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ORIGINAL ARTICLE

Dynamics of MTBE-degrading activity in porous media using a large-scale laboratory experiment

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Abstract Methyl tert-butyl ether (MTBE) is an oxygenated organic compound that tends to form large groundwater contamination plumes. If bioaugmentation is used as a remediation technique, the question of the mobility of the bioactive zone (BAZ) with time is of interest. The objective of this experiment was to study the spatial redistribution of MTBE-biodegradation activity through time, following the injection of a bacterial culture in a homogeneous porous media, at high pressures and concentrations. The experiment was performed using a large-scale aquifer physical model, which can incorporate physicochemical heterogeneities similar to those found in the field, under controlled laboratory conditions. The experimental tank was filled with 1.0-mm-diameter glass beads to represent a homogeneous high hydraulic conductivity porous medium. During inoculation, the bacterial culture was distributed in a circular pattern. Initially it appeared that the BAZ was located in the upstream portion of the inoculated zone, where oxygen was available in conjunction with the inoculated bacteria and MTBE. With time, the BAZ moved upgradient through the whole tank towards the inlet. This implies the successful movement of bacteria from the inoculation area against advective flow into previously sterile zones of the tank. A mass balance showed that dissolved oxygen concentrations were likely not a limiting factor during the experiments.

Keywords MTBE · Biodegradation · Transport · Bioaugmentation · Bioactive zone · Inoculation

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Introduction

Bioaugmentation is an aquifer remediation technique involving the delivery of a microbial culture into an aquifer and if necessary the accompanying nutrients. This approach is generally only used if indigenous degrading organisms lack sufficient or specific activity for the chemicals of concern (Alexander 1999). The processes influencing the transport of inoculated microorganisms through the subsurface can be physicochemical and biological (Jordan et al. 2004). In some cases, the expansion of bacterial colonization in porous media has been detected in all directions from the point of inoculation, including migration against advective water flow (Yarwood et al. 2002; Dorn et al. 2005). Some laboratory techniques employed to study the fate and transport of bacteria in porous media include: (1) luminescence methods (Yarwood et al. 2002; Yolcubal et al. 2003; Dorn et al. 2005; Oates et al. 2005), (2) substrate or terminal electron acceptor utilization (Odencrantz et al. 1990; Murphy et al. 1997; Yolcubal et al. 2003), and (3) laboratory visualization experiments (Thullner et al. 2002; Oates et al. 2005).

During bioaugmentation, the mobility of the degrading activity or bioactive zone (BAZ) with time is of interest. When the injected suspension is dilute and the pores are large relative to the microorganisms, these microorganisms can be readily carried through the aquifer by the groundwater flow. However, as the injected suspension becomes

more concentrated with microorganisms, flocs larger than aquifer pores are formed, and during injection much of the biomass is expected to be filtered and to clog the pores relatively near to the injection point. It is this second scenario that is being studied here.

The importance of mobility of microorganisms relies on the accessibility of bacteria to the substrate. The substrate is generally a compound which is the focus of the remediation efforts (Alexander 1999). MTBE is an oxygenated organic compound that tends to form large groundwater contamination plumes (Schirmer and Barker 1998; Mackay et al. 1999; Salanitro et al. 2000). Since the reduction of MTBE mass by physical process is relatively insignificant (Jacobs et al. 2001), bioremediation may play a significant role in the reduction of MTBE at contaminated sites. Although MTBE was originally thought to be highly resistant to biodegradation, currently the literature reporting biodegradation of MTBE is rather extensive (Deeb et al. 2000; Fiorenza and Rifai 2003; Schmidt et al. 2004 and others).

The objective of this experiment was to study the spatial redistribution of MTBE-biodegradation activity through time, following the injection of a bacterial culture in porous media, at high pressures and concentrations. Field investigations are costly and often fall short of rigorous testing; in contrast, most laboratory experiments do not incorporate the physical and chemical heterogeneities found in the field. Large-scale laboratory experiments provide a relatively inexpensive approach for studying complex processes in idealized porous media under controlled laboratory conditions. Therefore, these experiments were performed in large-scale aquifer physical models.

Materials and methods

Two-dimensional aquifer physical model

The aquifer physical model was designed to simulate a two-dimensional aquifer section. The tank was made of stainless steel (1.22 × 1.22 × 0.05 m). On one side it has a 0.9 × 0.9 m window made of 1.3-cm-thick Lexsan for visualization. The tank was designed with three removable steel support bars to support the window during the high-pressure injections. Four 0.13-cm-diameter inlet/outlet ports are found on each side and on the bottom. The back has a 5 × 5 array of sampling ports, each with a diameter of 0.3 cm. Each port has a Swagelock fitting, through which a 10.2-cm pipetting needle is inserted (16 gauge with blunted ends, VWR). A luer fitting plastic valve (VWR) is attached to the needle, and the water sample is withdrawn using a plastic syringe. The top is covered by a removable stainless steel cap that is bolted on. Each tank is supported in a stand that allows it to be tilted and moved.

The tank top has a 1.9-cm injection hole in the center, plugged except when inserting the drive rod during inoculation. Through this hole, and only during inoculation, a 1.6-cm-diameter soil-gas sampler rod (AMS, American Falls) is inserted. This hollow drive rod is used to inject the inoculation suspension. The injection pressure is monitored with a manual pressure gauge connected to the top of the injection rod. The inlet ports are manifolded together using 1 cm diameter copper tubing. Ports on the outlet and bottom are similarly manifolded. During inoculation of the tanks, these manifolds serve as fluid overflow outlets. A 1,000 psi (max), Hydra-cell, variable-speed, diaphragm pump (Warner Engineering, Inc., Minneapolis, MN, USA) is used to inject the bacterial inoculation suspension into the tank through the injection rod. The tanks were packed with glass beads (Potters Industries Inc., TX; Jaygo Inc). Water flow in the tanks is from left to right (when looking at the window) and is maintained by a constant head system.

The feed solution was prepared daily in a 50-L nalgene container with previously de-chlorinated tap water (de-chlorination by airsparging for 2 days). The water was then sparged with oxygen for 10 min to obtain a dissolved oxygen (DO) level of >30 mg/l. MTBE was then added, and the solution was stirred. DO levels dropped during the day due to volatilization; preparing a feed solution daily ensured that inflow concentrations did not drop below 10 mg/l. To maintain a high DO concentration as high as possible in the feed solution, a passive oxygen diffusion system was installed inside the container based on the work by Wilson et al. (2002) and Wilson and Mackay (2002). This passive diffusion system consists of a coil of a 0.9 cm ID–1.3 cm OD tube permeable to oxygen (Platinum-cured silicone, Tygon 3350, VWR) which is pressurized with oxygen at 15 psi. The feed container has a bottom outlet of 0.3 cm, and a peristaltic pump is used to feed a constant head device going first through a 0.2-μm filter (Whatman). The excess flow (return flow) from the constant head device discharges back into the feed tank. To avoid as much as possible MTBE and DO volatilization, only a small hole from the constant head device is open to the atmosphere. The tank has a pair of inflow and outflow tubing lines that work as piezometers to measure hydraulic head differences manually. Water collection for DO analyses was done using Tedlar bags connected to a valve located before the outlet discharge. Then the water from the Tedlar bag was slowly pumped into a DO measuring cell. The DO was measured using a dissolved oxygen probe (Model 550A, YSI Inc) with a maximum quantification limit of 40 mg/l.

Approach

The experimental tank was filled with 1.0-mm-diameter glass beads to represent a homogeneous high hydraulic conductivity (K) porous medium. The objective of this experimental tank was to determine the migration of MTBE-biodegradation activity or BAZ following an inoculation at the center of a homogenous tank.

After filling the tank with the porous media, the tank was rinsed five times with a 10% bleach solution for sterilization. Following this, rinsing was performed with deionized water until all measurable chlorine was gone. Flow was initiated 67 days prior to the inoculation date. Prior to and after inoculation two tracer tests were performed on each tank: one using Uranine as a visual tracer and another one using NaBr (analyzed with a Dionex DX40 ion chromatograph).

Measured parameters

Periodically, 20 ml samples were collected from the ports on the back of the tanks into 40 ml vials. The samples were analyzed for MTBE using headspace gas chromatography (GC) analysis. The GC (SRI Model 8610C) was equipped with a 0.53-mm ID × 60 m MXT-Vol glass capillary column with a 2.0-μm *df*. A flame ionization detector (FID) was used for most samples; a photo ionization detector (PID) was used to analyze low MTBE concentrations. The analyses were performed by injecting 0.5 ml of headspace, and the GC was set isothermally at 70°C for 4 min. For every ten samples, a standard was run. This method was not used to analyze for TBA because GC/MS analyses suggested co-elution of another chemical along with TBA (the chemical suggested by the GC/MS software was dimethyl sulphide).

Samples were also collected periodically from the back of the tanks for DO analysis. Sixty milliliters was withdrawn with a plastic syringe at each port and released slowly into a DO measuring cell until achieving a stable reading with a DO probe meter (Model 550A, YSI Inc.). Other measurements collected through the length of the experiment were hydraulic head differentials (using calipers), flow (measuring the water volume collected in different time periods), and MTBE and DO concentrations of tanks inflows, outflows and from the feed solution.

Results

The flow on the experimental tank was initiated 67 days prior to the inoculation date. Visualization tracer tests were performed with uranine 48 days prior to and 30 days after inoculation, revealing no change on the flowpath pattern. Bromide tracer tests were also performed 29 days before and 62 days after inoculation using NaBr salt. The results of the NaBr tracer test were interpreted using an analytical solution for solute transport of a conservative tracer in a one-dimensional flow field (Bear 1972). These tanks represent a two-dimensional flow experiment, but a one-dimensional analysis was selected because the samples were collected from the outflow line from the tank. The solution was fitted to the NaBr concentration data and determined values for pore-water velocity and longitudinal dispersion coefficient. Pore-water velocities obtained by this method were similar to the values calculated from flow measurements. The velocities obtained were 0.26 and 0.4 m/day for the prior to and after inoculation tests, respectively. The longitudinal dispersion coefficient increased from 0.005 m^2/day prior to the inoculation to 0.05 m^2/day after the inoculation.

The inoculation was performed with a mixed culture capable of degrading MTBE under aerobic conditions as described by Salanitro et al. (1994). The inoculation was performed with 9.0 l of the MC-culture (Salanitro et al. 1994) injected at a TSS of 2.3 g/l, with 0.44 mg/l of MTBE and at pressures from 12 to 30 psi. The injected microorganisms were stained red with Safranin O, and the carrier fluid was dyed green with Uranine. The culture was injected in the center of the tank, and the distribution attained was a circular pattern (Fig. 1). The stained microorganisms extended for 30.5 cm in diameter, and the dyed carrier fluid extended for 68.6 cm in diameter. After inoculation, the tank top was removed, and a small cavity was observed in the glass beads. This cavity was filled with 0.2 l of glass beads, and the tank was again sealed and flow restarted.

Fig. 1 Distribution achieved after injection (injected suspension dyed green; bacteria stained red). Some *red spots* on the edges of the window (especially on the left) are due to rust on the outside of the apparatus)

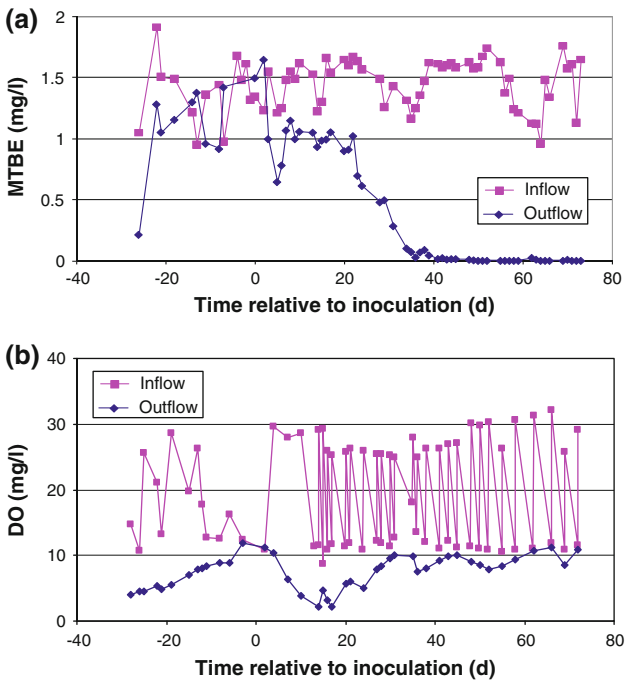

Fig. 2 **a** Inflow and outflow MTBE concentrations. **b** Inflow and outflow dissolved oxygen (*DO*) concentrations

The MTBE inflow concentrations were kept in the range of 1–2 mg/l through the length of the experiment. Outflow concentrations remained at those levels until 23 days after inoculation when they started to drop to <0.02 mg/l after about 50 days (Fig. 2a). Inflow DO levels had daily variations, decreasing from 30 mg/l in a fresh feed solution to 10 mg/l in an aged solution before being discarded. The outflow DO increased steadily from 5 to 12 mg/l prior to inoculation. After inoculation, DO levels decreased to 2 mg/l and steadily increased again to 11 mg/l (Fig. 2b). The estimated pore-water velocity was kept at approximately 0.3 m/day.

Periodically, samples were collected from the ports on the back of the tank. From these, snapshots of MTBE concentration patterns within the tank were obtained. Prior to inoculation, MTBE concentrations throughout the tank were >1 mg/l (Fig. 3a). MTBE concentrations decreased near the area of microbial inoculation 8 days after inoculation (Fig. 3b). After 22 days, the inoculation area turned dark, and most MTBE concentrations were >1 mg/l, except in the inoculated area and the zone downstream of it (Fig. 3c). After 35 days, MTBE levels dropped throughout most of the tank to <0.5 mg/l, except for the zone near the inlet (Fig. 3d). MTBE levels dropped throughout the tank to <0.1 mg/l, 49 days after inoculation (Fig. 3e). Dissolved oxygen levels increased throughout the tank with time. Thirty-five days after inoculation, most of the tank had DO concentrations >10 mg/l, except in the dark inoculated zone and downstream of it, which became anoxic (Fig. 4).

A mass balance was performed following the NRC (2000) guidelines using the following reaction for aerobic biodegradation of MTBE: $C_5H_{12}O + 7.5O_2 \leftrightarrow 5CO_2 + 6H_2O$. From this equation, it follows that DO consumption during aerobic biodegradation of MTBE is 7.5 mol O_2/mol MTBE or 2.73 g O_2/g MTBE (Rittmann 2002). The mass loading for DO varied from 24.0 to 162.6 mg/day if the daily low or high DO concentration is used (Fig. 2b). For MTBE, the average mass degradation rate was 12.0 mg/day, which using the stoichiometric relation for aerobic biodegradation of MTBE translates to a consumption of 32.9 mg/day of DO.

Discussion

After inoculation the inoculated area turned to a dark color. This effect was also observed in some preliminary experiments. This dark zone was anoxic and remained like this through the length of the experiment. Yarwood et al. (2002) also report the formation of an anoxic dark zone that extended through the inoculation zone, prevailing through the length of the experiment. Our dye tests showed that water passed through the entire inoculated area, similar to what was observed by Yarwood et al. (2002). Thullner et al. (2002) demonstrated that bioclogging must not necessarily change a given flow pattern or influence the properties of a system on a large scale. Then this dark color might indicate the zone where a high bacterial population remained after inoculation, even if it does not change the flow pattern. Even though MTBE has now been shown to degrade under anaerobic conditions (Bradley et al. 2001; Finneran and Lovley 2001; Kolhatkar et al. 2002), it is believed that in this experiment the BAZ first formed in a thin area upgradient of the dark inoculated zone, where oxygen was available in conjunction with the inoculated bacteria and MTBE, leaving clean water flowing downstream from it.

At around 4 days after inoculation there was a rapid drop and then rise in the MTBE outflow concentration (Fig. 2a). The only parameter that seems to be correlated to this MTBE drop is a drop in outflow DO concentrations to 4 mg/l.

From 8 to 22 days after inoculation, low MTBE concentrations were detected in the inoculated area and downstream of it (Fig. 3b, c). Then it is possible that initially the BAZ was located in the upstream portion of the inoculated zone where oxygen was available in conjunction with the inoculated bacteria and MTBE, similar to what was reported by Dorn et al. (2005). Then 35 days after inoculation, low MTBE concentrations were detected through most of the tank, except the whole upgradient zone (Fig. 3d). This could be caused by either of the two

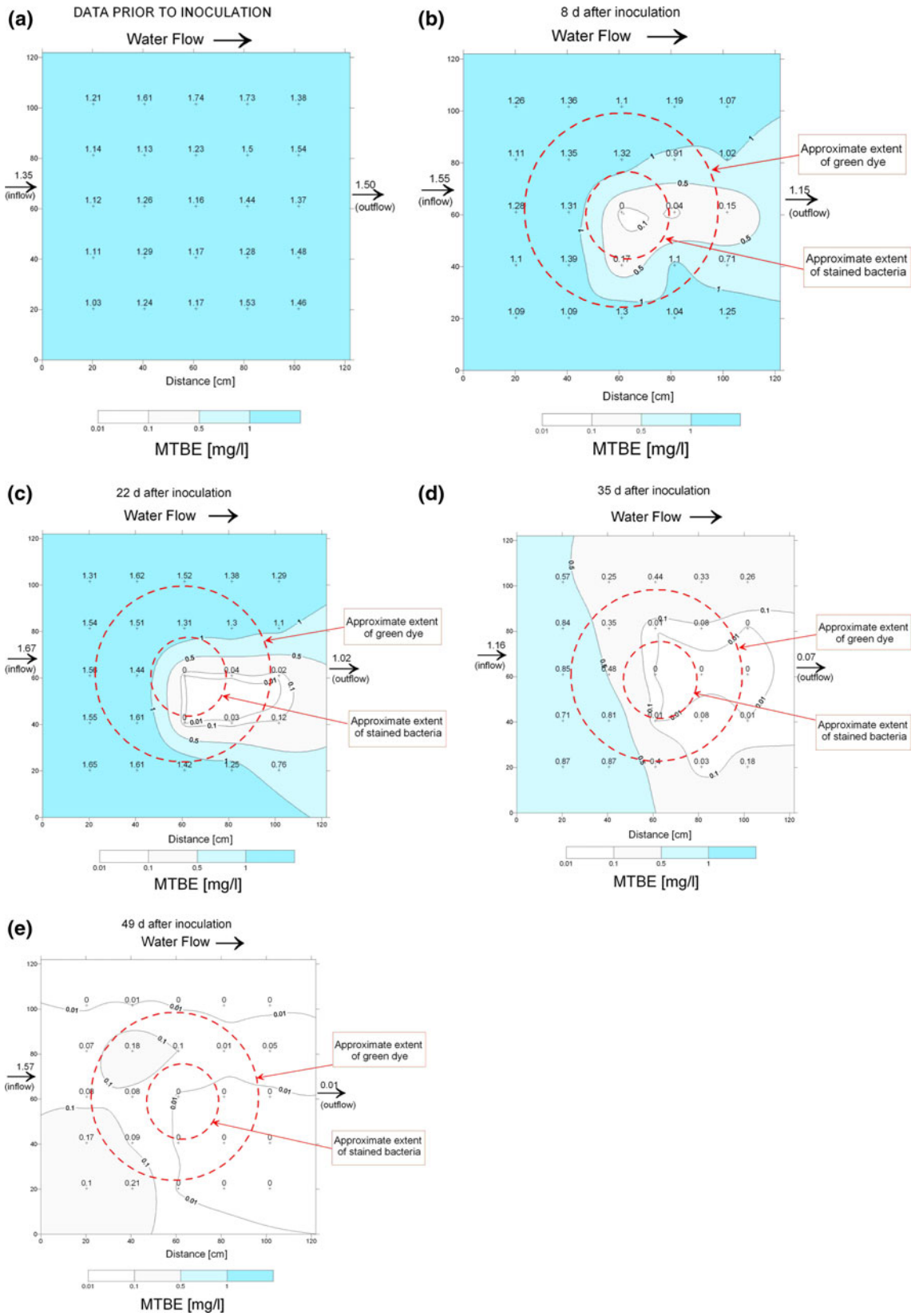

Fig. 3 **a** MTBE concentrations snapshot prior to inoculation. **b** MTBE concentrations snapshot 8 days after inoculation. **c** MTBE concentrations snapshot 22 days after inoculation. **d** MTBE concentrations snapshot 35 days after inoculation. **e** MTBE concentrations snapshot 49 days after inoculation

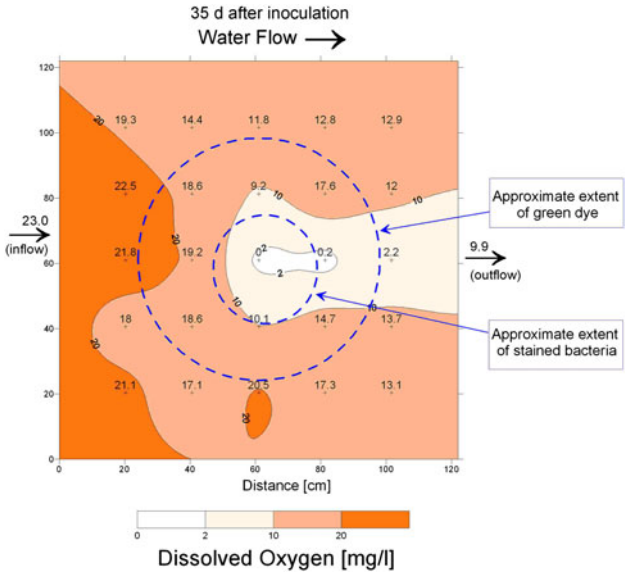

Fig. 4 Dissolved oxygen concentrations snapshot 35 days after inoculation

mechanisms: (1) bacteria traveling to all the zones in the tank creating a BAZ throughout most of the tank, or (2) having the BAZ moving to the upgradient portion of the tank, leaving clean water flowing downstream. Studies by Yolcubal et al. (2003) and Dorn et al. (2005) showed that the development of the BAZ near the inlet was not due to movement of bacteria but due to the growth of in situ bacteria population near the inlet (food source). This is different than what was observed in this study, where the compression of the BAZ towards the inlet implies the movement of bacteria from the inoculation area against advective flow into the previously sterile zones of the tank. The pattern of how the BAZ emanated from the inoculation area is strong evidence that the capable bacteria came originally from the inoculation as opposed to having been in the system at small numbers. This kind of movement has been reported with other kind of bacterial strains (Yarwood et al. 2002; Yolcubal et al. 2003; Dorn et al. 2005).

It appears that the BAZ or MTBE-degrading activity ended compressing near the inlet (Fig. 3e) similar to what has been observed by other researchers (Yolcubal et al. 2003; Dorn et al. 2005). Therefore, bacteria were successfully transported into sterile regions against advective flow. This does not prove, however, that other portions of the tank were not also capable of degrading MTBE (caused by the inoculation). Our results show that towards the end of the experiment, the tanks had mostly high DO levels (in general >6 mg/l) except in the inoculated zones and downgradient of them (Fig. 4). Yolcubal et al. (2003) showed that if DO was not the limiting factor, the BAZ could encompass the whole experimental area and that some biodegradation could occur downgradient.

A mass balance performed on the tank showed that the average DO mass loading for the tanks was 2.8 times the MTBE rate supply. This information, coupled with the DO effluent concentrations, suggests that DO concentrations were not a limiting factor on most of the experiment. There were, however, some anaerobic zones detected in some portions of the tank and a drop and rise on MTBE outflow concentration that occurred around 4 days after inoculation. The apparent excess DO consumption was probably lost by volatilization by the recirculation loop from the constant head device.

Bacterial activity occurs in zones where substrate and electron acceptors mix (Thullner et al. 2002; Murphy et al. 1997). Therefore, the formation of the BAZ is highly dependant on the method of substrate addition (Thullner et al. 2002). In aquifers, substrate and electron acceptors come from different sources. In this experiment, the feed solution contained both MTBE and oxygen, and these results may not be directly comparable to what may happen in the field. Therefore, the relevance of mixing of substrate and electron acceptors for real environmental scenarios remains a matter of discussion.

Previously, it had been shown that bacteria could get transported to the previously sterile zones, where electron acceptors and substrate are present, even against advective flow. Our results confirm this with an MTBE-degrading culture and at larger scales by the facilities provided by the size of the experiments. However, additional research is necessary to evaluate more in-depth bacterial migration into lower permeability regions, heterogeneous systems, and real hydrogeological settings. An open research question would be the reason for the formation of a dark (anoxic) zone in the inoculated area and its influence on the dynamics of BAZs in larger time frames.

Conclusions

- The BAZ formed first in a thin area upgradient of the inoculated zone, where oxygen was available in conjunction with the inoculated bacteria and MTBE, and clean water would flow downstream from it.
- The BAZ moved upgradient from the inoculation zone to the inlet, leaving clean water flowing downstream. This implies that the inoculated bacteria successfully migrated from the inoculation area into the previously sterile zones of the tank against advective flow.
- A mass balance performed on the tank showed that DO concentrations were likely not a limiting factor during the experiments. The apparent excess DO consumption was probably lost by volatilization by the recirculation loop from the constant head device.

- Additional research is necessary to evaluate more in-depth bacterial migration into lower permeability regions, heterogeneous systems, and real hydrogeological settings.
- After inoculation of the tank, the inoculated zone turned dark and anoxic. An open research question is the reason for the formation of this dark (anoxic) zone and its influence on the dynamics of BAZs in larger time frames.

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