Supporting Information

For

Methane Bioattenuation and Implications for Explosion Risk Reduction along the Groundwater to Soil Surface Pathway above a Plume of Dissolved Ethanol

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Flux Chamber Description

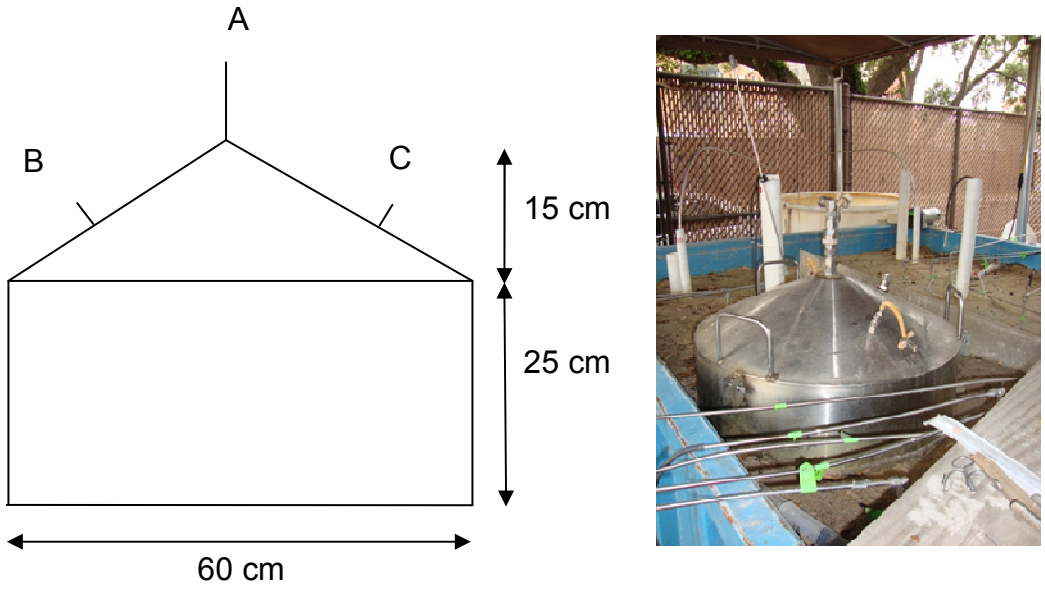

Figure S1. The structure schematic (left) and the photo of the flux chamber (right)

The flux chamber was made of stainless steel. The lower part of the chamber was a cylinder with the diameter of 60 cm and the height of 25 cm and the bottom is open. The top part of the chamber was a cone with the height of 15 cm. The chamber had an enclosed surface area of 2.8×10^3 cm^2 and total enclosed volumes of 8.5×10^4 cm^3. When emplaced on the soil surface, 8 cm of the chamber bottom was buried in the soil to make sure no gas leaked out from the bottom. We used this chamber as a "static-chamber" which means no anthropogenic introduction of gas into the chamber during the incubation period. There were three gas sampling ports (A, B and C). Our test showed that the gas samples collected from these three ports have same results, thus we used top sampling port (A) for our experiment. The sampling port contained one steel tube connected with an 8 cm rubber tube clamped by an open ended clamp (Pentair Technical Products, Anoka, MN). Testing for potential leaks from the chamber (including sampling ports), was conducted by inverting the chamber and filling with water. No leaks were observed.

qPCR analysis for *pmoA* gene

The target groups, primer sets and annealing temperatures for each assay are summarized in Table S1. The qPCR reaction mixture contained 12.5 μL Power SYBR® Green PCR Master Mix (Applied Biosystems; Foster City, CA), 500 nM each primers and 2μL template DNA in a total volume of 25 μL. An ABI 7500 Sequence Detector (Applied Biosystems, Foster City, CA) was used to perform qPCR reactions with the following temperature program: 50°C for 2 min, followed by 95°C for 10 min and 40 cycles at 95°C for 15 s, and 30 s at the annealing temperature for each primer set (Table S1), 40 s at 72 °C for extension. Melting curve analysis was conducted after the thermal cycle was completed to make sure no nonspecific PCR products were generated.

Table S1. Primers, target group, standard DNA and annealing temperature for qPCR

Assay	Primer	Primer sequences	Target group (genus)	Annealing temp. (ºC)	Calibration standard (genomic DNA) *
MBAC	A189F	5'-GGNGAC TGGGACTTCTGG-3'	*Methylobacter and Methylosarcina*	54	*Methylomicrobium album*
	Mb601R	5'-ACRTAGTGGTAACCTTGYAA-3'			
MCOC	A189F	5'-GGNGAC TGGGACTTCTGG-3'	*Methylococcus*	60	*Methylococcus capsulatus*
	Mc468R	5'-GCSGTGAACAGGTAGCTGCC-3'			
MCAP	A189F	5'-GGNGACTGGGACTTCTGG-3'	*Methylocapsa*	60	*Methylocapsa acidiphila*
	Mcap630R	5'-CTCGACGATGCGGAGATATT-3'			
FOREST	A189F	5'-GGNGACTGGGACTTCTGG-3'	Forest clones	60	DGGE band affiliated with forest clones
	Forest675	5'-CCYACSACATCCTTACCGAA-3'			
TYPEII	II223 F	5'-CGTCGTATGTGGCCGAC-3'	*Methylosinus*	60	*Methylocystis parvus*
	II664R	5'-CGTGCCGCGCTCGACCATGYG-3'			

*Except for the assay "FOREST" which uses DGGE band affiliated with forest clones

Conceptual model and details about "Biovapor"

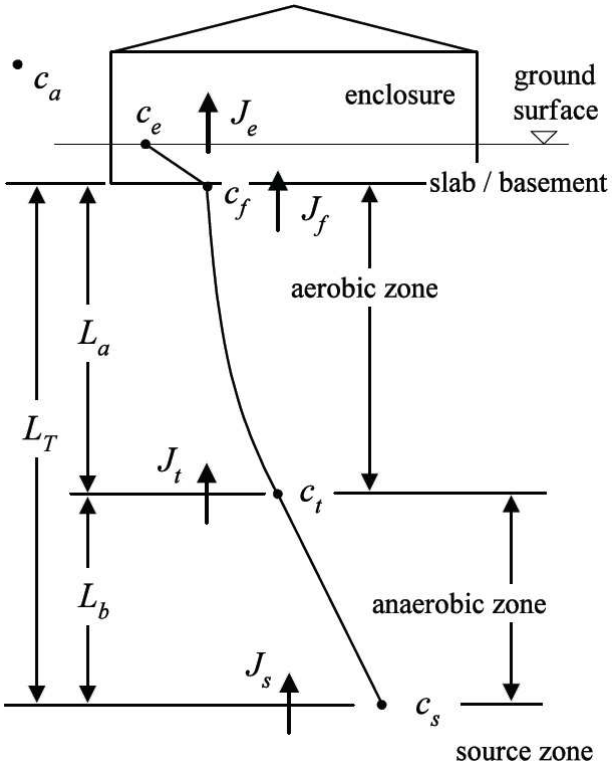

Figure S2. Conceptual model of "Biovapor".[1]

Figure S2 shows an illustrative conceptual model assumed in "Biovapor". The soil is divided into a shallow aerobic layer including biodegradation and a deeper anaerobic layer in which biodegradation is omitted. Oxygen demand is attributed to a sum of baseline respiration of soil organic matter and biodegradation of multiple chemicals assuming first-order degradation rates. The model is solved by iteratively varying the aerobic depth to match oxygen demand to oxygen supply.

Table S2. Model inputs for methane explosion simulation

Building parameters:
Indoor Mixing Height (L_{mix}): 244cm
Air Exchange Rate (ER): 6 day^{-1}
Foundation Thickness: 15cm
Foundation Area: 1,060,000 cm^2
Foundation Crack Fraction (η): 3.77×10^{-4} cm^2-cracks/ cm^2-total
Total Porosity (Soil-filled Cracks, $\theta_{T\text{-}cracks}$): 1 cm^3-void/cm^3-soil
Water Filled Porosity (Soil-filled Cracks, $\theta_{w\text{-}cracks}$): 0 cm^3-void/cm^3-soil
O_2 concentration below the foundation: 5% (v:v)
Aquifer parameters:
Soil Porosity ($\theta_{T\text{-}soil}$): 0.38 cm^3-void/cm^3-soil
Soil Water Content ($\theta_{w\text{-}soil}$): 0.05 cm^3-water/cm^3-soil
Soil Organic Carbon Fraction (f_{oc}): 5×10^{-3} cm^3-void/cm^3-soil
Soil Density-Bulk (ρ_s): 1.7 g-soil/cm^3-soil
Airflow Under Foundation (Q_f): 83 cm^3-air/sec
Annual Median Soil Temperature (T): 10ºC
Depth of Water Table (LT): change from 1m to 20m
Minimum O_2 Concentration For Aerobic Biodegradation: 1%
Chemicals:
Methane Concentration in Groundwater: change from 0 to 20 mg/L
1st order Biodegradation Rate of Methane*: 71 hr^{-1}
Attenuation Factor: 1

* The first order biodegradation rate constants is the default value used by Biovapor, and it represents a geometric mean from 17 data sets as described by DeVaull 2007[1].

Table S3. Model inputs for benzene vapor intrusion simulation

Building parameters:

Indoor Mixing Height (L_{mix}): 244cm

Air Exchange Rate (ER): 6 day^{-1}

Foundation Thickness: 15cm

Foundation Area: 1060,000 cm^2

Foundation Crack Fraction (η): 3.77×10^{-4} cm^2-cracks/ cm^2-total

Total Porosity (Soil-filled Cracks, $\theta_{T\text{-}cracks}$): 1 cm^3-void/cm^3-soil

Water Filled Porosity (Soil-filled Cracks, $\theta_{w\text{-}cracks}$): 0 cm^3-void/cm^3-soil

Airflow Through Basement Foundation (Q_S): 83 cm^3-air/sec

Aquifer parameters:

Soil Porosity ($\theta_{T\text{-}soil}$): 0.38 cm^3-void/cm^3-soil

Soil Water Content ($\theta_{w\text{-}soil}$): 0.05 cm^3-water/cm^3-soil

Soil Organic Carbon Fraction (f_{oc}): 5×10^{-3} cm^3-void/cm^3-soil

Soil Density-Bulk (ρ_s): 1.7 g-soil/cm^3-soil

Airflow Under Foundation (Q_f): 83 cm^3-air/sec

Annual Median Soil Temperature (T): 10ºC

Depth of Water Table (LT): 5m

Minimum O_2 Concentration For Aerobic Biodegradation: 1%

Chemicals:

Benzene Concentration in Groundwater: 5mg/L

Methane Concentration in Groundwater: Change from 0 to 20mg/L

1st order Biodegradation Rate*:

1st order Biodegradation Rate of Benzene: 0.79 hr^{-1}

1st order Biodegradation Rate of Methane: 71 hr-1

Attenuation Factor: 1

* The first order biodegradation rate constants is the default value used by Biovapor, and it represents a geometric mean from 17 data sets as described by DeVaull 2007[1].

Table S4. CH_4 concentrations in groundwater *

	February 2011 (7 ºC)		June 2011 (28 ºC)	
Sampling port	Before (mg/L)	After (mg/L)	Before (mg/L)	After (mg/L)
C1	8.9	7.6	21.6	23.7
C2	5.5	8.3	20.6	19.2
C3	6.3	5.8	22.5	20.0

* Groundwater methane was measured before and after the flux chamber was installed. CH_4 concentrations in groundwater remained stable during the flux chamber measurement period.

Estimation of the contribution of methanotrophic activity to methane flux attenuation in the vadose zone

The methane emission flux in the pilot-scale aquifer was significantly decreased along the water table to ground surface pathway due to biodegradation. We estimated this reduction in flux from the measured methane soil gas data (15 to 30 cm BGS， Figure 3). Assuming one-dimensional steady-state diffusive vapor flow with first-order reaction and the soil parameters over the 15 to 30 cm BGS remained constant, the methane concentration profile in this layer for $0 \le z \le L$ ($\alpha > 0$) is given by Eq. S1 [1]

$$\frac{c(z)}{c_0} = \frac{\left(e^{-\alpha} - \beta\right)\cdot e^{\alpha\cdot z/L} + \left(\beta - e^{\alpha}\right)\cdot e^{-\alpha\cdot z/L}}{e^{-\alpha} - e^{\alpha}} \quad \text{Eq. S1}$$

Where $c(z)$ is the methane concentration at z. c_0 is the methane concentration at $z = 0$ (the lower boundary at 30 cm BGS), and L is the depth of simulated soil layer (15 cm for the soil layer between 15 cm BGS and 30 cm BGS). The boundary conditions were specified as [1]

$$\frac{C(z/L=1)}{C_0} = \beta \quad \text{Eq. S2}$$

$$\frac{C(z/L=0)}{C_0} = 1 \quad \text{Eq. S3}$$

The value α^2 is a diffusive Damkohler number over the depth, L. Nonlinear regression is applied in estimating an empirical value of $\alpha = 6.96$ from the data. In the nonlinear regression, average methane concentration data from the soil layer (15 to 30 cm BGS, 4 measured data points) were normalized by the methane concentration at 30 cm BGS, c_0, to yield $c(z)/c_{0[\text{data}]}$ (Table S4). Fitted values, $c(z)/c_{0[\text{fit}]}$ (Table S5) were calculated from the concentration profile

equation with Eq. S2. The summed squared error between the data and fitted values $\Sigma\,[c(z)/c_{0[\text{data}]} - c(z)/c_{0[\text{fit}]}]^2$ was minimized by varying α, using the Microsoft Excel ® SOLVER function, to yield the $\alpha = 6.96$ optimum value.

The methane flux through this layer, for $0 \le z \le L$ ($\alpha > 0$) is calculated by Eq. S4.

$$J(z) = -D_{eff}\frac{dc(z)}{dz} = \frac{D_{eff}\cdot c_0}{L}\cdot\alpha\cdot\frac{\left(e^{-\alpha}-\beta\right)\cdot e^{\alpha\cdot z/L} - \left(\beta - e^{\alpha}\right)\cdot e^{-\alpha\cdot z/L}}{e^{-\alpha}-e^{\alpha}} \quad \text{Eq. S4}$$

The reduction in flux across the layer due to biodegradation is calculated from the fitted α value and the measured β value by Eq. S5. The results are listed in Table S4.

$$1-\frac{J(z=L)}{J(z=0)} = 1-\frac{2-\beta\cdot\left(e^{\alpha}+e^{-\alpha}\right)}{\left(e^{\alpha}+e^{-\alpha}\right)-2\cdot\beta} \quad \text{Eq. S5}$$

.Within the accuracy of the data, this analysis indicates that degradation within this 15 to 30 cm BGS layer reduces the upward flux of methane by a factor greater than approximately 99%. Additional reduction in flux due to biodegradation would also occur outside of the modeled layer.

Table S5. Nonliear regression and reduction in flux due to biodegradation

Sample Depth BGS (cm)	z/L	$c(z)/c_0$ [data]	$c(z)/c_0$ [fit]	1-J(z/L=1)/J(z/L=0)
15	1	2.06E-03	2.06E-03	1.000
20	0.667	6.60E-03	9.78E-03	0.990
25	0.333	9.90E-02	9.83E-02	0.902
30	0	1.00E+00	1.00E+00	0

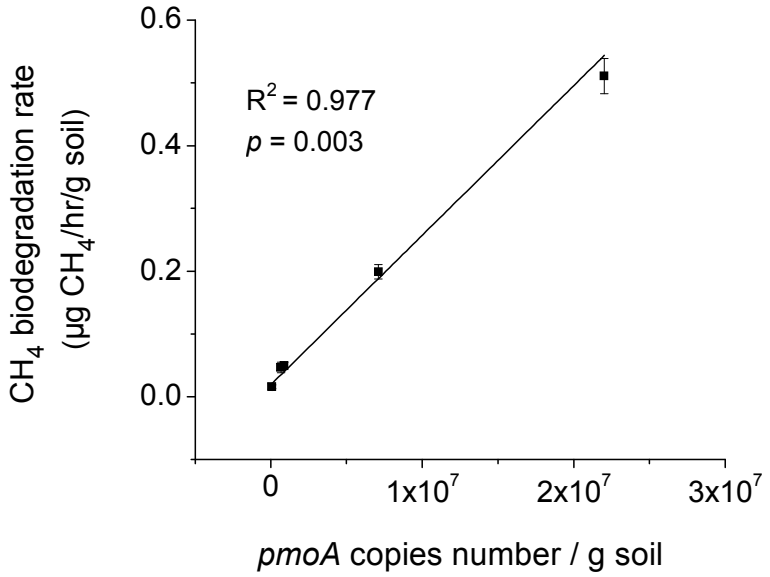

Figure S3. Correlation between methane biodegradation rate and *pmoA* gene concentration in the soil samples collected from different depths in the pilot-scale aquifer.

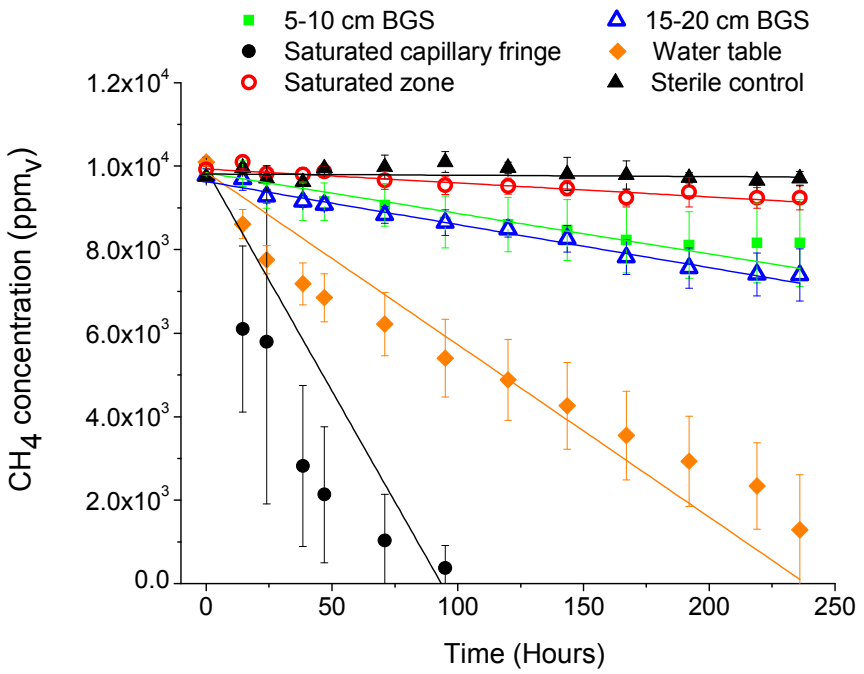

Figure S4. Methane biodegradation activity in microcosms prepared with soil samples from different depths. Error bars depict ± one standard deviation from the mean of triplicate microcosms, and solid lines are linear fits to estimate zero-order degradation rates.

REFERENCE

1. DeVaull, G. E., Indoor vapor intrusion with oxygen-limited biodegradation for a subsurface gasoline source. *Environmental Science & Technology* **2007,** *41*, (9), 3241-3248.