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ORIGINAL ARTICLE

Intrinsic potential for immediate biodegradation of toluene in a pristine, energy-limited aquifer

Agnieszka Herzyk · Piotr Maloszewski · Shiran Qiu · Martin Elsner · Christian Griebler

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Abstract Pristine and energy-limited aquifers are considered to have a low resistance and resilience towards organic pollution. An experiment in an indoor aquifer system revealed an unexpected high intrinsic potential for the attenuation of a short-term toluene contamination. A 30 h pulse of 486 mg of toluene, used as a model contaminant, and deuterated water (D_2O) through an initially pristine, oxic, and organic carbon poor sandy aquifer revealed an immediate aerobic toluene degradation potential. Based on contaminant and tracer break-through curves, as well as mass balance analyses and reactive transport modelling, a contaminant removal of 40 % over a transport distance of only 4.2 m in less than one week of travel time was obtained. The mean first-order degradation rate constant was $\lambda = 0.178\ day^{-1}$, corresponding to a half-life time constant $T_{1/2}$ of 3.87 days. Toluene-specific stable carbon isotope analysis independently proved that the contaminant mass removal can be attributed to microbial biodegradation. Since average doubling times of indigenous bacterial communities were in the range of months to years, the aerobic biodegradation potential observed is assumed to be present and active in the pristine, energy-limited groundwater ecosystems at any time. Follow-up experiments and field studies will help to quantify the immediate natural attenuation potential of aquifers for selected priority contaminants and will try to identify the key-degraders within the autochthonous microbial communities.

Keywords Natural attenuation · CSIA · Resilience · Resistance · Groundwater ecosystem

Introduction

The terrestrial subsurface harbors one of our most important resources for life, i.e. groundwater. Groundwater is a major source for drinking water in Europe and worldwide and supports a multitude of groundwater dependent ecosystems (Danielopol et al. 2003; EEA 1999). Natural attenuation of pollutants and production of clean water is a valuable service provided by groundwater ecosystems (NRC 2004; Röling and Verseveld 2002; Smets and Pritchard 2003). However, aquifers increasingly face severe impacts from contaminants that have been and are released into the subsurface as a result of deposition (landfills, gasworks plants), leakages of distribution

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A. Herzyk · P. Maloszewski · S. Qiu · M. Elsner · C. Griebler (✉)
Institute of Groundwater Ecology, Helmholtz Zentrum München – German Research Center for Environmental Health, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany
e-mail: christian.griebler@helmholtz-muenchen.de

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systems and storage tanks (petrol stations), or accidental spills (EEA 1999, 2007, 2010). Groundwater ecosystems intrinsic resilience and resistance potentials are of growing scientific and socioeconomic concern (EEA 2012a, b).

The subsurface and ecosystems therein are generally considered energy poor, due to the limited amounts of organic carbon introduced from surface systems. As a result, microbial cell numbers and activities are usually several orders of magnitude lower than in surface waters and top soils (Goldscheider et al. 2006; Griebler and Lueders 2009; Kieft and Phelps 1997). Being energy-poor and low in productivity, groundwater systems are expected to display a high vulnerability and thus a low resistance to disturbances, such as the contamination with organic chemicals. Yet, an organic contamination brings energy into the subsurface and at the same time creates a selective pressure to which microbial communities react by compositional reorganization, generally with the enrichment of specialized degrader populations. Indeed, organically contaminated sites typically harbor microbial biomass significantly above background levels and microbial communities reduced in diversity but with a relative high abundance of microbes involved in contaminant transformation (Anneser et al. 2010; Franzmann et al. 1996; Haack and Bekins 2000; Kostka et al. 2011; Winderl et al. 2008).

Adapted microbial communities significantly contribute to the attenuation and removal of organic contaminants (Berlendis et al. 2010; Richnow et al. 2003; Wiedemeier et al. 1999; Winderl et al. 2008; Yagi et al. 2010). However, since scientific investigations at contaminated sites are delayed by weeks to months or even decades with respect to the time of first contamination, our knowledge about the immediate biodegradation potential present in aquatic environments and specifically in pristine and energy-limited aquifers is rather poor. In fact, most information on biodegradation is derived from batch experiments and microcosm studies where freshly sampled groundwater or sediment material amended with individual contaminants or contaminant mixtures have been studied (Althoff et al. 2001; Gieg et al. 1999; Nielsen and Christensen 1994; Nielsen et al. 1996). These experiments rarely mirror in situ conditions, what may be the reason, for frequently observed lag phases in contaminant transformation (Aamand et al. 1989; Arvin et al. 1988; Nielsen et al. 1996) as well as the extraordinary high degradation rates (Alvarez and Vogel 1991; Angley et al. 1992) to name two extremes.

To close the gap between biodegradation of organic pollutants investigated in laboratory studies and a real in situ biodegradation potential, a meso-scale groundwater indoor aquifer was designed. Its size (several meters in length), its packing (unsorted natural sands), as well as the on-line recharge with real groundwater from the on-site Quaternary aquifer allowed to evaluate the immediate biodegradation potential at conditions comparable to the field. A toluene pulse was directed through this initially pristine, oxic, organic carbon and energy poor sandy aquifer. Injection of toluene for 30 h together with deuterated water (D_2O), as conservative tracer, as well as toluene-specific analysis of stable carbon isotope signatures allowed quantification and qualitative prove of an immediate biodegradation after a travel distance of 4.2 meters in less than one week of travel time.

Materials and methods

Experimental set-up

The study was conducted in a flow-through mesocosm constructed from stainless steel (indoor aquifer) with dimensions of 4.8 m × 0.8 m × 0.7 m for length, width, and height, respectively (see Fig. 1). It was filled with unsorted quaternary sediment (grain size ≤4 mm) from a local gravel pit and fed continuously by local groundwater. The average hydraulic conductivity was of $K_f = 5.3 \times 10^{-4}$ ms^{-1} (±10 %). A water level difference of 10 cm and the total flow length of 4.83 m led to a hydraulic gradient between the inflow and outflow of i = 0.0207. The average groundwater flow velocity was estimated to be v = 1.6 m day^{-1}. A highly conductive sediment lens (0.5 mL × 0.8 mW × 0.12 mH) composed of homogeneous quartz sand was embedded at the inflow region of the aquifer (transect B; see Fig. 1) with a measured $K_f = (1.11 \pm 0.13) \times 10^{-3}$ ms^{-1}. The purpose of this lens was to first focus water stream lines and further increase the transversal expansion of the contaminant pulse (see below). Water sampling ports made from stainless stell with sintered glass cylinders at their tip were distributed in 3 longitudinal rows (1–3) over 5 transversal transects (A; C; D; E; F)

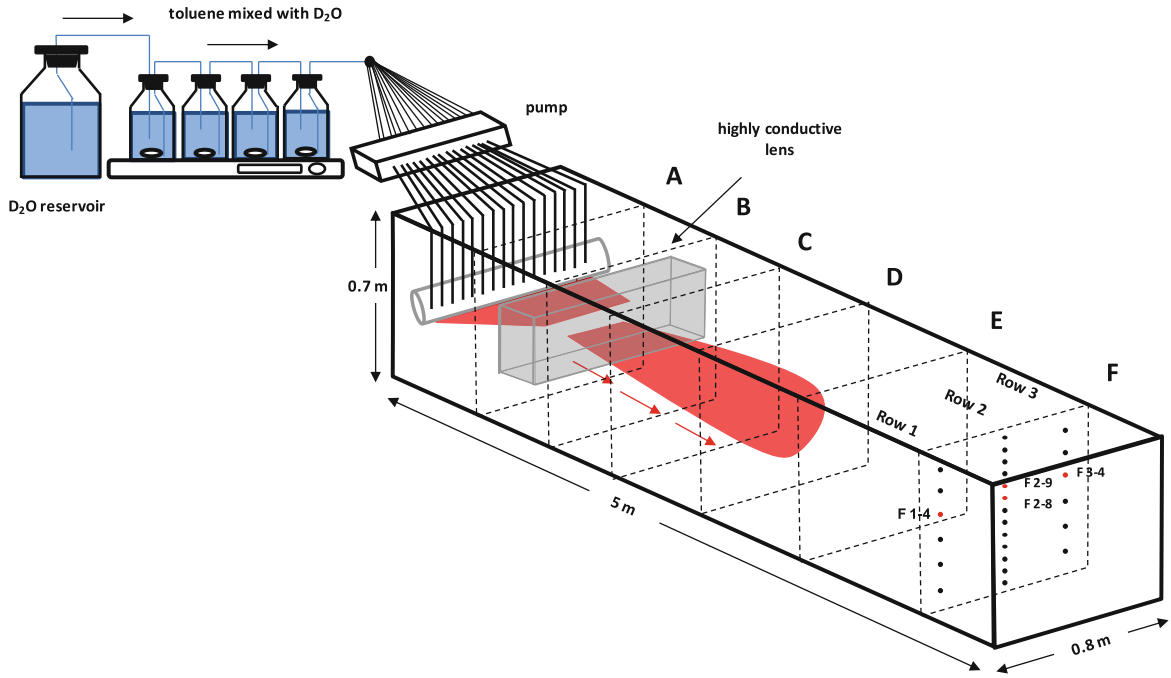

Fig. 1 Contaminant injection set-up and schematic overview of the indoor aquifer with 5 transects (*A*, *B*, *C*, *D*, *E*, *F*) , a highly conductive lens and sampling ports in different depths. Ports sampled are indicated in red and labeled

postionioned in different depths (see Fig. 1 and Table S1 providing coordinates of relevant sampling ports and sensors). Additionally, the aquifer was equipped with 18 oxygen sensors and 8 temperature sensors located along the water flow path (Huenniger 2011). The aquifer has been in a constant operation mode for 17 months (conditioning phase) free of contaminants. Prior to the toluene pulse experiment a sampling campaign was conducted to characterize the pristine stage of the system.

Contaminant injection system

Four 250 mL serum bottles were filled with 30 mL of pure toluene phase floating on 210 mL deuterated water (D_2O) each. The bottles were tightly sealed with viton stoppers, avoiding any headspace and connected with each other in line using steel capillaries and fluran tubings (Ismatec, Wertheim, Germany). The capillaries taking water were placed to the bottom of the bottles to avoid transfer of the floating toluene phase. To ensure that the injection solution was maximally saturated with toluene, bottles were placed on a magnetic stirrer during the time of injection. The first bottle in line were continuously fed with D_2O from a reservoir, and the last bottle was connected to a peristaltic pump feeding the toluene saturated D_2O into 16 injection ports made of stainless steel capillaries. These ports were connected with a horizontally oriented, perforated steel tube allowing infiltration of the contaminant solution and tracer across the entire width of the model aquifer, generating a contaminant plume. The injection front was placed vertically in the middle of the indoor aquifer (z [m bs] = 0.35). The injection rate for the feed solution was $q = 0.78$ L day^{-1} and lasted for 30 h Repeated measurements were performed to assure that the water infiltrating the aquifer system was toluene saturated. Along with the constant rate of injection, the time of the pulse and the constant temperature during the pulse, the total toluene mass imported was calculated, accounting for 0.486 and 204 g for toluene and deuterium, respectively.

Sampling

Water samples were repeatedly collected at four ports in transect F, 4.2 m downstream from the injection head. These four sampling ports were chosen based on a tracer test conducted prior to the experiment

(Huenniger, 2011), with the intention to capture the toluene pulse in a vertical and horizontal direction. Two ports, F2-8 and F2-9, were located in the central longitudinal transect (middle row, y [m] = 0.40) at a depth of z [m bs] = 0.36 and 0.32, respectively. Ports F1-4 (y [m] = 0.20) and F3-4 (y [m] = 0.60) were located in the longitudinal transects 1 and 3, left and right from the middle row 2, at a depth z [m bs] = 0.30 (Fig. 1). Water was sampled into glass syringes (50 mL) by means of a multichannel syringe pump. The pumping rate was 0.5 mL min^{-1}. The collected volume of water sample (50 mL) was subsequently divided into: (i) aliquots for toluene carbon isotope analysis, stored without headspace in 40 mL vials; (ii) aliquots for toluene concentration measurements (2 × 3.5 mL), transferred to a 10 mL headspace GC vials; (iii) aliquots for water isotopes analysis (1 mL) stored in 1.5 mL Supelco vials. Sample aliquots for toluene analysis were fixed with NaOH (100 mM). Additionally, samples for toluene concentration measurements were spiked with an aqueous ethylbenzene (0.01 mM) solution as internal standard.

Deuterium analysis

Water samples were analyzed for D_2O by pyrolysis in a reactor loaded with "glassy carbon" and Ni-coated carbon (EuroVector) at 1,480 °C. Separation of CO and H_2 was done in a molecular sieve column at 95 °C. Both gases were transferred to an isotope ratio mass spectrometer (IRMS) in a continuous He stream using a ConFlowIII system (Thermo Finnigan). δ^2H measurements were conducted with a IRMS (Thermo Finnigan MAT 253) with relation to VSMOW (Vienna Standard Mean Ocean Water), where δ^2H is expressed as:

$$\delta^2 H = \frac{\left(\frac{^2H}{^1H}\right)_{\text{sample}} - \left(\frac{^2H}{^1H}\right)_{\text{standard}}}{\frac{^2H}{^1H}_{\text{standard}}} \times 1000\ [‰] \qquad (1)$$

Toluene and oxygen measurements

Toluene concentrations were determined via headspace analysis on a Trace DSQ GC–MS instrument (Thermo Electron, Germany) equipped with a Combi PAL autosampler (CTC Analytics, Swizerland) as described in Anneser et al. (2008). Separation was done in a DB5 capillary column (J & W Scientific, USA) with helium used as the carrier gas. The detection limit for toluene was 10 µg L^{-1}. A range of specific toluene (Riedel-deHäen, 99.9 %, purchased from Sigma-Aldrich) concentrations (0–3 mg L^{-1}) in water was used to produce a standard curve applied for quantification.

Dissolved oxygen concentrations were measured in situ via an optode-array technique (FIBOX3, PreSens GmbH, Regensburg, Germany). Light conducting silica fibers with a spot of oxygen sensitive polymer foil glued to the tip were buried right from the beginning at different depths in the water saturated sand.

Compound specifics isotope analysis (CSIA)

Since during a chemical or biochemical reaction, molecules with light isotopes at the reactive site tend to react faster than molecules with heavy isotopes, an enrichment of heavy isotopes in the remaining parent compound takes place as the reaction progresses (e.g. Meckenstock et al. 2004). With our toluene pulse experiment, this fractionation phenomenon allows a direct prove of in situ biodegradation, since physical processes, such as sorption, and dispersion have only a minor effect to isotopic changes (Qiu et al. 2013).

In our experiment, only samples from the two ports in the central longitudinal transect were subjected to CSIA. The carbon stable isotope composition (δ^{13}C) of toluene was measured using a Velocity XPT purge and trap (P & T) sample concentrator with an AQUATek 70 liquid autosampler (Teledyne Tekmar) coupled to a gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS). The GC-C-IRMS system was composed of a TRACE GC Ultra gas chromatograph (GC) (Thermo Fisher Scientific, Milan, Italy) connected to a Finnigan MAT 253 isotope ratio mass spectrometer (IRMS) (Thermo Fisher Scientific, Bremen, Germany) via a FinninganTM GC Combustion III interface (C). An Optic 3 temperature programmable injector with LN2-cryofocusing option (ATAS GL International) was attached to the GC. The analytical column used, was a DB-5 (30 m × 0.25 mm; 1 µm film; J & W Scientific, Folsom, CA).

The δ^{13}C values are reported in permille [‰] relative to the Vienna PeeDee Belemnite (V-PDB) standard:

$$\delta^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{\text{sample}} - \left(\frac{^{13}C}{^{12}C}\right)_{\text{standard}}}{\frac{^{13}C}{^{12}C_{\text{standard}}}} \times 1000\,[‰] \quad (2)$$

CO_2 reference gas was calibrated to V-PDB and was introduced at the beginning and the end of each run. The δ^{13}C of toluene were determined for samples collected at the port F2-8. The resulting concentrations of ^{13}C and ^{12}C were calculated based on the following equations (Kopinke et al. 2005):

$$C^{13C} = C_{\text{total}} \times \frac{1}{1 + \frac{1}{\left(1 + \frac{\delta^{13}C_{\text{sample}}}{1000}\right) \times R_{\text{standard}}}} \quad (3)$$

$$C^{12C} = C_{\text{total}} - C^{13C} \quad (4)$$

The C^{12C} and C^{13C} stands for concentration of ^{12}C and ^{13}C whereas C_{total} is the total concentration of carbon; R_{standard} is the isotope ratio of the reference carbon source.

The mass balances of both isotopes ^{13}C and ^{12}C and their relative recoveries (RR) were further calculated by applying Eq. 11. The stable carbon isotope signature of toluene passing through sampling port F2-8 was calculated according to the following equation:

$$\delta^{13}C_{F2-8} = \frac{\frac{C^{13C}_{\text{initial}} \times RR^{13C}}{C^{12C}_{\text{initial}} \times RR^{12C}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000\,[‰] \quad (5)$$

Mathematical modeling

In general, the transport of solutes in a natural medium should be described by a three-dimensional (3-D) dispersion equation in which dispersion has a tensor form. However, the 3-D transport equation may be simplified to a 2-D model in the case of: (i) horizontal line-injection, performed on an infinitely long line, spread in the y-direction, and (ii) assuming a homogeneous granular porous medium, where the movement of the solute is considered along the x-axis parallel to the flow lines (Bear 1961, Scheidegger 1961). Such simplified 2-D equation, coupled with an instantaneous equilibrium sorption model and exponential degradation of pollutant, was applied and reads as follows:

$$D_L \frac{\partial^2 C}{\partial x^2} + D_T \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} + p \frac{\partial C_s}{\partial t} + \lambda C \quad (6)$$

with

$$C_s = k_3 C \quad (7)$$

$$p = \rho(1 - n)/n \quad (8)$$

where v [m d^{-1}] is the mean water flow velocity; D_L, and D_T are the longitudinal and the vertical transversal dispersion coefficients, respectively [L^{2}T^{-1}]; t is the time variable [T]; C is the solute concentration in water [ML^{-3}]; C_s is the concentration of solute adsorbed by aquifer material due to an instantaneous equilibrium reaction with a linear adsorption isotherm [M/M]; k_3 is the distribution coefficient for the instantaneous equilibrium sorption with a linear adsorption isotherm [L^{3}M^{-1}], p is the factor needed to express C_s in the same units as C in Eq. 6 [ML^{-3}] with n being the effective porosity of the granular medium, and ρ being the density of the aquifer material [ML^{-3}]; λ is the degradation constant [T^{-1}], which represents an irreversible first order reaction, and accounts for biodegradation in the liquid phase. For substances undergoing biodegradation, the value of λ depends on conditions existing in the investigated system; here λ was assumed to be constant.

In modeling pollutants which undergo an instantaneous equilibrium sorption (Eq. 7), the so-called retardation factor (R_3) is often used instead of k_3. R_3 is defined as follows (Maloszewski et al. 2003):

$$R_3 = 1 + pk_3 \quad (9)$$

In the case of non-sorptive and non-degradable substances ($k_3 = 0$; $\lambda = 0$), Eq. 6 reduces to:

$$D_L \frac{\partial^2 C}{\partial x^2} + D_T \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} \quad (10)$$

and describes the transport of a "so-called" ideal tracer.

The analytical solution of Eqs. 6 or 10, C(x, z, t), in the case of a pulse injection, as applied in the present study, is found in (Carnahan and Remer 1984).

The relative recovery (RR) of a tracer mass observed in the cross-section having the surface (F) perpendicular to the flow direction (x) after sufficiently long time ($t = \infty$) can be estimated as follows (Leibundgut et al. 2009):

$$RR(\infty) = Fvn \int_0^{\infty} C(x, z, t')dt'/M \quad (11)$$

where n is the effective porosity of the saturated zone and M is the mass of tracer injected.

The modeling was conducted with a program written in FORTRAN with graphics written in C. The best fit was obtained by a Trial-and-Error procedure combined with minimizing the sum of error squares. From the deuterium concentration curves mean water velocity and both dispersivities (longitudinal and transversal) were derived. Those three parameters were further applied as known parameters when modeling toluene concentration curves. Thus, toluene was modeled with two fitting parameters (R_3 and λ).

Results

Comparison of toluene and D_2O concentration data

Modeling of the deuterium break-through curves, by using the solution of Eq. 10, provided values for water flow velocity (v), as well as for both dispersivities, longitudinal and transversal ($\alpha_L = D_L/v$ and $\alpha_T = D_T/v$). The water velocities found in different observation ports varied between 1.58 m day^{-1} at F3-4 to 1.77 m day^{-1} at F1-4. Modeling results based on the deuterium tracer (D_2O) are summarized in Table 1. In the central longitudinal row of the indoor aquifer water flow velocities were very similar, i.e. $v = 1.61$ m day^{-1} and $v = 1.67$ m day^{-1} at F2-8 and F2-9, respectively. The longitudinal dispersivity $\alpha_L = 2.18$ cm was similar for all ports. The calculated mean water velocity $v = 1.65$ m day^{-1} was taken for further modeling of the transversal-vertical dispersivity as well as for calculating the mean porosity of the porous material from the observed volumetric flow rate of water through the system. The transversal vertical dispersivity was found to be $\alpha_T = 0.035$ cm (see Fig. 6) while the calculated effective porosity was equal to $n = 0.30$.

Taking those transport parameters into consideration, the solution of Eq. 5 was used to model the toluene concentration curves. Calibration of the model with measured concentrations of toluene was then performed with two fitting parameters: R_3 (Eq. 9) and λ. The results of the modeled toluene concentrations as observed in all ports are summarized in Table 2, while the curves with the best fit are shown in Figs. 2, 3, 4 and 5. Toluene followed an instantaneous equilibrium sorption with a retardation factor being equal to $R_3 = 1.26 \pm 0.01$. Derived from three sampling ports the first-order degradation rate (λ) found for toluene varied between 0.156 and 0.190 day^{-1}. Only the results from port F3-4, suggested the degradation rate to be more than one order of magnitude lower (0.014 day^{-1}). Such low degradation

Table 1 Transport parameters resulting from modelling of deuterium concentrations observed at different ports (depths) at the cross-section (transect) F at a flow distance of x = 4.2 m from the inlet. ME stands for model efficiency as defined by Hornberger et al. (1992)

Port	z (m bs)	T (h)	v (m day^{-1})	α_L (cm)	ME (%)
F2-8	0.36	62.5	1.61	2.18	99.0
F2-9	0.32	60.2	1.67	2.18	99.0
F1-4	0.30	57.0	1.77	2.18	97.5
F3-4	0.30	64.0	1.58	2.18	99.5

ME 100 % means an ideal fit

Table 2 Reactive parameters of modelled toluene concentrations for the different ports (depths) at cross-section (transect) F at a flow distance x = 4.2 m. ME stands for model efficiency as defined by Hornberger et al. (1992)

Port	z (m bs)	R_3 (−)	λ (h^{-1})	ME (%)
F2-8	0.36	1.26	0.156	98.0
F2-9	0.32	1.27	0.190	98.0
F1-4	0.30	1.26	0.190	92.0
F3-4	0.30	1.25	0.014	95.0

ME 100 % means ideal fit

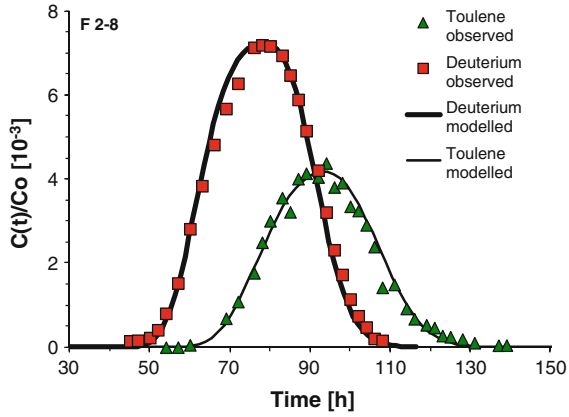

Fig. 2 Port F 2-8 (x [m] = 4.2, z [m bs] = 0.36); observed (*squares*, *triangles*) and modelled (*black solid* and *thin line*) break through concentration curves of deuterium and toluene, respectively

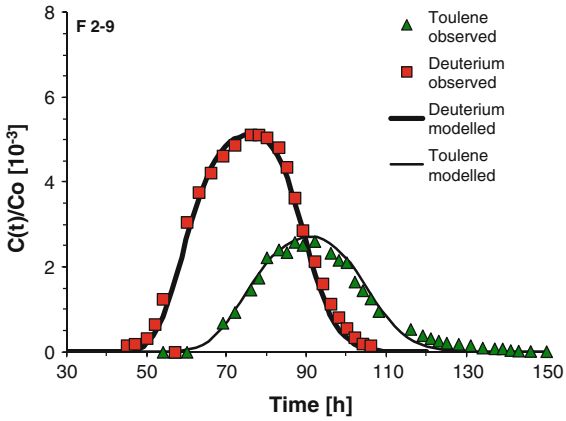

Fig. 3 Port F 2-9 (x [m] = 4.2, z [m bs] = 0.32); observed (*squares*, *triangles*) and modelled (*black solid* and *thin line*) break through concentration curves of deuterium and toluene, respectively

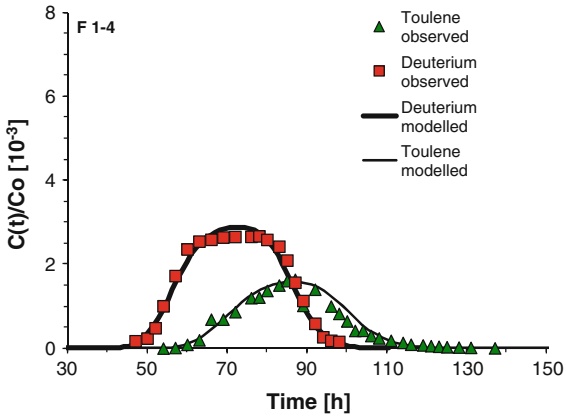

Fig. 4 Port F 1-4 (x [m] = 4.2, z [m bs] = 0.30); observed (*squares*, *triangles*) and modelled (*black solid* and *thin line*) break through concentration curves of deuterium and toluene, respectively

rate would mean that in this part of the system the toluene followed only an instantaneous equilibrium reaction without any significant degradation. Both hydraulic parameters determined with the conservative tracer, as well as the retardation factor R_3 agree well in all four ports, ruling out that water flow heterogeneity or sorption to the porous matrix are the reason for the abnormal behavior of toluene concentrations at sampling port F3-4. Finally, biological (bacterial) patterns as determined before and after the pulse experiment were not able to aid us with a satisfactory explanation. In conclusion, the patterns found at port F3-4 can at the moment not be

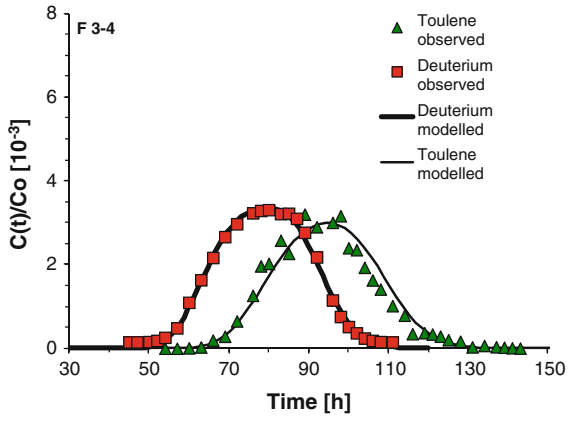

Fig. 5 Port F 3-4 (x [m] = 4.2, z [m bs] = 0.30); observed (*squares*, *triangles*) and modelled (*black solid* and *thin line*) break through concentration curves of deuterium and toluene, respectively

interpreted. Taking into account only three ports, the mean degradation rate was found to be $\lambda = 0.178\ \text{day}^{-1}$. This corresponds to a mean half-life time constant $T_{1/2}$ (= ln2/λ) of 3.87 days (Fig.6).

Based on transport and reaction parameters (Table 1, 2), the relative recovery of deuterium and toluene was calculated according to Eq. 11. For the cross-section area (F) being at a flow distance of x [m] = 4.2, the relative recoveries of deuterium and toluene were 100 and 61 %, respectively. Thus, along the flow distance of

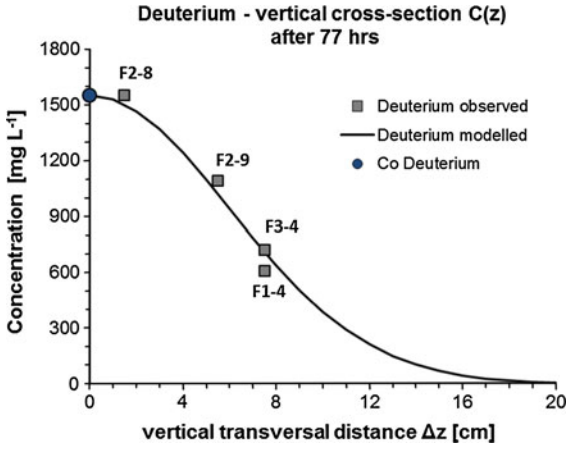

Fig. 6 Vertical distribution of deuterium concentrations C(z) measured (*squares*) and modelled (*line*) in the flow distance x [m] = 4.2 for the time of observation t [h] = 77 after starting of the injection. *The filled circle symbol* stands for calculated Co of deuterium leaving the horizontal mixing well

4.2 m and within 150 h, about 40 % of the toluene injected was immediately and irreversibly eliminated from the liquid phase. The elimination of toluene from the water and time-shifting of the toluene peak in comparison to the ideal tracer, as well as lack of a clear tailing underline the irreversible elimination of toluene via biodegradation only.

Concentration of dissolved oxygen dropped slightly during the course of experiment from 8.5 to 6.5 mg L^{-1} as measured in the toluene impacted zones.

Toluene carbon stable isotope composition

Independent of the total mass balance of toluene and D_2O, carbon stable isotope signatures from the central ports were used to calculate toluene ^{13}C and ^{12}C concentrations. Based on these data, a relative recovery of toluene ^{13}C and ^{12}C (Eq. 11) at port F2-8 was found to be 60.5 %, showing the overall enrichment of the heavy isotopes by 0.57 ‰.

Discussion

The enormous potential of oxic aquifers, and thus the ability of indigenous populations of aerobic bacteria to degrade a wide array of organic compounds, is well accepted and frequently reported in the literature (Aelion et al. 1987; Aelion and Bradley 1991; Alfreider and Vogt 2007; Barker et al. 1987; Cerniglia 1992; Dobbins et al. 1992; Gibson and Subramanian 1984; Gibson and Parales 2000). Moreover, the role of aerobic biodegradation as a fast and effective process in the initial removal of individual compounds from a bulk contamination has been underlined repeatedly (Kao and Wang 2001; Wiedemeier et al. 1999). However, in most cases investigations on the biodegradation potential of a given oxic aquifer was carried out in small scale batch or sediment column experiments using groundwater and/or sediment from the respective sites. Since the physical, geochemical and hydrological constrains for the transport and biodegradation of contaminants in field situations are complex and very different from small scale lab settings, very often these results cannot be easily extrapolated to the field. An exceptional approach was the one of Nielsen et al. (1996) who designed an in situ microcosm. Sediment entrapped by a core but left in place were donated to investigate aerobic degradation of aromatic hydrocarbons. However, although the sediment under investigation remained in situ, the corer system hydrologically disconnected it from the aquifer. Moreover, after the addition of organic contaminants, the sediment was aerated to avoid a shift to anoxic conditions, which most probably resulted in artificially high degradation rates. A weak point of many field studies is the inappropriate temporal and spatial resolution (Anneser et al. 2008, 2010) and one may fail to uncover the immediate in situ biodegradation potential present on a small scale.

The indoor aquifer system studied was intended to overcome most the limitations mentioned above. In a sampling campaign preceding the toluene pulse experiment, the pristine and energy-limited state of our experimental system was confirmed; concentrations of DOC were <1 mg L^{-1} throughout the aquifer and the total bacterial cell counts revealed $3 \pm 1.25 \times 10^4$ cells mL^{-1} groundwater and $1.28 \pm 0.40 \times 10^6$ cells cm^{-3} sediment. The 30 h pulse of a total of 486 mg toluene, injected into this oligotrophic (poor in productivity) and oligoalimonic (poor in nutrients) aquifer, were subject to an immediate degradation of 40 % of contaminant mass along a transport distance of 4.20 m and a mean travelling time of 150 h. Toluene and deuterium break-through curves followed at three selected sampling points close to the outlet of our indoor aquifer system (transect F) indicated toluene reduction to be almost exclusively attributed to biodegradation. Taking into account, that toluene is a compound that may also occur naturally (Head et al. 2003; Hornafius et al. 1999), we may assume that there are always microbes present in indigenous microbial communities, capable of aerobic toluene degradation. However, with respect to the moderate number of isolates of aerobic bacteria degrading toluene, as well as the lacking selective force for toluene oxidation before a contamination occurs the first time, these organisms should account for only low relative abundances within microbial communities in pristine aquifers. In our case, a follow-up experiment conducted with a continuous injection of contaminant, revealed a high relative abundance of key-degraders (e.g. *Pseudomonas* spp.) and considerable copy numbers of a marker gene (toluene monooxygenase, tmoA) after 16 days of exposure to toluene. In the pristine system at the time of first toluene injection tmoA was below detection in DNA extracts from water samples and the specific *Pseudomonas* strain (OTU bp 490) did

not play a prominent role in terms of abundance (Larentis and Lueders, unpublished data). There is no indication that during the short-term pulse experiment significant growth of microorganism took place. Mean bacterial growth rates in oligotrophic aquifers are considerably low, with community doubling times typically in the range of days to months or even years, rather than hours (Albrechtsen and Winding 1992; Kazumi and Capone 1994; Thorn and Ventullo 1988; Wilhartitz et al. 2009). Thus, the 30 h toluene pulse was most probably not long enough, to give sufficient time for indigenous communities to react, in terms of activation of dormant cells, and substantial growth of specific degraders, but biodegradation is attributed to an immediate response in activity (but not growth) of the aquifer's native microbial community. As estimated from incorporation of [^{3}H]-leucine into bacterial proteins and bacterial abundance data from a previous and a later sampling survey, the bacterial cell production in groundwater from the pristine indoor aquifer as well as 16 days after onset of a constant toluene injection, yielded generation times in the range of years (data not shown). Average community doubling times estimated from sediment samples were considerably higher compared to groundwater, but still in the range of months.

To our opinion, there is no doubt that the irreversible elimination of toluene as evaluated by breakthrough curves of toluene and deuterium as an ideal tracer, can be attributed to biodegradation. One qualitative argument is the observed change in oxygen concentration during the course of the experiment which clearly point at a microbially mediated aerobic toluene transformation. Moreover, the overall enrichment of δ^{13}C in the residual toluene at transect F is an independent prove for microbial degradation taking place apart from the mass balance calculations. Since the enrichment factors for aerobic toluene degradation are generally small (0.4–3.3 ‰) (Vogt et al. 2008) a shift of 0.57 ‰ is significant to demonstrate the occurrence of biodegradation and can indeed explain the 40 % of toluene loss. A Rayleigh-equation-based evaluation of toluene degradation, as conducted by Qui et al. (2013) and modeled by Eckert et al. (2013) for the same model system revealed between 33 and 22 % of toluene loss to be related to biodegradation. There is several examples from other aquatic ecosystems that showed the presence of hydrocarbon-degrading microorganisms prior to contamination, providing the backbone for a fast intrinsic biodegradation potential (Delille and Delille 2000; Hazen et al. 2010; Kostka et al. 2011; Margesin et al. 2003; Scow and Hicks 2005; Smets and Pritchard 2003).

We speculate that the potential to immediately attenuate the pulse of a significant amount of an organic contaminant in a pristine aquifer is limited over time and with respect to the total organic carbon load. Facing a continuous supply of a contaminant will most likely result in a ready depletion of dissolved oxygen which will turn the contaminated zones from oxic to anoxic. Since alternative electron acceptors are energetically less favorable and biodegradation via denitrification, iron reduction, sulfate reduction and methanogenesis is slower, the biodegradation potential will substantially decline. Moreover, in the case of high carbon loading microbial degradation in porous aquifers may become limited in any kind of dissolved electron acceptor over time (Bauer et al. 2008; Anneser et al. 2008, 2010).

The calculated first-order toluene degradation rate constant (day^{-1}) was estimated as high as $\lambda = 0.178$ day^{-1}. It is comparable with rates reported for aerobic toluene degradation from in situ mesocosms installed in an oxic, sandy aquifer (Nielsen et al. 1996), where degradation rates of toluene varied between 0.1 and 0.4 day^{-1}. In this study, however, a considerable lag phase (1–7 days) preceding the degradation of the aromatic compounds added were reported. Much higher toluene (input concentration = 2.9 mg L^{-1}) degradation rates ($\lambda = 12.5$–54.8 day^{-1}) were found in fast- and slow-velocity sediment column experiments performed by Angley et al. (1992). In contrast, a field study of Kao and Wang (2001), conducted in an oxic, porous aquifer contaminated with toluene in a concentration range between <0.0005–0.485 mg L^{-1}, provided degradation rate constants of $\lambda = 0.0042$–0.0043 day^{-1}, up to 2 orders of magnitude lower when compared to our findings. The huge variations in degradation rate constants among different studies might of course be due to the very individual natural attenuation potentials of the systems and material investigated, as well as a result of the initial concentration of the contaminant(s). The different experimental conditions (size of experiment, flow-through vs. closed system, temperature) and used approaches (sampling intervals, chemical analysis) substantially contributed to this discrepancy.

The toluene degradation rate constant, reported in the present study carried out in a flow-through system close to field scale and mimicking field conditions, contribute a fair approximation of a real field scenario.

Conclusions

This study reports on the presence of a surprising high potential for immediate, aerobic degradation of toluene, present in the indigenous microbial community of a pristine, energy-limited indoor aquifer system. The toluene virgin groundwater ecosystem was able to cope with a short pulse of toluene, degrading 40 % of a 486 mg toluene mass within the travel distance of only a few meters and the time frame of less than a week.

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