Lab-scale tests and numerical simulations for in situ treatment of polluted groundwater

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1. Introduction

Gasoline is one of the most widespread petroleum-derived products, whose accidental leaking can cause severe pollution of groundwater. Monoaromatic solvents (benzene, toluene, ethylbenzene, xylenes–BTEXs) and additives (e.g., Methyl tert-butyl ether – MTBE, Ethyl tert-butyl ether – ETBE) are the most critical compounds, due to their high mobility in the environment, toxicity and/or organoleptic modification of water. They can be biodegraded either under aerobic or anaerobic conditions [1–4].

Permeable reactive barriers (PRB) is an in situ passive treatment for groundwater remediation based on the emplacement of reactive materials to intercept and treat dissolved contaminants as they flow, typically under groundwater natural gradient. Biological PRBs, also know as “Biobarriers” (BBs), aim at promoting the biodegradation of pollutants by replacing part of the aquifer with a permeable porous medium to support autochthonous or allochthonous microorganisms and providing nutrients and/or electron acceptors to maintain optimal conditions for biomass. Compared to the traditional PRBs based on physical–chemical removal mechanisms, no regeneration of the porous medium is required over time as pollutants are degraded by biomass attached to the medium. Moreover, compared with aquifer biostimulation or bioaugmentation, the emplacement of a specific medium offers the

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possibility of creating a more homogeneous reactive zone both with respect to the hydraulic properties and to the presence of active microorganisms, and thus, can result in higher removal efficiencies of the pollutants of interest. The selection of filling material, of biomass, of type and amount of nutrients/electron acceptors (whether required) and of the dimension and configuration of the treatment zone are the key design factors for the success of this system \[5,6\].

This work reports on laboratory tests and numerical simulations carried out to design a BB system to remediate groundwater contaminated with gasoline-derived compounds. Experimental activities have been structured according to the main steps required for the BB design: (i) selection of a proper filling material; (ii) selection of a proper microbial culture in laboratory; and (iii) uninoculated and inoculated column tests to compare the pollutant removal efficiency with and without inoculation and to estimate the kinetic constants. The results were then used to model a BB system at the contaminated site described in \[7\].

2. Materials and methods

2.1. Filling materials

Based on existing literature, the following materials have been selected for evaluation as the BB’s potential filling material: activated carbon (PK 3–5 – NORIT), perlite (PEROIL T – Perlite Italiana), volcanic pumice (Euroterriflora), sieved to select the particle size range 6–10 mm (Fig. S1 in Annex 1), and expanded clay (termolite–laterite). They were tested in grain size distribution (ISO 11277:2009), porosity (ASTM D4404-10), bulk density (ISO 11272:1998), hydraulic conductivity (ISO 17312:2005) and organic carbon content (UNI EN 15169:2007). Mechanical tests were not performed as all these have been reported as suitable materials for BBs in the literature. However perlite tended to crack and crush during the characterization tests, exhibiting a poor mechanical behavior. As a consequence, it was not further tested.

_Rhodococcus_ sp. E25 and _Pseudomonas_ sp. XP452 were used to assess the capability of the material of being colonized by bacterial strains in adhesion tests. Fifty milliliters of inoculum (0.1 optical density at 540 nm, OD\textsubscript{540}) were mixed with 15 g of material on dry weight basis and incubated at 30 °C for 24 h. The amount of sorbed bacteria was quantified by dilution and plating on rich agar medium. The percentage of sorbed bacteria was calculated by dividing the amount of sorbed cells by the number of cells in the suspension used as inoculum.

2.2. Inoculum selection

Enrichment cultures were prepared in 120 ml serum bottles using an inoculum from gasoline-contaminated groundwater. Each serum bottle contained 25 ml of M9 mineral medium \[10\] amended with 3 μl of one of the following substrates: benzene (B), toluene (T), ethylbenzene (E), o-Xylene (o-X), m-Xylene (m-X), p-Xylene (p-X) and MTBE. Bottles were then incubated at 30 °C, refreshed several times, and pure cultures were isolated by plating on LB agar medium. A fragment of the gene 16S rRNA was PCR-amplified using Com primers \[11\] as previously reported \[12\]. The Riboso-mal Database Project (RDP) classifier \[13\] was used for taxonomic assignments of sequences. The cultures were then incubated 24 h in LB rich medium at 30 °C under shaking, washed and resuspended to OD\textsubscript{540} = 0.1 \[12\]. Subsequently the degradation capabilities of isolates were screened by liquid growth experiment. Each isolate was tested with all the substrates at the same concentrations reported above. Growth was considered positive when the OD\textsubscript{540} after 5 days of incubation was at least 0.15.

Referring to the growth experiment, three inocula were developed and tested (Table 1). Since no positive growth on MTBE was observed, _Methylibium petroleophilum_ LMG 22953 \[14\] was purchased from the Belgian Co-ordinated Collections of Micro-organisms (BCCM/LMG-Bacteria Collection, Gent, Belgium). The first inoculum (InoculumA) was the minimum number of strains capable of degrading all BTEX and MTBE. The second one (InoculumB) was made with all the selected strains. The last one (InoculumC) was chosen following two criteria: (i) each compound had to be degraded by at least two microorganisms and (ii) the taxonomic diversity had to be the greatest one. Mixing pure cultures to obtain the inocula allowed easily achieving high biomass yield in a relatively short time by growing the pure cultures in a rich medium. Conversely, the enrichment of a mixed culture directly from the contaminated site would have been more representative of the field conditions, but it would have been technically more difficult in real field application. Each test was prepared in triplicate in 120 ml serum bottles amended with: sterile tap water (30 ml), pumice (16 g), NH\textsubscript{4}NO\textsubscript{3} (3.31 mg/g), K\textsubscript{2}HPO\textsubscript{4} (0.32 mg/g), KH\textsubscript{2}PO\textsubscript{4}

<table>
<thead>
<tr>
<th>InoculumA</th>
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<th>InoculumC</th>
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</thead>
<tbody>
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<td><em>Pseudomonas</em> sp. CPX451 (T, m-X, p-X)</td>
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<td><em>Stenotrophomonas</em> sp. CPX452 (p-X)</td>
<td><em>Stenotrophomonas</em> sp. CPX452 (T, E)</td>
</tr>
<tr>
<td><em>Rhodococcus</em> sp. CB451 (B, T, E, p-X)</td>
<td><em>Stenotrophomonas</em> sp. CPX452 (T, E)</td>
<td><em>Rhodococcus</em> sp. CE461 (B, T, E, o-X)</td>
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<tr>
<td><em>Rhodococcus</em> sp. SA451 (B, T, E, m-X)</td>
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Table 1 Composition of the tested inocula. Acronyms between brackets state the substrates where a positive growth was observed in the growth tests with the exception of the _Methylibium petroleophilum_ LMG 22953 where the growth substrates from literature are reported \[32\].
(0.25 mg/g), the commercial oxygen-release compound “EHC-O®” provided by Adventus (0.18 mg/ml) and a mixture of the following substrates: MTBE (80 mg/l), benzene (20 mg/l), toluene (80 mg/l), ethylbenzene (40 mg/l), o-xylene (10 mg/l), m-xylene (10 mg/l) and p-xylene (10 mg/l). Pure cultures were prepared as previously described. Two experiments were set up without inoculum and with sterile pumice. Hydrocarbons concentration was monitored over time by GC-FID.

2.3. Column tests

The column tests (Fig. 1) were carried out in a Teflon-coated basin (250 cm long, 10 cm wide) filled with a 15 cm layer of pumice, saturated for 10 cm with water in the water flow. Above the pumice layer, 10 cm of activated carbon (Norit GF 40) were placed to avoid vapor emissions. Eight piezometers (“A” to “H”), sampling at half height of the pumice layer, were uniformly distributed along the column, with the last one (H) 240 cm far from the inlet point. Piezometers were made using Teflon tubes (extrernal diameter: 6 mm; internal diameter: 4 mm) inserted within the filling material; they were closed with steel caps during the test and opened only during the sampling.

In the inoculated test (IN), a microbial suspension containing Rhodococcus sp. CE461, Rhodococcus sp. CT451 and Methylobium petroleiphilum LMG 22953 (ODs40 = 0.1 each) was poured into the saturated zone of the basin before starting the test and recirculated for three days at 31°C.

The duration of the tests was 43 days for the un inoculated test (UN) and 85 days for IN. The IN test was longer than UN test in order to assess possible occurrence of bioclogging in the colonized pumice.

For both tests, tap water artificially contaminated with commercial gasoline was used as the inflow solution at 3.0 l/d; fresh free gasoline was maintained on the water level in the input tank to keep constant the pollutants’ dissolved concentrations over time. BTX, MTBE, ETBE and tert-butyl alcohol (TBA, the major MTBE by-product), were quantified under steady state conditions (Table 2).

To ensure aerobic conditions, a commercial oxygen-release compound (EHC-O, Adventus, 0.3% on dry weight basis of pumice) was uniformly distributed throughout the saturated layer of pumice before starting the tests. Further, amounts of EHC-O (250 mg per each piezometer twice a week) were added as a slurry during the IN test. Nitrogen (27.7 g of NH₄NO₃) and phosphorus (2.1 g of H₂PO₄ and 2.7 g of KH₂PO₄) were also added.

Water samples were collected from the piezometers twice a week to measure the pollutant concentrations, temperature, pH value (electronic pH-meter X5 pH 6, Oakton), dissolved oxygen (DO) concentration (OXI 340i probe, WTW) and specific electrical conductivity (SEC) (LM 8, EH Conducta). Pollutants in the water samples were extracted by solid-phase microextraction and quantified by GS–MS (UNICHIM 1210:1997). At the end of the tests, samples of activated carbon were collected and analyzed (CS₂ extraction and GS–MS analysis, ISO 16200-1:2001) to evaluate the pollutant loss due to volatilization.

Pumice samples were collected before and at the end of the tests next to each piezometer in order to characterize the microbial communities. DNA was extracted using a commercial soil kit FastDNA® Spin (BIO 101 Inc. Vista, CA). Denaturing gradient gel electrophoresis (DGGE) was performed on samples collected from the UN test and the IN test for the taxonomic characterization of the communities as described in [15]. DGGE was carried out in a D-Code System (Bio-Rad Laboratories, Hercules, CA, USA) using a denaturing gradient between 40 and 55%. Selected bands were excised, purified according to [10], sequenced and classified with the RDP classifier [13] for the taxonomic assignment. Terminal restriction fragments length polymorphism (T-RFLP) was carried out in order to compare the microbial communities of both IN test and UN test. 16s rRNA gene fragments were PCR-amplified with the primers FAM-Com1F and 1492R [16]. In a 20 μl reaction, containing 20 units of Alul and Haelll (Promega Corporation), 600 ng of amplified DNA were separately digested for 1 h at 37°C. A hierarchical cluster analysis of the relative peak area was performed with the HCLUST procedure in R 2.15.3 [17] with the complete linkage method on the Bray–Curtis distance between samples.

2.4. Numerical modeling

As a cost-effective method to optimize the design of the full-scale biobarrier at the site, numerical simulation of various BB scenarios was performed taking into consideration the results obtained in the laboratory tests. The site was an agricultural area in northern Italy, where the release of gasoline from an underground pipeline occurred in 2005. In March 2006, sampling results at full-screened monitoring wells revealed that, 45 m downgradient the residual source, MTBE, B, T, E and total X concentrations were approximately 7 mg/l, 1.6 mg/l, 6 mg/l, 0.45 mg/l and 4 mg/l, respectively. Groundwater flows at depth of approximately 4.5–5.5 m b.g.s. in a shallow (14 m high) medium to coarse sand aquifer, with silt lenses. Based on borehole logs and pumping tests, the average hydraulic conductivity is Kₘ₆ = 2.6 m/d. Groundwater flow is stable towards south, with an average hydraulic gradient of 0.0023.

The concentration profile of the organic compounds along the column were fitted to the model processed by AQUASIM v. 2.0 [18], as described in [19], to estimate the value of the first order

### Table 2
Mean concentrations (±standard deviation) in the inflow solution to the uninoculated test (UN) and to the inoculated test (IN).

<table>
<thead>
<tr>
<th>Compound</th>
<th>UN test (mg/l)</th>
<th>IN test (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>18.2 ± 3.7</td>
<td>22.8 ± 5.2</td>
</tr>
<tr>
<td>Toluene</td>
<td>78 ± 19</td>
<td>98 ± 40</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>7.9 ± 2.9</td>
<td>9.9 ± 2.1</td>
</tr>
<tr>
<td>m-p-xylene</td>
<td>11.6 ± 4.6</td>
<td>10.5 ± 2.0</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>3.5 ± 0.9</td>
<td>6.5 ± 2.5</td>
</tr>
<tr>
<td>MTBE</td>
<td>96 ± 19</td>
<td>141 ± 32</td>
</tr>
<tr>
<td>ETBE</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>TBA</td>
<td>7.9 ± 6.7</td>
<td>1.0 ± 0.7</td>
</tr>
</tbody>
</table>

Fig. 1. Column tests set-up.
Table 3

| Physical–chemical properties of the tested materials and percentage of sorbed bacteria referred to the amount of bacteria inoculated in the test (n = number of replicates). |
|-----------------|----------------|----------------|----------------|
|                  | Activated carbon | Perlite        | Pumice         | Expanded clay  |
| Particle size range (mm) | 3–6             | 1.5–4          | 6–10           | 6–20           |
| Porosity (–)      | 0.67 ± 0.02     | 0.55 ± 0.08    | 0.62 ± 0.01    | 0.47 ± 0.01    |
| Dry bulk density (kg d.w., m⁻³) | 460 ± 30       | 990 ± 170      | 910 ± 30       | 1010 ± 20      |
| Saturated hydraulic conductivity (m/s) | 4.4 ± 10⁻¹    | 6.3 ± 10⁻¹ ± 0.5 · 10⁻¹ | 5.2 ± 10⁻¹ ± 1.0 · 10⁻¹ | 4.1 ± 10⁻¹ ± 0.7 · 10⁻¹ |
| Organic carbon content (% on d.w. basis) | 0.34 ± 0.02   | (n=4)          | (n=4)          | (n=4)          |
| Pseudomonas sp. CPX452 (%) | <1              | –              | 60             | 3              |
| Rhodococcus sp. E252 (%) | <1              | –              | 74             | 4              |

Biodegradation rates of B, T, E, o-X, m + p-X and MTBE in the column. The pumice longitudinal dispersivity (a) was obtained by fitting the mono-dimensional transport model to the breakthrough curves of preliminary tracer tests with sodium chloride. Organic carbon content, porosity and saturated hydraulic conductivity (Kw) were set according to the pumice characterization results. Soil to water distribution coefficient of each compound was calculated as the product of water–organic carbon partition coefficient (MTBE: 15 l/kg, B: 62 l/kg, T: 140 l/kg, E: 240 l/kg, o-X: 241 l/kg, m + p-X: 254 l/kg) and the organic carbon of the filling material, as reported in [20].

The BB system can be located: (i) within the dissolved plume or at its leading edge, to prevent further migration downslope; or (ii) downslope the soil source, in order to reduce pollutant concentrations recharging the plume. According to the approach described in [7], the USGS MODFLOW computer code [21] was used to reproduce the natural steady state flow field at the contaminated site and to assess the aquifer response to various BB scenarios under steady state conditions. The biobarrier was located 5 m downslope the pollution source and designed to intercept the aquitard. Two BB configurations were modeled: (i) a continuous wall (CW), entirely composed by the inoculated pumice, catching the plume perpendicularly to the groundwater flow and (ii) a funnel and gate (F&G) system, composed by impermeable funnels oriented perpendicularly to the groundwater flow to direct groundwater through the inoculated gate. The main advantage of the F&G over the continuous wall configuration is that a smaller volume of the reactive zone is required to treat the plume, even though the add–tion of the impermeable funnels generates major disturbance in the natural groundwater flow patterns compared to the CW configura-tion. Capabilities of the proposed BB systems to capture the plume were assessed by the MODPATH particle-tracking code [22]. The BB length along the groundwater flow direction was based on the contaminant residence time within the reactive zone, estimated by the groundwater residence time from MODPATH and the pollutant retardation factors.

MT3DMS [23] was used to simulate the plumes of MTBE and BTEXs at the site and pollution attenuation while migrating through the biobarrier. The longitudinal dispersivity from the laboratory tests was increased to 0.2 m in order to account for scale effects. The vertical and transverse dispersivity ratios to the longitudinal dispersivity were set to 0.05 and 0.1, respectively; transverse to longitudinal dispersivity ratio was lower than that in the aquifer, due to the homogeneity of the BB filling material. Diffusion was neglected [24]. MTBE, B, T, E, o-X and m,p-X degradation constants Ki in the BB were based on laboratory test results; however, they were reduced by a factor three, according to the van’t Hoff–Arrhenius equation [25], to account for the temperature under field conditions (10 °C).

3. Results and discussion

3.1. Filling materials

Table 3 summarizes the results about the physical–chemical properties and the percentage of bacteria sorbed on the materials at the end of the adhesion tests. Activated carbon, pumice and expanded clay exhibited high hydraulic conductivity; as pumice had the best biomass sorption capacity, it was selected as the filling material for the column tests.

3.2. Inoculum selection

Ten strains were isolated from the enrichments cultures. Taxonomic assignments showed that the pure cultures belong to Rhodococcus, Stenotrophomonas and Pseudomonas genera. The results of growth tests are reported in Table S1 in Annex 1. The isolated strains were capable of growing on the whole range of BTEX. Despite the fact that strains Rhodococcus sp. CE461 and Stenotrophomonas sp. CXO452 did not reach the growth threshold, they were considered capable of growing on p-xylene (the former) and o-xylene (the latter) because of the presence of colored metabolites in the growth medium. However, it is worth considering that, an increasing in the biomass was not observed and the presence of metabolites would have been most likely due to biologically catalyzed reactions that did not produce energy for the microbial growth.

Referring to the three inocula prepared, InoculumA showed the best capabilities of degrading benzene, toluene, ethylbenzene and m + p-xylene after 3 days. o-Xylene was degraded after 7 days. After 25 days the residual concentration of MTBE was 80% and a small amount of TBA was produced (Fig. 2). The other inocula and pumice without inoculum degraded the hydrocarbons with slower rates. In pumice without inoculum all BTEX were degraded after 14 days. InoculumB degraded ethylbenzene after 3 days, while the other BTEX were degraded after 7 days. After 25 days no significant degradation of MTBE was shown but a small amount of TBA was produced. InoculumC degraded ethylbenzene and m,p-xylene after 3 days. Benzene, toluene and o-xylene were degraded after 7 days. After 25 days the residual concentration of MTBE was 80% and TBA was produced.

3.3. Column tests

3.3.1. Physical–chemical parameters

Laboratory temperature (21/26 °C) was measured in all samples from both column tests (Fig. S2 in Annex 1). In the UN test, the pH value increased up to a maximum value of 9.4 in the piezometer
Fig. 2. Degradation capabilities of the selected inocula.

H during the first 10 days of operations; during the remaining part of the test, pH values of about 7.5–8.0 were measured throughout the entire column length. A similar trend was observed for SEC, with values up to 2000 μS/cm in the last piezometer for the first 10 days and a constant value of 750 μS/cm in the following period in all piezometers. The trends of the physical–chemical parameters measured in water samples were related to the oxygen-releasing compound added at the beginning of the test. The DO confirmed
aerobic conditions along the column for the entire duration of the test (5.7 ± 0.5 mg/l).

In the IN test, DO values dropped from the starting value of 8.5 mg/l after 6 days of operations. The periodical addition of EHC-O ensured aerobic conditions within the column. Lower DO values were measured in the first part of the column (2.5–3.0 mg/l up to piezometer D) compared to those observed from D to H (3.5–4.5 mg/l). In general, pH values between 7 and 8 were observed in the column without significant variations over time, but values up to 9.0 were measured in G and H after the addition of EHC-O. SEC values at the beginning of the test were strictly related to the inoculum (SEC = 10800 µS/cm); a decreasing trend over time was observed in all piezometers, down to values similar to the input solution (about 700 µS/cm). SEC was not affected by the addition of EHC-O.

3.3.2. Chemical results

Fig. 3 shows the mean concentration of MTBE and toluene at steady-state conditions along the columns; additional contaminants are reported in Fig. S3 (Annex 1). Significant removal was observed in both tests. In the UN test, the concentration of MTBE decreased linearly along the column from IN to C, it was nearly constant between C and F and decreased from F and H. Toluene decreased exponentially along the column. The removal efficiency at steady-state conditions (referred to the average concentration measured in the input solution) in the piezometers C and H were 42% and 72% for MTBE, and 78% and 97% for toluene. ETBE behavior was similar to MTBE, while BEX was similar to toluene. In the IN test, an exponential decrease in concentrations along the flow path was observed for all compounds. The UN and IN tests exhibited similar removal efficiencies.

TBA concentrations in both the tests (data not shown) were unstable, even in the inflow solution; it was not possible to point out a clear trend as the concentrations in the outflow were not significantly different to the inflow.

Chemical analyses on activated carbon pointed out that MTBE and BTEX loss due to volatilization in the UN test was respectively 11% and 16% on mass basis, while it was negligible (0.2%) in the IN test.

3.3.3. Biological results

DGGE provided information about the beta-diversity of microbial community within the same column and the taxonomic classification of the main bands in the gels while T-RFLP data were more suitable for the comparison of the communities in IN and UN tests.

DGGE gel of UN test is shown in Fig. 4. Two bands were detected in the pumice samples collected before the test, whereas 6–11 bands were identified at the end of the treatment. Bands 1–3 and 5 were related to bacterial populations located in the first part of the column, whereas band 6 was found just in the second part of the column. Bacterial population of band 4 was detected in all samples. The population of band 1 was classified as Comamonas. Microorganisms belonging to Comamonas genus have been reported to be capable of degrading BTEX under aerobic conditions [26]. The population of band 4 was classified as Hydrogenophaga, whose Hydrogenophaga flava ENV735 was shown to be able to degrade MTBE as pure cul-ture [27]. Band 8 was related to Thauera, whose Thauera Aromatico K172 was capable of degrading aromatic hydrocarbons under both denitriﬁying and aerobic conditions [28]. DGGE analysis of IN test conﬁrmed that the added inoculum colonized the pumice and per-sisted during all the experiment along the column (data not shown).

T-RFLP showed that the communities grown in the UN test during the treatment formed a different cluster from the communities developed in the IN test (Fig. S4 in Annex 1). Furthermore the communities in the UN test were sorted out throughout the column while there was no spatial organization in the communities developed in the IN test. These results suggested that the bioaugmentation changed deeply the microbial communities in the column. Furthermore, the presence of bacteria related to the facultative anaerobe Thauera in the samples collected from the piezometer D to the piezometer H in UN, may reﬂect both the presence of microenvironments in which the oxygen concentration was low, despite the ORC addition and the adaptation of these popula-tions to the contaminant mixture composition in this part of the column.

No clogging of the ﬁlling material, due to excessive biomass growth, was observed throughout the entire IN test. No signiﬁcant changes were observed on the water ﬂows between UN and IN tests.

3.4. Numerical modeling

3.4.1. Parameter estimation

The estimate (± standard error, s.e.) of the ﬁrst order biodegradation rate of B, T, E, o-X, m + p-X and MTBE resulting from the model for both the columns is reported in Table 4. The experimental data fitting for the UN test, showing a different decline in the compound concentrations in the ﬁrst part of the column (from IN to C),
was improved by assuming that the two parts might exhibit different values in the biodegradation rates. Minor differences between the \( K_r \) values for the two column experiments were obtained for B, T and MTBE, while significantly higher values were obtained for E and \( o-X \) in the second part of the uninoculated column and for \( m.p.X \) in the first part of the column. In both columns, \( K_m \text{MTBE} \) was in the wide range (0.0003 d\(^{-1}\) to 5.3 d\(^{-1}\)) reported for MTBE metabolism in bench scale experiments or during in situ treat-ments [29–31]. The different biodegradation rates might be due to the presence of microorganisms with a different metabolism in the first part of the column and in the second part of the column. Micro-biological data suggest that the microbial communities enriched form the piezometer A to the piezometer C were dominated by aerobic bacteria, whereas facultative anaerobes were abundant in the communities for the piezometer D to the piezometer H. BTEXs are preferential substrates compared to MTBE under aerobic conditions and their biodegradation results in depletion of electron acceptors; at any rate, the fitting between the breakthrough curves and model prediction suggested no significant interaction in MTBE and BTEX biodegradation in the columns, where enough oxygen was present to support aerobic conditions. Similar removal for MTBE and BTEX were obtained over the entire length of the UN and the IN systems. Differently from the results reported in [19], the dispersivity value estimated in the preliminary tracer tests for the uninoculated system (7.52 ± 0.83 cm) was comparable to that obtained for the inoculated column (7.09 ± 1.48 cm), probably as a coarse material such as pumice is less affected by biomass growth than sand. Moreover, the biodegradation rate observed in the tests with pumice occurred at a faster rate than that observed in the tests with sand. For instance, the values reported in [19] for MTBE (0.031 ± 0.001 d\(^{-1}\)), B (0.045 ± 0.002 d\(^{-1}\)) and T (0.08 ± 0.004 d\(^{-1}\)) in the inoculated sandy soil were 5 to 10 times lower than those obtained with pumice in this work.

3.4.2. Biobarrier scenarios

The BB dimensions were calculated based on MTBE performance in the inoculated column, as this was the limiting compound among those investigated. In order to get the target concentration downgradient the site boundary, a 99.9% abatement of MTBE was required in the plume. This removal resulted in a required residence time in the BB system of about 115 d. The plume was about 15 m wide. Based on flow and transport simulations for the site specific flow field and a \( K_{app} \text{MTBE} \) ratio of 15, two different BB configurations were investigated: (i) CW 1.5 m long and 14 m wide \( \times \) 10 m high (Fig. 5a) and (ii) F&G with two funnels 12 m wide, and one gate 5 m long \( \times \) 3.5 m wide \( \times \) 10 m high (Fig. 5b). Both systems resulted in a local decrease of the natural hydraulic gradient to values in the range 0.0002–0.0012 for CW and 0.0005–0.0010 for the F&G BB; similarly, seepage velocity in proximity of the BB spanned in the range 0.015–0.06 m/d for the CW and 0.045–0.20 m/d for the F&G. These values were up to two orders of magnitude lower for the CW BB and up to one order of magnitude for the F&G BB than in the bench scale test. The discrepancies in the flow regime between field and bench scale tests and the lower inlet contaminant con-centrations in the field explain the different BB lengths in the two configurations. For the case study, in the hypothesis of placing the BB immediately downgradient the residual source in soil, where the associated dissolved plume still presents a limited width, the F&G

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**Fig. 4.** DGGE gel of pumice samples before (PUM) and after the UN test at different locations (piezometers A to H). The numbers associated to the bands represent the taxonomic classification (family and gender) of microorganisms (confidence level in brackets): (1) Comamonadaceae [100%], Comamonas [99%]; (2) Xanthomonadaceae [100%], Thermomonas [100%]; (3) Comamonadaceae [100%], Acidovorax [99%]; (4) Comamonadaceae [100%], Hydrogenophaga [100%]; (5) Xanthomonadaceae [100%], Luteimonas [92%]; (6) Rhodocyclaceae [100%], Thauera [100%].
configuration allowed to save just 20% of the volume of the biomass support compared to the CW BB (175 m$^3$ for the F&G, 210 m$^3$ for the CW); therefore, the F&G BB did not seem so convenient, taken into consideration the need of funnel installation. This finding may no longer hold in case another location of the BB is proposed at the site, and specific assessment is required. Probably the F&G configuration may be a convenient option in case the treatment of a wider plume is required.

Compared to the preliminary design reported in [7] for a sandy biobarrier (Fig. 6a and b), pumice allowed smaller BBs, for both CW and F&G configurations, than by using sand, own to the effects of the high hydraulic conductivity and the biomass sorption capacity of this filling material. With reference to the volume of the filling material, sand would require an in situ volume about 6 times higher than pumice.

4. Conclusions

Laboratory tests were performed to assess the performance of selected microbial consortia in degrading MTBE and BTEXs in groundwater in view of a BB installation. Compared to the inoculation performed in the undisturbed aquifer, BBs help solve problems in aquifer heterogeneity, poor hydraulic conductivity and biomass attachment/detachment. Experimental activities for the BB design were carried out following these steps: (i) selection of a proper filling material; (ii) selection of a proper microbial culture in laboratory; (iii) uninoculated and inoculated column tests; and (iv) numerical modeling.

In this study, pumice was used as the microbial consortium support. A mixed culture composed by Rhodococcus sp. CE461, Methylibium petroleiphilum LMG 22953 and Rhodococcus sp. CT451 (InoculumA) was selected as the inoculum for the column test due to the best capabilities of degrading the pollutants of interest among the tested consortia.

The breakthrough curves obtained for MTBE and BTEXs in column tests allowed the estimate of the biodegradation rates of the compounds in the BB system. A removal of about 70% was obtained at the end of the column for the most recalcitrant compound MTBE and up to 97% for BTEX.

The numerical codes (MODEFLOW, MODPATH and MT3DMS) were used to simulate 3-D groundwater flow, particle tracking and multispecies reactive transport. The biobarrier proper location, width and thickness for the complete capture of the plume and the abatement of pollutants to the target concentration at the point of compliance were obtained. For the case study, the regulatory standard for MTBE could be assured by a continuous wall 14 m wide, 1.5 m long and 10 m high, placed immediately downgradient the residual source in soil. Compared to the outcomes of [19], pumice showed better performance and seems more promising in view of field scale applications at the site. A F&G system did not exhibit advantages both in term of plume capture and gate volume. Based on the results of this work, field pilot tests are necessary to assess the behavior of the selected inocula in a real aquifer and to verify the performances of the treatment anticipated from this study are also confirmed at field conditions.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

References