Nitrate addition to groundwater impacted by ethanol-blended fuel accelerates ethanol removal and mitigates the associated metabolic flux dilution and inhibition of BTEX biodegradation

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HIGHLIGHTS:

- The attenuation of two controlled ethanol-blended fuel releases were monitored
- One release was biostimulated with nitrate as terminal electron acceptor
- This accelerated removal of ethanol and its inhibitory effect on BTEX degradation
- Preferential ethanol degradation was explained by the metabolic flux dilution model
- Thus, bioremediation efforts targeting ethanol first would also remove BTEX earlier

ABSTRACT

A comparison of two controlled ethanol-blended fuel releases under monitored natural attenuation (MNA) versus nitrate biostimulation (NB) illustrates the potential benefits of augmenting the electron acceptor pool with nitrate to accelerate ethanol removal and thus mitigate its inhibitory effects on BTEX biodegradation. Groundwater concentrations of ethanol and BTEX were measured 2 m downgradient of the source zones. In both field experiments, initial source-zone BTEX concentrations represented less than 5% of the dissolved total organic carbon (TOC) associated with the release, and measurable BTEX degradation occurred only after the ethanol fraction in the multicomponent substrate mixture decreased sharply. However, ethanol removal was faster in the nitrate amended plot (1.4 years) than under natural attenuation conditions (3.0 years), which led to faster BTEX degradation. This reflects, in part, that an abundant substrate (ethanol) can dilute the metabolic flux of target pollutants (BTEX) whose biodegradation rate eventually increases with their relative abundance after ethanol is preferentially consumed. The fate and transport of ethanol and benzene was accurately simulated in both releases using RT3D with our general substrate interaction module (GSIM) that considers metabolic flux dilution. Since source zone benzene concentrations are relatively low compared to those of ethanol (or its degradation byproduct, acetate), our simulations imply that the initial focus of cleanup efforts (after free-product recovery) should be to stimulate the degradation of ethanol (e.g., by nitrate addition) to decrease its fraction in the mixture and speed up BTEX biodegradation.

Keywords: Mixed substrates, BTEX, fuel ethanol, metabolic flux, natural attenuation, GSIM.

INTRODUCTION

Environmental contamination by single compounds rather than mixtures is uncommon, and microorganisms often face complex mixtures of potential organic substrates. For example, fuel releases contain a wide variety of compounds, including the relatively water-soluble and toxic BTEX compounds (e.g., benzene, toluene, ethylbenzene and xylenes). Unlike the less soluble hydrocarbons in gasoline that exhibit limited migration potential, BTEX concentrations in impacted groundwater are commonly monitored to assess risk and determine the need for remediation.

Ethanol is increasingly being used as a fuel additive to alleviate dependence on imported oil and greenhouse gas emissions due to fossil fuel combustion (Goldemberg, 2007), which increases the likelihood of ethanol-blended fuel releases. Preferential degradation of ethanol and its high electron acceptor demand may decrease the rate of BTEX degradation and increase plume reach (Chen et al., 2008; Corseuil et al., 1998; Da Silva & Alvarez, 2004; Gomez et al., 2008; Mackay et al., 2006; Ruiz-Aguilar et al., 2002; Schaefer et al., 2010).

Although the molecular structure of a compound can significantly influence its susceptibility to degradation (Alvarez & Illman, 2006), the relative biodegradability of a compound in a mixture of alternative substrates (as reflected by degradation rates) is often a concentration-dependent phenomenon (Egli, 1995). Specifically, the relative abundance of alternative substrates in a mixture can significantly influence biodegradation rates of the compound of interest (Lendenmann et al., 1996; Bielefeldt & Stensel, 1999; Lovanh et al., 2002; Dou et al., 2008; Freitas et al. 2011). For example, higher proportions of toluene in various mixtures of toluene and ethanol result in higher toluene biodegradation rates (Lovanh et al. 2002). This phenomenon, termed metabolic flux dilution (Lovanh & Alvarez, 2004), is a form of noncompetitive inhibition of the degradation of a target substrate due to the metabolism of another (likely initiated by different enzymes). This

hindrance by alternative substrates in the mixture (e.g., ethanol) can be accounted by using the metabolic flux dilution factor, which is calculated as the aqueous concentration of a target substrate divided by the total concentration of other dissolved species, expressed as total organic carbon (TOC):

$$f = S_{TOC} / T_{TOC} = ri / r_0 \qquad \text{Equation (1)}$$

where f is the metabolic flux dilution factor (dimensionless), S_{TOC} is the available concentration of a specific substrate as total organic carbon (mg L^{-1}), T_{TOC} is the total available organic carbon concentration (mg L^{-1}), r_i is the degradation rate of compound i when present in a mixture, and r_0 is its degradation rate when present alone. Thus, $r_i = f \times r_0$ (Gomez et al., 2008).

Principles and concepts regarding the use of multiple substrates are more easily developed under simple laboratory conditions; however, they have to be validated in real, natural environments. Conditions leading to sequential substrate degradation have different time scales, and therefore, might lead to different observations in the field. In addition, due to differences in the dilution processes, rates of biodegradation and changes in redox conditions, it is very difficult to design laboratory experiments to encompass the complex dynamics of biodegradation in the subsurface. This suggests the need for long-term field studies to determine the effect of ethanol preferential degradation on the attenuation of BTEX compounds.

This study evaluates at the field scale how substrate proportions influence biodegradation of ethanol and BTEX in groundwater, in two controlled release experiments under different redox conditions. Field data are compared with computer simulations using the Modular Three-Dimensional Finite Difference Groundwater Flow (MODFLOW; Harbaugh et al., 2000), Reactive Transport in 3 Dimensions (RT3D; Clement, 1997), and the General Substrate Interactions Module (GSIM; Gomez et al., 2008). These simulations of the dynamics of ethanol

and BTEX plumes were used to assess the validity of the metabolic flux dilution model, and to gain fundamental insight to recommend practical solutions to accelerate BTEX biodegradation (e.g., add nitrate to increase the electron acceptor pool and stimulate faster degradation of ethanol and other easily degradable substrates that hinder BTEX biodegradation).

MATERIALS AND METHODS

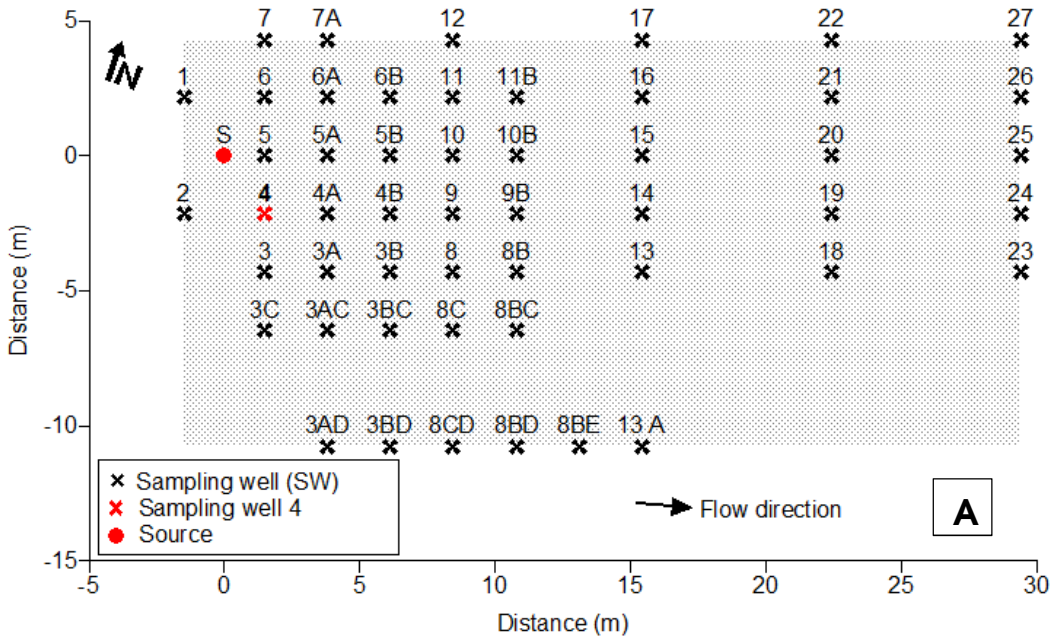
Two controlled releases of Brazilian gasoline (ethanol content 24% v/v) were considered: one under natural attenuation (MNA) and the other under nitrate biostimulation (NB) conditions (Figure 1). A detailed description of the experiments is presented elsewhere (Corseuil et al., 2011; Da Silva and Corseuil, 2012). Briefly, the experimental areas are located in the Ressacada Farm, Florianópolis, SC, Brazil (Latitude: 27°30'S, Longitude: 48°30'W). Climate in the region is mesothermic humid with an annual average precipitation of 1600 mm. Regional geology is characterized by unconsolidated deposits of eolian, alluvial, lacustrine and marine sands with less than 5% of silt and clay. Each release consisted of one pulse of 100 L at the groundwater table level. Initial masses of benzene, toluene, ethylbenzene and total xylenes released were 355, 2,234, 680 and 3,756 g, respectively. The releases were 400 m apart to avoid co-mingling. Groundwater seepage velocity was approximately 3.1 m $year^{-1}$ and 5.1 m $year^{-1}$, respectively, for the MNA and NB experiments. For the NB experiment, 5 L of a solution containing sodium nitrate and potassium dihydrogen phosphate (4 g L^{-1}) was injected into six injection wells upstream of the source zone three times a week for approximately 9 months after the release.

Groundwater monitoring was performed using multilevel sampling wells (SW) installed perpendicular to groundwater flow direction. Data used to evaluate multiple substrate

interactions were located at SW4 approximately 2.0 m downgradient of the source at 2.0 m below ground surface (bgs) for the MNA experiment and 2.3 m bgs for the NB experiment.

Dissolved total organic carbon (T_{TOC}, expressed as mg TOC L^{-1}) included ethanol, BTEX and acetate, which is a common byproduct of ethanol degradation. However, acetate represented less than 2% and 1% of T_{TOC} in the MNA and NB experiments, respectively, thus exerting minor contribution to metabolic flux dilution.

Pseudo-first-order decay coefficients were determined for BTEX after the lag period (about 1.7 years for MNA and 3 years for the NB release), by fitting the concentration versus time data from SW4 to an exponential decay model. Background groundwater concentrations of geochemical parameters are presented in Table 1.



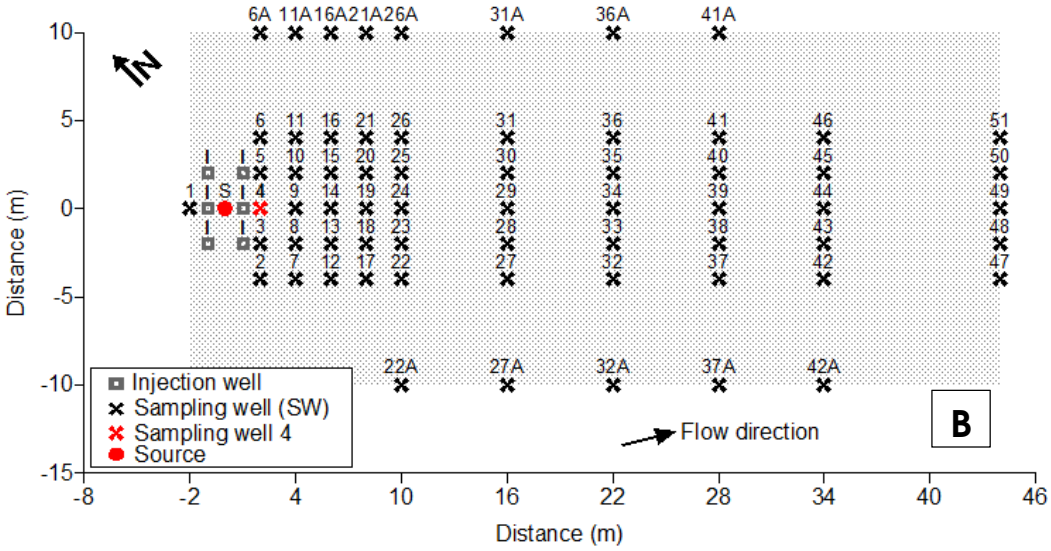


Figure 1. Schematic view of MNA (A) and NB (B) experimental areas configuration.

Table 1 – Background groundwater characteristics.

Variable	Value
Temperature (^{o}C)	17 – 25
Redox Potential (ORP) (mV)	32 - 524
pH	4.0 – 5.3
Dissolved oxygen (DO) (mg L^{-1})	2.3 – 7.0
Nitrate (mg L^{-1})	0.05 – 1.6
Sulfate (mg L^{-1})	0.3 – 4.4
Iron (II) (mg L^{-1})	0.0 – 1.6
Phosphate (mg L^{-1})	0.1 – 0.5
Methane (mg L^{-1})	< 0.01

A General Substrate Interaction Model (GSIM), using Monod multiplicative kinetics, was used to evaluate 3-dimensional interactions between substrates (ethanol and BTEX) and microbial populations present in the soil. The GSIM model (Gomez et al., 2008) works as a module for RT3D (Clement, 1997), and requires groundwater flow conditions from MODFLOW

(Harbaugh et al., 2000). Together, these models incorporate common fate and transport processes such as: advection, dispersion, adsorption, biodegradation and depletion of available electron acceptors. The GSIM model further incorporates important, and often ignored, substrate interaction processes between ethanol and BTEX compounds, which can cause slower degradation rates of BTEX at sites with high ethanol concentrations (Lovanh and Alvarez, 2004). These interactions include: (a) metabolic flux dilution (MFD; Lovanh and Alvarez , 2004), (b) catabolite repression (CR; Madigan et al., 2000), and (c) changes in specific microbial populations of ethanol/BTEX degraders. Metabolic flux dilution refers to a lower specific BTEX utilization rate by microorganisms due to non-competitive inhibition by ethanol or other preferentially degraded substrate (Lovanh and Alvarez, 2004). The model is capable of simulating contaminant plume elongation and degradation over time, with the associated changes in microbial populations following the traditional sequential development of different electron accepting conditions (aerobic, nitrate reducers, iron reducers, sulfate reducers and methanogenic microbes), associated with the depletion of corresponding electron acceptors. The model requires as input contaminant concentrations over time at the source zone, and does not consider LNAPL dissolution dynamics (Gomez et al., 2008).

The Ressacada field site conditions were simulated in the RT3D/GSIM domain considering a hydraulic conductivity of 1.1×10^{-4} cm s^{-1}, effective aquifer porosity of 0.2, average groundwater velocity of 3.1 m $year^{-1}$ and average hydraulic gradient of 0.018 m/m (Corseuil et al., 2011; Da Silva and Corseuil, 2012). Hydraulic flow conditions in the simulated domain, as generated by MODFLOW, were corroborated by simulations with the MODPATH software (Pollock, 1994). Particles generated by MODPATH similarly travelled 3.1 m over the course of 1 year of simulation time. Due to the complex source zone physical conditions, including a

137 variable height water table, source zone dissolution rates for ethanol and BTEX were estimated
138 using initial total mass (355 g for benzene and 18,900 g for ethanol). Biokinetic parameters for
139 ethanol and benzene degradation were based on those described by Gomez & Alvarez (2010),
140 modified for calibration of specific Ressacada site conditions (Table 3). The aquifer material has
141 an organic content (f_{oc}) of 0.06% (Corseuil et al., 2011), suggesting linear partitioning
142 coefficients (K_d) of 3.55×10^{-13} mg m^{-3} for ethanol and 4.24×10^{-11} mg m^{-3} for benzene.
143 Partitioning coefficients were calculated using the relationship $K_d = 0.63 \times f_{oc} \times K_{ow}$ (Karickhoff
144 et al., 1979) with water-octanol partition values obtained from the literature (Hilal et al., 2004).

145

146 **RESULTS AND DISCUSSION**

147 Converging lines of evidence indicate that biodegradation was largely responsible for the
148 significant decreases in ethanol and BTEX concentrations in both releases, after initial increases
149 associated with the dissolution and migration of the released fuel (Figure 2).

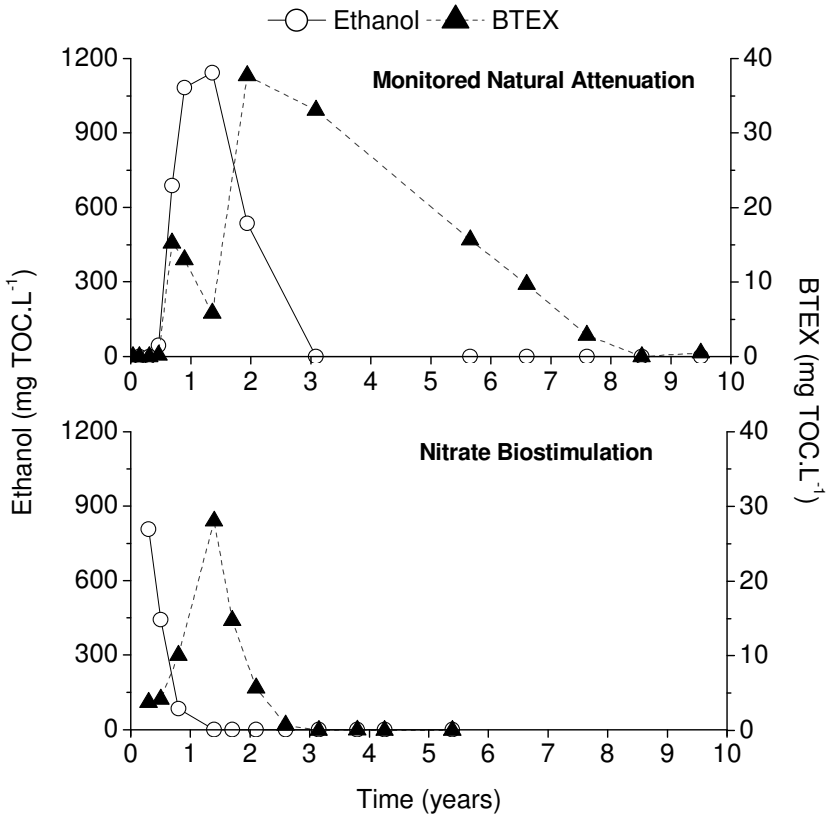


Figure 2. Dissolved TOC concentrations for ethanol and BTEX for the MNA (SW4, 2.0 m bgs) and NB (SW4, 2.3 m bgs) experiments.

This evidence includes (1) the geochemical footprint; i.e., simultaneous consumption of released substrates (Figures 2) and electron acceptors with accompanying decrease in ORP and methane generation (for the MNA experiment) before rebounding to initial conditions after the release was attenuated (Figure 3); (2) an adequate fit of the data by model simulations (Figures 5 and 6) using typical biokinetic coefficients (Table 2); and (3) past studies demonstrating the corresponding indigenous biodegradation capabilities at this site (Corseuil et al., 2011; Da Silva et al., 2012).

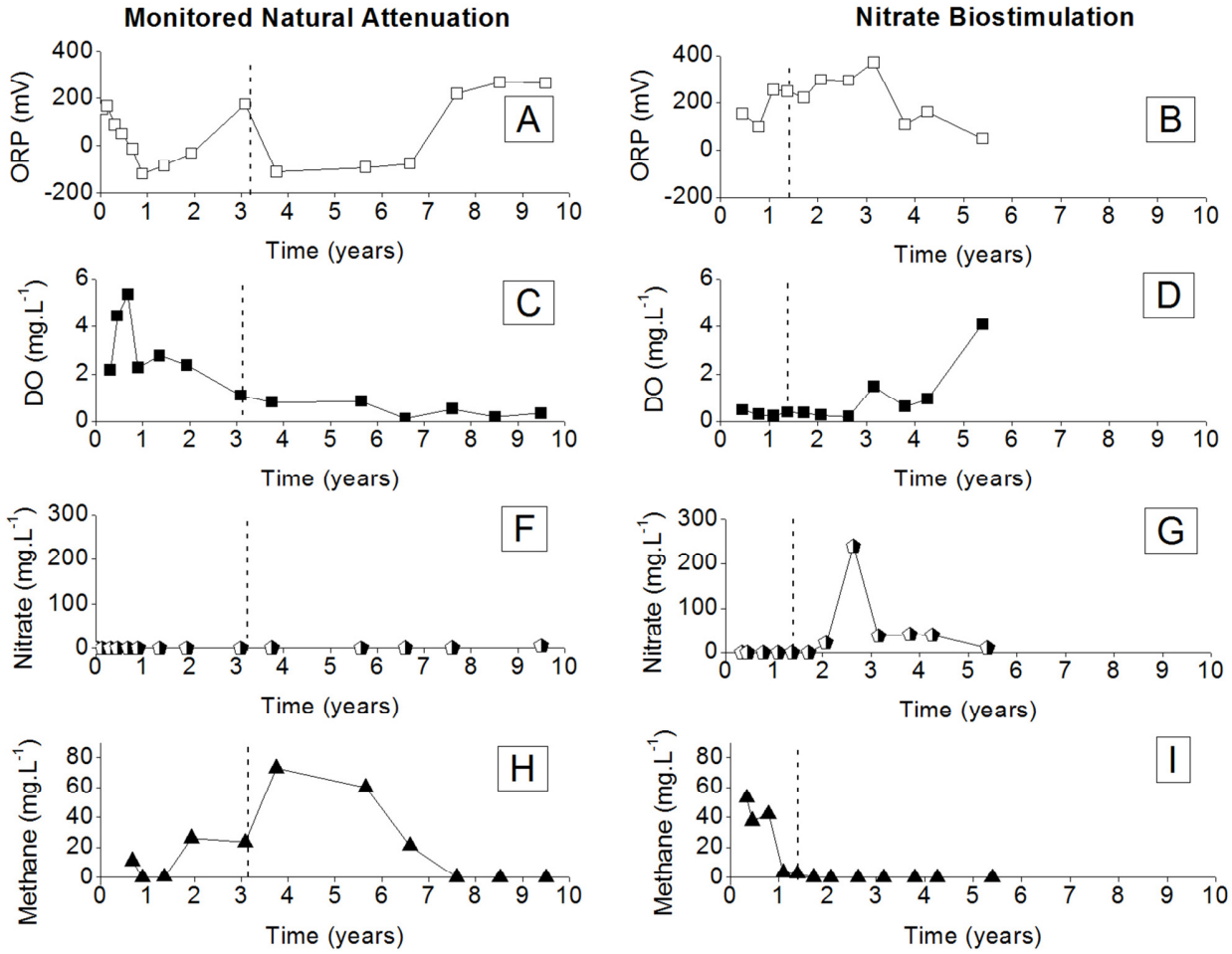


Figure 3 – Redox potential (ORP) (A and B), dissolved oxygen (DO) (C and D), nitrate (E and F) and methane (G and H) for the monitored natural attenuation experiment (SW4 2.0 m bgs) and nitrate biostimulation experiment (SW4 2.3 m bgs). Dashed lines indicate onset of BTEX decay.

171 Table 2 - Biokinetic Parameters of Modeled Compounds.

172

	First-order degradation rate coefficient, λ (1/d)	Source	Maximum specific growth rate, μ_m (1/d)	Source	Half saturation constant, K_s (mg/l)[3]	Source	Microbial cell yield, Y (g/g)	Source
Ethanol								
Aerobic	0.35	*Powers et al., 2001b*	11.04	*Lovanh et al., 2002*	63.09	Calculated[3]	0.50	*Heulekian and Manganelli, 1951*
Nitrate Reducing	0.53	*Corseuil et al., 1998*	0.35	Calculated[2]	0.25	Calculated[3]	0.26	Calculated[2]
Sulfate Reducing	0.10	*Corseuil et al., 1998*	0.21	Calculated[2]	1.17	Calculated[3]	0.18	Calculated[2]
Iron Reducing	0.17	*Corseuil et al., 1998*	0.21	Calculated[2]	0.67	Calculated[3]	0.18	Calculated[2]
Methanogenic	0.2	*Powers et al., 2001b*	0.16	Calculated[2]	0.12	Calculated[3]	0.07	*Lawrence and McCarty, 1969*
Benzene								
Aerobic	0.68	*Alvarez et al., 1991*	3.24	*Alvarez et al., 1991*	1.22	Calculated[3]	0.39	*Grady et al. 1989*
Sulfate Reducing	0.02	*Kazumi et al., 1997; Wiedemeier et*	1.25	*Godeke et al, 2008*	3.25	Calculated[3]	0.24	Calculated[2]
Iron Reducing	0.00	*Wilson et al, 1996; Rifai et al., 1995*	0.15	*Lovley and Lonergan, 1990*	3.05	Calculated[3]	0.14	Calculated[2]
Methanogenic	0.003	*Wilson et al, 1990; Kazumi et al, 1997*	0.30	*Ulrich and Edwards, 2003*	2.00	*O'Rourke, 1968*	0.05	*O'Rourke, 1968*

	Value	Source
Other Parameters		
Aerobic Microbial Decay Rate (b_{Aer})	0.2 1/d	Based on mix culture aerobic systems [*McCarty and Brodersen, 1962*]
Anaerobic Microbial Decay Rate (b_{An})	0.03 1/d	Based on methane fermentation [*Lawrence and McCarty, 1969*]
Alcohol aerobic degraders initial population	10^6 cells/g-soil	*Chen et al., 1992*
Alcohol anaerobic degraders initial population	10^5 cells/g-soil	10% of alcohol degrading aerobic populations
Benzene aerobic degraders initial population	10^5 cells/g-soil	10% of total populations
Benzene anaerobic degraders initial population	10^3 cells/g-soil	1% of Benzene degrading aerobic populations

[1] *First order degradation rates estimated on water half-lives of the compounds. [Howard et al., 1991]*

[2] *Values estimated using the Thermodynamic Electron Equivalents Model for Bacterial Yield Prediction [McCarty, 2007].*

173 [3] *Values estimated on the basis of the relationship* $\lambda = (\mu X/Y Ks)$ *[Alvarez and Illman, 2006].*

174

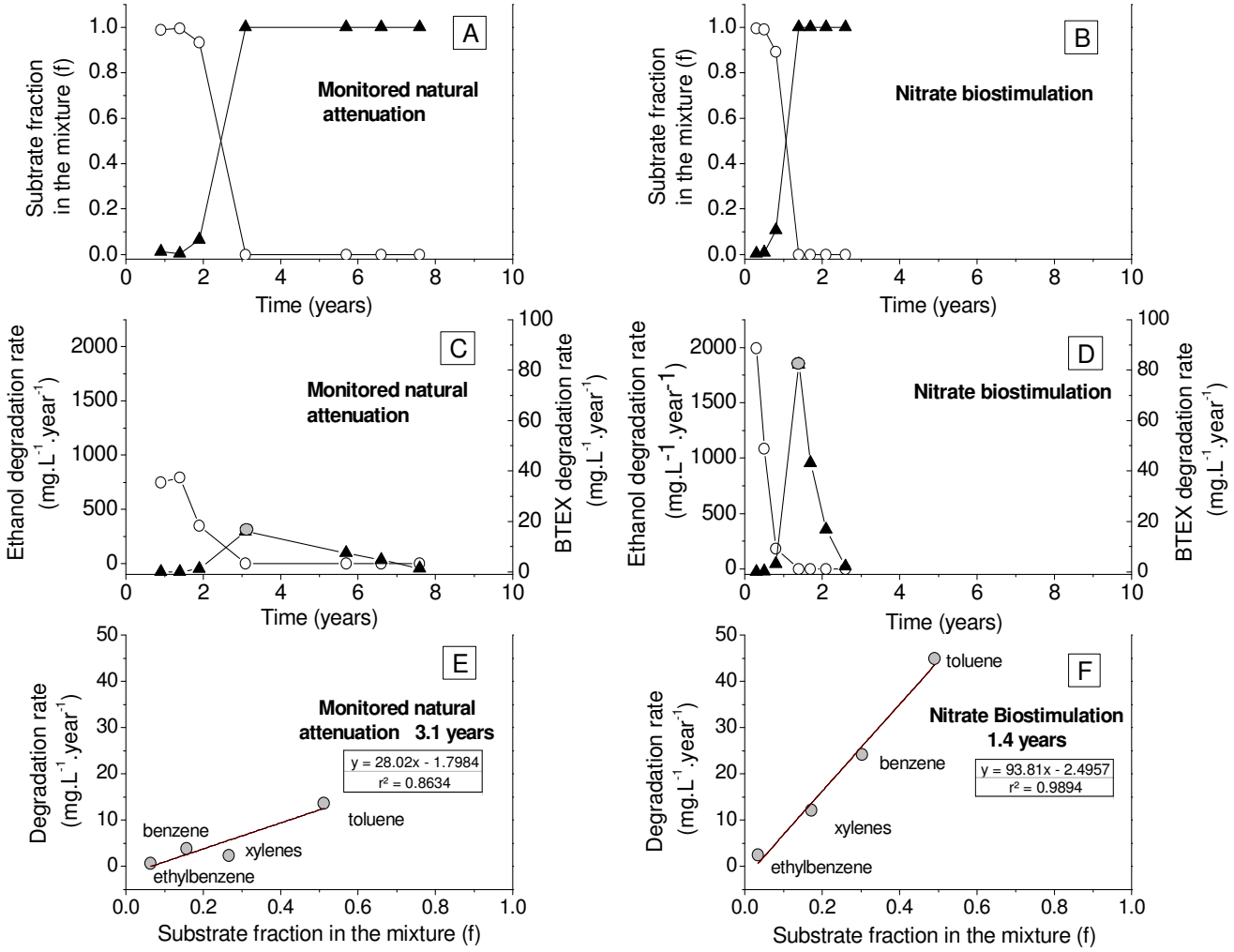
175 When an ethanol-blended fuel is released to the environment, the preferential degradation
176 of ethanol (which has a relatively simple molecular structure) can hinder BTEX degradation.
177 Potential inhibitory mechanisms include the depletion of favorable terminal electron acceptors

178 (e.g., dissolved oxygen) and nutrients during ethanol consumption, proliferation of microbial
179 populations that grow on ethanol but cannot degrade BTEX, thermodynamic constraints posed
180 by accumulation of fermentation products that are common to ethanol and BTEX degradation
181 pathways (e.g., acetate and H_2), catabolite repression and metabolic flux dilution (Gomez et al.,
182 2008; Cápiro et al., 2007; Lovanh & Alvarez, 2004; Da Silva & Alvarez, 2002). Previous
183 modeling studies showed that metabolic flux dilution can be the most influential inhibitory
184 mechanism for BTEX biodegradation in ethanol-blended fuel spills scenarios (Lovanh &
185 Alvarez, 2004; Gomez et al., 2008). Furthermore, the concept of metabolic flux dilution is
186 relatively simple to model at the reactor and field scales (Equation 1). Therefore, the metabolic
187 flux dilution model was used to analyze the release data.

188 For ethanol-blended fuel spills, initial aqueous concentrations of ethanol tend to be much
189 higher than BTEX concentrations. Ethanol concentrations near the source zones of the MNA and
190 NB releases reached about 2,000 mg L^{-1} (1,100 mg-TOC L^{-1}), while maximum BTEX
191 concentrations were approximately 40 mg L^{-1} (38 mg-TOC L^{-1}) (Figure 2), representing only 3%
192 of the total available substrate as dissolved TOC. Consequently, significant BTEX metabolic flux
193 by the more abundant ethanol would be expected, which would result in the apparent preferential
194 biodegradation of ethanol. Accordingly, BTEX concentrations started to decrease only after the
195 onset of ethanol removal, which occurred earlier in the NB experiment (after 1.4 years) than the
196 MNA experiment (after 3.0 years). The onset of BTEX degradation in both experiments occurred
197 when the metabolic dilution factor (*f*) approached 1 (Figure 4A and Figure 4B).

198 First-order decay constants (determined after the compound-specific onset of
199 biodegradation) are summarized in Table 3. These rate constants were significantly lower for the
200 MNA experiment (k= 0.76 ± 0.60 for benzene; 0.81 ± 0.97 for toluene; 0.33 ± 0.13 for

ethylbenzene, 0.28 ± 0.14 $year^{-1}$ for xylenes and 0.71 ± 0.43 $year^{-1}$ for ethanol) than for the NB experiment (k= 2.84 ± 2.01 for benzene; 3.27 ± 1.10 for toluene; 2.58 ± 0.52 for ethylbenzene, 2.52 ± 0.48 $years^{-1}$ for xylenes, and 2.48 ± 1.96 $years^{-1}$ for ethanol). Nitrate addition to increase the electron acceptor pool accelerated the biodegradation of ethanol, which resulted in earlier onset of BTEX biodegradation (Figure 2). Nitrate amendment also resulted in a higher redox potential and no methane was detected in the NB release (Figure 3). Moreover, nitrate consumption was consistent with the onset of ethanol and BTEX biodegradation (Figure 3), providing evidence that nitrate reduction was the main biodegradation pathway in NB experiment.



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212 Figure 4. Ethanol (○) and BTEX (▲) as TOC fractions in the mixture (calculated according to
213 equation 1) for the MNA (SW4 2.0 m bgs) (A) and NB (SW4 2.3 m bgs) (B) experiments.
214 Ethanol (○) and BTEX (▲) degradation rates ($r_i = f \times r_0$) as a function of time after the release in
215 MNA (C) and NB (D) experiments. BTEX compounds degradation rate (r_0 = k C) after ethanol
216 depletion (●) and their relative fraction in the mixture for the MNA (E) and NB (F) experiments.

217

218 The metabolic flux dilution model predicts that specific removal rates should be
219 proportional to the fraction of the available substrates in the mixture (equation 1). BTEX

degradation follows this trend (Figure 4, panels E and F; $r^2 \geq 0.9$). More favorable thermodynamic conditions for biodegradation under NB conditions resulted in higher degradation rates (maximum of 2,000 and 82 mg L^{-1}.$year^{-1}$ for ethanol and BTEX, respectively) relative to MNA (maximum of 800 and 16 mg L^{-1}.$year^{-1}$ for ethanol and total BTEX, respectively). Furthermore, the faster removal of ethanol in the NB experiment (at 1.4 years compared to 3.1 years for MNA) resulted in a faster disappearance of its inhibitory effect (i.e., metabolic flux dilution) and an earlier onset of BTEX biodegradation. These observations are in accordance with previous laboratory studies (Dou et al., 2008; Lendemann et al. 1996; Lovanh et al. 2002; Lovanh & Alvarez, 2004).

Table 3 – BTEX Pseudo-first-order attenuation constants (*k*) near the source zones (SW4) for the natural attenuation and nitrate biostimulation experiments

Experiment	**Substrate**	**Timeframe[a] (years)**	***k* ($year^{-1}$)**	**R^2**	**n**	**p-value**
Monitored Natural Attenuation	Benzene	3.1 to 7.6	0.76 ± 0.60	0.84	5	< 0.05
	Toluene	3.1 to 7.6	0.81 ± 0.97	0.94	5	< 0.05
	Ethylbenzene	3.1 to 7.6	0.33 ± 0.13	0.95	5	< 0.05
	Xylenes	3.1 to 7.6	0.28 ± 0.14	0.93	5	< 0.05
	Ethanol	1.0 to 3.7	0.71 ± 0.43	0.96	4	< 0.05
Nitrate Biostimulation	Benzene	1.4 to 2.6	2.84 ± 2.01	0.95	4	< 0.05
	Toluene	1.4 to 2.6	3.27 ± 1.10	0.99	4	< 0.05
	Ethylbenzene	1.4 to 2.6	2.58 ± 0.52	1.00	4	< 0.05
	Xylenes	1.4 to 2.6	2.52 ± 0.48	1.00	4	< 0.05
	Ethanol	0.3 to 2.1	2.48 ± 1.96	0.94	4	< 0.05

[a] Period after gasohol release. The timeframes correspond to the highest compound concentration until its depletion.

Field data from both experiments were simulated using GSIM model. Using data from the MNA experiment to calibrate the source zone LNAPL dissolution rates and the GSIM model half saturation coefficients for Ethanol and Benzene (K_S, Table 2), the calculated total plume mass over time was accurately simulated for both ethanol and benzene (Figure 5). Calculated R^2 values are 0.87 and 0.80 for ethanol and benzene respectively. Simulated contaminant concentrations at SW4 (Figures 5c and 5d) fit the observed patterns with R^2 of 0.69 and 0.51 for ethanol and benzene respectively. Modeled accuracy was hindered by coarse model domain cell resolution (1 m × 1 m in XY plane), which are required for feasible execution times, and to significant spread of the field data, particularly for benzene measurements. Nevertheless, the model adequately simulated peak concentration values at point SW4 and concentration evolution over time (Figure 5).

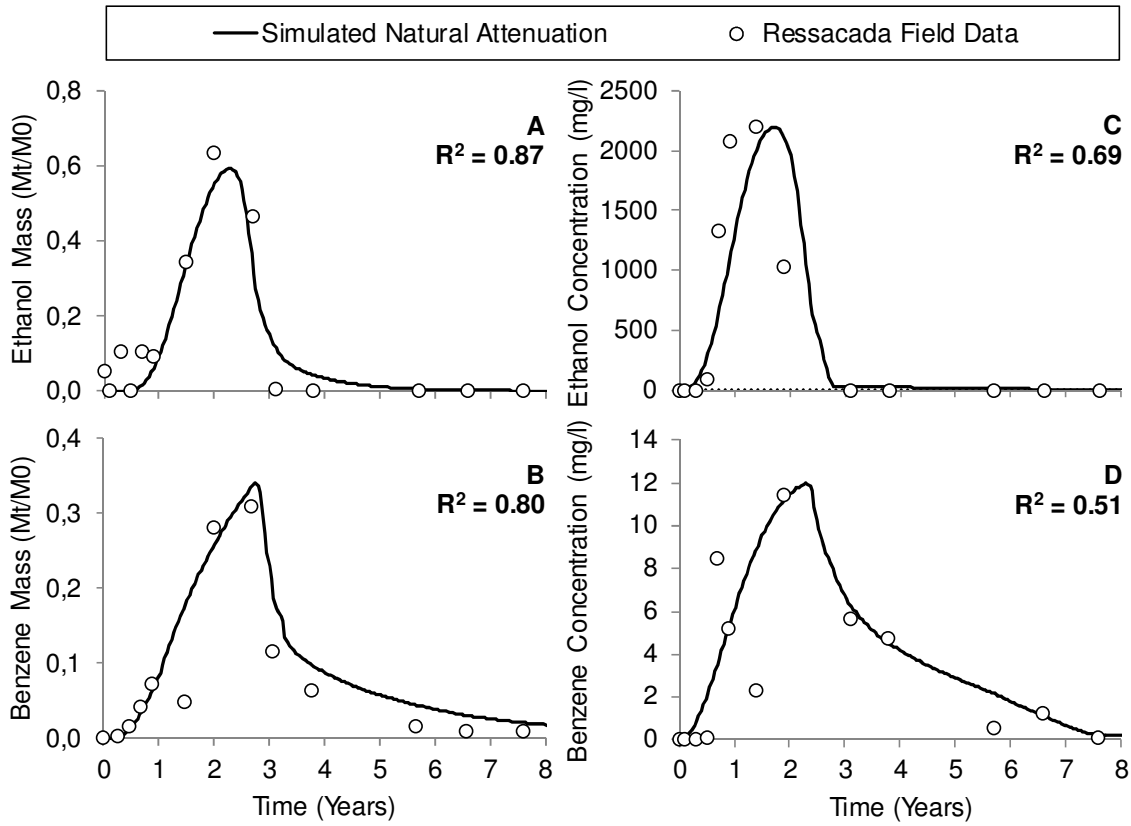
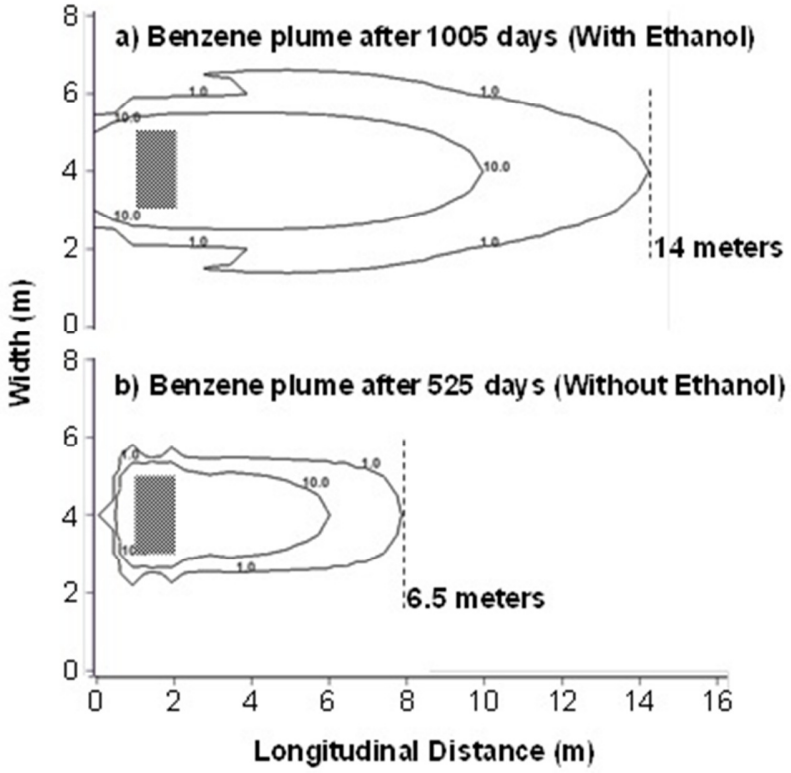


Figure 5 – Comparison of natural attenuation simulation using GSIM (solid line) with Ressacada field data (dots), considering: (A) Ethanol plume total mass (**R^2 = 0.87**); (B) Benzene plume total mass (**R^2 = 0.80**); (C) Ethanol concentration over time at point SW4 (**R^2 = 0.69**); and (D) Benzene concentration over time at Point SW4 (**R^2 = 0.51**).

The total contaminant plume mass in the system (Figures 5a and 5b) was correctly simulated to increase initially due to ethanol and benzene source zone dissolution, reaching a peak after about 2 years of simulation time, at which point degradation and diffusion processes dominate and the total dissolved mass begins to decrease. The total dissolved ethanol mass is significantly reduced by year 3, and ethanol disappears from the system by year 5. Benzene mass

269 removal increases markedly once ethanol disappears from the system. The simulated maximum
270 plume lengths (1 mg L^{-1} contours) reach 19 m for ethanol and 14.5 m for benzene. This is
271 significantly longer (8 m increase) than the simulated plume length for benzene alone at these
272 concentrations (Figure 6).



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274 Figure 6. Maximum benzene plume length reached using the GSIM model, with and without
275 ethanol in the system: (a) 14 meters for the 1 mg L^{-1} contour with ethanol present after 1005
276 days; (b) 6.5 meters for the 1 mg L^{-1} contour without ethanol present after 525 days. Source zone
277 is shown as grey area.

278

279 The benefits of nitrate addition to enhance ethanol biodegradation and mitigate its
280 inhibitory effect on BTEX degradation are evident by comparing GSIM simulations of MNA
281 versus NB conditions (Figure 7). Using a constant near-source zone concentration of 80 mg L^{-1}

of nitrate results in a reduction of 74% for ethanol total plume mass (Figure 7a). A 53% reduction of the benzene total plume mass present in the system over time was also observed (Figure 7b). Benzene mass reduction corroborates that the presence of ethanol in the system hinders benzene natural attenuation. These results are in agreement with the metabolic flux dilution model (Lovanh and Alvarez, 2004; Madigan et al., 2000; Gomez et al., 2008), which adequately simulated ethanol and benzene plume dynamics for these two releases under different redox conditions. Since metabolic flux dilution proved to be suitable to model MNA and NB field data and that proportionality among substrates fraction in the mixture and degradation rates was demonstrated, it is plausible to infer that the late BTEX biodegradation relative to ethanol was influenced by its relative contribution to the mixture.

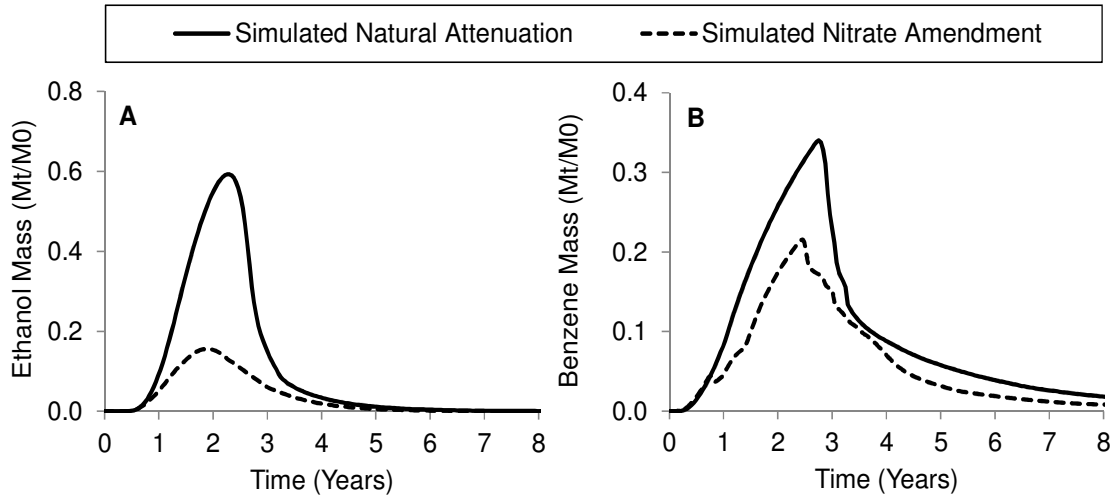

Figure 7. Comparison of total plume mass (M_t) present in the simulation domain over time, shown as a ratio of the total contaminant mass injected in the system (M_0), for simulated natural attenuation versus nitrate biostimulation. (A) Nitrate amendment results in a 74% increase in ethanol degradation; (B) Nitrate amendment results in a 53% increase in benzene degradation.

CONCLUSIONS

Previous studies of ethanol-blended fuel releases indicated that ethanol can delay BTEX degradation independently of redox conditions. This study shows that the apparent preferential degradation of ethanol may be largely explained by the metabolic flux dilution model, which considers the simultaneous degradation of multiple substrates with compound-specific degradation rates being proportional their relative abundance in the mixture. Accordingly, the metabolic flux dilution factor used to simulate non-competitive inhibition in the presence of alternative substrates (previously presented for lab-scale batch and continuous flow experiments) was shown to be applicable to complex, field-scale systems involving subsurface spills of ethanol-blended fuel. However, caution should be exercised in recognizing the limitations of this model (Gomez et al., 2008): microbial growth is assumed to as fully penetrated biofilms in the aquifer matrix with no attachment/detachment kinetics (Chen et al., 1992, Harvey et al., 1984; Lehman et al., 2001); biological degradation activity occurs in the liquid phase, and decay of sorbed constituents is ignored; dissolved total organic carbon is assumed to be fully available to microorganisms; the model does not consider degradation byproducts such as acetate or propionate; the model does not consider complex capillary zone transport behavior of high alcohol content mixtures.

One important practical implication of this work is that the initial focus of effort to remediate releases of ethanol-blended fuel (after free-product recovery) should be to accelerate the degradation of ethanol (e.g., by augmenting the electron acceptor pool through stoichiometric nitrate addition). This would more rapidly decrease the fraction of ethanol in the mixture and speed up the biodegradation of BTEX that are the main regulatory drivers.

ACKNOWLEDGMENTS

This research was funded primarily by Petróleo Brasileiro S/A – PETROBRAS. Additional funds (scholarships) were provided by Coordination of Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq). The authors also thank students, technicians and researchers that directly or indirectly helped for the development and monitoring of the experiments.

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