 population. Given favorable hydrogeologic and geochemical conditions, the use of recirculating well pairs to introduce dissolved oxygen into the subsurface is a viable method to stimulate in situ biodegradation of TBA or other aerobically degradable aquifer contaminants.

Introduction

Tert-butyl alcohol (TBA) is a suspected human carcinogen but its fate and transport in the subsurface environment has only recently received much attention (Schmidt et al. 2004). TBA is typically present in groundwater as an intermediate biodegradation product of methyl *tert*-butyl ether (MTBE) (DeVaull et al. 2003). In addition, TBA may be introduced to groundwater if present as an impurity in spilled MTBE or by other fuel oxygenates, for example, ethyl *tert*-butyl ether (ETBE; ITRC 2005).

Contamination of groundwater by TBA is strongly linked to MTBE use and contamination. MTBE began to be added to gasoline in the late 1970s as a performance-boosting replacement for alkyl lead additives and then as a fuel oxygenate to reduce emissions under the Clean Air Act Amendments of 1990 (Fiorenza et al. 2002). Oxygenated gasoline contains 15% v/v MTBE and reformulated gasoline contains 10% to 11% v/v MTBE (Clawges et al. 2000). In the 1990s, MTBE from oxygenated gasoline became recognized as a widespread groundwater contaminant. The United States Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) found that, for groundwater wells in urban areas sampled from 1985 to 1995, 16.9% had detections of MTBE (Squillace et al. 1999). More recently, Moran et al. (2005) found a similar detection frequency, 13%, of MTBE in urban groundwater wells not near known point sources. Of all volatile organic compounds (VOCs), MTBE was found by Carter et al. (2008) to be the third most frequently detected VOC in U.S. groundwater, after chloroform and tetrachloroethylene (PCE).

TBA is increasingly detected despite the fact that many states took action to discontinue use of MTBE in gasoline formulations in the early 2000s (Fiorenza et al. 2002). Over the last decade, complete in situ mineralization of MTBE by microbes under aerobic conditions has been demonstrated (Schirmer and Barker 1998; Schirmer et al. 1999; Wilson et al. 2002; Johnson et al. 2003). However, TBA can accumulate during biodegradation of MTBE, especially under anaerobic conditions (Wilson and Adair 2007) and TBA is increasingly recognized as an enduring daughter product at gasoline spill sites (Kolhatkar et al. 2000; Shih et al. 2004). In a survey of 390 gasoline-contaminated sites conducted by the U.S. EPA, TBA was detected in 11% of the sites (Fiedler et al. 2004). Currently there is no maximum contaminant level defined for TBA; in California, the notification level is 12 µg/L (California OEHHA 1999).

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Conventional physical/chemical treatment techniques applied to ex situ treatment of MTBE-contaminated groundwater are generally considered ineffective for treatment of TBA-contaminated groundwater. TBA is not readily air stripped from water due to a low Henry's constant (1.4×10^{-5} atm·m^3/mol) and high water solubility (fully miscible) (Schmidt et al. 2004; Sutherland et al. 2005). In addition, TBA does not readily sorb to granular-activated carbon or other media (Bi et al. 2005; Sutherland et al. 2005). In situ bioremediation can be an effective treatment approach for TBA contamination in the subsurface; however, the rates of TBA biodegradation are slow under the anaerobic conditions that often prevail at fuel spill sites (Wiedemeier et al. 1995, 1999; Wilson and Adair 2007). Biodegradation of TBA is more efficient under aerobic conditions, which can be achieved in situ using methods such as air sparging or passive oxygen diffusion (Johnson et al. 2003). However, additional methods are needed to allow effective and affordable in situ aerobic treatment when conditions are not ideal for current methods, for example, when surface constraints or depth to water prevent or raise the cost of installation of sparge or passive release wells at close spacing.

Biodegradation of TBA

In microcosm studies, TBA has been shown to be degraded under aerobic conditions by indigenous microbial communities in sediments from two sites in South Carolina (Bradley et al. 1999) and sediments from Vandenberg Air Force Base (VAFB) Site 60, California (Mackay et al. 2004). At VAFB Site 60, release of oxygen to groundwater using diffusive emitters was found to lead to rapid establishment of steady-state conditions and in situ degradation of MTBE and TBA by the native microbial community (Mackay et al. 2001; Wilson et al. 2002). In the experimentally created oxygenation zone at VAFB Site 60, a strain of bacteria was detected with a 16S ribosomal DNA (rDNA) sequence greater than 99% identical to the 16S rDNA region of bacterium *Methylibium petroleiphilum* strain PM1 (Hristova et al. 2003). Strain PM1 is capable of degrading MTBE and its degradation intermediates, including TBA, when supplied with oxygen as an electron acceptor (Hanson et al. 1999; Deeb et al. 2000).

The fate of TBA under anaerobic conditions is less clear (Wilson and Adair 2007). Monitoring at VAFB Site 60 from 2004 through 2009 suggested that no significant degradation of the TBA plume occurred under the natural sulfate-reducing conditions (Chakraborty 2011). Laboratory studies have indicated that TBA is recalcitrant in sulfate-reducing and methanogenic microcosms (Bradley et al. 2002). Research at several field sites has suggested the presence of microbes capable of anaerobic transformation of TBA, based on incorporation of ^{13}C-enriched TBA into cellular material (K. Sublette, personal communication, 2008), but microcosm studies have found that anaerobic transformation of TBA in VAFB Site 60 sediments does not occur at the low TBA concentrations (<2 mg/L) found in the plume in recent years (Chakraborty 2011).

Use of Recirculating Well Pairs for Bioremediation

The use of well pairs to recirculate groundwater within an aquifer or between separate water-bearing layers is a bioremediation alternative that eliminates the need for aboveground treatment (Christ et al. 1999). This method supports in situ biotreatment by capturing all or part of a contaminant plume with an extraction well, adding electron acceptors and nutrients, if desired, to the extracted groundwater via aboveground systems and reintroducing the amended water into the aquifer via an injection well. A portion of the reinjected water is recaptured by the extraction well while the remainder escapes the recirculation zone to flow downgradient. The proportion of water recirculated vs. escaped depends on recirculation rates, aquifer properties, and groundwater flow rates. The recirculation creates a zone of biotreatment that extends downgradient of the recirculation wells if amendments are not consumed completely within the recirculation zone.

The use of recirculating well pairs for in situ bioremediation of groundwater contaminated with chlorinated organics is well-documented (ESTCP 2001). McCarty et al. (1998) used a recirculation and amendment system between two contaminated aquifers to degrade trichloroethylene (TCE). Indigenous toluene-degrading bacteria aerobically cometabolized TCE, reducing concentrations by 97% to 98% throughout the treatment zone. Hyndman et al. (2000) used a series of injection and extraction wells to create a treatment zone, or "biocurtain," across a carbon tetrachloride (CT) plume. Through bioaugmentation with *Pseudomonas stutzeri* and a nitrate-rich substrate, the mean CT removal efficiency approached 99% after 4 years of operation, with median groundwater concentrations decreasing from 31.8 µg/kg to a minimum of 0.12 µg/kg (Dybas et al. 2002). Wu et al. (2006) used recirculating well pairs to immobilize uranium in the subsurface by injecting ethanol to promote the microbial reduction of mobile uranium (VI) to insoluble uranium (IV).

The primary objectives of this work were to demonstrate (1) biodegradation of TBA by a single well pair recirculation/oxygenation system and then (2) scale-up of a recirculation/oxygenation system with two well pairs. To our knowledge, our study is the first to report the successful application of recirculating well pairs for the aerobic biotreatment of TBA.

Materials and Methods

Study Site

Site 60 is a former service station on VAFB, Lompoc, CA (Figure 1). A brief release history and hydrological characterization of the site is provided here; a detailed description can be found in Mackay et al. (2006).

After discovery of a gasoline leak from underground storage tanks and pipelines approximating 2150 L (570 gallons), the service station was closed in 1994. Tanks and piping were removed in 1995 and the excavation was backfilled with relatively permeable sand and gravel. The resulting BTEX plume was never detected beyond 25 m from the source, whereas the accompanying MTBE plume extended to 520 m in length, based on monitoring by consultants to VAFB. Since 1999, when our monitoring began, BTEX concentrations rapidly diminished to below detectable levels while MTBE and TBA concentrations persisted above

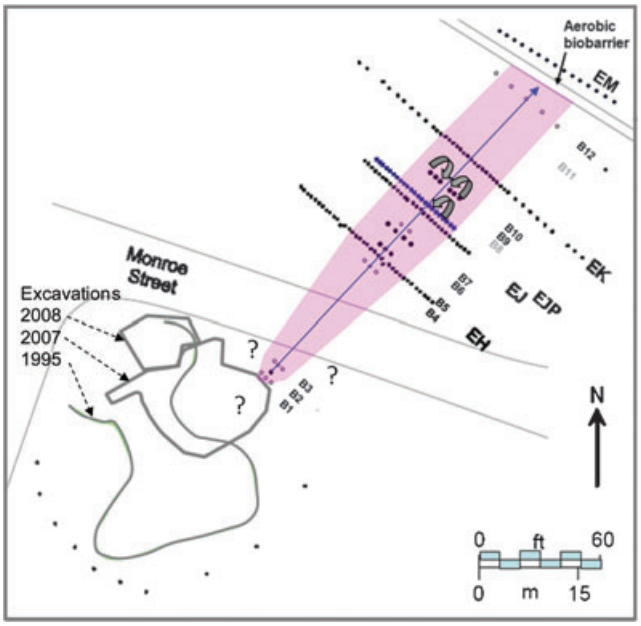

Figure 1. VAFB Site 60 site map indicating the location of excavations, monitoring well transects, and the approximate location and centerline of the TBA plume at the time of this research. The single well pair and double well pair recirculation/oxygenation systems are indicated with curved arrows in the EJP and B9 transects, respectively. Note EH and EJ transects, upgradient of both systems, were used for monitoring during both experiments.

acceptable levels until 2010 (Mackay et al. 2006; Rasa et al. 2011). The MTBE and TBA plume migrated through a thin permeable layer located approximately 2.4 to 3.4 m below ground surface (bgs) (Wilson et al. 2002). Aquifer parameters are presented in Table 1.

Background geochemical parameters, based on groundwater samples collected downgradient of the excavated source area (Mackay et al. 2006), are presented in Table 2; background bromide was determined by Mackay et al. (2012). Aquifer sediments are not significant reservoirs of solid-phase electron acceptors (e.g., ferric iron and manganese (IV)) and laboratory microcosm studies suggest that the solid-phase electron acceptors are not readily bioavailable (Wood 2004). Dissolved sulfate is the predominant electron acceptor controlling microbiological reactions under natural conditions at Site 60.

TBA was the primary VOC detected at the experimental site during this research. Five months prior to the first

Table 1

VAFB Site 60 Aquifer Characteristics

Parameter	Value
Hydraulic conductivity	5.2–27.1 m/d
Average bulk density	1.54 g/cm^3
Average porosity	0.34
Average groundwater velocity	0.5 m/d

Table 2

VAFB Site 60 Background Geochemical Characteristics

Parameter	Range
Temperature	14.5–17.5 °C
pH	6.8–8.1
Alkalinity as $CaCO_3$	161–378 mg/L
Total carbon	0.36%
Dissolved organic carbon	0.11%
Dissolved oxygen	<0.5 mg/L
Dissolved iron	0.02–6.9 mg/L
Sulfate	100–200 mg/L
Bromide	3.1 mg/L

experiment, maximum concentrations of TBA and MTBE at the EJ transect were 835 and 1.9 µg/L, respectively. At the time of the second experiment, started 14 months later, the TBA concentration maximum at the EJ transect had decreased to 193 µg/L and MTBE was not detectable (≤0.8 µg/L), presumably due to source exhaustion.

TBA and MTBE are negligibly sorbed to sediments in the contaminated aquifer at Site 60 (Mackay et al. 2006). Thus, it was reasonable to assume that all MTBE or TBA mass was dissolved and the average migration rate for both was essentially identical to groundwater flow rate.

Single Recirculating Well Pair System Design

The first field experiment was conducted with the goal of exploring biodegradation and distribution of dissolved oxygen (DO). For this study, we used groundwater monitoring wells in three transects orthogonal to the mean direction of groundwater flow. The injection and extraction wells used to establish the recirculation treatment zone were located in the EJP transect (Figure 1). The injection (EJP-18) and extraction (EJP-20) wells were spaced 1.5-m apart, with a monitoring well (EJP-19) located centrally between them (Figures 2 and 3). EJP-19 was assumed to be representative of in situ geochemical conditions in the recirculation and treatment zone.

The EJ transect, located 1.8 m upgradient of the recirculation wells, was used to monitor TBA concentrations approaching the recirculation well pair. The EK transect, located 7.9 m downgradient of the recirculation system, was monitored to provide information on groundwater velocity and treatment efficacy. Prior to the experiment, all recirculation and monitoring wells were developed by surging/brushing and repeated pumping. All wells were 2.5 cm (1 inch) diameter Schedule 40 PVC, screened from 2.4 to 3.4 m bgs with 0.5 mm slots.

Groundwater was extracted through 9.5 mm internal diameter (ID) LDPE tubing from the top of the extraction well screen at a flowrate of 1 L/min with a peristaltic pump (Masterflex model HV-07591-00, Cole Parmer, Vernon Hills, Illinois). The groundwater was filtered with a 10-µm filter and then spiked with a bromide tracer (potassium bromide, 98% extra pure, Acros Organics, Pittsburgh, Pennsylvania). A second pump injected the 28,000 mg/L

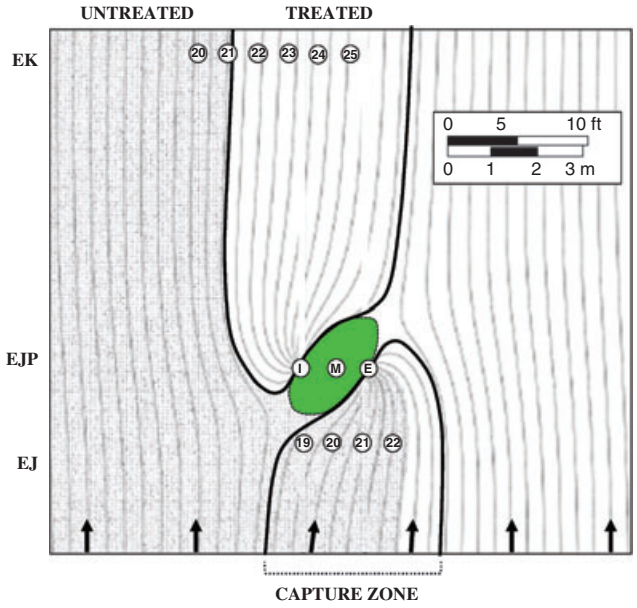

Figure 2. Plan view of well layout with simulated groundwater flowlines. Vertical scale is identical to horizontal scale. The TBA plume was captured by the extraction well EJP-20 ("E"), oxygenated aboveground, and injected into well EJP-18 ("I"). The injected water was partly recaptured by the extraction well and thus recirculated (green area), while a portion escaped recirculation and continued downgradient ("treated"). The middle well, EJP-19 ("M") was used for recirculation zone monitoring; the EJ transect was used for upgradient monitoring; the EK transect was used for downgradient monitoring.

bromide spike solution into the groundwater recirculation line at approximately 3.5 mL/min.

Oxygen was added to the bromide-spiked recirculating groundwater via a 30-L diffusion reactor. The reactor contained 91 m of Tygon® (Saint-Gobain Performance Plastics, Beaverton, Michigan) 3350 platinum-cured silicone tubing wound around a PVC support and pressurized to 20 psi with oxygen (Praxair, >99.9% purity). The tubing was vented biweekly to ensure optimal oxygen release (Wilson and Mackay 2002). The bromide-spiked and oxygenated water flowed into the injection well via 9.5 mm ID LDPE tubing.

Data Collection and Analytical Methods

We monitored groundwater concentrations of VOCs, bromide, and DO as well as piezometric head. All monitoring wells had dedicated LDPE sampling tubes through which samples were collected. The ends of the sampling tubes were placed approximately 5 cm above the top of the screened section. Using a peristaltic pump, approximately 400 mL of groundwater (approximately two times the internal volume of the screened section, as found adequate in prior work) was purged in order to collect a vertically integrated composite sample across the well screen (Mackay et al. 2006).

Groundwater samples for VOC analysis were collected in 22-mL glass hypovials containing 0.5 g of powdered trisodium phosphate dodecahydrate. Subsequent handling, storage, transport, and analysis was as described by Mackay et al. (2006). The detection limits were 3 µg/L for TBA and 0.8 µg/L for MTBE.

Groundwater samples for bromide analysis were collected into 8 mL LDPE bottles and stored at 4 °C until analyzed via ion chromatography. The chromatograph was equipped with an HPLC pump (ConstaMetric IIIG, LDC/Milton Roy, Riviera Beach, Florida), a self-regenerating suppressor (ASRS-ULTRA-II, 4 mm, operated at 100 mA, Dionex Corporation, Inc.), and an electrical conductivity detector (ED40, Dionex Corporation, Inc., Sunnyvale, California). The system was operated at room temperature

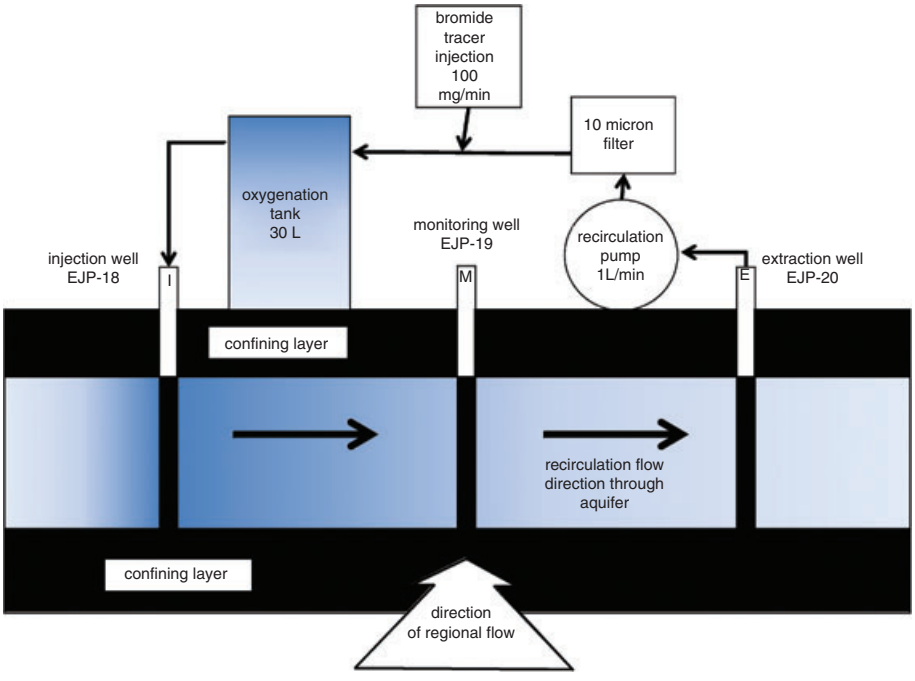

Figure 3. Schematic of the single well pair recirculation system (not to scale). Areas of expected higher oxygen concentration are shaded dark blue. Regional groundwater flow direction is indicated by the arrow pointing into the page at the bottom of the diagram.

(22 ± 2 °C). The samples were centrifuged to remove particulate matter; the supernatant was injected using an automated sample injector (7126, Rheodyne, IDEX Corporation, Oak Harbor, Washington) through a 7-µL injection loop. Anions in the samples were separated on an IonPac AS22 analytical column (4 × 250 mm, Dionex Corporation Inc.) in tandem with an IonPac AG22 guard column (4 × 50 mm) with an eluent (4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$) at a flow rate of 1.5 mL/min. The detection range was 0 to 100 µS and data were acquired by a Chromatopac integrator (C-R501, Shimadzu Corporation, Columbia, Maryland). The bromide detection limit for this method was 0.3 mg/L.

For field confirmation of system operation, on-site bromide measurements were made with a Cole-Parmer Bromide Ion Electrode connected to an ORION Model 250A ion meter, calibrated using bromide solutions made on site. These results were not used for calculations or in reports or manuscripts.

DO concentrations were measured using an YSI Model 95 digital meter with an attached YSI microelectrode array (MEA) DO probe (0 to 50 mg/L quantitation range). Groundwater was slowly pumped into a 2-L graduated cylinder, with the probe at the bottom of the cylinder. The probe was moved gently inside the bottom of the full cylinder until the reading stabilized.

Piezometric head was measured from the top of the surveyed well casing with a Solinst 101 Mini Water Level Meter (Georgetown, Ontario, Canada).

Groundwater samples for quantitative polymerase chain reaction (qPCR) analysis were collected in sterile 500-mL polyethylene bottles and stored at 4 °C. Two groundwater samples from each well, totaling 1 L, were filtered in the lab through one sterile 0.22-µm filter (47 mm diameter, type GTTP 2500; Millipore Corporation, Billerica, Massachusetts). DNA was extracted from the filters with FastDNA SPIN Kits (Qbiogene, Inc., Solon, Ohio) according to manufacturer specifications. qPCR was performed in 25-µl volumes with MicroAmp optical 96-well reaction plates and MicroAmp optical caps (Applied Biosystems, Inc., Carlsbad, California) on an Applied Biosystems 7300 Real-Time PCR System. For PM1 quantification, 16S rDNA was amplified by using *Taq*Man PCR PM1 forward primer 963F (5′-CCT TGA CAT GTC TAG AAG TTA CCA GAG A-3′), *Taq*Man PCR PM1 reverse primer 1076R (5′-GCG GGA CTT AAC CCA ACA TCT-3′) and *Taq*Man PCR probe 1030T (5′-ACA CGA GCT GAC GAC GGC CAT G-3′). For total bacteria, 16S rDNA was amplified using *Taq*Man PCR universal forward primer 1369F (5′-CGG TGA ATA CGT TCY CGG-3′), *Taq*Man PCR universal reverse primer 1492R (5′-GGW TAC CTT GTT ACG ACT T-3′), and *Taq*Man PCR universal probe 1389F (5′-CTT GTA CAC ACC GCC CGT C-3′). qPCR conditions and data analyses were performed as described by Suzuki et al. (2000) for total bacteria and by Hristova et al. (2001) for PM1.

Double Recirculating Well Pair System Design

A second field experiment was conducted with the goal of exploring recirculation scale-up by attempting to create a symmetric treatment zone using two well pairs for recirculation. A site-calibrated groundwater model developed in MODFLOW (Harbaugh et al. 2000) was used to assist in selecting well spacing, planning recirculation rates, and predicting capture and release zones (Figure 4). The predicted capture zone was verified in the model using an analytical solution for well pairs by Cunningham et al. (2004). The experiment was oriented toward confirming that the lateral impact of DO fit modeling expectations.

On the basis of model results, six new wells were installed at VAFB Site 60 in July 2008 to create the B9 transect, 5.6 m downgradient of the EJP transect used for the single well recirculation experiment previously described (Figure 1). Extraction (B9-1 and B9-6), injection (B9-3 and B9-4), and monitoring (B9-2 and B9-5) wells were 5.1-cm (2 inch) diameter Schedule 40 PVC, screened from 2.4 to 3.4 m bgs with 0.5 mm slots. The EH and EJ transects, located 16.9 and 7.5 m upgradient of the recirculation wells, respectively, were used to monitor TBA concentrations in the plume entering the recirculation zone (Figure 1). As for the first recirculation/oxygenation experiment, the EK transect, located 2.3 m downgradient of the B9 wells, was monitored for TBA, MTBE, and electron acceptors to confirm efficacy of recirculation/oxygenation. At the request of regulators, no tracer was used in this study and the recirculation system was not turned off for confirmation, as in the first experiment. At a groundwater velocity of 0.5 m/d, there was an approximate 40-d plug flow travel time between the upgradient EH and downgradient EK transects; there was an approximate 22-d plug flow travel time between the upgradient EJ and downgradient EK transects.

Groundwater was extracted from wells B9-1 and B9-6 through 0.95 cm (3/8 inch) outer diameter (OD) LDPE

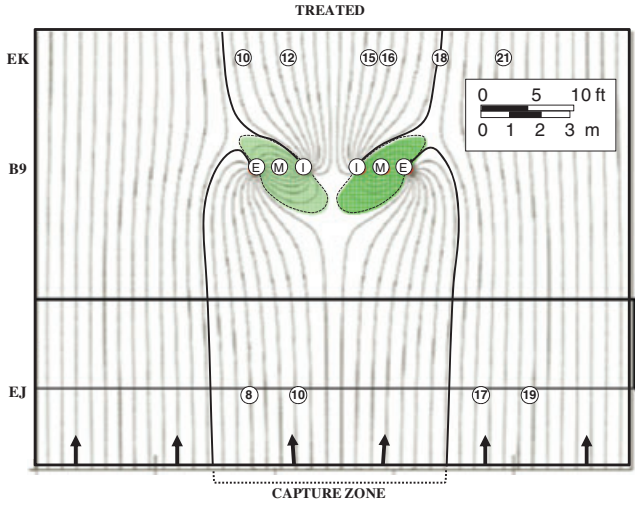

Figure 4. Plan view of well layout with simulated groundwater flow directions for the double well pair recirculation/oxygenation. Vertical scale is identical to horizontal scale. The TBA plume was captured by the extraction wells B9-1 and B9-6 ("E"), oxygenated aboveground, and injected into wells B9-3 and B9-4 ("I"). The captured water was recirculated through the system (green area), some portion escaping the recirculation zone to continue downgradient ("treated"). The "M" wells within the B9 transect were used for recirculation zone monitoring; the EJ transect was used for upgradient monitoring; the EK transect was used for downgradient monitoring.

tubing at a flow rate of 1 L/min with a peristaltic pump (Masterflex model HV-07591-00). Groundwater was filtered with a string-wound polypropylene cartridge filter (50 µm, McMaster Carr) then directed through an oxygenation chamber equipped with the same silicone tubing system used in the prior experiment. The tubing was pressurized at 23 to 26 psi with oxygen (Praxair, >99.9% purity). The oxygenated groundwater flowed into wells B9-3 and B9-4 via 0.95 cm (3/8 inch) OD LDPE tubing.

Core samples were collected 178 d after recirculation and oxygenation were initiated. Cores were collected manually from locations 0.5 m downgradient (north) of five monitoring wells (EJ-19, EJ-23, B9-5, EK-16.5, and EK-19). Locations were selected to sample environments within and outside of both the TBA plume and the aerobic treatment zone. After hand augering to 2.74 m (9 feet) bgs, a 15.2-cm (0.5 feet) coring device with stainless steel liner (0.46 m or 1.5 inch OD) was driven from 2.74 to 2.89 m bgs (9 to 9.5 feet). Immediately after collection, the liner containing the core sample was removed from the device, capped, and kept on ice until arrival at the laboratory. The process was repeated twice to collect samples from the intervals 2.89 to 3.05 m bgs (9.5 to 10.0 feet) and 3.05 to 3.20 m bgs (10.0 to 10.5 feet). Between sample locations, the core barrel was cleaned with water and a 70% ethanol solution. In the laboratory, core samples were subsampled and stored in 50 mL graduated polypropylene tubes (430829, Corning Inc., Corning, New York) at −80 °C until DNA extraction and analysis were performed.

Other data collection and analytical methods for the double well pair system were the same as for the single well pair system with the exception of DO, which was measured in the field with the HACH Dissolved Oxygen Test Kit (Model OX-2P, HACH Company, Loveland, Colorado) according to manufacturer specifications. In addition, samples were collected for analysis of nitrate, sulfate, and total and dissolved iron and manganese at the EH and EK transects. The nitrate and sulfate samples were unpreserved and analyzed within 28 d. The samples for dissolved metals were filtered with a 0.45 µm reinforced membrane filters (47 mm diameter, Millipore Corporation); dissolved and total metal samples were both acidified to a pH < 2 with nitric acid. All samples were stored at 4 °C until analysis at UC Davis. Nitrate was analyzed by flow injection analyzer with a detection limit of 0.05 mg/L. Sulfate as sulfur and total and dissolved iron and manganese were analyzed by ICP-AES, with detection limits of less than 0.1 mg/L.

Results and Discussion

System Performance: Single Well Pair Test

Constant rate recirculation between the injection and extraction wells was sustained for approximately 42 d. The recirculated water was oxygenated except from time zero to 6.3 d and 26.4 to 31.3 d (117-h duration). Bromide spiking commenced 12 h after groundwater recirculation began; the initiation of bromide spiking is defined as time zero for subsequent figures and discussions. Field monitoring detected elevated bromide levels in the extracted water within 24 h, confirming that injected water was being recirculated between the injection and extraction wells as expected based on initial modeling. More detail is provided by Kayne (2008).

Bromide concentrations steadily increased and, by 17.3 d, reached an apparent steady-state concentration of 200 mg/L in the groundwater at monitoring well EJP-19, midway between the injection and extraction wells (data not shown). DO in the recirculating water was increased by an average 16 mg/L during the test; the maximum injected concentration of DO was 27 mg/L. As shown in Figure 5, DO at EJP-19 rose to 8 to 10 mg/L by 26.4 d, then dropped during the subsequent 117 h during which oxygen release was stopped. After resumption of oxygen release, DO at EJP-19 was measured at about 10 mg/L and rose to 18 mg/L by the end of the test (42 d). The breakthrough of DO was more rapid during the resumption of oxygenation than it had been at the beginning of oxygenation, which suggests that solid oxygen demands in the recirculation zone were satisfied during the initial oxygenation. A similar observation was made in prior oxygen release tests at the site (Wilson et al. 2002) and lab evaluation of solid oxygen demands in VAFB sediments (Gandhi 2001).

Average and maximum TBA concentrations in the four monitoring wells (EJ-19, EJ-20, EJ-21, and EJ-22) upgradient of the recirculation well pair were 324 and 474 µg/L, respectively. Average TBA concentrations in the center of the recirculation zone (EJP-19) were 208 mg/L prior to oxygenation, suggesting that uncontaminated water to the east of the TBA plume was captured and diluted the plume concentrations to some extent (as illustrated by the expected capture zone in Figure 2). Because the background conditions are anaerobic, in situ biotreatment was not expected to be stimulated by recirculation alone. However, by Day 12 of the test (6 d after oxygenation began), TBA concentrations in EJP-19 decreased to below detection limits (Figure 5), indicating that in situ biodegradation was stimulated by oxygen addition.

Confirmation of Biodegradation: Single Well Pair Test

To test that TBA removal was associated with oxygen amendment, the oxygen supply and bromide tracer were shut

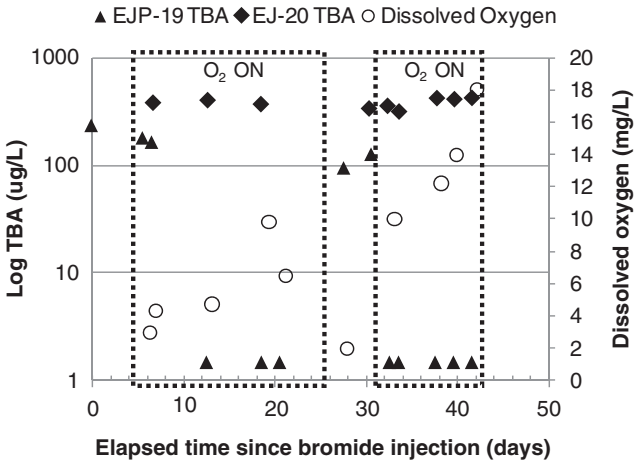

Figure 5. Concentrations of TBA and dissolved oxygen in monitoring well EJP-19 over time. Oxygenation began at 6.3 d and ceased for 117 h starting at 26.4 d, allowing the TBA concentration to rebound. TBA concentrations in upgradient monitoring well EJ-20 is shown for comparison.

off at 26.4 d, as mentioned above, but recirculation was held constant at 1 L/min. In EJP-19, DO and bromide concentrations dropped and TBA concentrations rebounded from below detection to above 100 µg/L (Figure 5). Once oxygenation was restored and DO increased to above 10 mg/L in EJP-19, TBA levels rapidly decreased to nondetectable concentrations and remained nondetectable throughout the remainder of the test period. This rise of TBA concentrations during the cessation of oxygen delivery to the subsurface and the decline of TBA concentrations during subsequent restarting of oxygen delivery indicate that the disappearance of TBA was due to aerobic biodegradation by native bacteria.

Figure 6 depicts the changes in TBA and bromide concentration that were detected 7.9 m downgradient of the recirculation wells at six wells spaced at approximately 0.8-m intervals in the EK transect. Bromide concentrations began to rise above background levels in the EK transect at 12.5 d, consistent with an approximate groundwater velocity (including local impact of recirculation) of 0.6 m/d, which agrees well with previous measures of velocity at Site 60 (Mackay et al. 2006). Comparing concentration data for 13.2, 33.2, and 60.2 d indicates that bromide concentrations in the EK transect rose above 200 mg/L while TBA concentrations dropped below detection limits (Figure 6). High bromide and nondetectable TBA concentrations were observed for the remainder of the 42-d study (Kayne 2008).

The simultaneous rise of bromide concentrations and fall of TBA concentrations at the EK transect is evidence that the TBA-contaminated water was being treated as a result of passing through the oxygenated recirculation zone and that it continued to migrate downgradient at approximately the regional groundwater velocity. Bromide distribution in the EK transect suggests stable groundwater flow direction during the 42-d study, as the maximum concentration remained in roughly the same location throughout (Kayne 2008). The bromide plume was approximately 5.4 m wide at the EK transect, confirming the expectations from the flownet model (Figure 2).

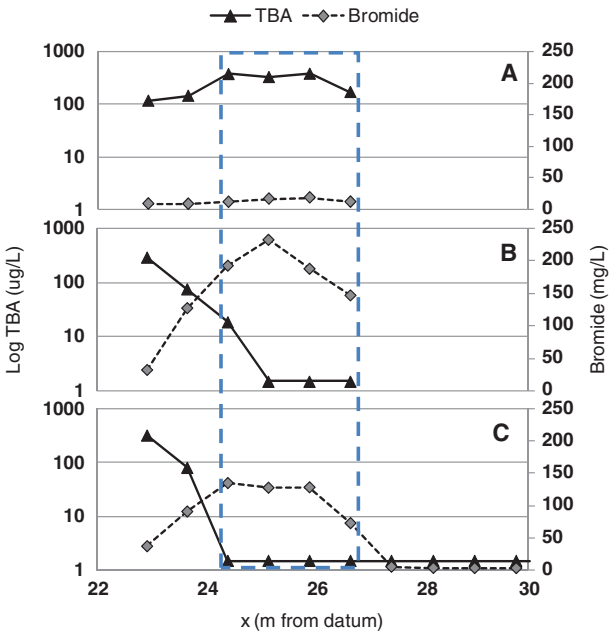

Figure 6. TBA and bromide tracer concentrations at three times in the EK transect, located 7.9 m downgradient of the single well pair recirculation/oxygenation. Frame (A) represents 13 d; Frame (B), 33 d; Frame (C), 60 d. Monitoring well locations are defined by distance from a reference point to the west. The approximate extent of the treatment zone is indicated by the dashed box.

Quantification of Microbial Populations: Single Well Pair Test

DNA extracted from groundwater samples collected at 5.6, 12.6, and 34.6 d from the treatment zone monitoring (EJP-19) and extraction (EJP-20) wells was analyzed using qPCR. Average measured population densities of strain PM1 and total bacteria at the study site are summarized in Tables 3 and 4. Our findings are consistent with results of prior aerobic bacterial biodegradation studies, both laboratory microcosms using Site 60 sediments and in situ experiments at VAFB Site 60 (Gandhi 2001; Wilson et al. 2002; Hristova et al. 2003).

In the sulfate-reducing region upgradient of the treatment zone, PM1 density was 5.4×10^4 copies/L and total bacteria 4.6×10^7 16S copies/L. In the aerobic treatment zone, the PM1 population density ranged between 1.3×10^5 and 1.6×10^6 16S copies/L; the total bacterial population density ranged between 4.9×10^7 and 1.7×10^8 16S copies/L (Table 3). The 16S genes in a bacterium may range from 1 to 13 copies per cell and thus contribute variation to the conversion from copy number to cell number (Bach et al. 2002); however, the PM1 genome contains only one copy of 16S rDNA. It can thus be reasonably assumed that each PM1 copy number is equivalent to an individual PM1 bacterial cell whereas the copy numbers of total 16S rRNA may slightly underestimate the total bacterial population.

The normalized PM1 density was consistently three to four orders of magnitude lower than the total bacterial density. Normalization was achieved by dividing PM1 copy numbers by total bacteria copy numbers. The normalized PM1 density ranged from 0.16% to 0.58% in the aerobic treatment zone and 0.05% to 0.18% in the sulfate-reducing zone. Although the normalized PM1 density is slightly

Table 3

Average PM1 and Total Bacterial Copy Numbers in Recirculation Monitoring Well EJP-19 at Three Times for the Single Well Pair Recirculation/ Oxygenation System

Day	TBA (µg/L)	Universal Bacteria 16S (Copy Number/L)	PM1 16S (Copy Number/L)	Percentage of Total Bacteria That Is PM1
5.6	180.3	1.7E+08	1.5E+06	0.88
12.6	ND (<3 µg/L)	7.4E+07	1.3E+05	0.18
34.6	ND (<3 µg/L)	1.1E+08	3.2E+05	0.28

ND = not detectable.

Table 4
Comparison of PM1 Population Densities in Two Field Studies at VAFB Site 60

Contaminant and Reference	Contaminant Concentrations	DO Concentrations in Treatment Zone (mg/L)	PM1 Inside Treatment Zone (Aerobic)	PM1 Outside Treatment Zone (Sulfate-Reducing)
MTBE (Hristova et al. 2003)	~1300 µg/L MTBE degraded to <20 µg/L	3–5	10^5–10^7 cells/L	10^4 cells/L
TBA (this study)	~250 µg/L TBA degraded to <3 µg/L	5–10	10^5 cells/L	10^4 cells/L

higher in the aerobic zone than in the sulfate-reducing zone, the difference is minor and, regardless, PM1 made up only a small portion of the total bacterial population even under aerobic conditions. This suggests that even though TBA could serve as a substrate due to the presence of oxygen, there was not enough of it to support an increase in PM1 population density that would typically be considered significant in practice (i.e., at least one order of magnitude).

On the basis of previous observations, we predicted there to be a relationship between PM1 densities and TBA concentration. Hristova et al. (2003) found a correlation between the distribution of native PM1 density with oxygen presence and higher MTBE concentrations in situ during prior experiments at VAFB Site 60. In Table 4, the PM1 density and DO and TBA concentrations detected both inside and outside the aerobic zone in this study are compared with those detected by Hristova et al. (2003). TBA was not detectable in the Hristova et al. (2003) study; the concentrations of MBTE are presented instead. Table 4 shows that background PM1 densities were similar in the two studies, but the increase in PM1 density in the prior study was in the order of 1 to 100 times higher than that in this study, presumably in part because the substrate concentration (MTBE or TBA) was higher by a factor of five in the former study than in the current study.

Unlike the results of Hristova et al. (2003), correlation analysis of normalized PM1 densities and concentrations of TBA and of DO from this study did not show strong relationships. For example, linear regression of normalized PM1 concentration vs. TBA and DO concentrations gave respective R^2 values of 0.34 and 0.11 for well EJP-19. Neither the disappearance of TBA nor the presence of DO made a significant difference in the percentage of PM1 in the total bacterial density detected in the sampled groundwater.

System Performance: Double Well Pair Test

The areal distribution of tracer and treated water from the recirculation and oxygenation of groundwater by a single well pair fit model expectations quite well, as noted previously. We therefore assume accurate predictions from similar modeling of two recirculating well pairs in the same aquifer. Thus, although use of bromide tracer was not allowed for the double well pair test, we can reasonably expect recirculation to occur and treated water to appear where expected based on our modeling.

The double well pair recirculation ran successfully for 291 d; brief system shutdown was required for regular maintenance (replacement of filters, cleaning of lines, redevelopment of injection wells). The diffusive oxygenation system added 10 mg/L DO to the recirculating water (data not shown). This concentration is far above the theoretical oxygen demand (0.5 mg/L), assuming complete mineralization of the average extracted TBA concentration (208 µg/L), so the system was expected to sustain in situ aerobic TBA degradation and lead to extra DO migrating downgradient from the recirculation zone.

DO snapshots collected at the EK transect, 2.3 m downgradient from the recirculation wells, indicated throughout system operation that an aerobic zone (DO ≥ 2 mg/L) extended to at least the EK transect; Figure 7 presents results for 232 d after start-up, but similar results were seen in other samplings (data not shown). The aerobic zone was on the order of 7 m (23 feet) wide, consistent with model predictions (~6.7 m or 22 feet) at the EK transect (Figure 4). TBA was below the detection limit (<3 µg/L) at the EK transect during this test, suggesting complete treatment of TBA within the recirculation/treatment zone.

Between Days 268 and 290, snapshot sampling for nitrate, sulfate, and total and dissolved iron and manganese was conducted at the EH (upgradient of recirculation/oxygenation) and EK (downgradient of recirculation/oxygenation) transects. At the EH transect, both species of iron and manganese were slightly elevated within the plume compared to surrounding groundwater; the maximum total iron concentration was 10.0 mg/L and the maximum total manganese concentration was 5.0 mg/L. Where aerobic conditions prevailed in the EK transect, the iron and

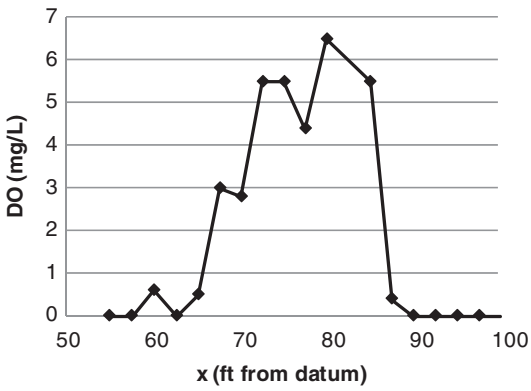

Figure 7. Dissolved oxygen concentrations in the EK transect 2.3 m downgradient of the double well pair recirculation/oxygenation on June 23, 2009, 232 d after system start-up. Monitoring well locations are defined by reference to a datum to the west, as in Figure 6.

manganese concentrations were below 1.0 and 0.5 mg/L, respectively, suggesting that the reduced forms of these metals had been oxidized within the treatment zone (data not shown). Interestingly, the dissolved iron and manganese presented a higher oxygen demand than the TBA. Manganese has an oxygen demand of 0.29 g/g and iron has a demand of 0.14 g/g, assuming reaction stoichiometry presented by Stumm and Morgan (1970). Thus, complete oxidation of dissolved iron and manganese consumed a maximum of 2.9 mg/L of the 10 mg/L oxygen added via recirculation, suggesting that more than enough oxygen would have been available to support biodegradation of the TBA, which had an oxygen demand less than 0.5 mg/L.

Quantification of Microbial Populations: Double Well Pair Test

As in the single well pair recirculation experiment, groundwater samples were collected for microbial analysis. However, total DNA extracted from 2-L samples, twice the groundwater volume used in the single well pair experiment, was not detectable on a gel, thus there was not enough DNA to conduct qPCR for total bacteria and PM1 on groundwater samples. The small amount total DNA extracted indicates that bacterial population densities were lower during the second than the first experiment.

Sediment samples from the anaerobic area within the plume (EJ-19), the aerobic treatment zone (B9-5), and the anaerobic area outside the plume (EK-19) contained detectable amounts of DNA and were analyzed for total bacteria 16S and PM1 with qPCR. Average measured population densities are summarized in Table 5.

The normalized PM1 density was at least two orders of magnitude lower than the total bacterial density. The ratios of PM1 16S to total bacterial 16S copy numbers were 1.0 and 3.8% in the anaerobic and aerobic treatment zones of the plume, respectively. These results may indicate growth of PM1 within the aerobic treatment zone, but not the anaerobic aquifer, although qPCR results for PM1 are all below the practical quantification limit. As in the single well pair test, these results suggest that PM1 made up a small portion of the total bacterial population and the concentration of the substrate (TBA) was too low to support a quantifiable increase in PM1 population density.

Table 5
Average PM1 and Total Bacteria Copy Numbers from Sediment Cores

Core Location	Universal Bacteria 16S	PM1 16S	Percentage of Total Bacteria That Is PM1
B9-5 in treatment zone	1.2E+05	4.5E+03	3.8
EJ-19 in plume	4.7E+05	4.8E+03	1.0
EK-19 outside plume	1.0E+08	Undetermined[1]	Undetermined[1]

[1]Copy numbers are below the practical quantitation limit.

Summary and Conclusions

The results of these studies suggest that recirculating well pairs can create efficient, sustainable aerobic treatment zones for in situ biodegradation of TBA or other contaminants readily biodegraded under aerobic conditions. Depending on the hydrogeologic and geochemical properties of a contaminated aquifer, this technique could be applied to wider plumes by using multiple well pairs across the plume or by increasing flow rates so that each well pair captures larger portions of the plume.

For the recirculation/oxygenation method to work, the recirculation rate must be sufficiently high to ensure that a fraction of the injected water is captured by the extraction well and recirculated. It is possible to adjust the fraction of injected water that is recaptured by adjusting the recirculation (i.e., pumping) rate and thus, the average residence time of plume water within the recirculation zone. A distinct advantage of the recirculating well pair system is that recapturing of injected water allows for the addition of more oxygen with each recirculation cycle. The total amount of oxygen that can be added is thus a function of the average number of cycles that groundwater is recirculated before flowing downgradient. This can be an important advantage over "single pass" systems (e.g., passive oxygen diffusion), allowing adjustment of total oxygen addition and accommodation of higher concentrations of organic substrates or abiotic oxygen demands such as dissolved metals or reduced species on sediments.

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