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Groundwater remediation by an in situ biobarrier: A bench scale feasibility test for methyl tert-butyl ether and other gasoline compounds

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ABSTRACT

Most gasoline contains high percentages of methyl tert-butyl ether (MTBE) as an additive. The physico-chemical properties of this substance (high water solubility, low sorption in soil) result in high mobility and dissolved concentrations in soil. In situ permeable biological barriers (biobarriers, BBs) can remediate MTBE polluted groundwater by allowing pure cultures or microbial consortia to degrade MTBE when aerobic conditions are present, either by direct metabolism or cometabolism. Lab-scale batch and column tests were carried out to assess a selected microbial consortium in biodegrading MTBE and other gasoline compounds (benzene B, toluene T, ethylbenzene E, xylenes X) and to measure the parameters affecting the efficacy of a BB treatment of polluted groundwater. During the aerobic phase of the batch tests, the simultaneous biodegradation of MTBE, tert-butyl alcohol (TBA), B, T, E and *o*-X was observed. The rapid biodegradation of BTEXs resulted in decreased oxygen availability, but MTBE degradation was nevertheless measured in the presence of BTEXs. Stationary concentrations of MTBE and TBA were measured when anoxic conditions occurred in the systems. Values for a first order kinetic removal process were obtained for MTBE ($0.031 \pm 0.001\ d^{-1}$), B ($0.045 \pm 0.002\ d^{-1}$) and T ($0.080 \pm 0.004\ d^{-1}$) in the inoculated column tests. The estimate of the BB design parameters suggested that inoculation could significantly modify (double) the longitudinal dispersivity value of the biomass support medium. No effect was observed in the retardation factors for MTBE, B and T.

1. Introduction

Petroleum products fall into three major categories: (i) fuels; (ii) solvents and lubricating oils; and (iii) feedstock for the petrochemical industry. Demand is greatest (more than 40%) for products in the fuel category, especially motor gasoline. Gasoline is a mixture of hydrocarbons with carbon atoms between 5 and 12, composed by approximately 40% of linear and branched alkanes, 20% of cycloalkanes, 25% of aromatic compounds (benzene B, toluene T, ethylbenzene E, xylenes X, naphthalenes). The remaining percentage is composed of additives that increase the octane rating of the mixture [1].

Since the early seventies, ethers and alcohols have replaced toxic additives such as tetraethyl lead or tetramethyl lead. Methyl tert-butyl ether (MTBE) is added to approximately 80% of the gasoline used worldwide; it is preferred to other possible additives (ethanol, ethyl tert-butyl ether, tert-amyl methyl ether, etc.) for its low reactivity under standard conditions and costs [2,3].

MTBE water solubility (23 200–54 000 mg l^{-1} at 25 °C) and its percentage in unleaded gasoline result in a very high effective solubility in water (up to 7500 mg l^{-1}). Moreover, sorption in soil is limited by its low water–organic carbon distribution coefficient K_{OC}. In groundwater where gasoline leaks have occurred [4,5], long contaminated plumes often develop.

Due to issues of odor and taste, health concerns, and uncertainty about the carcinogenic effects of MTBE [6], many governmental agencies have issued drinking water standards for MTBE. For example, in the New York State public drinking water systems 10 μg l^{-1} has been issued [7]. The U.S. EPA has issued an interim measure of 20–40 μg l^{-1}. In the State of California 13 μg l^{-1} has been issued as a primary standard [8]. In some European countries, guideline values between 2 and 30 μg l^{-1} have also been issued [9].

Many researchers [10–12] reviewed MTBE degradation studies. Biodegradation under anaerobic conditions was reported. However, depending on the microorganisms involved in the process, biodegradation was more frequently observed under aerobic conditions by pure or mixed cultures, either through direct metabolism [13–15], or cometabolism on different primary substrates, such as short chain alkanes [16,17], monoaromatic compounds [18], glycerol, lactate, etc. [19]. Inhibition or competitive use with BTEXs was reported in [20,21]. However, Deeb et al. [22] suggested that BTEX

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and MTBE degradation can occur via independent and inducible pathways.

Intermediate compounds of MTBE biodegradation under aerobic or microaerobic conditions were observed in both laboratory and field studies and included tert-butyl alcohol (TBA), and tert-butyl formate (TBF) [10–12,23]. TBA can be produced directly in waters by means of microbially mediated hydrolysis of TBF or oxidation of MTBE [24].

The different technologies that have been applied to remediate groundwater polluted by MTBE and other gasoline compounds are Pump and Treat, Air Sparging, Multi-Phase Extraction, and In Situ Chemical Oxidation [25]. One innovative approach involves permeable reactive barriers (PRBs). In this approach, groundwater flows through a high permeable reactive zone of the system, under the effect of the natural hydraulic gradient, so that the dissolved contaminants in the plume can be captured by sorption in the barrier filling material (e.g. on activated carbon), or removed by chemical reactions (e.g. by reduction with zero valent iron) or biodegraded by the microorganisms attached to the biobarrier (BB). PRBs are considered passive systems, that is to say that only limited maintenance is necessary and no on-going energy input is required [26]. The U.S. EPA database on MTBE treatment profiles [27] reports 3 full scale "biobarriers" for 317 cases in which groundwater treatment was applied. However, at these sites, inoculation or electron acceptor/nutrient supply was performed in a natural aquifer. As such, limitations due to soil heterogeneities, hydraulic conductivity, and biomass attachment/detachment could not be avoided. It is possible to reduce these problems by using a proper biomass support. For attaining the ultimate goal of degrading MTBE by a BB system, several filling materials were considered [13], including peat, perlite, pozzolan, and pumice. Vesela et al. [28] reported a BB field pilot scale test, where oxyhumolite (oxidized young lignite) was used as a biofilm carrier to biodegrade gasoline compounds in groundwater, including BTEXs.

The design of a BB system requires the following steps [29,30]: (i) site characterization, especially concerning geology and hydrogeology (i.e. groundwater level, aquifer thickness, effective porosity, hydraulic conductivity, flow direction, hydraulic gradient), and groundwater physico-chemical parameters (pH, redox potential, dissolved oxygen, nutrients, temperature, iron, manganese and sulfate concentrations); (ii) lab-scale batch tests (to select the proper biomass and conditions for the process), and column tests (to estimate the longitudinal dispersivity of the BB filling material, the pollutant retardation coefficient in the system, and the biodegradation kinetic constants); (iii) 3D numerical modeling, in order to select the optimal BB location and size (affecting the residence time of pollutants in the reactive zone), and to evaluate its effect on water flow.

This paper focuses on step (ii) where a site with an unleaded gasoline leak produced MTBE and BTEX groundwater pollution. Step (iii) has been described in [31]. The work done during the experiment focused on assessing some of the problems that may arise when designing a biobarrier: (a) variation of the hydraulic conductivity during the treatment, highly affecting the hydraulics of the system; (b) variation of the longitudinal dispersivity and retardation coefficients, affecting the transport of pollutants; (c) delivery of the proper amount of electron acceptors and side effects.

2. Materials and methods

2.1. Polluted groundwater

In order to perform the experiments under realistic but stable conditions (gasoline spill along an underground pipeline), the polluted groundwater was prepared at the laboratory. Fresh unleaded gasoline (MTBE content: 3% on volume basis) was periodically spilt over not-sterilized unpolluted groundwater in a closed tank, in order to get a Non-Aqueous Phase Liquid (NAPL) to groundwater ratio of approximately 1:10 on a volume basis. At equilibrium, reached within 3 d of contact at the laboratory temperature ($23 \pm 1\,^{\circ}C$), NAPL resulted in dissolved concentrations of MTBE, BTEXs, and n-octane constant with time; measurements ($n = 25$) performed throughout the experiments resulted in the mean values reported in Table 1. Other short chain linear alkanes and TBF remained always below their analytical detection limit (DL). Stationary conditions were not obtained for TBA, whose irregular variation over time (2.8–13 mg l^{-1}) was ascribed to biotic mechanisms occurring in the dissolved phase of the tank [24]. The solution had 22.1 ± 0.5 mg NO_3^- l^{-1}, 65 ± 2 mg SO_4^{2-} l^{-1}, 18.0 ± 0.6 μg PO_4^{2-} l^{-1}, 28.3 ± 0.3 μg Fe l^{-1}, 2.15 ± 0.02 μg Mn l^{-1}, total alkalinity 284 ± 3 mg $CaCO_3$ l^{-1}, pH value 8.2 ± 0.1 and dissolved oxygen DO 4.5 ± 0.1 mg l^{-1} (as mean values ± standard error of the means—s.e.m., $n = 22$).

Magnesium peroxide MgO_2 (Aldrich) at saturation concentration and Bacto Bushnell Haas Broth (Difco) mineral medium were added to the polluted groundwater for the inoculated column testing, in order to increase the DO and to balance the carbon to nitrogen ratio (10:1 on a weight basis, w w^{-1}) for the aerobic biodegradation. Preliminary tests showed that no chemical oxidation reactions occurred on compounds reported in Table 1. The pH value and the DO in the modified polluted groundwater were 7.8 ± 0.1 and 6.5 ± 0.2 mg l^{-1} respectively (as mean values throughout the experiments ± s.e.m., $n = 21$).

2.2. Inocula

The biomass was obtained by enrichment cultures inoculated with samples of soils polluted by aromatic compounds and wastewater from an industrial treatment plant. The incubation medium was prepared by dissolving 1.08 g of Bacto Bushnell Haas Broth (Difco) in 3.3 l of polluted groundwater obtained as previously described. Successive transfers of the microbial consortium (20 ml) into fresh medium (180 ml) and incubation at 28 °C under aerobic conditions for 15 d at a time were carried out. Five transfers were performed to obtain batch test inocula, resulting in a microbial density of 5.4×10^5 CFU ml^{-1}. For column tests, 100 ml of this inoculum were further suspended in fresh medium (900 ml) and incubated at 28 °C for 7 d under aerobic conditions; then this suspension was inoculated in 11.0 l of polluted groundwater and incubated for 4 d under the same conditions. Finally, air stripping was applied for 3 d, in order to remove purgeable gasoline compounds. All compounds reported in Table 1, TBF and TBA in the final suspensions were below their DL.

2.3. Batch tests

Batch tests were performed at laboratory temperature in 1.1 l closed glass bottles, by suspending the inoculum (30 ml) in the polluted groundwater (270 ml). Biomass inhibition in the abiotic bottles was obtained by NaN_3 (2 M, as final concentration). Two

Table 1
Dissolved concentration (mean values) of gasoline compounds at the equilibrium condition; short chain linear alkanes other than n-octane, and TBF were always below the analytical detection limit (DL). The standard error of means was below the coefficient of variance (CV) of the analytical method applied.

Compound	Concentration (mg l^{-1})	Compound	Concentration (mg l^{-1})
MTBE	950	n-Octane	5.6×10^{-2}
Benzene	7.8	Toluene	21
Ethylbenzene	2.0	*o*-Xylene	1.7
m-Xylene	5.4	*p*-Xylene	3.8

Table 2
Biobarrier filling material properties and MTBE retardation factor by Eq. (1).

Parameter	Mean value ± standard error
Organic carbon f_{OC} (% w w^{-1})	0.22 ± 0.01 (n = 4)
Porosity f	0.40 ± 0.02 (n = 4)
Saturated hydraulic conductivity K (m s^{-1})	$2.2 \times 10^{-6} \pm 0.5 \times 10^{-6}$[a] ($n$ = 14)
Longitudinal dispersivity α_L (mm)	4.0 ± 0.9[a] (n = 10)
MTBE retardation factor R_{MTBE}	1.08 ± 0.02 (n = 4)

[a] Standard error of the mean.

biotic and two abiotic bottles were prepared for each time of analysis (days 0, 3, 6, 10 and 13 of incubation). At which times, the concentrations of specific compounds and the microbial density were measured.

2.4. Column tests

The biobarrier filling material used in the column tests was characterized according to Table 2. It was sand with 90% w w^{-1} in the particle size range 355–600 μm (effective diameter D_{10} = 0.38 mm, and D_{60} = 0.5 mm; uniformity coefficient U = 1.32). Porosity (f) was calculated on the basis of dry bulk density (1590 ± 80 kg m^{-3}) and particle density ρ_s (assumed to be 2650 kg m^{-3}). The preliminary estimate of the longitudinal dispersivity fell within the literature's range for sand (0.6–8.0 mm, reported in [32,33]. MTBE retardation factor was close to the value estimated by:

$$R = 1 + \rho_s \frac{(1-f)}{f} K_d = 1 + \rho_s \frac{(1-f)}{f} f_{OC} K_{OC} \quad (1)$$

where K_d is MTBE distribution coefficient (2.25×10^{-2} l kg^{-1}), obtained as the product of the organic carbon f_{OC} of the filling material (Table 2) and MTBE water–organic carbon partition coefficient K_{OC} (10.23 l kg^{-1} [34]).

The experimental equipment (Fig. 1) included a hollow glass column (inner Ø = 11 cm, length L = 120 cm), which was silylated with $(CH_3)_2SiCl_2$, and it included an inlet port for the polluted groundwater (IN), lateral sampling ports along the column (A–F), and an outlet port (OUT). Water flow was along the x axis, with the origin (x = 0 cm) at IN. A PTFE tube (inner Ø = 2.0 mm) was installed in each lateral port, for sampling water from the center of the column. The glass tank containing the inlet polluted groundwater (Tank IN) was connected to a Teflon® diaphragm pump (Telab) by a PTFE tube used for adjusting the volumetric water discharge.

The filling material was introduced into the column in the vertical position; the column was closed, rotated horizontally, and saturated with unpolluted groundwater 48 h before starting the experiments. A vertical setup had been checked before carrying out the experiments, but it did not allow to get water flowing towards the top of the column, because of the permeability of the filling material and the low seepage velocity applied (similar to that estimated for the aquifer). The diameter of the column was small enough to get a uniform flow through the horizontal column. The inoculation of the filling material for the inoculated column testing was performed before the tests began, recirculating the suspension at 62.0 l d^{-1} for 4 d, and then at 12.0 l d^{-1} for 20 h. The mean biomass concentration in the inoculated column (1.5×10^3 CFU ml^{-1} of filling material) was estimated by a mass balance on the microbial density in the suspension before and after recirculation. Two replicates were prepared for both the inoculated and uninoculated system. The volumetric water discharge through each column was Q = 223 ml d^{-1} (±5%, pump precision), resulting in a residence time of water in the column of 20.3 d and water seepage velocity of 5.9 cm d^{-1}. This order of magnitude for water seepage velocity was necessary in order to assess the behavior of the system under a hydraulic regime similar to that in the aquifer at the site. The duration of each test was 40 d.

During the experiments, groundwater (15 ml) was collected at all ports to quantify the pollutant concentrations. In order to prevent water desaturation, pH and DO measurements were performed in ad hoc samples (20 ml) along the columns, which were then poured back into the systems after the measurements were taken. In the inoculated tests, 4 mg MgO_2 were added to each sample before it was poured back, in order to provide further oxygen to the biomass. At the end of the tests, the microbial density in the liquid samples, and the total organic content of the filing material were measured.

The concentration values $C_Z(x, t)$ (mean values on duplicates) measured at time t for the organic compounds Z for which station-

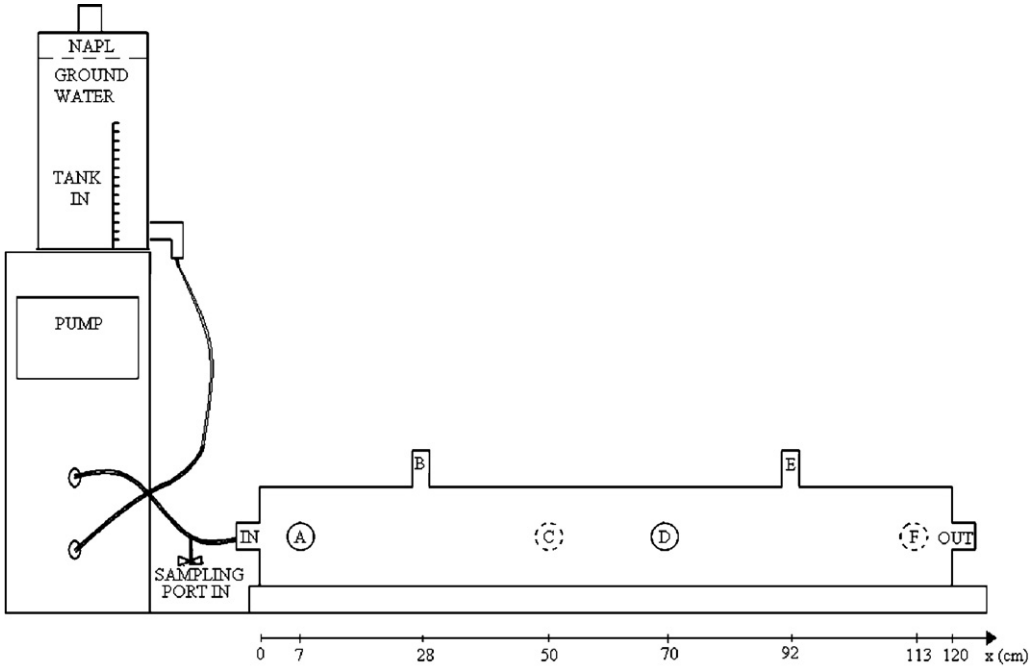

Fig. 1. Column test equipment.

ary conditions were obtained at the end of the columns (MTBE, B, and T) were processed by AQUASIM v. 2.0 [35]. This numerical code can provide a model for a saturated porous medium, accounting for advection, hydrodynamic dispersion, sorption, and degradation of pollutants. $C_Z(x, t)$ can be used as input data sets to estimate the transport parameter values. The filling material in the columns was assumed to be homogeneous and incompressible, and constant over time for water-filled porosity (as f in Table 2), and ρ_S as previously reported (2650 kg m^{-3}). The volumetric water discharge through the column cross section was constant over time and set as the experimental value. The pollutant inlet concentration was set as reported in Table 1. Distribution between phases was assumed to be instantaneous, completely reversible and described by a linear isotherm with $K_{d,Z}$ as the distribution coefficient. Degradation was modeled as a first order kinetic reaction with constant $K_{r,Z}$ with respect to the dissolved concentration. The porous medium longitudinal dispersivity α_L, the retardation factor R_Z, and $K_{r,Z}$ were estimated by the code.

2.5. Chemicals and analytical methods

All chemicals were of analytical grade purity (99.0%).

The biobarrier filling material was characterized as follows: (i) moisture content by the gravimetric method ASTM D 2216; (ii) organic carbon by dry combustion according to ISO 10694; (iii) particle size distribution by dry sieving (ASTM D 422 method; (v) dry bulk density as mass of a known volume of material, according to the ASTM 2854 method; (vi) hydraulic conductivity of the saturated material by a rigid-wall permeameter, according to the ISO 17312 method. Preliminary values for the longitudinal dispersivity and

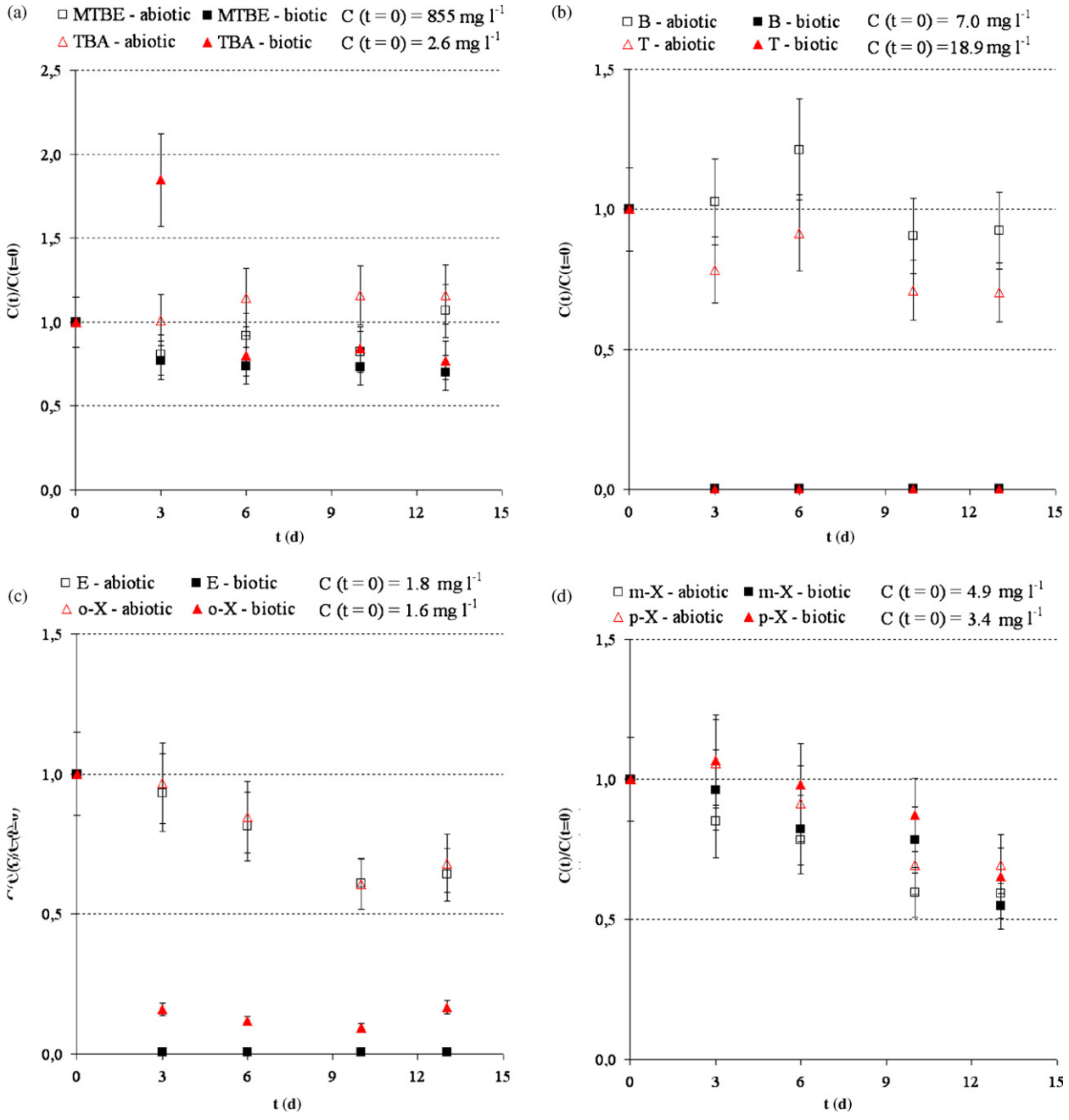

Fig. 2. Concentrations (mean values of replicates) as a function of time measured in batch tests: (a) MTBE and TBA; (b) B and T; (c) E and *o*-X; (d) *m*- and *p*-X. Error bars represent the analytical method CV.

the MTBE retardation factor, necessary to design the column tests, were obtained by using the experimental equipment and procedure reported in [36] in order to determine breakthrough curves for NaCl and MTBE respectively. As the precision of the method is limited by the precision in determining the concentration of the chemical in the effluent, NaCl was preferred as a tracer for the preliminary evaluation of the longitudinal dispersivity. The glass column had an inner diameter of 10.5 ± 0.5 mm and was 60 cm long, with PTFE end caps. Steady flow rates in the range 7.2–22.0 l d^{-1} were applied. Water effluent samples were collected regularly for the measurement of specific conductance when NaCl was used, whereas MTBE concentration was the benchmark in the other tests. Breakthrough curves were processed to estimate values which best fit the analytical solution of the one-dimensional fate and transport problem for a continuous source reported in [37].

Liquid samples were chemically analyzed as follows. MTBE was measured by direct injection in a GC-FID according to EPA 8015D method (coefficient of variance CV: ±10%; DL = 0.3 mg l^{-1}). Short chain linear alkanes, BTEXs, TBF, and TBA were measured by headspace solid-phase microextraction, according to ASTM D 6520 method, followed by GC-MS analysis (CV: ±10%; DL = 2.0×10^{-2} mg l^{-1} for n-alkanes and BTEXs, 7.0×10^{-2} mg l^{-1} for TBF and TBA). The GC-MS was equipped with a Supelcowax column (30 m, 0.25 mm, 0.5 μm), a programmed temperature vaporization injector and a mass selective detector (40–350 a.m.u.). Analytical conditions were: (i) injector temperature = 220 °C; (ii) ionization energy: 70 eV; (iii) thermal ramp: 37 °C for 5 min, from 37 °C to 240 °C at 10 °C min^{-1}, 10 min at 240 °C. Dissolved oxygen concentration was measured according to ISO 5814 (CV: ±10%), pH was measured according to ISO 10532 (CV: ±5%). Specific conductance was measured according to ISO 7888 (CV: ±5%).

Microbial density in liquid samples was spectrophotometrically measured as absorbance at 600 nm A_{600} (CV: ±5%). A calibration curve A_{600} vs. colony-forming units CFUs per unit volume of liquid sample was obtained for the microbial culture used in the inoculated experiments. Microbial counts in standard samples were determined by plating tenfold serial dilutions of the suspension in duplicate using Plate Count Agar (Merk), and colonies were counted following 4 days of incubation at 28 ± 1 °C.

3. Results and discussion

Experimental data are reported as mean value on replicates. For all measurements, the standard error (s.e.) of replicates was below the CV of the analytical method applied.

3.1. Batch tests

MTBE concentrations at time t, $C(t)$, normalized to the concentration at the beginning of the tests $C(t = 0)$ are shown in Fig. 2a. During the abiotic tests, MTBE concentration was constant within the CV of the analytical method applied, suggesting that abiotic losses could be neglected. In the biotic tests, MTBE decreased within 3 days of incubation, but after this time a nearly stationary condition was reached.

Fig. 2a also shows TBA concentration normalized to the value measured at the beginning of the tests (2.8 ± 0.3 mg l^{-1}), resulting in not significantly different concentrations in the abiotic samples at all incubation times. At day 3 of the biotic tests, the concentration was 1.85 times its value at the beginning of the tests. In the following samples however it decreased to a nearly constant value, not significantly different from $t = 0$. Therefore TBA was produced during MTBE biodegradation, and it could be biodegraded as well. TBF was below DL in all analyzed samples, and whenever it was produced during MTBE degradation, it was rapidly transformed.

In both types of tests, n-octane was constant over time, so that either biotic or abiotic removal mechanisms could be excluded.

Under biotic conditions, benzene, toluene (Fig. 2b) and ethylbenzene (Fig. 2c) were removed to values below the DL within 3 days of incubation. Benzene was constant throughout the abiotic tests, whereas toluene and ethylbenzene were removed at 30 and 36% respectively following 13 days of incubation. Over the same period, a comparable abatement (32%) was obtained for *o*-xylene (Fig. 2c) in the abiotic tests. However the biotic tests resulted in 84% abatement of *o*-xylene, constant over time after 3 days of incubation. *m*- and *p*-xylene (Fig. 2d) removal over time during the biotic tests was the same of that in the abiotic tests, resulting in about 43% for *m*-xylene and 32% for *p*-xylene at the end of the experiments. Based on these results, benzene removal could be completely ascribed to biological mechanisms, whereas *m*- and *p*-xylene removal was due only to abiotic mechanisms, and each process occurred for the other monitored aromatic solvents. According to the chemical–physical properties of these compounds, sorption on unsilylated glass is expected to be the cause of the abiotic losses observed.

DO measurements performed during the experiments showed that anoxic conditions (DO < 2 mg l^{-1}) occurred between day 3 and day 6 of incubation in the biotic tests. This was due to the oxygen consumption in the bottles for MTBE, T, TBA, B, E and *o*-X biodegradation, according to their concentration, oxygen demand and biodegradation kinetics.

The microbial density (Fig. 3) in the biotic tests varied with time, resulting in the maximum value (5.9×10^6 CFU l^{-1}) at day 6 of incubation. This result suggests that if a biodegradation process (under anoxic conditions) was still occurring after this time, it was not able to maintain the microbial density in the bottles.

BTEXs and TBA are usually co-contaminants at MTBE polluted sites. Unfortunately for MTBE, BTEXs are preferential substrates for aerobic and anaerobic metabolism, and their degradation result in depletion of the available electron acceptors. However, some aerobic microbial communities degrading BTEXs can degrade MTBE too, so that BTEX degradation can increase the cell mass for MTBE metabolism [22]. TBA can be a preferred substrate relative to MTBE, when present as initial pollutant in groundwater. However, as by-product of MTBE degradation, it will not likely inhibit MTBE biodegradation [10]. During the aerobic phase of the batch tests described in this paper, the simultaneous biodegradation of MTBE, TBA, B, T, E and *o*-X was observed. However B, T, and E were completely removed within 3 days of incubation, and MTBE was not further biodegraded after this time, when an increase of TBA concentration was measured. The rapid biodegradation of BTEXs resulted in decreased oxygen availability, but MTBE degradation was nevertheless measured in the presence of BTEXs. Following 3

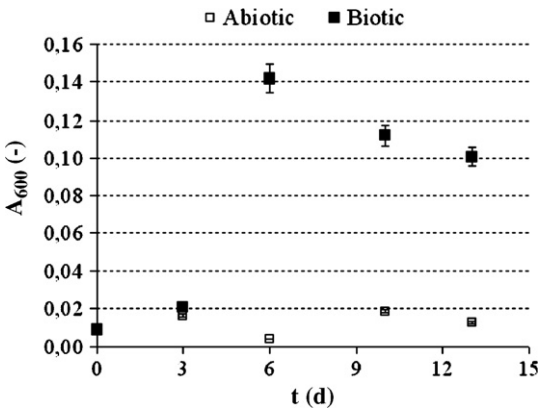

Fig. 3. Absorbance at 600 nm (A_{600}) in batch test samples (mean value of replicates) as a function of time. Error bars represent the CV of the applied analytical method.

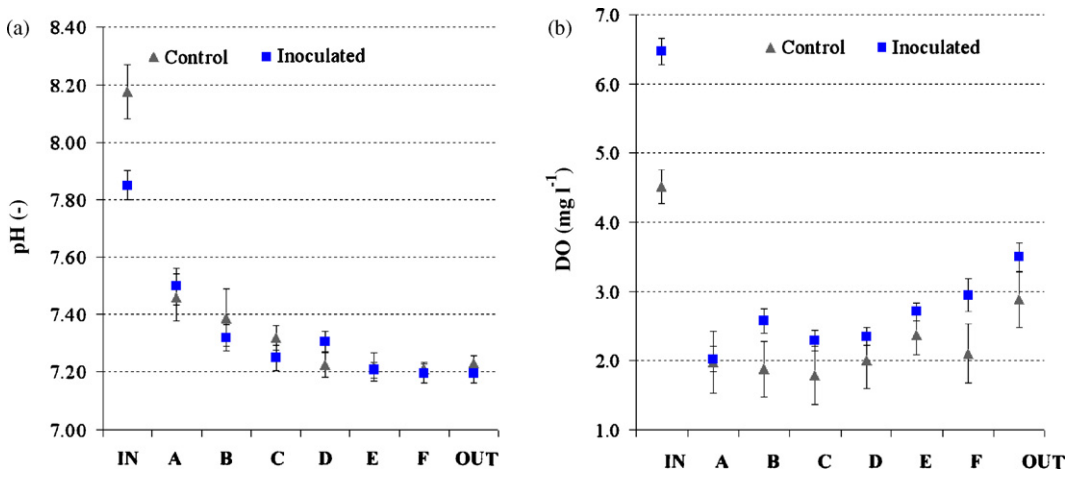

Fig. 4. pH (a) and DO (b) mean values throughout the time of the column tests as a function of the distance (x) from the column inlet; error bars represent the s.e.m.

days of incubation, stationary concentrations for MTBE could be ascribed to the preferential use of TBA as a substrate for the aerobic process, up until anoxic conditions were reached and the degradation of both MTBE and TBA ceased. Different results were reported in [38], where MTBE degraded to completion, followed by the disappearance of the intermediate TBA. Moreover, when MTBE and TBA were contemporary added at almost the same concentration (5–46 mg l^{-1}), MTBE was degraded significantly prior to TBA, and the TBA degradation rate increased significantly once MTBE was completely degraded.

3.2. Column tests

Fig. 4a and b respectively show pH and DO measured at each sampling location as a mean value ± s.e.m. throughout each type of experiment. The pH value decreased within 70 cm of filling material to a constant value (7.2 ± 0.1), both in the inoculated and in the uninoculated columns. The difference in the inlet groundwater in the two types of systems reduced to a not significant value within 7 cm, due to the filling material buffer capacity to offset the effect of the additives along the inoculated columns. The groundwater DO decreased to about 2 mg l^{-1} within 7 cm of filling material in both types of systems, but the addition of MgO_2 in the inoculated columns maintained aerobic conditions over the entire column. Therefore, oxygen availability was not a limiting factor for MTBE biodegradation, as it occurred during the batch tests.

Fig. 5 shows MTBE concentration $C_{MTBE}(x)$ at some sampling locations normalized to the mean inlet value $C_{MTBE}(x=0)$, as a function of the number of pore volumes of groundwater flowed through the column tests. Continuous and dotted lines show the breakthrough curves best fitting the experimental data, as provided by the numerical model. Among compounds reported in Table 1, a S-shaped curve with stationary conditions at the end of the columns was observed for MTBE, B and T. The parameter estimate (±s.e.) resulting from the model for the uninoculated and the inoculated columns is reported in Table 3. The dispersivity value estimated for the uninoculated columns is comparable to that obtained in the preliminary assay of the filling material. A significant increase (more than a 2 factor) was observed for α_L in the inoculated columns compared to those uninoculated. This effect has already been reported in Bielefeldt et al. [39] and ascribed to the biomass. However Bielefeldt et al. [39] observed a significant reduction of K, which was not observed in this study, as verified at the end of the experiments (data not reported). For both types of column experiments, the MTBE retardation factor matched that in Table 2; calculation of log K_{OC} by Eq. (1) in the uninoculated and the inoculated columns

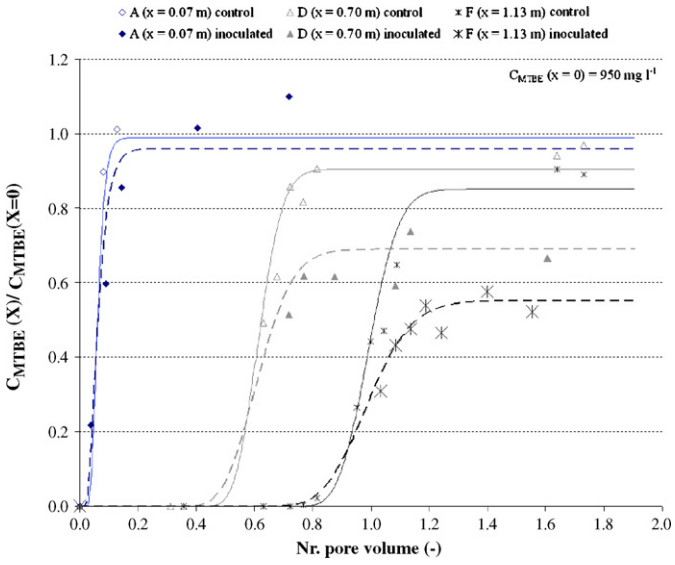

Fig. 5. MTBE concentrations, C_{MTBE} (mean of replicates), measured in the column tests, normalized to the input concentration, $C_{MTBE}(x=0)$, as a function of pore volumes flowed in the columns.

for B (1.65 and 1.71 respectively) and T (1.79 and 1.78 respectively) resulted in values within the ranges reported in [40] (1.09–2.53 for B, 1.12–3.28 for T). Therefore, possible effects on MTBE, B, and T sorption and distribution coefficient due to biomass or other gasoline compounds could be neglected. For these compounds, a significant difference between the K_r values for the two types of column experiments was obtained, resulting in a not significant removal (below 16%) for MTBE, B, and T over the entire length of the uninoculated systems, and 47% for MTBE, 71% for B, and 90% for T in those inoculated. K_{rMTBE} in the inoculated columns was in the wide

Table 3
Parameter estimate (mean value ± s.e.) resulting from the model for the uninoculated and the inoculated columns.

Parameter	Uninoculated columns	Inoculated columns
α_L (mm)	3.6 ± 0.3	8.0 ± 3.0
R_{MTBE}	1.06 ± 0.01	1.06 ± 0.03
R_B	1.39 ± 0.01	1.45 ± 0.04
R_T	1.54 ± 0.01	1.53 ± 0.04
K_{rMTBE} (d^{-1})	0.009 ± 0.003	0.031 ± 0.001
K_{rB} (d^{-1})	0.000 ± 0.002	0.045 ± 0.002
K_{rT} (d^{-1})	0.005 ± 0.002	0.080 ± 0.004

range (0.0003–5.3 d^{-1}) reported in [41–43] for MTBE metabolism under bench scale or in situ treatments. The fitting between the experimental breakthrough curves and the model prediction suggested that no significant interactions during MTBE, B, T, and TBA biodegradation occurred in the columns, that had enough oxygen to support aerobic conditions.

For the inoculated systems, the linear regression of $\ln[C_Z(x, t\rightarrow\infty)/C_Z(x=0)]$ (Z = MTBE, B, T) as a function of x, where $C_Z(x, t\rightarrow\infty)$ is the stationary concentration of Z at x, was used to get a preliminary estimation of the thickness X of a biobarrier installed at a short distance from the source area at the site, which was necessary to reduce the pollutant concentrations to a target value. For example, removals to approximately 1% of the inlet concentration to the inoculated biobarrier would require X = 8.8, 4.4 and 2.4 m for MTBE, B, and T respectively. Whenever a 90% removal was required, X could be reduced to 4.4, 2.2, and 1.2 m respectively. These removal percentages are expected to be valid over a wide range of inlet concentrations. In fact, they only depend on the barrier thickness and the degradation constant.

The unstationary conditions in the TBA inlet concentration were reflected in the irregular trend observed at different times and locations in the columns. Peaks in the inlet groundwater were amplified by a factor of up to 6 (measured in OUT) during the transport through the uninoculated columns, and by a factor of 2 (measured between IN and C) for the inoculated columns, suggesting that TBA was produced during MTBE degradation in both the uninoculated and the inoculated columns. However, in the inoculated systems, stable TBA concentration (2.6 ± 0.3 mg l^{-1}) was measured in OUT following 24 days of groundwater injection in the columns. This suggests that the intermediate TBA could be biodegraded within the column length. TBF was below the DL at all sampling times and locations in the inoculated columns. In the uninoculated columns, concentration peaks were observed with TBA peaks (with ratios TBA/TBF increasing from 45 at A to 350 at OUT), suggesting a link between the compounds in these tests. In [10], TBF was reported as MTBE metabolite produced during bacterial or fungal aerobic cometabolism on alkanes. However, in this study, stationary conditions were assessed for n-octane only in A, due to the retardation of this compound in the columns. No significant variations were measured relative to its concentration in the untreated groundwater. Therefore, if a cometabolic process occurred due to the biomass in groundwater or in the not inoculated filling material, then compounds other than n-octane were used.

The microbial density in the groundwater along the columns did not vary significantly during the experiments. This suggests that the biomass produced during the biodegradation attached to the filling material. Unfortunately, the monitoring performed on solid samples from the columns resulted in a not significant difference between the two types of systems, due to its poor sensitivity.

4. Conclusions

In this study, bench scale tests were performed to assess the performance of a selected microbial consortium in degrading MTBE and BTEXs in groundwater by a biobarrier system. Compared to the inoculation performed in the undisturbed aquifer, problems related to heterogeneities, low hydraulic conductivities, and biomass attachment/detachment can be reduced by selecting the proper filling material and biomass. In this study, sand with narrow particle size distribution was used to support the microbial consortium selected on gasoline compounds dissolved in groundwater.

In batch tests, the biomass was suspended in the polluted groundwater. It was able to simultaneously degrade MTBE and its intermediate TBA, benzene, toluene, ethylbenzene and *o*-xylene under aerobic conditions. When oxygen was depleted in the inoculated systems, biodegradation of these compounds ceased. TBF was not detected.

Biobarriers are designed for in situ remediation of contaminated groundwater. For most of cases, the subsurface possesses a reducing condition. In this study, MTBE could only be degraded under aerobic conditions. Therefore oxygen should be delivered in the biobarrier reactive zone, for instance by passive systems, such as socks containing oxygen-releasing compounds located in wells; some registered products are available.

In the inoculated columns tests, aerobic conditions were obtained by periodically adding MgO_2 through the systems. Stationary conditions at the end of the columns were obtained for MTBE, B and T, which allowed values to be estimated for the parameters affecting groundwater flow and pollutant transport through the BB system. The longitudinal dispersivity of the inoculated columns was twice the value of those uninoculated, but no significant variation of the hydraulic conductivity was observed. Sorption of MTBE, B, and T was not affected by the inoculated biomass or other gasoline compounds. Removals of 47, 71 and 90% were obtained at the end of the inoculated columns for MTBE, B, and T respectively. This was due to biodegradation (for all compounds). TBA formation was observed in both types of columns, but where inoculation had been performed, the intermediate could be biodegraded within the column length. TBF was detected only in the uninoculated systems, where a cometabolic degradation of MTBE could have occurred on gasoline compounds not monitored during the experiments.

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