Biodegradation of petroleum hydrocarbon vapors: laboratory studies on rates and kinetics in unsaturated alluvial sand

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Abstract

Predictions of natural attenuation of volatile organic compounds (VOCs) in the unsaturated zone rely critically on information about microbial biodegradation kinetics. This study aims at determining kinetic rate laws for the aerobic biodegradation of a mixture of 12 volatile petroleum hydrocarbons and methyl tert-butyl ether (MTBE) in unsaturated alluvial sand. Laboratory column and batch experiments were performed at room temperature under aerobic conditions, and a reactive transport model for VOC vapors in soil gas coupled to Monod-type degradation kinetics was used for data interpretation. In the column experiment, an acclimatization of 23 days took place before steady-state diffusive vapor transport through the horizontal column was achieved. Monod kinetic parameters $K_s$ and $v_{max}$ could be derived from the concentration profiles of toluene, $m$-xylene, $n$-octane, and $n$-hexane, because substrate saturation was approached with these compounds under the experimental conditions. The removal of cyclic alkanes, isooctane, and 1,2,4-trimethylbenzene followed first-order kinetics over the whole concentration range applied. MTBE, $n$-pentane, and chlorofluorocarbons (CFCs) were not visibly degraded. Batch experiments suggested first-order disappearance rate laws for all VOCs except $n$-octane, which decreased following zero-order kinetics in live batch experiments. For many compounds including MTBE, disappearance rates in abiotic batch experiments were as high as in live batches indicating sorption. It was concluded that...
the column approach is preferable for determining biodegradation rate parameters to be used in risk assessment models.

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Keywords: Petroleum hydrocarbons; Natural attenuation; Vadose zone; Bioremediation

1. Introduction

Due to the widespread use of fuels, fuel components such as petroleum hydrocarbons and methyl tert-butyl ether (MTBE) are among the most frequent groundwater contaminants (Baehr et al., 1999). Accidental release of fuel to the subsurface results in residual pools retained in the unsaturated zone (Mercer and Cohen, 1990). This residual fuel can generate organic vapors in the soil gas phase that can migrate through the unsaturated zone by diffusion and advection (Scanlon et al., 2000). Gaseous transport of volatile organic compounds (VOCs) through the unsaturated zone has been identified as a serious threat for groundwater quality (Baehr et al., 1999; Pasteris et al., 2002). VOC vapors may also volatilize into the atmosphere, thereby creating a potential health threat to individuals living in the vicinity of emission sources (Jin et al., 1994). However, the unsaturated zone is a porous filter layer in which microbiological degradation naturally attenuates pollutants. It has been observed that, under favorable conditions, some petroleum hydrocarbons are rapidly and completely biodegraded in the unsaturated zone (Ostendorf and Kampbell, 1991; Hinchee and Ong, 1992; Lahvis et al., 1999). Vapor transport is also influenced by partitioning between liquid and gas phases, and by sorption onto soil particles (Jin et al., 1994; Li and Voudrias, 1994a,b; Baehr et al., 1999; Kim et al., 2001). Improved knowledge of the different processes operating in the soil and governing reactive gas transport is essential to estimate the migration of VOC vapors from contaminated sites through the unsaturated zone.

Several mathematical models have been proposed to describe the reactive transport of VOCs in the unsaturated zone (Jury et al., 1983; Jin et al., 1994; Baehr and Baker, 1995; Baehr et al., 1999). These models include typically first-order kinetics to represent biodegradation. First-order reactions are popular because of simplicity. They assume constant biomass, but do not reflect biological phenomena such as dependence on substrate concentration, inhibition or preferential substrate utilization (Schirmer et al., 1999). These factors can, however, be incorporated in the Monod equation (Monod, 1949). Monod kinetic parameters have been determined for various microorganisms growing in liquid cultures on VOCs such as BTEX (Robertson and Button, 1987; Alvarez et al., 1991; Chang et al., 1993; Duetz et al., 1997; Schirmer et al., 1999) or n-alkanes (Button, 1985). Also, the effects of substrate interactions on the Monod kinetic parameters have been described (Bielefelt and Stensel, 1999a,b; Reardon et al., 2000). All this work was based on microorganisms that have been isolated from aquatic ecosystems such as, e.g., sewage or aquifer sediments and were cultivated in the laboratory, usually in liquid media at relatively high carbon substrate concentrations. The transfer of these results to microorganisms in unsaturated soil is therefore not easily achieved. Autochthonous microorganisms have to cope with carbon limitation, exhibit moderate to low specific activity,
and may comprise a large number of inactive (dormant) cells (Dobbins et al., 1992; Haack and Bekins, 2000). Moreover, in the unsaturated zone, microbial populations generally live attached to surfaces, which may result in drastically reduced substrate availability (Harms and Zehnder, 1994; Simoni et al., 2001).

As noted by Jin et al. (1994), the experimental basis to understand biodegradation kinetics of VOC vapors in the unsaturated zone is still limited. A number of previous studies was based on laboratory batch microcosm experiments (English and Loehr, 1991; Allen-King et al., 1994a; Zhou and Crawford, 1995; Freijer et al., 1996; Ostendorf et al., 2000; Baker et al., 2000). Zhou and Crawford (1995) determined Monod kinetic parameters with total petroleum hydrocarbon in batch experiments with soils that were acclimated for 1.5 months to gasoline vapors. Drawbacks of batch techniques include the disruption of soil aggregates, changing boundary conditions (e.g. O2), and the need to apply much higher contaminant-to-soil ratios than observed in natural soil. Laboratory soil column experiments can avoid some of these limitations and can account for transport and degradation. Unsaturated laboratory column experiments have been performed with vapor transport being solely diffusive (Baehr and Baker, 1995) or advective and diffusive (Jin et al., 1994; Moyer et al., 1996). Other experiments included also aqueous transport in unsaturated laboratory columns (Allen-King et al., 1994b, 1996). Conflicting findings were obtained with unsaturated experimental systems, as first-order (Allen-King et al., 1994a; Jin et al., 1994; Moyer et al., 1996; Lahvis et al., 1999) as well as zero-order biodegradation kinetics (Baehr and Baker, 1995; Freijer et al., 1996; Baker et al., 2000) were reported for various compounds.

We report here on laboratory column and batch experiments to study the biodegradation kinetics of fuel VOCs in homogeneous unsaturated alluvial sand. The contaminant was a mixture of 13 VOCs typical for gasoline or kerosene with two recalcitrant fluorinated tracers. Kinetic parameters for individual compounds degraded from the mixture are reported. An outdoor lysimeter study was previously conducted with the same sand and contaminant source (Pasteris et al., 2002). In that study, first-order biodegradation rates were estimated from concentration vs. depth profiles of VOC vapors. However, the analytical certainty and the spatial and temporal resolution of data in that experiment were insufficient to gain detailed insight into biodegradation kinetics. This manuscript aims to determine biodegradation kinetic constants under well-defined conditions in the laboratory, as part of a larger European project developing an experimental base, models, and guidelines for groundwater risk assessment at contaminated sites (GRACOS).

2. Materials and methods

2.1. Fuel compound mixture

A mixture of 13 typical fuel compounds (Johnson et al., 1990; Cline et al., 1991; Potter and Simmons, 1998) and 2 chlorofluorocarbons (CFCs) as volatile organic tracers (Table 1) was prepared from products of >99% purity obtained from Fluka (Buchs, Switzerland). Trichlorofluoromethane (CFC-11) and 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113) were chosen because of their persistence under aerobic conditions (Höhener et al.,
The alluvial sand used in this study was extracted from Lake Geneva near the Rhone river delta, de-watered, and sieved <4 mm. No microorganisms were added before or during the experiments. Characteristics of the sand were reported previously (Pasteris et al., 2002).

2.3. Exposure experiment to quantify biomass changes during exposure to VOCs

Small glass dishes filled with 20 g of sand were incubated for 1–38 days in a closed glass jar containing humidified air and an open vial with 50 ml of the VOC mixture. The air phase was thus saturated with VOC vapors (see Table 1 for vapor concentrations). O₂ partial pressure was always >20%. Total microbial cell numbers were determined after extraction from the sand fraction <2 mm by shaking 10 g soil and 5 g glass beads (⌀=3 mm) in 50 ml distilled, cell-free water on a rotary shaker for 30 min. Extracts were stained with 1:10,000 diluted fluorescent dye Sybr Green II (Molecular Probes, Eugene, US). Twenty microliters of extract were filtered through a white polycarbonate filter (0.2 µm, Sartorius, Göttingen, Germany) and stepwise dried with ethanol (50%, 80%, and 96%).
Bacteria were counted in 16 randomly selected fields of 0.0001 cm$^2$ per sample with a microscope equipped for epifluorescence (Olympus BX-60, Olympus Optical, Tokyo, Japan). The variance ($\sigma$) is reported for counts of three samples from one extraction. Total protein in the sand was determined using the Bradford assay as described in Hess et al. (1996). Microbial biomass was calculated from the protein content using a conversion factor of 0.55 g protein g$^{-1}$ cells.

### 2.4. Laboratory column experiment

A one-dimensional horizontal column experiment was carried out during 51 days at room temperature ($23 \pm 2 \, ^\circ$C). The laboratory column (Fig. 1) of 120 cm length and 8.1 cm internal diameter made of acrylic glass was homogeneously packed with sand to a soil density of 1.49 g cm$^{-3}$. Voids of 3 cm length remained on both ends of the column. Tight packing and constant moisture self-stabilized the sand–air interface. The sand had previously been moistened with distilled water to the volumetric water content of 0.118 m$^3$ m$^{-3}$. This water content corresponds to 28% of the total porosity ($n_{tot} = 0.42$) and is above the water retention capacity (0.03 m$^3$ m$^{-3}$) for this coarse-textured sand. Due to the horizontal position of the column, no hydraulic gradient causing water advection along the column axis was present. A small hydraulic gradient that established across the column diameter was assumed to be of limited influence, as vapor transport was studied in the center of the column. The sand and its indigenous microbial community were left undisturbed during 20 days to acclimatize after column packing. After that period, designated day 0, the column was connected to a reservoir containing 10 ml of VOC mixture (Fig. 1), in a way that one end of the sand column was in direct contact with the fuel headspace. Fresh fuel was added on days 9 and 28, since by then some of the very volatile compounds were slightly depleted. The void space on the other end of the column was purged with a water-saturated airflow at a rate of 5 ml min$^{-1}$, in order to chase the fuel vapor without drying the sand. Fluxes of VOC vapors escaping from the column were quantified by multiplying vapor concentrations with the air flow rate. Periodical weighing of the column showed that loss of water was negligible. The column was equipped with 13 sampling ports positioned every 10 cm.

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**Fig. 1.** Schematic drawing of the column experimental set-up.
cm (Fig. 1). These ports were made with GC septa (Injection rubber plugs, Part No. 201-35584, Shimadzu, Kyoto, Japan) fitted into a hole of 4.8 mm diameter. Two ports allowed measurements of gas concentrations in the air at both ends outside of the sand and the others were for sampling of the gas phase in the sand. Gas samples were taken with gas-tight syringes equipped with a two-way valve and stainless steel hypodermic needles (length 50 mm; i.d. 0.15 mm).

2.5. Laboratory batch microcosm experiments

Bottles of 63 ml volume \((H \times \Omega = 90 \times 35 \text{ mm})\) closed with Teflon Mininert® valves (Supelco, Buchs, Switzerland) were used for microcosm experiments. After bringing the sand to the desired moisture content, it was filled into the bottle with a spoon and packed to a total porosity of 0.42 ± 0.02 without leaving any headspace in the bottle. Before adding the VOCs, the microcosms were stored at 25 °C for 24 h. Then, 2 ml of the VOC-saturated headspace of a bottle containing the fuel mixture at 25 °C were injected by using a stainless steel hypodermic needle (50 mm length) fitted to a gas-tight syringe. The injection was directed at the center of the bottle in the sand. Diffusion was the process responsible for the homogenization of vapor concentrations in the microcosm. Since diffusion is fast over short distances, homogeneous distribution was expected within a few minutes after vapor addition. Abiotic controls were prepared by autoclaving the sand three times at 120 °C for 20 min at intervals of 24 h and adding thereafter 0.2 g of NaN₃ per 100 g of sand. The sand used in this study was also separated into sieve fractions of 150–200 and 500–1000 μm. These fractions were treated separately as abiotic controls and abiotic losses were studied therein. Further controls were performed by injection of 10 ml of VOC vapor into empty bottles to account for gas leaks and sorption to glass and stoppers.

2.6. Analytical methods

Gas concentrations of volatile organic compounds were analyzed by injecting 50 μl of gas into an HP-6890 Series gas chromatograph (Agilent Technologies, USA) using gas-tight syringes with Teflon plungers. The GC method and detection limits were reported previously (Pasteris et al., 2002). The GC was calibrated by diluting the fuel mixture in cyclohexane, whereas calibration for cyclohexane was performed by diluting it in toluene. Partial pressures of CO₂ and O₂ were analyzed by injecting 100 μl of gas into a GC-8AIT gas chromatograph (Shimadzu) equipped with two PORAPAK Q columns (3 m × 1.6 mm) and a thermal conductivity detector operated at 55 °C, using N₂ as carrier gas. Dilutions of pure gases of CO₂ and O₂ were used for calibration.

3. Theory

3.1. Biodegradation kinetics

The basic assumptions underlying this work are that microorganisms are living in the aqueous phase of the unsaturated zone, that VOC vapors need to dissolve in the aqueous
phase before biodegradation can occur, and that biodegradation follows Monod kinetics. The hyperbolic function proposed by Monod (1949) to describe microbial growth as a function of the aqueous substrate concentration was modified by Lawrence and McCarthy (1970) to describe the removal rate of a growth limiting substrate as a function of the substrate concentration:

$$r_w(C_w) = \frac{v_{\text{max}}X C_w}{(K_s + C_w)}$$

where $$r_w(C_w)$$ (g substrate m\(^{-3}\) day\(^{-1}\)) is the reaction rate in the aqueous phase, $$v_{\text{max}}$$ (g substrate g\(^{-1}\) cells day\(^{-1}\)) is the maximum specific substrate utilization rate at infinite substrate concentration, $$X$$ (g cells m\(^{-3}\)) is the biomass in the aqueous phase, $$C_w$$ (g m\(^{-3}\)) is the carbon substrate (VOC) concentration in the aqueous phase, and $$K_s$$ (g m\(^{-3}\)) is the half-saturation constant in the aqueous phase. Note that use of $$C_w$$ in Eq. (1) assumes that carbon is the limiting element and that other elements such as oxygen, nitrogen, or phosphorus are assumed to be present in excess.

Concentrations of VOCs in soil water are difficult to measure directly. However, it can be assumed that $$C_w$$ is proportional to the concentration in soil air $$C_a$$ (g m\(^{-3}\)) via

$$C_a = HC_w$$

where $$H$$ is the dimensionless form of Henry’s law constant (g m\(^{-3}\) air/g m\(^{-3}\) water).

Combining and transforming Eqs. (1) and (2) gives the biodegradation rate in the aqueous phase as a function of the concentration in soil gas:

$$r_w(C_a) = \frac{v_{\text{max}}X C_a}{(HK_s + C_a)}$$

Eq. (3) assumes instantaneous equilibration of VOC between soil air and water. It furthermore regards reaction rates observed with constant biomass $$X$$. Growth was not regarded in this study but can be accounted for by the Monod-with-growth model (Simkins and Alexander, 1984; Kelly et al., 1996).

### 3.2. Coupling biodegradation, sorption, and diffusive transport

The transport model used in this study is a modified form of the diffusive reactive transport model used by Jin et al. (1994). In addition of the assumptions regarding the biodegradation processes, the following further assumptions were made: (1) diffusion is the dominant transport process and Fick’s law applies, (2) all solid surfaces are wetted, i.e., air/solid interfaces are absent, (3) sorption is linear and reversible, (4) volatilization obeys Henry’s law (Eq. (2)), (5) diffusion in soil water is very slow as compared to diffusion in soil air and thus negligible, and (6) the diffusion coefficient in soil air, as opposed to air, is reduced by a tortuosity factor $$\tau_a$$ as given by Millington and Quirk (1961):

$$\tau_a = \theta_a^{233}/n_{\text{tot}}^2$$

Here, $$\theta_a$$ (m\(^3\) air m\(^{-3}\) total) is the volumetric soil air content and $$n_{\text{tot}}$$ is the total porosity. Modifications from Jin et al. (1994) are assumption 5 and the omission of decay in the
sorbed phase. With these assumptions, the reactive transport of VOC vapors in soil can be expressed in terms of the soil air concentration $C_a$ as:

$$ R_a \frac{\partial C_a}{\partial t} = D \frac{\partial^2 C_a}{\partial z^2} - \theta_w r_w(C_a) $$

(5a)

where the capacity (or retardation) factor $R_a$ ($\text{m}^3 \text{ air m}^{-3} \text{ total}$) and the diffusion coefficient in soil $D$ ($\text{m}^2 \text{ day}^{-1}$) are defined as follows:

$$ R_a = (\rho_b K_d + \theta_w + \theta_a H)/H $$

(5b)

$$ D = \theta_a \tau_a D_a $$

(5c)

where $\rho_b$ (g m$^{-3}$) is the soil bulk density, $K_d$ ($\text{m}^3 \text{ g}^{-1}$) is the distribution coefficient between dissolved and solid phase, $D$ ($\text{m}^2 \text{ day}^{-1}$) is the effective diffusion coefficient of a fuel compound in soil air, $D_a$ ($\text{m}^2 \text{ day}^{-1}$) is the molecular diffusion coefficient in air calculated according to the method of Fuller as outlined in Schwarzenbach et al. (1993), and $\theta_w$ ($\text{m}^3 \text{ water m}^{-3} \text{ total}$) is the volumetric soil water content.

### 3.3. Solutions applying to the column experiments

#### 3.3.1. First-order

Due to the non-linearity of the Monod equation, analytical solutions of Eq. (5a) generally cannot be found. However, solutions for first-order and zero-order kinetics are available. At steady state, the left-hand side of Eq. (5a) is zero and the capacity factor $R_a$ has no influence. When $HK_s \gg C_a$, Eq. (3) becomes a first-order rate law. For the column experiment, an analytical solution describing the special case of first-order biodegradation at steady-state was published by Wilson (1997) for the boundary conditions

$$ C_a = C_{a,0} \text{ at } z = 0 $$

(6a)

$$ C_a = 0 \text{ at } z = L $$

(6b)

$$ C_a = C_{a,0} \frac{\sinh \left[ \sqrt{\mu_c^1} (L - z) \right]}{\sinh \left[ \sqrt{\mu_c^1} L \right]} $$

(6c)

$$ \mu_c^1 = \frac{v_{\text{max}} \theta_w X}{HK_s} $$

(6d)

Here, $\mu_c^1$ (day$^{-1}$) is a lumped first-order rate coefficient applying to the column experimental setup, $C_{a,0}$ is the concentration in the source headspace, and $L$ (m) is the length of the soil column. Note that $\theta_w X$ is the biomass per unit volume of the column (g cells m$^{-3}$). $\mu_c^1$ can be determined for each compound in this study by fitting this solution to the measured $C_a(z)$ profiles.
3.3.2. Zero-order

When $HK_s \ll C_a$, Eq. (3) becomes a zero-order rate law. An analytical solution for Eqs. (5a)–(5c) and zero-order kinetics at steady-state is available (Eweis et al., 1998) for the boundary conditions:

$$C_a = C_{a,0} \text{ at } z = 0$$  \hspace{1cm} (7a)

$$\frac{dC_a}{dz} \to 0 \text{ at } z \to \lambda, \text{ with } \lambda = \sqrt{\frac{2C_{a,0}D}{\mu_c^0}}$$  \hspace{1cm} (7b)

$$C_a = C_{a,0} \left[ 1 + \frac{z^2 - 2z\lambda}{\lambda^2} \right]$$  \hspace{1cm} (7c)

$$\mu_c^0 = v_{\text{max}}\theta_wX$$  \hspace{1cm} (7d)

Here, $\mu_c^0 \text{ (g substrate m}^{-3} \text{ day}^{-1})$ is a lumped zero-order rate coefficient applying to the column experimental setup. Note that the $\lambda$ (Eq. (7b)) corresponds to the penetration depth of VOC vapors into the column (Eweis et al., 1998). Note also that the assumption of zero-order kinetics is violated near this penetration depth where $C_a$ becomes small.

3.3.3. Determination of Monod parameters

Following Suidan and Wang (1985) as described in Ostendorf and Kampbell (1991), Monod kinetic parameters can be obtained from VOC fluxes inferred from hydrocarbon concentration profiles. Therefore, concentration versus distance profiles have to be transformed to flux versus distance profiles. Diffusive fluxes $F \text{ (g m}^2 \text{ day}^{-1})$ of hydrocarbon vapors through the soil column are calculated using Fick’s law:

$$F = -D \frac{\partial C_a}{\partial z}$$  \hspace{1cm} (8)

Fluxes for each compound are calculated from $C_a(z)$ profiles from the concentration gradient at two adjacent sampling ports. By coupling Eqs. (3), (5a)–(5c), and (8) and deriving $F^2$ as a function of a dimensionless concentration, the following equation can be written (Ostendorf and Kampbell, 1991):

$$\frac{\partial (F^2)}{\partial C^*_a} = 2Dv_{\text{max}}HK_s\theta_wX \frac{C^*_a}{(1 + C^*_a)}$$  \hspace{1cm} (9)

where $C^*_a = C_a/HK_s$ is a dimensionless form of the VOC concentration.

Following Ostendorf and Kampbell (1991), the variables in Eq. (9) can be separated and integrated:

$$F = \left[ 2Dv_{\text{max}}\theta_wXHK_s(C^*_a - \ln(1 + C^*_a)) \right]^{1/2}$$  \hspace{1cm} (10)

When $D$, $H$, and $\theta_wX$ are known, $K_s$ and $v_{\text{max}}$ can be derived by fitting Eq. (10) to $(F, C^*_a)$, using the solver modules provided by commercially available software. In this study, Excel (Microsoft) and Kaleidagraph (Abelbeck) yielded the same results.
3.4. Solutions applying to batch experiments

For batch experiments, the solutions of Eqs. (5a)–(5c) for first-order and zero-order case are

First / order : \( C_a = C_{a,0} \exp \left\{ -\mu_{c}^l t / R_a \right\} \)  \hspace{1cm} (11a)

Zero / order : \( C_a = C_{a,0} - \frac{\mu_{c}^l t}{R_a} \) \hspace{1cm} (11b)

where \( C_{a,0} \) is the concentration in the soil gas after initial homogenization of vapors. It should be noted that, for the batch experiments, the capacity factor \( R_a \) accounts for abiotic losses of VOC vapor. The first-order exponential loss rate in a batch equals \( \mu_{c}^l / R_a \) and the zero-order loss rate equals \( \mu_{c}^0 / R_a \).

4. Results

4.1. Biomass formation in sand upon exposure to VOCs

Before exposure to VOC vapors, the sand used for all experiments contained \( 3 \pm 0.6 \times 10^8 \) cells g\(^{-1}\) dry sand \((n=6)\). This corresponded to \( 0.24 \pm 0.05 \) mg protein g\(^{-1}\). During exposure to VOC vapor in the closed jar, cell numbers rose without significant lag (Fig. 2) and reached \( 8.8 \pm 0.8 \times 10^8 \) cells g\(^{-1}\) and \( 0.71 \pm 0.18 \) mg protein g\(^{-1}\) on day 38.

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Fig. 2. Microbial cell numbers in sand as a function of exposure time to VOC vapors, measured during the exposure experiment in the closed glass jar.
Fig. 3. Evolution of concentration profiles of selected VOC compounds in the column. Solid squares: day 1, open triangles: day 7, solid circles: day 23.
4.2. Column experiment

The evolution of longitudinal VOC concentration profiles in the column was monitored during 56 days. Profiles of four selected compounds obtained after 1, 7, and 23 days are shown (Fig. 3). The concentration of CFC-113 decreased linearly after 1 day. No significant changes were observed thereafter. MTBE profiles were curved on day 1 and to a lesser extent on day 7 with concentrations below detection limit at the column end. On day 23 and thereafter, the profile was linear. Concentration profiles of n-octane and m-xylene remained curved throughout the experiments with no compound entering the columns further than approximately 50 cm.

Fig. 4. Evolution of the partial pressures of carbon dioxide (CO$_2$) and oxygen (O$_2$) between 3 days before and 44 days after contamination. (○) day −3, (■) day 1, (▲) day 7, (×) day 21, (□) day 32, (●) day 44.
The corresponding partial pressures of CO2 and O2 are given in Fig. 4. Three days before exposure to fuel vapor, the CO2 partial pressure was in the range of 0.3%. During exposure to fuel vapor, it rose steadily and reached a maximum of 4.6% close to the fuel inlet on day 21 before decreasing again. Partial pressures of O2 were recorded from day 21 on. They showed the corresponding inverse trend with lowest concentrations of 13.3% on day 21 at the fuel inlet of the column (Fig. 4). The volumetric water and soil air contents remained constant at \( \theta_w = 0.118 \) and \( \theta_a = 0.302 \) throughout the experiment.

On day 56, the protein content in a sand sample taken at 0.53 m distance from column end was \( 0.78 \pm 0.58 \) mg protein g\(^{-1}\) dry sand. Assuming a bulk density of 1480 kg dry sand m\(^{-3}\) and a conversion factor of 0.55 g protein g\(^{-1}\) microbial cells, the microbial cell mass per unit volume in the column (equalling \( \theta_w X \)) is estimated to be \( 0.30 \pm 0.05 \) g cells m\(^{-3}\) before contamination and \( 0.96 \pm 0.71 \) g cells m\(^{-3}\) on day 56.

Fig. 5. VOC profiles along the column after 23 days. Symbols: measured concentrations. Solid lines: first-order model (Eqs. (6a)–(6d)). Broken line: zero-order model (Eqs. (7a–7d); \( n \)-octane only).
Table 2  
Summary of rate laws and rate constants obtained for column and batch experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Column on day 23</th>
<th>Batch</th>
<th>Lysimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate law giving best fit</td>
<td>First-order rate $\mu_I$ (day$^{-1}$)</td>
<td>Capacity factor$^a$, $R_a$</td>
</tr>
<tr>
<td>$n$-Pentane</td>
<td>–</td>
<td>&lt; 0.01</td>
<td>0.43</td>
</tr>
<tr>
<td>$n$-Hexane</td>
<td>Monod$^d$</td>
<td>0.26</td>
<td>0.54</td>
</tr>
<tr>
<td>$n$-Octane</td>
<td>Monod$^d$</td>
<td>5.0</td>
<td>1.33</td>
</tr>
<tr>
<td>$n$-Decane</td>
<td>first-order</td>
<td>13.5</td>
<td>5.08</td>
</tr>
<tr>
<td>Methylcyclopentane</td>
<td>first-order</td>
<td>0.1</td>
<td>0.48</td>
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<tr>
<td>Methylcyclohexane</td>
<td>first-order</td>
<td>0.16</td>
<td>0.67</td>
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<td>Cyclohexane</td>
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<tr>
<td>Isooctane</td>
<td>first-order</td>
<td>0.09</td>
<td>0.42</td>
</tr>
<tr>
<td>(2,2,4-trimethylpentane)</td>
<td>first-order</td>
<td>1.31</td>
<td>5.09</td>
</tr>
<tr>
<td>Toluene</td>
<td>Monod$^d$</td>
<td>3.28</td>
<td>11.1</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>Monod$^d$</td>
<td>4.98</td>
<td>26.3</td>
</tr>
<tr>
<td>1,2,4-Trimethyl-benzene</td>
<td>first-order</td>
<td>–</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MTBE</td>
<td>–</td>
<td>&lt; 0.01</td>
<td>0.44</td>
</tr>
<tr>
<td>CFC-113</td>
<td>–</td>
<td>&lt; 0.01</td>
<td>0.56</td>
</tr>
<tr>
<td>CFC-11</td>
<td>–</td>
<td>&lt; 0.01</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$ Measured with Eq. (5b) using $n = 1.48$ kg l$^{-1}$, $\theta_s$ = 0.302, $\theta_w$ = 0.118, and $K_d$ values taken from Pasteris et al. (2002).

$^b$ Mean ± standard deviation of two live and five abiotic batch experiments.

$^c$ Rate in live sand not significantly different from rate in abiotic sand.

$^d$ Data shown in Table 3.

$^e$ Better fit: zero-order rate in live batches $1.68 ± 0.24$ g m$^{-3}$ day$^{-1}$. 

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Concentration versus distance profiles of all VOCs are shown for day 23 (Fig. 5). This day was chosen because CO₂ and O₂ partial pressures (Fig. 4) indicated maximum biodegradation and VOC migration data (Fig. 3) indicated the establishment of a steady state of substrate VOC transport and consumption. Besides linear profiles of CFC-113 and MTBE, and n-dodecane concentrations below detection limit at all sampling ports, all other VOC profiles were curved. Most of these profiles were reasonably well explained by the reactive transport model with first-order biodegradation (Eqs. (6a)–(6d)). Vapors of six compounds (toluene, n-octane, n-decane, n-dodecane, m-xylene, and 1,2,4-trimethylbenzene) did not extend to the column outlet on days 23 (Fig. 5) and 44 (data not shown). Hence, fluxes of these compounds across the sand–air interface at the outlet were zero. For all the other VOCs, fluxes at the column outlet could be determined (Table 2). The total flux of all VOCs at the column inlet on day 21 was 3.9 ± 0.8 g m⁻² day⁻¹. The corresponding flux of CO₂ was 3.0 ± 0.6 g C m⁻² day⁻¹, while the flux of O₂ into the column was 12.4 ± 2.5 g m⁻² day⁻¹.

All concentration profiles except those of CFC-11 and n-dodecane were transformed into flux versus concentration profiles using Eqs. (8) and (9) (data not shown). The parameters $K_s$ and $v_{\text{max}}$ were estimated from such profiles by curve fitting using Eq. (10). Monod kinetic parameters for toluene, m-xylene, n-octane, and n-hexane could be obtained this way and compiled with literature data (Table 3). For the other compounds, the flux was constant indicating no degradation activity (CFC-113, MTBE, and n-pentane), or flux versus concentration profiles were linear indicating first-order kinetics over the entire range of concentrations (cyclic alkanes, isooctane, and 1,2,4-trimethylbenzene), or they had too few data points to be interpreted (n-decane). Monod parameters could not be obtained in any of these cases.

Table 3
Comparison of Monod coefficients obtained in this study with selected literature values

<table>
<thead>
<tr>
<th>VOC</th>
<th>Experimental system</th>
<th>Temperature (°C)</th>
<th>$K_s$ (g m⁻³)</th>
<th>$v_{\text{max}}$ (g g⁻¹ cells day⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Xylene</td>
<td>saturated batch, pristine sandy aquifer</td>
<td>10</td>
<td>0.79</td>
<td>7.9 ± 2.3a</td>
<td>Schirmer et al., 1999</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>batch, gasoline-contaminated soil</td>
<td></td>
<td>16</td>
<td>6.2b</td>
<td>Goldsmith and Balderson, 1988</td>
</tr>
<tr>
<td>Xylene</td>
<td>batch, creosote-contaminated soil</td>
<td>24–26</td>
<td>1.17 ± 0.38</td>
<td>15.8 ± 2.6b</td>
<td>Kelly et al., 1996</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>alluvial sand column</td>
<td>23 ± 2</td>
<td>1.04 ± 0.70</td>
<td>0.96 ± 0.39</td>
<td>this study</td>
</tr>
<tr>
<td>Toluene</td>
<td>batch, creosote-contaminated soil</td>
<td>24–26</td>
<td>0.039</td>
<td>2.2b</td>
<td>Kelly et al., 1996</td>
</tr>
<tr>
<td>Toluene</td>
<td>saturated sandy aquifer</td>
<td>25</td>
<td>17.4</td>
<td>9.9</td>
<td>Alvarez et al., 1991</td>
</tr>
<tr>
<td>Toluene</td>
<td>alluvial sand column</td>
<td>23 ± 2</td>
<td>&lt; 0.3</td>
<td>0.29 ± 0.05</td>
<td>this study</td>
</tr>
<tr>
<td>n-Octane</td>
<td>P. putida (oleovorans) GP01</td>
<td>0.0008</td>
<td></td>
<td>22b,c</td>
<td>Lageveen, 1986</td>
</tr>
<tr>
<td>n-Octane</td>
<td>alluvial sand column</td>
<td>23 ± 2</td>
<td>0.004 ± 0.001</td>
<td>2.45 ± 0.45</td>
<td>this study</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>alluvial sand column</td>
<td>23 ± 2</td>
<td>0.005 ± 0.002</td>
<td>0.21 ± 0.16</td>
<td>this study</td>
</tr>
</tbody>
</table>

a Using reported cell yield to convert from $\mu_{\text{max}}$.  
b Assuming a cell yield of 0.5 g cells g⁻¹ substrate degraded.  
c $v_{\text{max}}$ obtained in pure culture studies may not be comparable to $v_{\text{max}}$ in mixed cultures.
4.3. Batch experiments

In control experiments using bottles without sand, gaseous concentrations of all VOCs except \( n \)-dodecane (data not shown) stayed within \( \pm 10\% \) of the initial concentrations for 15 days (Fig. 6). In abiotic controls, concentrations of all VOCs decreased between 0.5

![Graph showing concentration ratios of various VOCs over time for different conditions: empty control, abiotic sand, and biotic sand.](image)
and 5.5 h after VOC injection following first-order rate law (Fig. 6) with rate constants ranging between 0.13 ± 0.13 day⁻¹ for methylcyclohexane and 0.91 ± 0.52 day⁻¹ for toluene (Table 2). The gaseous concentrations of n-dodecane and to a lesser extent also of n-decane and 1,2,4-trimethylbenzene (data not shown) decreased in an erratic manner, suggesting condensation and sorption. No CO₂ production or O₂ consumption (data not shown) was found. Total losses in sterile sand within 14 days ranged between 5% for CFC-11 (Fig. 6) and 90% for m-xylene. In live sand, all compounds except n-octane and MTBE decreased with first-order kinetics between 0.5 and 5.5 h after injection. First-order rate constants evaluated from data points after 0.5 h in live sand are shown in Table 2. n-Octane disappearance in live sand appeared to be fitted slightly better with a zero-order than with a first-order rate law (Fig. 6), although the last data point may indicate that the degradation rate declined slightly at a concentration below 0.03 g m⁻³. MTBE decreased for 2.5 h with neither a zero- nor a first-order law and was constant thereafter. CO₂ production and O₂ consumption within 5.5 h in live batch experiments were below detection limit.

5. Discussion

5.1. Biomass formation with VOC vapors

An exposure experiment was carried out to follow changes in microbial biomass in sand as a function of exposure to VOC vapors. A two-fold increase in microbial numbers occurred within only 5 days of exposure (Fig. 2), suggesting that growth initially is not limited by nutrients and that toxicity is not a major problem. As a consequence, batch degradation experiments conducted to infer degradation kinetic parameters should be significantly shorter to avoid influences of changing biomass. During longer exposure to VOC vapors, the microbial numbers rose more slowly and the assumption of constant biomass for exposure times of a few weeks may be justified.

5.2. Column experiment

A column experiment was conducted to study steady state VOC degradation after a period of acclimatization dominated by sorption and air–water partitioning of VOC, which is accounted for by the capacity factor Rᵦ. During this period, some bacterial growth took place as was seen from protein measurements in the column. Values for Rᵦ were calculated according to Pasteris et al. (2002) (Table 2). High Rᵦ values are a result of a strong tendency for of the compounds partitioning either into the aqueous phase (hydrophilic compounds such as MTBE) or into the solid phase (hydrophobic compounds such as n-dodecane). The time needed to reach steady state is linearly related to Rᵦ. CFC-113 (Rᵦ = 0.44) and MTBE (Rᵦ = 5.6) reached linear concentration profiles after 1 and 21 days, respectively (Fig. 3). The steady state is characterized by stable VOC concentration profiles indicating that the sum of biomass production and specific degradation activity and abiotic removal rate is constant and equals the mass-transfer rate. At steady state, the Monod-no-growth model was applied to infer kinetic constants from instantaneous
concentration versus distance profiles, provided that certain assumptions (listed in the theory section) are fulfilled. Some of these assumptions could be verified. An independent gas tracer experiment performed in the column as described in Werner and Höhener (2002) allowed the calculation of a tortuosity factor of 0.37 ± 0.02, which is in good agreement with a value of 0.35 obtained using the Millington–Quirk relationship (Eq. (4)). The dominance of diffusion as the transport process for VOCs was demonstrated by consistent fluxes of the two inert compounds CFC-113 and MTBE through the column with respect to Fick’s law. On day 21, fluxes of 0.17 and 0.91 g m⁻² day⁻¹ were measured at the column outlet (Table 2), which were in agreement with calculated diffusion fluxes of 0.13 and 1.17 g m⁻² day⁻¹ for CFC-113 and MTBE, respectively.

VOC profiles along the column were compared with the analytical solution (Eqs. (6a)–(6d)) of the coupled transport biodegradation model using first-order kinetics for biodegradation (solid lines in Fig. 5). The good match of most profiles suggests that the reactive transport model with first-order degradation is a useful approximation. Recalcitrant or slowly biodegraded VOCs such as CFC-113 and MTBE exhibited linear profiles with distance, in accordance to μ₁ values < 0.01 day⁻¹ (Fig. 5). Easily biodegraded VOCs such as n-octane and toluene exhibited strongly curved profiles with μ₁ values larger than one per day (see Table 2). A calculated n-octane profile obeying zero-order kinetics (Eqs. (7a)–(7d)) was compared with the measured profile (Fig. 5) since n-octane degradation had followed zero-order kinetics in batch experiments. However, the first-order model fit measured data slightly better than the zero-order model (Fig. 5).

The biodegradation rates of n-octane, n-hexane, toluene, and m-xylene and compounds deviated from first-order kinetics (Fig. 5) near the column inlet, thus allowing the calculation of Monod kinetic constants. Fitting the data with the Ostendorf and Kampbell (1991) approach (Eq. (10)) yields the lumped parameters HK and vₘₐₓDX₀w from which Kₛ and vₘₐₓ were obtained by dividing by known H (Table 1) or estimates of DX₀w, respectively. Table 3 compares the values obtained with those collected by a number of other investigators using various experimental setups. There is considerable variability in the data for each compound, which may be due to the kind of microbial community or the experimental conditions. Nevertheless, a few general trends can be observed. For n-octane and n-hexane, lower Kₛ values are reported than for BTX. Among the BTX, Kₛ values for toluene are frequently smaller than for xylenes (Table 3). The results for the n-alkanes, toluene, and m-xylene in this study show the same trend. The somewhat lower vₘₐₓ for the xylenes compared to toluene and n-octane may be explained by substrate toxicity at high concentrations (Kelly et al., 1996). It should be noted that vₘₐₓ express substrate utilization rate on a basis of total cells, but, in studies with mixed substrates, not all cells may be involved in the degradation of one specific substrate. This explains the relatively low vₘₐₓ obtained in our study and other mixed culture studies compared to values from pure cultures growing on one substrate.

5.3. Batch experiments

The batch experiments in this study were designed to keep the ratio of soil to soil air as close as possible to that in real soils, reducing the influence of sorption to stopper or to glass, which was reported to be significant over long time scales (Ostendorf et al., 2000).
The duration of all experiments involving live sand was kept shorter than 0.3 days in order to avoid also significant bacterial growth. As in the column experiment, vapor concentrations were monitored instead of aqueous concentrations. Occasional leaks in stoppers were identified using the CFC data. Analytical problems were encountered for the three compounds with the highest boiling temperatures, \( n \)-dodecane (216 °C), \( n \)-decane (174 °C), and 1,2,4-trimethylbenzene (169 °C), probably due to condensation of the vapors in batch bottles and syringes used for sampling. It is concluded that the batch experimental technique in this study can be applied only to VOCs with boiling temperatures smaller than about 160–170 °C.

After the initial addition of VOC vapors to sand, 0.5 h (2.5 h for MTBE) were needed for diffusive mixing of the vapors in the bottle. The concentration versus time profiles obtained thereafter were generally too inaccurate to distinguish unambiguously between zero- or first-order kinetic rate laws for degradation (Fig. 6). However, the interpretation of batch experimental data in terms of kinetic constants was seriously complicated by the fact that only four VOCs had disappearance rates in live sand which were significantly larger than disappearance rates in abiotic controls (Table 2). The use of large soil air (headspace) to soil volumes ratios as, e.g., in the experiments by Allen-King et al. (1994a) or Zhou and Crawford (1995) would minimize the importance of abiotic loss as compared to biodegradation, but require much longer incubation, thereby increasing the probability of bacterial growth or toxic effects.

For most compounds, two phases of disappearance were observed. Rapid abiotic losses due to sorption and partitioning took place during the first 30 min after addition. They were followed by slower sorption as can be seen from the abiotic controls and biodegradation. Even for the recalcitrant CFCs, abiotic disappearance rates of 0.3 day\(^{-1}\) were measured (Table 2). The slow ongoing sorption in abiotic batches may be interpreted as intraparticle diffusion-limited approach of equilibrium between soil water and soil particles. A characteristic of intraparticle diffusion is its dependency on the particle radius \( a \) with faster sorption kinetics obtained with coarser materials (Grathwohl and Reinhard, 1993). In order to test this, abiotic batch experiments were performed using sieved sand fractions (Fig. 7). Again rapid initial decline occurred within the first 0.5 h. Thereafter, abiotic losses were more pronounced in the fraction 500 < \( a < 1000 \) μm and followed first-order kinetics (Fig. 7), whereas in the sieve fraction 150 < \( a < 200 \) μm sorption obviously nearly reached equilibrium within the first 30 min. Intraparticle diffusion is thus a likely mechanism for abiotic loss of VOCs during short-term batch experiments. In a similar batch experiment with poisoned sandy soil (Allen-King et al., 1994a), toluene vapor concentration was found to decrease rapidly during the first hour, then more slowly during the next 60 h and finally stayed constant during 600 h.

5.4. Comparison of data from column and batch experiments

The kinetic data obtained from both experimental approaches cannot be compared directly due to the different nature of the experiments. Acclimatization of the sand in the column led to growth of microorganisms and to a closer approach to sorption equilibrium. At steady-state conditions in the column, a lumped first-order rate coefficient \( \mu_c^1 \) independent of sorption, partitioning, and retardation can be calculated. To estimate
first-order rates in the column approach, only the knowledge of diffusion coefficients is needed. In batch experiments, VOC disappearance depends on $R_a$ (Eqs. (11a) and (11b)) and the parameters therein ($H, K_d, \rho_b, \theta_a, \theta_w$). Estimation of the biodegradation rate in batch experiments requires thus that all these parameters are accurately known. Furthermore, the gaseous concentrations in the column experiment at the column inlet (Fig. 5) were about 10 times higher than the initial gaseous concentrations in the live batch experiments (Fig. 6). At low concentrations, first-order rate laws are more likely to be expected. All this makes it a priori difficult to compare kinetic rate data obtained with these experiments. The first-order rate constants obtained in the column experiment can, however, be compared with those obtained in the field lysimeter experiment (Pasteris et al., 2002). A good correlation of first-order rate constants for all compounds is found for those two experiments (Table 2).

6. Conclusions

Kinetic rate laws of VOC biodegradation in unsaturated alluvial sand were determined in column and batch laboratory experiments. First-order kinetics was a good approximation for most of the compounds in both experimental systems, with $n$-octane as the only exception out of 10 VOCs that were biodegraded. Only the column approach allowed us to
measure Monod kinetic parameters. The correct interpretation of kinetic biodegradation parameters in unsaturated batch experiments remains a difficult task. Abiotic losses pose problems when working in short incubations with large soil/headscape ratios and changes in microbial communities pose problems when working in long-term incubations with low soil/headscape ratios. The study confirms furthermore the recalcitrance of MTBE vapors. Unlike, e.g., toluene or \textit{m}-xylene vapors, MTBE vapors are not attenuated within 1.14 m of homogeneous unsaturated alluvial sand.

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**References**


