

Supporting Information

BTEX Plume Dynamics Following an Ethanol Blend Release: Geochemical Footprint and Thermodynamic Constraints on Natural Attenuation

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SITE DESCRIPTION

The experiment was carried out at Ressacada, the Federal University of Santa Catarina experimental farm, in Florianópolis (SC-Brazil), which is located on the southwestern island of Santa Catarina, near the International Airport Hercílio Luz (Figure S1).

The climate is mesothermal with rainfall distributed throughout the year. The average annual air temperature measured at the meteorological station of Florianópolis is 21°C. The average annual precipitation is 1,600 mm and the average relative humidity is approximately 80%. The seasonal water table varies approximately 0.8-1.8 m and the average groundwater temperature in the aquifer is approximately 22°C. The regional geology is characterized by unconsolidated deposits of eolian, alluvial, lacustrine and marine sands. The Quaternary deposits occur predominantly in the region where the Ressacada farm is located. Holocene deposits are the most significant coastal marine sediments and the Pleistocene sediments are represented locally by colluvial-alluvial-eluvial undifferentiated. The experimental farm is located in the plain that is compartmentalized by the hills of East Santa Catarina in its central and southern sectors, within the domain of the Marine Plains (1).



FIGURE S1: The Experimental Ressacada Farm (highlighted by dotted yellow line) and test area (inset) located in Florianópolis, SC, Brazil

SUBSURFACE CHARACTERIZATION

Percussion drilling and textural analysis was used to characterize soil layers at the site. Surface soil is gray-brown loamy sand and the subsurface layer is a uniform gray fine sand with less than 5% of silt and clay.

The characterization of soil on the centerline of the groundwater flow (from sampling well (SW) 04 to SW24) is shown in Figure S2. The vertical lines represent sampling well

locations. Most of the area is characterized by sand, with the presence of clay and loamy sand between the monitoring wells SW19 and SW24.

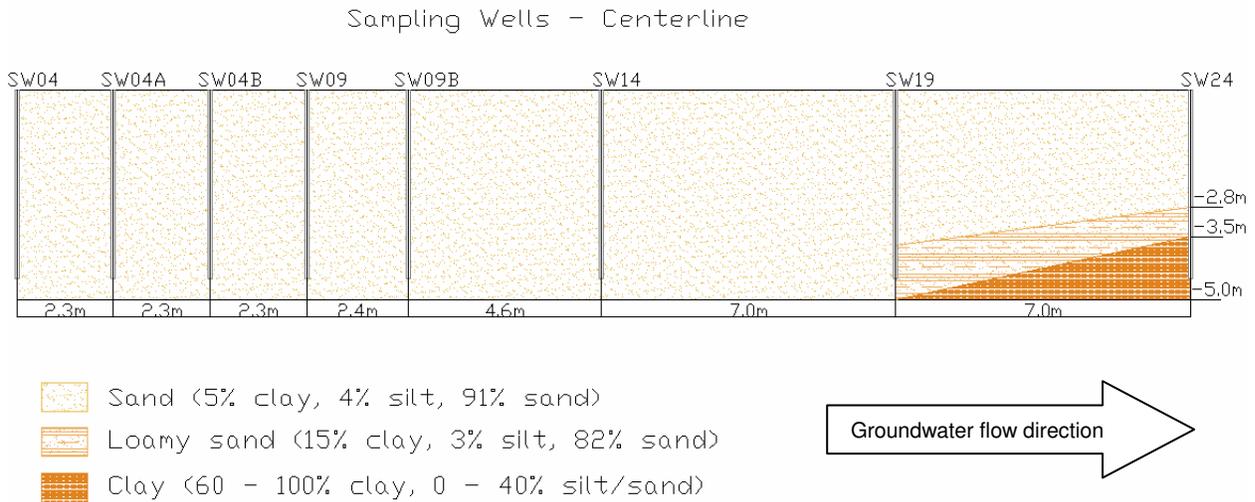


FIGURE S2: Vertical Section of Centerline

MONITORING WELL INSTALLATION

The multilevel wells used for monitoring the dissolved contaminant plume were installed in July 1998 prior to the release. The well locations were selected based on hydrogeological information obtained at the planning stage of the experiment. The multilevel wells were installed using percussion drilling with recirculated water from the aquifer. During drilling, soil samples were collected (using a standard sampler with inner diameter of 34.9 mm and external of 50.8 mm) to characterize the area. Multilevel wells with five depth ranges (1m, 2m, 2.5m, 3.5m and 4.5 m) were placed in the boreholes. To protect each well's multilevel mesh, a 50 mm PVC pipe was inserted along with a removable cap.

Each multilevel well consists of bundle of five 3/16" ID polyethylene tubing with each tube cut to a length that corresponds to the zone of interest. The terminal end of each tube was thermally bonded to a narrow diameter, 20 cm long, 200-mesh stainless steel wire-cloth screen. Each well was cleared periodically with a peristaltic pump until the water was free of visible sediment.

GROUNDWATER SAMPLING

The experiment began in December 1998. Over a 10 year period, 18 sampling events occurred, (months (years)): 0.5m (0.0 yr); 1m (0.1yr); 3m (0.3 yr); 5 (0.5 yr); 8m (0.7 yr); 10m (0.9 yr); 16m (1.5 yr); 23m (2.0 yr); 32m (2.7 yr); 37m (3.1 yr); 46m (3.8 yr); 61m (5.0 yr); 68m (5.7 yr); 79m (6.6 yr); 91m (7.6 yr); 102m (8.5 yr); 114m (9.5 yr); 126m (10.5 yr).

A MicroPurge[®] Flow Cell model MP20 and a peristaltic pump (Millipore Easy-Load model XX80EL000) were used for groundwater collection and onsite monitoring. Before sampling, all equipment was cleaned and calibrated in the laboratory. The MicroPurge[®] Flow Cell was calibrated according to manufacturer's specifications and. was used to measure the following parameters: temperature, pH, conductivity, redox potential and dissolved oxygen. Values were recorded after read-out stabilization.

The groundwater samples were collected using a peristaltic pump and Masterflex[®] Tygon tubes at a flow rate of approximately 60 mL min⁻¹; sampling was always performed from low to high concentrations to avoid cross contamination. Groundwater samples for BTEX, ethanol and methane were transferred to glass bottles with 40 mL with Teflon septum (Agilent) containing three drops of 1:1 hydrochloric acid at pH < 2 as a preservative. For anion analysis, the samples

were collected in 200 mL amber glass bottles. For the preservation and transport of samples, the bottles were packed in coolers containing ice (Termogel Ice-X mini) and kept at 4°C until the analysis could be performed.

CHEMICALS AND MATERIALS

B, T, E, and X were obtained from UST Modified Gasoline Range Organics (Supelco) with following purity degrees: benzene (99.9% purity), ethylbenzene (99.8%), toluene (99.8%), and p-, m-xylene (99%) and o-xylene (99.5%). Ethanol was obtained from JT Baker Industry, with 99.9% purity. Methane standard solution was prepared from natural gas obtained from White Martins Company (99.5% purity). The following reagents were used for anion standard solutions (2) and were obtained from JT Baker. Sodium chloride (99.9% purity), potassium bromide (99.5%), sodium nitrate (100%), sodium nitrite (99.5%), sodium sulfate (100%), phosphate potassium phosphate (99.0%) and sodium acetate (99.9%).

LABORATORY GROUNDWATER ANALYSES

Acidity and alkalinity analyses were performed immediately following sample collection using the standard titration methods 2310B and 2320B respectively (3) . The ferrous iron and sulfide analyses were performed with HACH kits using the colorimetric method 3500-Fe D - 1.10 phenanthroline for iron (II) and 4500-S2-D - methylene blue for the sulfide (3).

Volatile organic compounds analyses (BTEX, ethanol and methane) were performed using an Agilent Technologies gas chromatograph 6890N GC equipped with a flame ionization detector (FID) and an Agilent Technologies 7694 headspace auto-sampler. Chromatographic

analyses were obtained using Agilent Technologies Chemstation® software. The analytical column used for separation of compounds was an Agilent Technologies HP 1 (crosslinked methyl siloxane, 30m x 0.53 mm internal diameter and 2.65 µm film thickness).

Operating conditions for headspace sampling were as follows: temperature at 75°C including 20 min equilibrium time, loop temperature at 85°C; transfer line temperature at 85°C; loop fill and equilibrium time of 6s, injection time of 30s, low agitation and bottle pressure at 19.9 psi. Injection and detector temperatures for the analysis of ethanol and BTEX samples were 260°C and 280°C, respectively. Injection and detector temperatures for methane sample analysis were 190°C and 280°C, respectively. The initial oven temperature for BTEX and ethanol was 70°C held for 2 minutes, then raised at 5°C/min to 120°C, and at 30°C/min to 210°C. EPA/5021A and EPA/8015D methods were used for organic volatile compounds (4).

Methane analysis was performed under isothermal conditions at 40°C. For a standard solution, methane gas was bubbled into a container containing ultrapure deionized water until equilibrium between the gaseous and aqueous phase was reached. The methane concentration in the aqueous phase was calculated using Henry's Law (5).

Anion analysis was performed according to U.S. EPA method 300 (2) using an ion chromatograph Dionex model ICS-1000, equipped with a conductivity detector and AS40 auto-sampler, a 25 ml loop injection, a guard column IonPac AG22 (4 x 50 mm) and an analytical column AS22 (4 x 250 mm). The system operated at 30°C with a suppressor (ASRS - Ultra II 4 mm) and the following eluents: sodium carbonate (Na_2CO_3 - 4.5 mM) and sodium bicarbonate (NaHCO_3 - 1.5 mM). Chromeleon 6.4 chromatography software was used for instrument control, data acquisition, and processing. The analytical methods and detection limits for analyses are listed in Table S1.

TABLE S1: Summary of methodology employed for analysis in field and in the laboratory

Analysis	Method	Detection Limit
<i>Analyses on site</i>		
Temperature °C	Micropurge® Flow Cell	NA
Dissolved Oxygen	Micropurge® Flow Cell	0.5 mg/L
pH	Micropurge® Flow Cell	NA
Redox potential (mV)	Micropurge® Flow Cell	NA
Conductivity (µS/cm)	Micropurge® Flow Cell	NA
Iron II	Colorimetric, 1,10-Phenanthroline	0.01 mg/L
Sulfite	Colorimetric, Methylene Blue	5 µg/L
Alkalinity	Potentiometric Titration	1 mg/L
Acidity	Potentiometric Titration	1 mg/L
<i>Laboratory Analysis</i>		
BTEX and ethanol	Headspace analysis by Gas Chromatograph with FID detector	1 µg/L and 1 mg/L
Methane	Headspace analysis by Gas Chromatograph with FID detector	10 µg/L
Acetate	ICS 1000 conductivity detector	0.1 mg/L
Bromide	ICS 1000 conductivity detector	0.1 mg/L
Nitrate	ICS 1000 conductivity detector	0.1 mg/L
Phosphate	ICS 1000 conductivity detector	0.1 mg/L
Sulfate	ICS 1000 conductivity detector	0.1 mg/L

NA = Not Applicable

TABLE S2: Background and maximum concentrations of selected analytes

Analytes	Well Location ^c (year sampled)	Maximum Concentration	Baseline conditions *
Alkalinity (mg L ⁻¹ CaCO ₃)	SW9 (1.4)	338 **	4-30
Bromide (mg L ⁻¹)	SW4 (3.1)	54	0
Acetate (mg L ⁻¹)	SW5, SW4 (1.9)	167 , 112	0
Fe ⁺⁺ (mg L ⁻¹)	SW5 (2.7)	206	0-1.64
Methane (mg L ⁻¹)	SW4A (2.7)	170.24	< 0.01
Ethanol (mg L ⁻¹)	SW4A (1.4)	2,503	0
Benzene (µg L ⁻¹)	SW4 (1.9)	11,411	0
Toluene (µg L ⁻¹)	SW4A (2.7)	26,694	0
Ethylbenzene (µg L ⁻¹)	SW5 (2.7)	3,489	0
Xylene (µg L ⁻¹)	SW5 (2.7)	13,670	0
BTEX Total (µg L ⁻¹)	SW4A (2.7)	50,803	0

^c Figure 1 in the manuscript provides a map of sampling well locations.

*Baseline values were obtained from well SW1 prior to the ethanol-blend release.

** The alkalinity at SW5 was 54 mg L⁻¹ as CaCO₃ when the peak acetate concentration was measured, and this value was used for the thermodynamic calculations at the end of the SI document.

ESTIMATION OF GROUNDWATER VELOCITY

The hydrogeological parameters of the experimental area were determined by planialtimetric surveying and *in situ* permeability tests to determine hydraulic conductivity.

Hydraulic conductivity was determined by failing-head tests (performed by Geodésia, Estudos, Projetos and Assessoria Ltda) at three points and various depths near the experimental area. The average hydraulic conductivity was determined to be $1.1 \times 10^{-4} \text{ cm s}^{-1}$. The effective porosity (η_e) of the saturated soil was 0.2 (6).

The average velocity of the groundwater at the site is 3.1 m yr^{-1} . The average hydraulic gradient (0.018 m/m) was calculated using 422 points collected from piezometers installed in the area (Figure S1) between 1998 and 2010. Applying Darcy's law with hydraulic conductivity $K = 1.1 \times 10^{-4} \text{ cm s}^{-1}$ and a porosity = 0.2, the average groundwater velocity was obtained. Bromide tracer tests determined a preferential flow from point A to B (Figure S3) with a variation of

approximately 40° . This variation was the result of recharge and possibly tidal influence, and is reflected in the value of the hydraulic gradient over time.

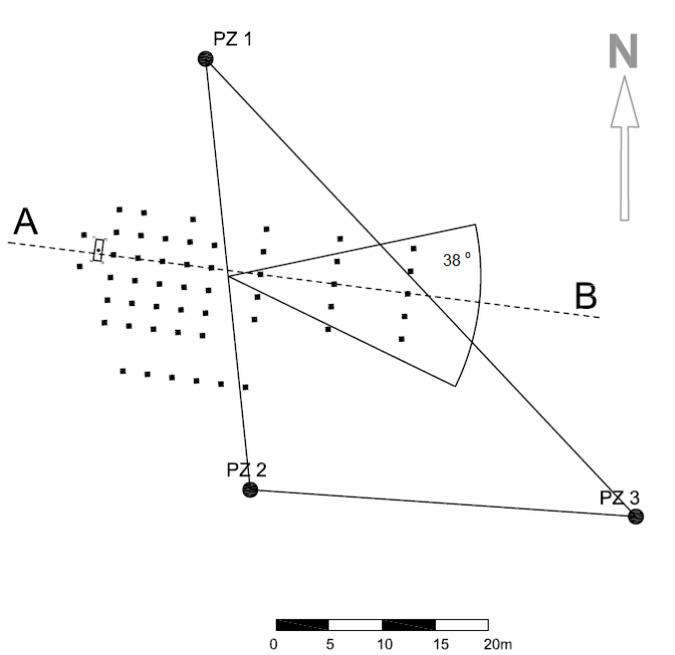


FIGURE S3: Groundwater flow path (A to B) and piezometer triangulation (PZ1, PZ2, and PZ3) used to determine hydraulic conductivity.

INTERPOLATION METHOD

The interpolation method was based on the recovery of a released water soluble, conservative tracer (potassium bromide). The ratio between the interpolated mass and released mass represented the total error of the method. The total error includes the error of the interpolation method, laboratory analytical errors and bromide offsite migration by advective transport. The error of interpolation was calculated using the mean square error (RMSE) and was compared to two deterministic interpolation methods: inverse distance weighted and minimum curvature. The linear interpolation method was not considered because the sampling grid was irregular resulting in interpolation points outside the boundary (out of bounds) of the area defined for interpolation (polygon interpolation), which lead to a loss of sample points. The method of Kriging (probabilistic approach) was not used because some requirements for the method could not be circumvented (i.e., temporal analysis of the biodegradation process requiring a minimum number of samples (> 30), and low coefficient of variation (ratio of standard deviation and mean). Since the distribution of sample values (BTEX, methane, iron, acetate) are lognormal, it was not always possible to obtain low coefficients of variation to enable the use of Kriging method (7) for a long-term temporal analysis. Therefore, the choice of deterministic interpolations represented the best option to meet the goal of long-term analysis of this work.

TRACER MASS

Potassium bromide (1 kg) was used as tracer and the initial mass of bromide ion dissolved in water was 671 grams. During the transport process, the greatest mass obtained for the bromide was at 2.7 years after the ethanol-blend release. The minimum curvature mass obtained for this period was 56% (378 g) of the released mass and with the inverse distance weighted was 54% (363 g). The mean squared error (RMSE) for bromide was calculated for the two methods using eq. 1:

$$RMSE = \sqrt{\frac{1}{n_{est}} \sum_{j=1}^{n_{est}} [d_{est}(x) - d_{obs}(x)]^2} \quad \text{eq. 1}$$

Where n_{est} is the number of estimated values; $d_{est}(x)$ is density of solute per unit area of the aquifer ($\text{mg}\cdot\text{m}^{-2}$ or $\text{g}\cdot\text{m}^{-2}$) estimated for each locality and $d_{obs}(x)$ is density of solute per unit area of the aquifer ($\text{mg}\cdot\text{m}^{-2}$ or $\text{g}\cdot\text{m}^{-2}$) observed for each location. For interpolating, the measured concentrations represented only a portion of the contaminant dissolved in groundwater and exclude the portion sorbed in porous media. The sorbed portion was evaluated separately.

The mean square error (RMSE) for the minimum curvature and the inverse distance weighted are 0.04 and 0.11, respectively. Since the error was calculated from the density of solute per unit area (eq. 1, (7)), (a transformed unit using a conversion factor less than 1) the resulting absolute value for RMSE was very low and the relative percentage variation $[(IPD-MC)/MC]*100$ was used rather than absolute variation (IDP-CM) for comparison. The relative

percentage variation between the two methods was around 175%, demonstrating the superiority of minimum curvature compared to the inverse of the distance.

BROMIDE OFFSITE MIGRATION

The bromide offsite migration was ascertained by the high concentrations of bromide measured in the last row (Figure S3) of sampling wells (SW24, SW25, SW26 and SW27) between 2 and 9.5 years after the ethanol-blend release. The maximum bromide concentrations (Figure S4) were measured in sampling wells SW24, SW25, SW26 and SW27 taking into account all monitoring levels (1 m; 2 m; 2.5 m; 3.5 m; 4.5 m). Regarding BTEX compounds, only the benzene reach the sampling well SW24 within 2.7 years at a concentration of $22 \mu\text{g L}^{-1}$.

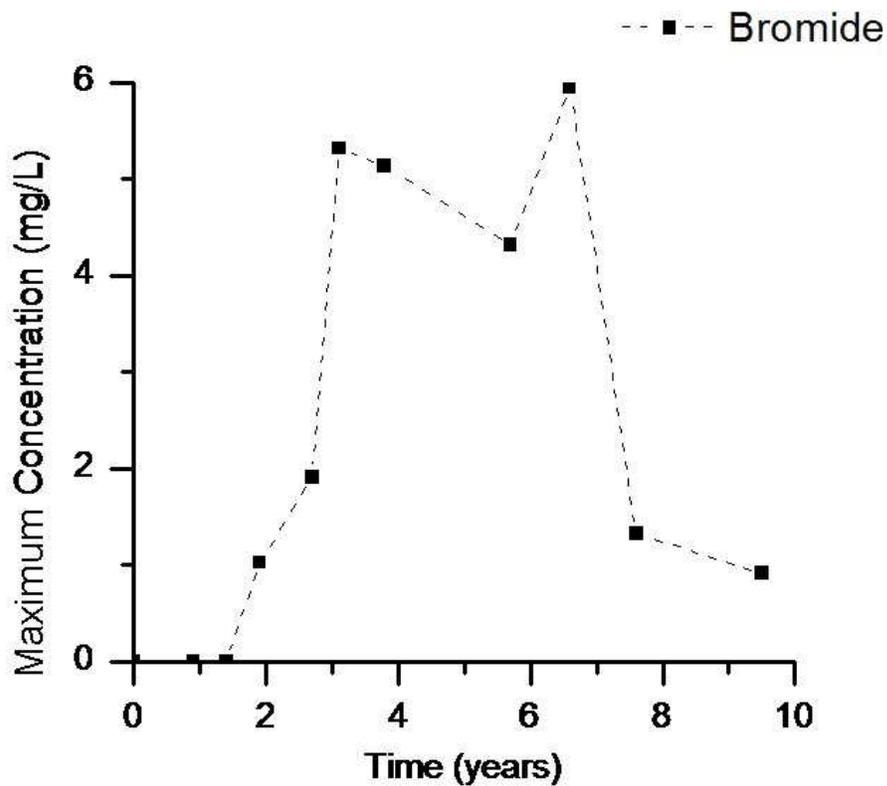


FIGURE S4: Bromide Maximum concentration at SW24, SW25, SW26, SW27

ESTIMATION OF CONTAMINANT MASS

The steps of interpolation (using the contour and surface mapping software Surfer 8.0 (8)) are as follows: a) redefinition of the interpolated area; b) definition of the tributary area; c) conversion of groundwater concentration in densities per area using corresponding conversion factor as shown above; d) use the function $C [= \max (a, 0)]$, to reset the values below zero, which are generated during the interpolation, since the minimum curvature function generates values above and below the monitored values; e) mass estimation with the Simpson algorithm,

available in the volume function of the program Surfer 8.0; g) sum of the masses obtained at each depth to obtain the total plume mass.

The mass calculation considered the volume occupied by the contaminate plume in the monitored area. A two-dimensional analysis was performed and the plume mass was defined as the collective sum of the five layers (Figure S5). It is important to remember that the estimated mass represents only the dissolved portion in groundwater, since this calculation is based on measured concentrations in the dissolved phase of groundwater. The sorbed portion was estimated from the retardation factor (R, eq. 5). The total mass of the plume was estimated as the sum of dissolved and sorbed mass. The mass calculation considered only concentrations above the detection limit for each compound (Table S1).

Surfer 8.0, by default, uses rectangular areas for interpolation (eq. 2) and the sampling well locations did not coincide with this preformatted area therefore, it was necessary to redefine the area to be interpolated. This was done to avoid artifacts in the corners of the rectangular interpolation area where monitoring wells do not exist. A new polygon (Figure S5) was drawn (using Surfer command blankfile outside) with the limits set at the 19 outer wells (SW1, SW2, SW3C, SW3AC, SW3BC, SW8C, SW8BC, SW13, SW18, SW 23, SW24, SW25, SW26, SW27, SW22, SW17, SW12, SW7A, SW7) of the monitored area.

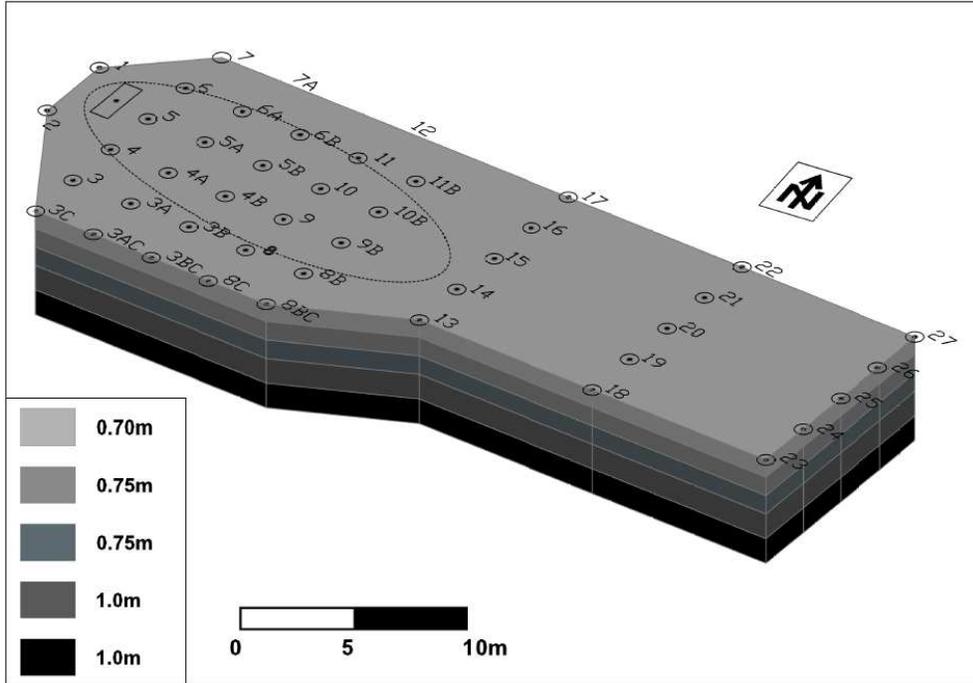


FIGURE S5: Map of experimental area with sampling wells (SW), interpolated area boundaries and tributary areas

The layer thickness was defined as tributary areas. The tributary areas were based on the midpoint between adjacent monitoring depths (1.0 m, 2.0 m, 2.5 m, 3.5 m, 4.5 m) outlined in Figure S5. Thus, the midpoints for the different depths were 1.5 m (1 m and 2 m), 2.25 m (2 m and 2.5 m), 3 m (2.5 m and 3.5 m) and 4 m (3.5 m and 4.5 m). The numerical difference between the values of adjacent midpoints resulted in the thickness of the tributary areas. For the upper level, near the water table, we used the average value (0.8 m) as the upper limit. The thicknesses of the tributary areas: 0.7 m, 0.75 m, 0.75 m, 1 m and 1 m.

For mass calculations Surfer 8.0 considers a surface defined by a rectangular region extends from x_{\min} to x_{\max} and y_{\min} and y_{\max} , according to eq. 2 (9).

$$\text{Mass} = \int_{x_{\min}}^{x_{\max}} \int_{y_{\min}}^{y_{\max}} f(x, y) dx dy \quad \text{eq. 2}$$

Where x and y are geographical coordinates and f (x, y) is the function used for interpolation. This is computed by integrating over X (the columns) to get the areas under the individual rows, and then integrating over Y (the rows) to get the final mass. Surfer 8.0 approximates the necessary one-dimensional integrals using numerical integration algorithms, in this case Simpson's algorithm was used (10).

In the two-dimensional analysis the contaminants were assumed to be uniformly distributed within the tributary area (7). The concentrations (expressed per unit volume of water from the aquifer ($\mu\text{g L}^{-1}$ or mg L^{-1})) were transformed into additive variables by multiplying by the porosity. The bulk densities were converted to densities per area of the aquifer (mg m^{-2} or g m^{-2}) by multiplying by the thickness of the plume tributary area. Porosity and the thickness of each tributary area were used to convert groundwater concentrations ($\mu\text{g L}^{-1}$ or mg L^{-1}) to contaminant densities per area (mg m^{-2} or g m^{-2}). The conversion factors are 0.14, 0.15, 0.15, 0.20, 0.20 for depths of 1 m, 2 m, 2.5 m, 3.5 m and 4.5 m respectively.

ESTIMATION OF ETHANOL ZERO-ORDER DEGRADATION RATE AND ITS HALF LIFE

The total dissolved plume mass can be used to estimate the zero-order degradation rate of ethanol, using following equation:

$$M = M_0 - kt$$

eq. 1

where M is the total plume mass (dissolved) at a given time, M_0 is the released mass (18.9 kg), k is the zero order rate, and t is the time after the release. The sorbed mass was neglected because of the very low ethanol partitioning coefficient (0.0003 L kg^{-1}). The reaction rate was determined by a linear regression analysis at a 95% confidence interval. The zero-order degradation rate (k) was $6.6 \pm 2.4 \text{ kg year}^{-1}$ as illustrated in Figure S6 ($R^2 = 0.96, p < 0.05$).

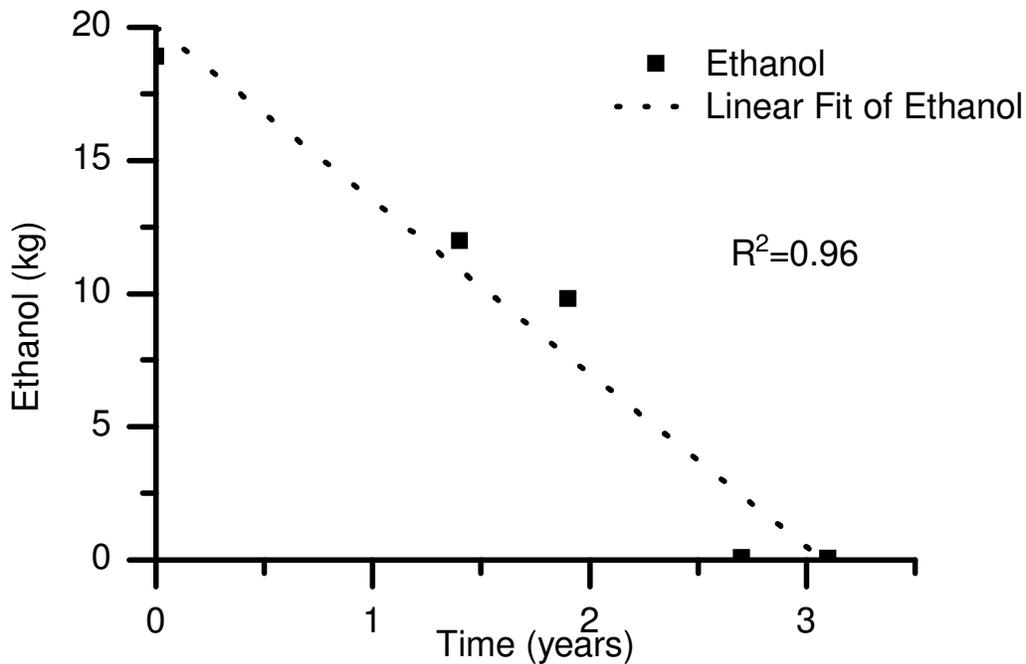


FIGURE S6: Linear regression of zero-order degradation rate for ethanol.

The half life of a compound undergoing linear (zero-order) degradation kinetics is

$$t_{1/2} = (0.5 \cdot M_0) / k$$

where M_0 is the mass released and $k = 6.6 \text{ kg/year}$ (Figure S6)

$$\text{Thus, } t_{1/2} = (0.5 \cdot 19.8 \text{ kg}) / 6.6 \text{ kg/year} = 1.5 \text{ years}$$

ESTIMATION OF PSEUDO FIRST ORDER REACTION RATES FOR TOTAL BTEX PLUME MASS

The calculated total plume mass (dissolved plus sorbed) was used to estimate pseudo first-order reaction rates for the degradation of BTEX, using following equation:

$$M = M_0 e^{-kt} \quad \text{eq. 2}$$

where M is total plume mass at a given time, M_0 is the maximal total mass, k is the pseudo first-order reaction rate, and t is the time after the ethanol-blend release. Thus, the pseudo first order reaction rates can be calculated using the eq. 2 from an exponential regression analysis at a 95% confidence interval. Table S3 summarizes the attenuation rate k for each BTEX compound, the time frame (years) and statistical values (R^2 , n and p-value).

The maximum total BTEX mass occurred at 2.7 years following the release. This value was used for characterizing the initial period in which to evaluate the attenuation coefficient. For benzene the final period was 6.6 years since from that period on, the mass decay was very slow (Figure 4- paper) and its weight in the final figure could misrepresent the attenuation coefficient value. For other compounds, the final period was 8.5 years.

TABLE S3: Pseudo first order reaction rates (k) of aromatic hydrocarbons (exponential regression analysis at 95% confidence level).

Compounds	Time frame(years)	pseudo first order biodegradation coefficient (year ⁻¹)	R ²	n	p-value
benzene	2.7 to 6.6	0.81±0.34	0.95	6	< 0.05
toluene	2.7 to 9.5	0.73±0.11	0.98	8	< 0.05
ethylbenzene	2.7 to 9.5	0.55±0.15	0.93	8	< 0.05
xylenes	2.7 to 9.5	0.48±0.11	0.95	8	< 0.05

ESTIMATION OF RETARDATION OF BTEX

The retardation factor R was determined using eq. 5:

$$R = 1 + \frac{\rho_b}{n} * K_d \quad \text{eq. 3}$$

Where ρ_b is the bulk density of the medium (g/cm³), n is the porosity (-) and K_d the distribution coefficient.

The distribution coefficient (K_d) is determined by the following equation:

$$K_d = K_{om} * f_{om} \quad \text{eq. 6}$$

Where K_{om} is the organic-matter-normalized distribution coefficient and f_{om} is the fraction of organic matter in solids. The K_{om} is estimated using the

eq. (11)

$$\log K_{om} = 1.01 (\log K_{ow}) - 0.72 \quad \text{eq. 7}$$

Where K_{ow} is the octanol-water partition coefficient of the sorbing chemical.

The sorbed mass was estimated by the following equation (12):

$$M_S = (R - 1)M_D \quad \text{eq. 8}$$

Where M_S is the sorbed mass, M_D , the dissolved mass, and R , the retardation factor. The fraction of organic carbon to the area of Ressacada was 0.06%. The total plume mass of BTEX (dissolved plus sorbed mass) was 1,095 g (Table S4). The 80% total benzene mass removal within one year (2.7 years to 3.8 years) following the onset of its degradation was determined as follow: $M/M_i = 0.3$ at 2.7 years and $M/M_i = 0.06$ at 3.8 years. [$0.8 = (0.3 - 0.06)/0.3$]

TABLE S4: BTEX Mass (Dissolved and Sorbed) and Iron II and Methane Dissolved Masses

Compounds	Dissolved Mass (g)	log K_{ow} ^(a)	log K_{om}	K_d	R	Sorbed Mass (g)	Total Plume Mass(g)
Benzene	95.3	2.13	1.43	0.0016	1.14	13.1	108.4
Toluene	293.7	2.69	2.00	0.060	1.51	148.7	442.4
Ethybenzene	44.8	3.15	2.46	0.174	2.48	66.1	110.9
Xylenes	167.8	3.18	2.49	0.186	2.58	265.5	433.3
Iron II	2638.4	-	-	-	-	-	2638.4
Methane	3180.0	-	-	-	-	-	3180.0

(a) We used the K_{ow} of m-Xylene, the highest value of the three isomers (o-,m-,p-Xylene) to calculate Xyenes sorbed mass. (13).

MICROBIAL ANALYSIS

DNA was extracted from groundwater samples using a vacuum filter (0.22 μm , Osmonics Inc., Minnetonka, MN) and the filter served as the matrix for the MoBio Power Soil™

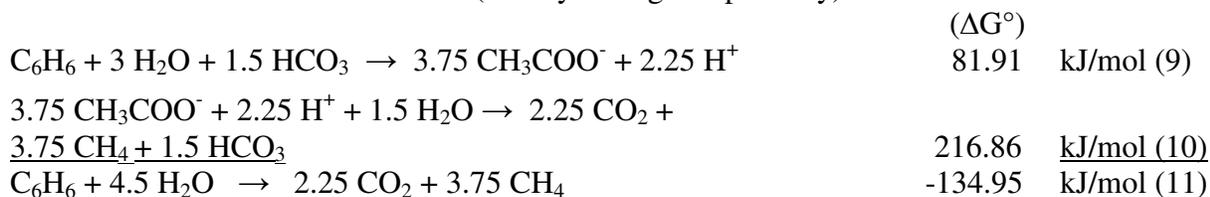
kit (Carlsbad, CA). The primers and probes used for quantification were obtained from IDT Technologies (Coralville, IA). Calibration curves (10^1 to 10^8 gene copies mL^{-1}) were prepared for all genes under consideration, yielding r^2 values of at least 0.99. The detection limit of each assay was approximately 10^2 gene copies mL^{-1} . The RTQ-PCR mixture contained 0.9 μM of each primer (0.45 μM of each forward primer for archaea), 0.25 μM of the respective probe, 1x TaqMan Universal PCR Master mix (Applied Biosystems), 2.5 μl of DNA extract, and nuclease-free sterile water to a final reaction volume of 25 μl . RTQ-PCR was conducted using an Eppendorf Mastercycler® ep realplex (Bio Research do Brasil, Sao Paulo, SP) with the following temperature conditions: 50°C for 2 min, followed by 95°C for 10 min and 40 cycles at 95°C for 15 s, and 60°C for 1 min.

THERMODYNAMIC CALCULATIONS

ΔG_f values from Thauer et al (14):

H_2	0	kJ/mol
H^+	0	kJ/mol
CH_3COO^-	-369.41	kJ/mol
C_6H_6	124.6	kJ/mol
H_2O	-237.17	kJ/mol
CH_4	-50.75	kJ/mol
CO_2	-394.359	kJ/mol

For benzene fermentation to acetate (strictly acetogenic pathway):



For benzene fermentation to acetate and hydrogen (acetogenic and hydrogenogenic pathway):
(ΔG°)



Using the Nernst equation:

$$\Delta G \text{ (kJ/mol)} = \Delta G^\circ + RT \cdot \ln\left(\frac{[\text{Products}]^a}{[\text{Reactants}]^b}\right)$$

Where a and b are the stoichiometric coefficients in the balanced equations and R is the universal

gas constant ($0.008134 \text{ kJ mol}^{-1} \text{ K}^{-1}$) and the temperature value is the median temperature of

Ressacada groundwater ($21^\circ\text{C} = 294 \text{ K}$). The pH near the source zone was 5.2 therefore the H^+ concentration was calculated: $10^{-5.2} = 6.30957 \cdot 10^{-6} \text{ M}$. Acetate and benzene concentrations were converted from mg L^{-1} to M. The alkalinity value at well SW5 (near the source where the highest acetate concentrations were observed) at year 1.9 was used, which was 54 mg L^{-1} as CaCO_3 . This was converted to M of HCO_3^- using $(\text{Alkalinity mg L}^{-1} \text{ as CaCO}_3 \cdot 61) / ((1 + (2 \cdot 10^{-10.3}) / (10^{-\text{pH}})) \cdot 50)$. Threshold inhibition acetate values were determined for $\Delta G = 0$. Hydrogen was not measured at the Ressacada site, so to evaluate the thermodynamic feasibility of equation 12, a range of plausible hydrogen concentrations for methanogenic conditions were selected from the literature (15-18) (i.e., from the 10^{-8} M threshold to 10^{-3} M (near the saturation limit for H_2)).

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