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Research article

Ethanol content in different gasohol blend spills influences the decision-making on remediation technologies

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ABSTRACT

Gasohol blend spills with variable ethanol content exert different electron acceptor demands in groundwater and the distinct dynamics undergone by these blends underscores the need for field-based information to aid decision-making on suitable remediation technologies for each gasohol blend spill. In this study, a comparison of two gasohol releases (E10 (10:90 ethanol and gasoline, v/v) and E25 (25:75 ethanol and gasoline, v/v) under monitored natural attenuation (MNA) and nitrate biostimulation, respectively) was conducted to assess the most effective remediation strategy for each gasohol release. Microbial communities were assessed to support geochemical data as well as to enable the characterization of important population shifts that evolve during biodegradation processes in E25 and E10 field experiments. Results revealed that natural attenuation processes sufficiently supported ethanol and BTEX compounds biodegradation in E10 release, due to the lower biochemical oxygen demand they exert relative to E25 blend. In E25 release, nitrate reduction was largely responsible for BTEX and ethanol biodegradation, as intended. First-order decay constants demonstrated that ethanol degradation rates were similar ($p < 0.05$) for both remediation technologies (2.05 ± 0.15 and 2.22 ± 0.23, for E25 and E10, respectively) whilst BTEX compounds exhibited different degradation rates ($p > 0.05$) that were higher for the experiment under MNA (0.33 ± 0.06 and 0.43 ± 0.03, for E25 and E10, respectively). Therefore, ethanol content in different gasohol blends can influence the decision-making on the most suitable remediation technology, as MNA processes can be applied for the remediation of gasohol blends with lower ethanol content (i.e., 10% v/v), once the aquifer geochemical conditions provide a sufficient electron acceptor pool. To the best of our knowledge, this is the first field study to monitor two long-term gasohol releases over various time scales in order to assess feasible remediation technologies for each scenario.

1. Introduction

The dependence of fossil fuels and the potential threats they can pose to the environment have boosted the development and use of alternative renewable fuels (Schnoor, 2006). Ethanol has been increasingly added to the worldwide energy matrix, typically through gasoline-blended formulations to alleviate dependence on fossil fuels and reduce the environmental issues associated with fossil fuels (Goldemberg, 2007). In Brazil, commercial gasoline has an ethanol mandatory blending percentage of 27% (Brazil, 2015), while in the United States 10% of ethanol is blended into gasoline formulations (US EPA, 2015). In EU member states, the current blending percentage of ethanol to gasoline is up to 10% (European Parliament, 2009) but countries such as Spain, Germany, Italy and the United Kingdom opted for a 5% ethanol percentage to the commercial gasoline (European Environmental Agency, 2015). In the Asian continent, China primarily uses pure gasoline and diesel as commercial fuels (USDA, 2007) followed by E10 blends (10:90 ethanol and gasoline, %) that are used in 9 of their 22 provinces (Pang et al., 2008). In India, 5% of ethanol is blended into gasoline in 11 states (Sukumaran et al., 2010). As fuel leaks and spills are commonly observed during storage and transport (Das and Chandran, 2011), this can lead to increasing contaminated sites by the widely used ethanol-blended gasoline fuel. Since these

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formulations contain priority contaminants such as BTEX (benzene, toluene, ethylbenzene and xylenes), they require remedial actions when released to the environment.

Monitored natural attenuation (MNA) is a well-established strategy to remediate contaminated sites that relies on natural attenuation processes to achieve remediation goals within a reasonable time frame. MNA is minimally invasive and the cost of implementation and monitoring is relative low (Adriano et al., 2004; Blum et al., 2009; Corseuil et al., 2011; Kao et al., 2006; Khan and Husain, 2003; Mackay et al., 2006; Naidu et al., 2012). The efficiency and applicability of MNA depends primarily on the site characteristics, the time needed to remove contaminants and potential risks to human health (Khan et al., 2004). When natural attenuation processes are insufficient to reduce contaminants concentration or when the time required or risk involved are not compatible with natural attenuation processes, active remediation technologies (i.e., biostimulation) can be applied to speed up contaminants attenuation and meet remediation goals.

The high biochemical oxygen demand (BOD) commonly exerted by ethanol leads to the exhaustion of the available electron acceptors (Da Silva and Alvarez, 2002) and thereby gasohol blends with higher ethanol content would require a greater stoichiometric electron acceptor demand. To exemplify, for a 100L-spill of E25 and E10 blends, the theoretical BOD for ethanol biodegradation (reaction (1)), according to McCarty (1969) model, would be 2.5 times higher for E25 as compared to E10 blend (calculations provided in SI). Therefore, the enhanced consumption of electron acceptors can make natural attenuation processes unfeasible to deal with gasohol blends spills with high ethanol content. In this case, engineering interventions (i.e., active remediation technologies) may be required to avoid persistent contaminants concentrations.

$$CH_3CH_2OH + 1.1O_2 + 0.4HCO_3^- + 0.4NH_4^+ \rightarrow 0.4C_5H_7O_2N + 0.5CO_2 + 2.8H_2O \quad (1)$$

Remediation technologies can be either aerobic or anaerobic and the decision-making is dependent on the scenario of the contaminated site. Although aerobic strategies generally exhibit faster degradation rates (Corseuil et al., 1998; Ruiz-Aguilar et al., 2003), they are not universally applicable as hydrocarbons contaminated sites are invariably anaerobic due to the rapid oxygen consumption by indigenous microorganisms. Therefore, the majority of hydrocarbon contaminants are degraded by anaerobic microorganisms, which makes anaerobic technologies more suitable to deal with gasohol releases.

Among the existing anaerobic strategies, nitrate biostimulation that refers to the use of nitrate as terminal electron acceptor to enhance the conversion of organic compounds into carbon dioxide and water (Wilson and Bouwer, 1997), is widely applied for the remediation of aromatic compounds (Cunningham et al., 2001; Da Silva et al., 2005; Hutchins et al., 1991; Schreiber and Bahr, 2002; Wilson and Bouwer, 1997). The broad use of nitrate biostimulation can be explained by (1) the higher oxidation potential provided (0.25–0.85 V) as compared to other anaerobic processes such as iron reduction (0.10 to −0.50 V), sulfate reduction (−0.20 to −0.70 V) or methanogenesis (−0.25 to −0.75 V) (Christensen et al., 2000; Stumm and Morgan, 1996), (2) the high solubility of nitrate salts that facilitates the injection through the site and (3) the relatively low cost (Hutchins et al., 1998; Korda et al., 1997).

Nitrate biostimulation usually involves the continuous injection of nitrate salts into the groundwater. Nevertheless, some aquifers may already have considerable background concentrations of nitrate, as is the case of areas under agricultural activities that usually exhibit significant amounts of fertilizer-derived nitrate (Galloway et al., 2004; Sebilo et al., 2013). Thus, depending on the scenario of the contaminated site, nitrate reduction or other redox processes (such as iron or sulfate reduction) could occur naturally and this must be taken into account before deciding whether to apply active remediation technologies or to rely on monitored natural attenuation processes.

Given the several gasohol blends that are currently used worldwide and the associated risk of spills that require remedial actions, these field studies will advance the current understanding on the complex dynamics undergone by different gasohol releases in groundwater and overall site management. Furthermore, the information obtained can be potentially used for the development of risk assessment models to confidently predict the behavior and biodegradation of different gasohol blends in real environments, thus underscoring the need for field-based information. This can aid decision-making process on the most suitable remediation strategy by enabling a more cost-effective and targeted response to different gasohol blends spills, which correspond to the main concerns of cleanup decisions.

This study presents two long-term field experiments (monitored over 11 and 6 years) of different gasoline-ethanol blends (E25 (25:75 ethanol and gasoline v/v) and E10 (10:90 ethanol and gasoline v/v)) under nitrate biostimulation and natural attenuation that were conducted to assess the most effective remediation strategy. To the best of our knowledge, this is the first field study to monitor two different gasohol releases over various time scales in order to assess feasible remediation technologies for each scenario.

2. Materials and methods

2.1. Field experiments

Two field experiments were conducted in neighboring areas (located at a distance of 23 m) at Ressacada Experimental Farm in Florianópolis, SC, Brazil. The experiments were established by the release of 100 L of E25 (25:75 ethanol and gasoline, v/v) and E10 (10:90 ethanol and gasoline, v/v) into source-zone areas of 1.0 m × 1.0 m for E25 and 1.5 m × 1.0 m for E10, at the water table level (Fig. 1). Geological characterization of the sites were previously described (Da Silva and Corseuil, 2012). Multilevel wells were installed in E25 (6 injection wells and 64 sampling wells) and E10 (58 sampling wells). A peristaltic pump and Teflon tubing were used to collect samples at different depths (2, 3, 4, 5 and 6 m below ground surface [bgs] for E10 and 2.3, 2.8, 3.8, 4.8 and 5.8 m bgs for E25) to capped sterile vials without headspace. The levels that exhibited the most significant concentration of ethanol and BTEX were presented in the results.

Ressacada Experimental Farm has a natural availability of electron acceptors (Table 1). The background nitrate concentrations are possibly present due to previous cattle farming activities in the area, while sulfate is likely related to minerals (i.e. pyrite) that infiltrate from soil and are dissolved into the groundwater. These background concentration of electron acceptors were already presented in other Ressacada field studies (Corseuil et al., 2011; Müller et al., 2017; Ramos et al., 2013). Therefore, in E10 site, monitored natural attenuation was conducted to evaluate whether the natural availability of electron acceptors (i.e., nitrate and sulfate) could be sufficient to support ethanol and BTEX biodegradation. In E25 site, nitrate was injected as a supplementary source of electron acceptor to stimulate nitrate reduction processes and enhance organic contaminants biodegradation. Injections initiated 2 months after E25 was released and were performed by the release of 5 L of $NaNO_3$ (4 g L^{-1}) into the injection wells three times a week

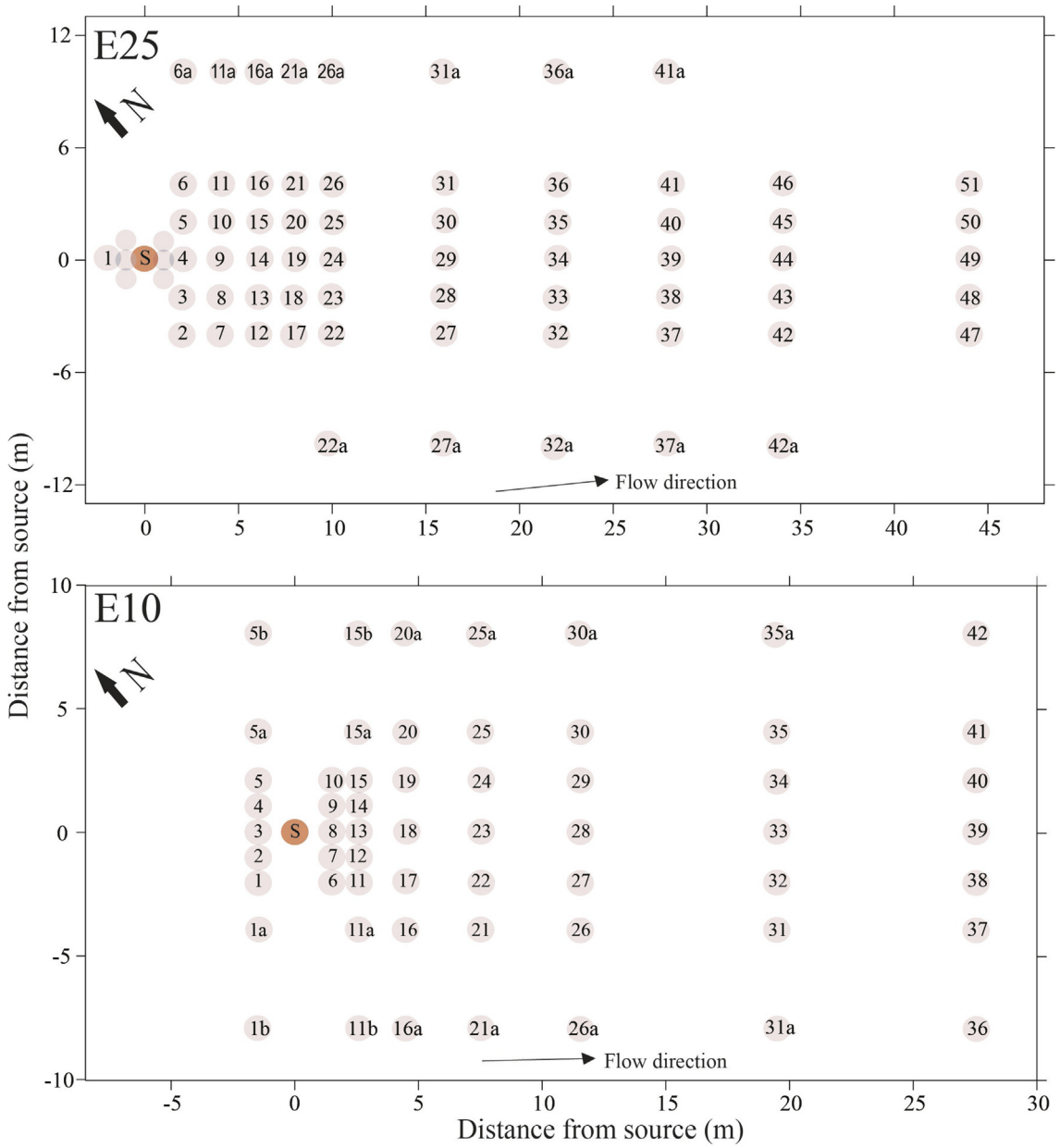

Fig. 1. Schematic view of both experimental sites. Empty circles (without numbers) represent injection wells, numbered circles represent sampling wells and red circles (S) correspond to the source area. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Background geochemical groundwater conditions at the source-zone of both E25 and E10 experiments.

Variables	E25		E10	
	Level 2.8	Level 3.8	Level 3.0	Level 4.0
Temperature (°C)	25.20	23.92	24.37	24.19
pH	4.15	4.09	4.06	4.10
Redox potential (ORP) (mV)	+519	+522	+404	+402
Dissolved oxygen (DO) (mg L^{-1})	2.31	3.30	2.85	2.48
Nitrate (mg L^{-1})	0.58	1.18	8.10	2.84
Sulfate (mg L^{-1})	3.92	4.44	3.33	3.02
Iron II (mg L^{-1})	<0.10	<0.10	<0.10	0.22
Phosphate (mg L^{-1})	<0.01	<0.01	0.56	<0.01
Methane (mg L^{-1})	<0.01	<0.01	N.A.[a]	N.A.[a]

[a] N.A.: not analyzed.

over 9 months. E25 experiment started 5 years prior to the start of E10. The field studies were monitored over 11 and 6 years for E25 and E10, respectively.

2.2. Chemical analyses

Groundwater samples were analyzed for pH, redox potential, dissolved oxygen, acetate, nitrate, sulfate, methane, ethanol, BTEX and ferrous iron. The ions acetate, nitrate, sulfate were analyzed by ion chromatography using a Dionex ICS-3000 equipped with a conductivity detector and an AS19 column. Dissolved oxygen, redox potential and pH were measured on site with a portable Micro-purge Flow Cell (MP20) analyzer. BTEX, ethanol and methane were measured by gas chromatography with a HP 6890 II

chromatographer coupled with flame ionization detector (FID). Ferrous iron was measured by using the 1,10-phenanthroline method with a HACH DR/2500 spectrophotometer (APHA, 1998). Detection limits were (in parenthesis): DO (0.5 mg L^{-1}), acetate (0.1 mg L^{-1}), nitrate (0.1 mg L^{-1}), sulfate (0.1 mg L^{-1}), methane (10 μg L^{-1}), ethanol (1 mg L^{-1}), BTEX (1 μg L^{-1}) and ferrous iron (0.01 mg L^{-1}).

2.2.1. Degradation rates (k)

First-order kinetics are commonly applied to model biodegradation rates of hydrocarbons in aquifers where mass transfer mechanisms are often rate-limiting as they undergone desorption, dissolution and diffusion to the cell surface (Alvarez and Illman, 2006). Thus, first-order decay coefficients were determined for ethanol and BTEX after the onset of their degradation, by fitting their concentration versus time to an exponential decay model.

2.3. Microbial analysis

Microbial communities were assessed to support geochemical data as well as to enable the characterization of important population shifts that evolve during the biodegradation processes in E25 and E10 field experiments. For DNA extraction, 1L of groundwater was collected and samples were subsequently filtered with a 0.22 μm Millipore membrane filter (Sartorius Stedim Biotech, Göttingen, Germany). The filters were weighed before and after filtration and qPCR results were expressed in gene copies per gram of total suspended solids, since groundwater bacteria are mostly associated with solid surfaces rather than suspended in water (Harvey et al., 1984; Lehman et al., 2001). DNA was extracted using a MoBio Power Soil™ (Carlsbad, CA) kit, following the manufacturer's protocol.

2.3.1. Real-time quantitative polymerase chain reaction (qPCR)

Real-time quantitative polymerase chain reaction (qPCR) analysis were conducted for E10 site. Real-time qPCR assays for E25 site were performed and described elsewhere (Da Silva and Corseuil, 2012). PCR mixture contained 1× Taqman Universal PCR Master Mix or SYBR Green, 0.5 μM of forward and reverse primers and 0.25 μM of probes and 2 μL of extracted DNA. The final volume of the solution (25 μL) was completed with Milli-Q DNAse-free water. Target microbial groups, primers and probes are described in Table 2. PCR assays were performed on an Eppendorf Mastercycler ep realplex (Thermal Cycler, CA, USA) with the following conditions: 50 °C for 2 min, followed by 95 °C for 10 min and 40 cycles at 95 °C for 15 s ending with 60 °C for 1 min (Da Silva and Alvarez, 2002; Da Silva and Corseuil, 2012). The detection limits for each qPCR assay were about 10^3 gene copies g^{-1}. The standard DNA curves were conducted by serial dilutions of the genomic DNA of the following microorganisms: *Pseudomonas aeruginosa* (DSM 50071) for total bacteria and nitrate reducers, *Geobacter metallireducens* (DSM 7210) for iron- and sulfate reducers and *Methanococcus maripaludis* (DSM 2067) for archaea.

2.3.2. 16S rRNA gene sequencing

Next-generation sequencing (16S rRNA gene sequencing) were conducted for both E10 (at 4 and 5.7 years after the release) and E25 (at 6.7 and 9.6 years after the release) experiments. 16S rRNA sequencing was performed using the Illumina Miseq platform (Illumina, 2013). Bacteria 16S rRNA gene was amplified within V3 and V4 regions by PCR (Klindworth et al., 2013). PCR assays were conducted in a Biometra Tpersonal Thermal Cycler and the primers with adaptors as well as the PCR conditions are listed elsewhere (Müller et al., 2017). A FastX-Toolkit was used to trim the first 20 nucleotides obtained and its quality was verified using the Quantitative Insights Into Microbial Ecology – QUIIME (v1.9.0, http://qiime.org/index.html) software. Illumina paired-end reads were assembled by PANDAseq (Masella et al., 2012), and the sequences were clustered into operational taxonomic units (OTUs). The sequences were blasted against Greengenes database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) for taxonomic information.

3. Results and discussion

3.1. Groundwater chemical analysis

The higher BOD exerted by E25 blend relative to E10, contributes to the rapid depletion of background electron acceptors, thus engineering interventions were conducted to enhance organic contaminants biodegradation. Nitrate was added as a supplementary source of as electron acceptor to stimulate nitrate-reducing conditions. Accordingly, nitrate reduction was largely responsible for organic compounds biodegradation at E25 site, which was evidenced by the decrease in ethanol and BTEX concentration that was accompanied by the depletion of nitrate (Fig. 2). While in E10 site, background electron acceptors (nitrate, ferric iron and sulfate) were sufficient to support organic compounds biodegradation due to the reduced ethanol content in E10 blend that exerts a lower BOD relative to E25. Therefore, results suggest that three different terminal electron-accepting processes (TEAP) likely occurred in E10 as opposed to E25 where nitrate reduction prevailed.

Ethanol was preferentially biodegraded in both experiments and the onset of BTEX biodegradation was observed only after ethanol depletion (Fig. 3). In E25 plot, ethanol biodegradation via nitrate reduction was demonstrated by their complete removal along with the production of the anaerobic metabolite – acetate (up to 118.2 mg L^{-1} at level 3.8 m) at the same time frame (3.2 years after release) (Figs. 2 and 3). In E10 plot, at the beginning of the experiment (at 0.28 and 0.46 years), considerably high concentrations of ferrous iron were detected (between 106.8 and 43.4 mg L^{-1} at levels 3.0 and 4.0 m, respectively), which chronologically

Table 2
Target groups, primers and probes sequences for E10 qPCR analysis.

Target	Forward Primer	Reverse Primer	Probe	References
Total Bacteria	5′-CGGTGAATACGTTCYCGG-3′ (BACT1369F)	5′-GGWTACCTTGTTACGACTT-3′ (PROK1492R)	FAM-5′CTTGTACACACCGCCCGTC3′-BHQ-1 (TM1389F)	Suzuki et al. (2000)
Nitrate reducers	5′-CCTAYTGGCCGCCRCART-3′ (NIRS1F)	5′-CGTTGAACTTRCCGGT-3′ (NIRS6R)	–	Braker et al. (1998)
Iron reducers	5′-GCGTGTAGGCGGTTTCTTAA-3′ (561F)	5′-TACCCGCRACACCTAGTTCT-3′ (825R)	–	Stults et al. (2001)
Sulfate reducers	5-′AAGCCTGACGCASCAA-3′ (361F)	5′-ATCTACGGATTTCACTCCTACA-3′ (685R)	–	Stults et al. (2001)
Archaea	5′-CGGTGAATACGTCCCTGC-3′ (ARCH1-1369F) 5′-CGGTGAATATGCCCCTGC-3′ (ARCH2-1369F)	5′-AAGGAGGTGATCCTGCCGCA-3′ (PROK1541R)	FAM-5′CTTGTACACACCGCCCGTC3′-BHQ-1 (TM1389F)	Suzuki et al. (2000)

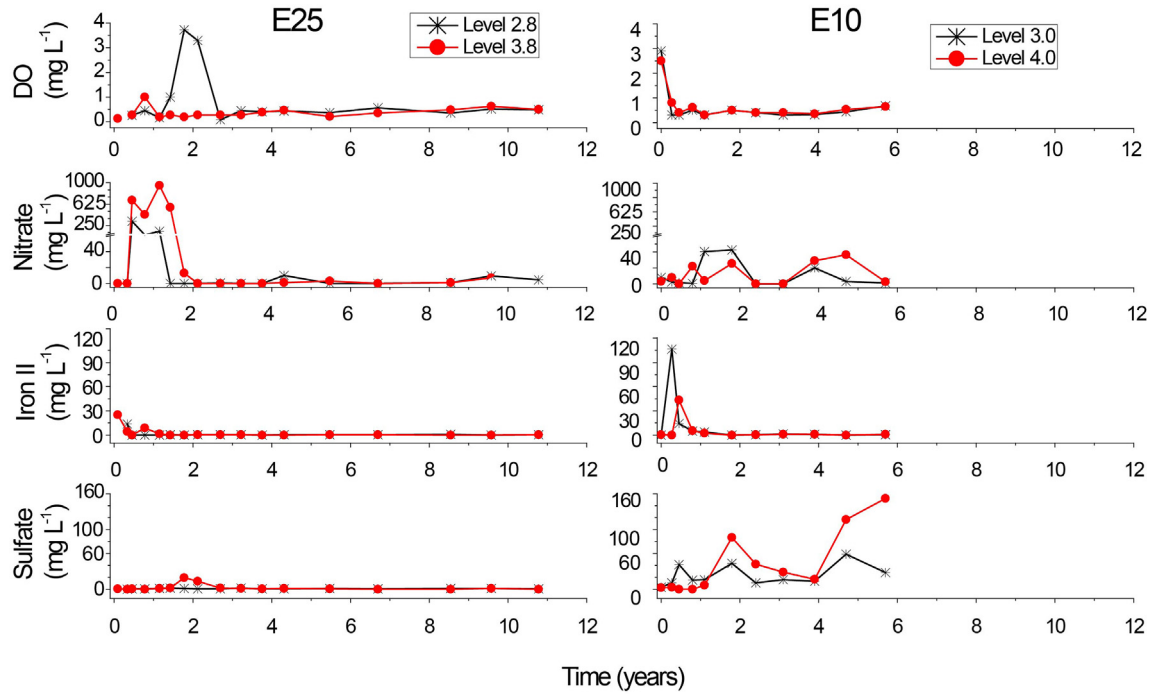

Fig. 2. Time series of dissolved oxygen (DO), nitrate, iron II and sulfate at the source-zones from both experimental sites.

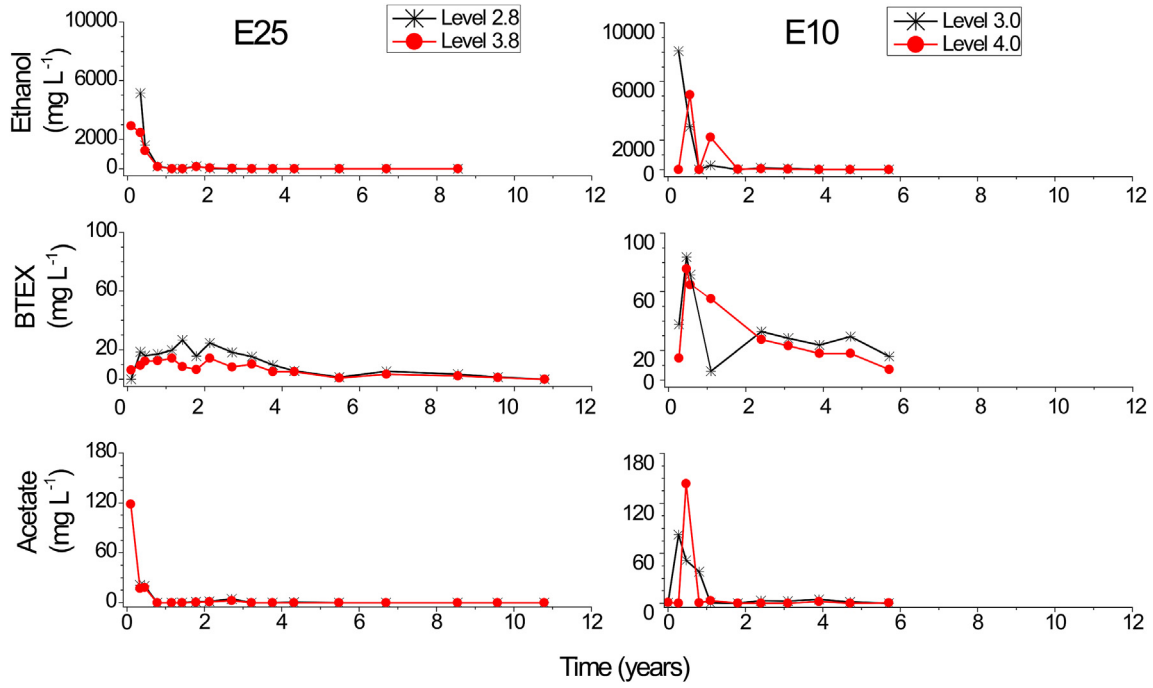

Fig. 3. Ethanol, BTEX and acetate concentrations (mg L^{-1}) at the source-zones from both experimental sites.

coincided with ethanol removal, thus providing evidence of iron reduction as the main TEAP during ethanol biodegradation (Fig. 3). Variable nitrate concentrations were observed over the 6 years of monitoring, which likely reflected the cycling of nitrate through rainfall infiltration (Ressacada Experimental Farm mean annual precipitation corresponds to 1334 mm (±83.82)) from the soil into the groundwater and its consumption by ethanol or BTEX oxidation. Moreover, significant amounts of sulfate were consumed after 2 years following the release, suggesting that sulfate reducers possibly played a role on BTEX biodegradation. Considering the thermodynamic hierarchy (Chapelle, 2001; Wiedemeier et al., 1999), it is reasonable to assume that sulfate reduction would be a preferential pathway after nitrate and ferric iron depletion.

Table 3
First-order degradation constants (k) at the source-zone for E25 and E10 experiments.

Experiment	Substrate	Time (years)	k (year^{-1})	R^2	n[a]
E25	Ethanol	0.1 to 2.1	2.05[a] (±0.15)	0.99	4
	BTEX	2.1 to 6.7	0.33[b] (±0.06)	0.94	4
E10	Ethanol	0.6 to 3.1	2.22[a] (±0.23)	0.98	4
	BTEX	0.5 to 5.7	0.43[c] (±0.03)	0.99	6

a,b,c. Different letters within the same column indicate significant differences between the mean k values ($p < 0.05$) according to *t*-test.
[a] n. Number of observations.

3.2. Degradation rates

Ethanol and BTEX revealed different patterns for the degradation rates evaluated in both E25 and E10 experiments. Results shown in Table 3, demonstrate that degradation rates were similar ($p < 0.05$) for ethanol in both experiments (2.05 ± 0.15 and 2.22 ± 0.23, for E25 and E10, respectively) whilst BTEX compounds revealed different degradation rates ($p > 0.05$) that were higher for the experiment under MNA (E10) as compared to nitrate biostimulation (E25) (0.43 ± 0.03 and 0.33 ± 0.06, for E10 and E25, respectively). Comparatively, a study of a 100L-spill of two similar gasohol blends (E24 and E25) conducted by Corseuil et al. (2015), demonstrated that degradation rates were consistently higher for nitrate biostimulation as compared to monitored natural attenuation. Given that all these field experiments are located in neighboring areas at Ressacada Experimental Farm and thereby exposed to similar hydrogeological conditions, it can be concluded that natural attenuation can be an effective strategy to deal with gasohol spills with lower ethanol content (10% v/v). This can be attributed to the considerably lower BOD exerted by E10 (≈ 2.5 times) relative to E25 and to the natural groundwater geochemical conditions that were sufficient to support contaminants biodegradation. It is worth noting that groundwater geochemical characteristics are utterly important to effectively apply monitored natural attenuation as a bioremediation technology.

3.3. Groundwater microbial analysis

Microbial response in E10 plot was consistent with the geochemical changes previously mentioned. E10 release enhanced total biomass growth (from 10^7 to 10^9 gene copies g^{-1}) as well as iron (from 10^4 to 10^6 gene copies g^{-1}) and sulfate reducers (from 10^2 to 10^7 gene copies g^{-1}) (Fig. 4). Nitrate reducers were consistently detected in all sampling events, reaching concentrations as high as 10^9 gene copies g^{-1} after 4 years following the release, which suggests their participation in E10 compounds biodegradation. The absence of archaea was corroborated by the negligible methane production (<0.1 mg L^{-1}) observed over the 6 years of monitoring. In E25 site, the increased nitrate consumption coincided with zones with enhanced concentration of iron reducers (Da Silva and Corseuil, 2012). Nonetheless, iron reducers (such as *Geobacter* spp.), sulfate reducers and archaea were not detected (Da Silva and Corseuil, 2012), which is consistent with the preferential growth of nitrate reducers relative to other outcompeted microbial communities and nitrate reduction as the main TEAP in E25 site.

16S rRNA gene sequencing in E10 plot revealed a microbial community structure mainly constituted by the genera *Telmatospirillum*, *Salinispora*, *Erwinia* and *Burkholderia* (Fig. 5). These genera were detected at 4.0 and 5.7 years after E10 release, when ethanol was already depleted, though significant BTEX compounds concentrations were still detected (between 24 and 7 mg L^{-1} at levels 3.0 m and 4.0 m) along with nitrate (between 28.8 and 2.5 mg L^{-1} at level 4.0 m) and sulfate (between 151.9 and 13.3 mg L^{-1} at levels 3.0 m and 4.0 m) as the available electron

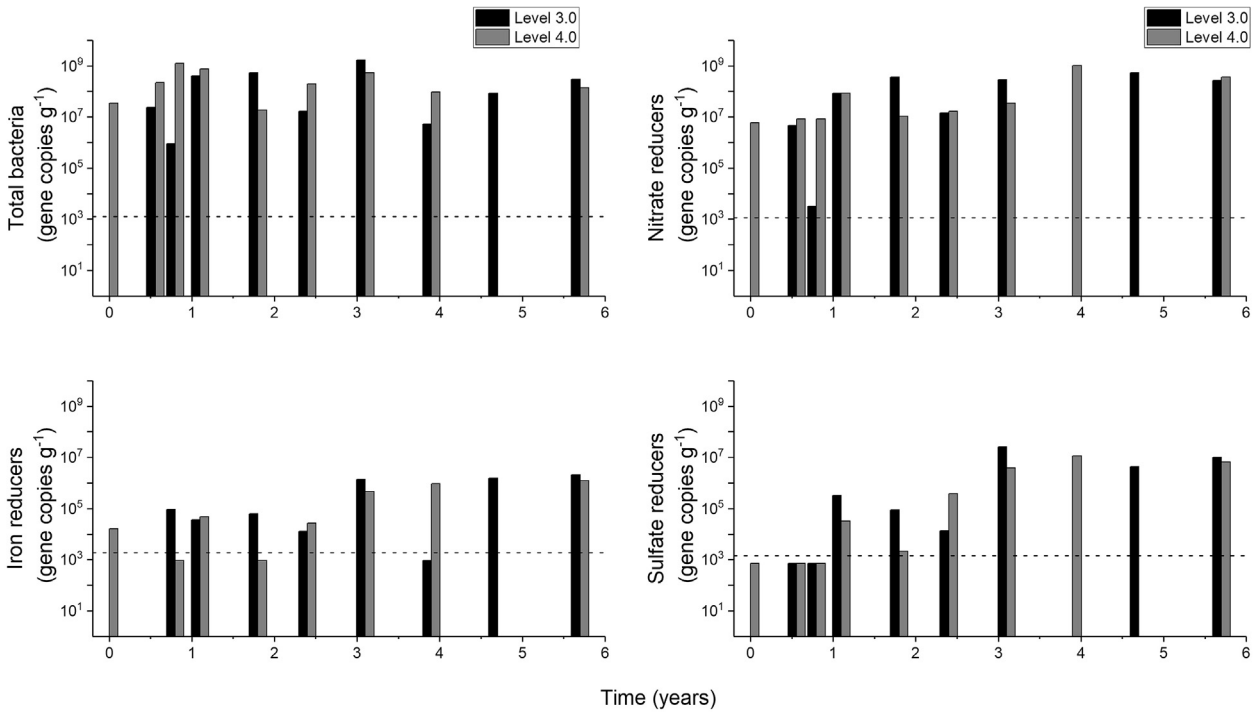

Fig. 4. Concentrations of total bacteria, nitrate reducers, iron reducers and sulfate reducers, at the source-zone of the E10 experiment. Dashed lines represent detection limits for the microbial analysis.

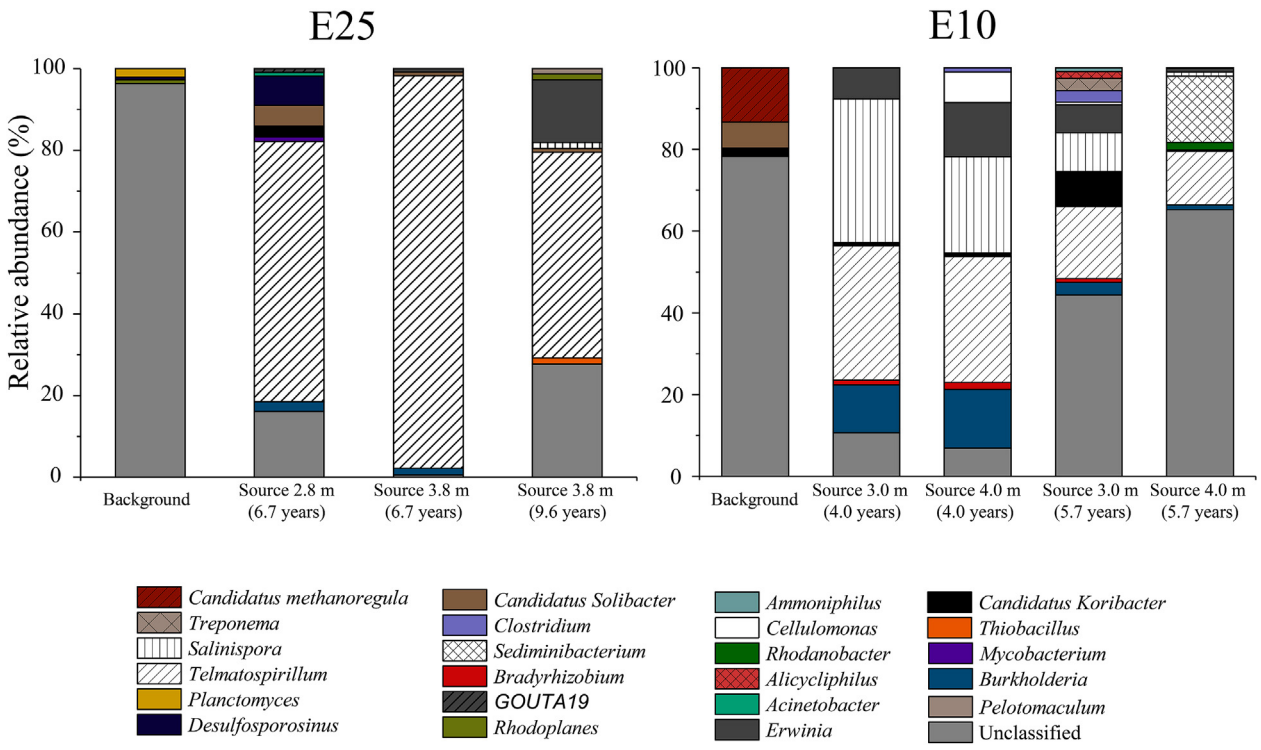

Fig. 5. 16S rRNA gene relative abundance (%) of microbial communities at the E25 and E10 experiments at levels 2.8; 3.8 m and 3.0, 4.0 m bgs, respectively. Charts depict microbial genera with a relative abundance of ≥0.6%.

acceptors. *Burkholderia* spp. have been implicated in aromatic hydrocarbons biodegradation, under nitrate (Leahy et al., 1996) or aerobic conditions (Tillmann et al., 2005), which suggests their role in BTEX compounds anaerobic biodegradation during the observed sampling events. Although the marine genus *Salinispora* possesses the metabolic machinery that elicits the assimilatory nitrate reduction owing to the existence of *nas*A gene on its genome (Cai and Jiao, 2008) and can also degrade complex organic compounds such as starch (Ahmed et al., 2013), these species have not yet been linked to BTEX biodegradation under nitrate-reducing conditions. *Telmatospirillum* are microaerophilic species capable of degrading organic compounds such as butyrate or acetate and can alternatively use sulfate as electron acceptor, which can justify its abundance (Hausmann et al., 2016; Schmidt et al., 2016). *Erwinia* spp. are ecologically associated with plants (Hauben et al., 1998; Starr and Chatterjee, 1972) and though they have not been directly associated with hydrocarbons biodegradation, they were found in a consortia able to metabolize petroleum hydrocarbons (Díaz et al., 2002). Furthermore, *Erwinia* spp. were recently found in a microbial community profile of palm biodiesel B100-contaminated groundwater (Fedrizzi et al., 2016), which suggests they might play a role in complex organic compounds degradation reactions. Other genera detected such as *Rhodanobacter*, *Candidatus Koribacter*, *Alicycliphilus* and *Bradyrhizobium* are nitrate reducers commonly associated with hydrocarbons biodegradation (Lafortune et al., 2009; Le Digabel et al., 2013; Müller et al., 2017; Weelink et al., 2007) which may indicate their possible role on BTEX biodegradation. Furthermore, nitrate reducers and microaerophilic bacteria (i.e., *Telmatospirillum* spp.) are frequently observed to co-occur in organic compounds-contaminated environments (Hemme et al., 2010). Therefore, sequencing profile demonstrated that the transient geochemical conditions in E10 site can shape microbial community structure.

In the E25 site, the microbial community was mainly composed by the phylum Proteobacteria and Firmicutes, as observed by the abundant genera *Telmatospirillum*, *Erwinia*, *Burkholderia* and *Desulfosporosinus* (Fig. 5). The association of these genera with organic compounds-contaminated environments and the significant concentrations of BTEX detected in E25 site even after 6.7 and 9.6 years after release (between 5.46 and 0.99 mg L^{-1}), suggest that they could be deriving energy from BTEX compounds oxidation. The negligible concentrations of the anaerobic electron acceptors nitrate (<0.1 mg L^{-1}), iron II (<0.3 mg L^{-1}), sulfate (<1.5 mg L^{-1}), the absence of methane and the increase in ORP values (from −51 to +199 mV at level 2.8 m and from −59 to +209 mV at level 3.8 m) at 6.7 and 9.6 years suggest that the aquifer might be slowly rebounding to the geochemical conditions encountered prior to the release. In addition, the low DO concentration (<0.7 mg L^{-1}) indicate the establishment of microaerophilic conditions which is consistent with the dominance of *Telmatospirillum* spp. relative to the other genera. These findings reflect the importance of microbial sequencing as a valuable tool for environmental monitoring as communities can shift according to the different ongoing geochemical conditions. The metabolic characteristics of all genera detected in both experiments are described in Table S2 (supporting information).

4. Conclusions

The natural geochemical conditions in E10 site were sufficient to support ethanol and BTEX compounds anaerobic biodegradation, which likely occurred under nitrate, iron and sulfate reduction processes, as evidenced by groundwater geochemical footprint and microbial communities. In E25 site, although nitrate biostimulation was conducted to enhance contaminants biodegradation, BTEX biodegradation rates were lower than E10 site under MNA and ethanol was biodegraded at similar rates. Thus for gasohol blends with lower ethanol content (i.e., 10% v/v),

monitored natural attenuation can provide statistically higher degradation rates compared to nitrate biostimulation and alleviate the overall cost of remediation and ecosystem disturbances. Nevertheless, caution should be exercised against generalizations, as the feasibility of monitored natural attenuation technologies is invariably scenario-dependent and the electron acceptor pool must support contaminants biodegradation at relatively fast rates to avoid long-lasting contamination and delays on site closure.

In summary, the contaminated site geochemical conditions (e.g. electron acceptors availability) and the content of ethanol in gasohol blends are important factors for the decision-making process as they can determine the most cost-effective and targeted remediation technology for different gasohol spills. To the best of our knowledge, these are the first field studies to assess feasible technologies for the cleanup of sites impacted by gasohol blends with different ethanol content. These findings support decision-making processes on suitable remediation strategies and advance the current understanding on overall contaminated sites management.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.01.071.

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