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

A. Careghini [a], S. Saponaro [a,*], E. Sezenna [a], M. Daghio [b], A. Franzetti [b], I. Gandolfi [b], G. Bestetti [b]

[a] Politecnico di Milano, DICA Sez. Ambientale, Piazza Leonardo da Vinci 32, 20133 Milano, Italy
[b] Università degli Studi Milano-Bicocca, Dipartimento di Scienze dell'Ambiente e del Territorio, Piazza della Scienza 1, 20126 Milano, Italy

HIGHLIGHTS

- Biobarriers (BBs) are in situ passive systems.
- BBs gain in homogeneity, hydraulic conductivity and biomass attachment.
- Laboratory tests and numerical simulations were carried out to design a BB system.
- Pumice was chosen as the filling material.
- About 70% removal was obtained for the most recalcitrant compound investigated (MTBE).

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ABSTRACT

Methyl *tert*-butyl ether (MTBE) is used at significant percentages as an additive of unleaded gasoline. The physical–chemical properties of the substance (water solubility, soil organic carbon–water partition coefficient) cause high mobility and high concentrations in groundwater. Laboratory scale batch and column tests and mathematical modeling were performed to study the feasibility of a biobarrier (BB), that is an in situ permeable biological barrier with or without inoculation, for the remediation of MTBE and other gasoline-derived pollutants (benzene, toluene, ethylbenzene, *o*-xylene and *m* + *p*-xylenes, BTEXs) polluted groundwater and to estimate kinetic constants. The experimental results showed simultaneous biodegradation of MTBE and BTEXs, with similar removals in the uninoculated and the inoculated systems. Ranges for the first order kinetic removal were obtained for MTBE ($(0.18 \pm 0.02)/(0.28 \pm 0.11\ d^{-1})$), B ($(0.39 \pm 0.12)/(0.56 \pm 0.12\ d^{-1})$), T ($(0.51 \pm 0.03)/(0.78 \pm 0.15\ d^{-1})$), E ($(0.46 \pm 0.18)/(1.57 \pm 0.21\ d^{-1})$), *o*-X ($(0.24 \pm 0.08)/(0.64 \pm 0.09\ d^{-1})$) and *m* + *p*-X ($(0.20 \pm 0.04)/(1.21 \pm 0.04\ d^{-1})$). The results of the laboratory tests allowed to improve mathematical modeling in order to design a full-scale BB at a gasoline-contaminated site.

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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

* Corresponding author. Tel.: +39 0223996432/00; fax: +39 0223996499.
E-mail address: sabrina.saponaro@polimi.it (S. Saponaro).

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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 petroleiphilum LMG 22953 where the growth substrates from literature are reported [32].

InoculumA	InoculumB	InoculumC
Rhodococcus sp. CE461 (B, T, E, *m*-X)	*Pseudomonas* sp. CXP451 (T, *m*-X, *p*-X)	*Rhodococcus* sp. CE461 (B, T, E, *m*-X)
Methylibium petroleiphilum LMG 22953 (MTBE, B, T, *o*-X)	*Pseudomonas* sp. CXP452 (T, *m*-X, *p*-X)	*Methylibium petroleiphilum* LMG 22,953 (MTBE, B, T, *o*-X)
Rhodococcus sp. CT451 (B, T, E, *o*-X)	*Pseudomonas* sp. CXM451 (T, *m*-X, *p*-X)	*Stenotrophomonas* sp. CXO452 (T, E)
	Stenotrophomonas sp. CXM452 (*p*-X)	*Pseudomonas* sp. CXP452 (T, *m*-X, *p*-X)
	Stenotrophomonas sp. CXO452 (T, E)	*Rhodococcus* sp. CT451 (B, T, E, *o*-X)
	Rhodococcus sp. CB451 (B, T, E, *p*-X)	
	Rhodococcus sp. CE461 (B, T, E, *m*-X)	
	Rhodococcus sp. CT451 (B, T, E, *o*-X)	
	Methylibium petroleiphilum LMG 22,953 (MTBE, B, T, *o*-X)	
	Rhodococcus sp. E252 (B, T, E)	

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 [8,9]: 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. CXP452 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_{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 Ribosomal 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 $OD_{540} = 0.1$ [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_{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 petroleiphilum* LMG 22953 [14] was purchased from the Belgian Co-ordinated Collections of Microorganisms (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_4NO_3 (3.31 mg/g), K_2HPO_4 (0.32 mg/g), KH_2PO_4

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