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Soil Physical Constraints on Intrinsic Biodegradation of Petroleum Vapors in a Layered Subsurface

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Abstract

Naturally occurring biodegradation of petroleum hydrocarbons in the vadose zone depends on the physical soil environment influencing field-scale gas exchange and pore-scale microbial metabolism. In this study, we evaluated the effect of soil physical heterogeneity on biodegradation of petroleum vapors in a 16-m-deep, layered vadose zone. Soil slurry experiments (soil/water ratio 10:30 w/w, 25°C) on benzene biodegradation under aerobic and well-mixed conditions indicated that the biodegradation potential in different textured soil samples was related to soil type rather than depth, in the order: sandy loam > fine sand > limestone. Similarly, O₂ consumption rates during in situ respiration tests performed at the site were higher in the sandy loam than in the fine sand, although the difference was less significant than in the slurries. Laboratory and field data generally agreed well and suggested a significant potential for aerobic biodegradation, even with nutrient-poor and deep subsurface conditions. In slurries of the sandy loam, the biodegradation potential declined with increasing in situ water saturation (i.e., decreasing air-filled porosity in the field). This showed a relation between antecedent undisturbed field conditions and the slurry biodegradation potential, and suggested airfilled porosity to be a key factor for the intrinsic biodegradation potential in the field.

Accidental release of petroleum hydrocarbons to terrestrial environments is typically related to leaking pipelines and storage tanks buried near ground level in the vadose zone (USEPA, 2004). Risks posed by a spillage in this heterogeneous multiphase system depend on naturally occurring processes influencing the fate of individual pollutants (Fischer et al., 1996). Petroleum hydrocarbons in the ground involves generation of a vapor plume of volatile organic compounds (VOCs) that spread rapidly through unsaturated soil pores and

fractures (Christophersen et al., 2005). As a result, key gas transport pathways of concern include potential intrusion of VOCs into residential buildings (Fischer et al., 1996; Hers et al., 2000; Patterson and Davis, 2009) and downward VOC migration to groundwater aquifers (Baehr et al., 1999; Pasteris et al., 2002; Christophersen et al., 2005).

In general, the dominating transport mechanism of VOCs in the deep vadose zone is gas diffusion, governed by temperature and soil physical properties including total soil porosity, water content, and tortuosity of the soil pore network (Buckingham, 1904; Moldrup et al., 2001; Choi and Smith, 2005). Besides acting as a transport medium, under aerobic conditions the vadose zone functions as a biological air filter in which petroleum vapors can be degraded by native soil microbes (Höhener et al., 2006; Abreu and Johnson, 2006; DeVaul, 2007). Contrary to immobilization by sorption and dilution by transport processes, biodegradation can reduce the total mass of contaminants in the environment. Thus, the potential for natural biodegradation merits attention when setting up conceptual models for risk assessment and management of petroleum spill sites (Höhener et al., 1998; Downey et al., 1999; DeVaul, 2007). Moreover, aerobic in situ bioremediation has proven to provide a cost-effective and environmentally friendly alternative to conventional excavation and pump-and-treat techniques (Balba et al., 1998; Boopathy, 2000; USEPA, 2004). Bioremediation technologies applicable for treating vadose zone contamination include bio-venting and monitored natural attenuation, both of which rely on indigenous microbial populations to transform organic pollutants into less toxic metabolites (Downey et al., 1999). As a result, to ensure successful bioremediation, it is necessary to possess a solid understanding of microbial soil ecology and its complex interrelation with the physical and chemical conditions prevailing within the soil pore space (Balba et al., 1998).

The abundance, diversity, and functions of soil microorganisms are governed by factors that control microbial growth and activity in general, i.e., the availability of water, a substrate, terminal electron acceptors, and nutrients and environmental factors such as pH, salinity, toxic compounds, and temperature (Holden and Fierer, 2005). These factors are again closely related to the physical soil formation, defined by the size, shape, and arrangement of particles, aggregates, voids, and water films (Smiles, 1988; Young and Crawford, 2004; Or et al., 2007). In general, finetextured soils tend to harbor larger microbial populations than coarser soils, as organic matter and nutrients can attach to the significantly higher surface area associated with clay and silt particles (Taylor et al., 2002; Young and Crawford, 2004). An additional feature of soil texture is its relation to soil moisture conditions, and thereby the potential liquid- and gas-phase diffusion of O₂ and substrate to the soil bacteria (Stark and Firestone, 1995; Hers et al., 2000; Holden et al., 2001; Davis et al., 2003, 2005). Skopp et al. (1990) suggested a conceptual model with a condition of “optimal” water saturation, where the addition of water will limit the gaseous O₂ supply, while the loss of water will limit substrate diffusion to the bacteria attached in water films on the particles. In very dry soil, for example at water potentials lower than -600 kPa, physiological effects associated with dehydration will prevent significant microbial activity (Stark and Firestone, 1995). The optimal water saturation is typically assumed to be around 60% of the total soil porosity (Greaves and Carter, 1920; Linn and Doran, 1984), although Schjonning et al. (2003) showed that it varies with soil texture. This emphasizes the strong link existing between soil texture, water content, transport properties, and the activity of soil microbes, for which reason geologic heterogeneity has a profound effect on microbial processes (Or et al., 2007) and the fate of petroleum vapors in the vadose zone (Davis et al., 2005; Bazkurt et al., 2009).

Studies of hydrocarbon biodegradation have traditionally been based on microcosms (English and Loehr, 1991; Zhou and Crawford, 1995; Xu and Obbard, 2003). In most cases, the experiments are performed using slurry systems, where optimal mixing, aeration, and

improved substrate bioavailability is achieved. Although slurry reactors rarely reflect in situ conditions, slurries are commonly applied at hydrocarbon spill sites to screen for possible limitations of bioremediation, such as nutrient deficiency and insufficient populations of degrading bacteria (Balba et al., 1998). Field investigations of hydrocarbon biodegradation have predominantly been performed based on in situ respiration tests (Davis et al., 1998; Pearce and Pretorius, 1998; Aichberger et al., 2005), and O₂ and CO₂ gas profile analysis (Lahvis and Baehr, 1996; Lahvis et al., 1999; Hers et al., 2000; Davis et al., 2009). These studies have mostly used shallow and homogeneous vadose zones, but since geochemical and biophysical conditions governing biodegradation processes at the field scale are strongly related to depth and soil type, it has been suggested that studies focusing on deep, heterogeneous subsoils are needed (Taylor et al., 2002; Holden and Fierer, 2005). In addition, only a limited number of studies have addressed biodegradation processes in unsaturated soil deeper than 5 m belowground (e.g., Konopka and Turco, 1991; Fredrickson et al., 1995; Davis et al., 2009).

We conducted a laboratory and field investigation assessing the effects of geologic heterogeneity on biodegradation in a 16-m-deep, layered vadose zone contaminated with petroleum hydrocarbons. The site is interesting because (i) hydrocarbon-degrading bacteria are dominant due to low natural organic matter contents, and (ii) the distribution of petroleum vapors is highly heterogeneous as a result of the site geology. Our objective was to link the potential for subsurface biodegradation to the physical, chemical, and biological characteristics. First, soil samples were collected from all major soil layers and characterized in the laboratory in terms of soil texture, water content, air-filled porosity, concentration of total petroleum hydrocarbons (TPH), nutrient availability, soil pH, gas diffusivity, and direct counts of soil bacteria. Second, soil slurry experiments using benzene amendment of suspended soil samples were performed to determine aerobic biodegradation potentials under well-mixed conditions, reflecting the size of benzene-degrading populations at the time the experiments were set up. Third, in situ respiration tests were conducted in different-textured soil layers to compare with laboratory data.

Materials and Methods

Site Description

The study was performed in Nyborg (55°18'42" N, 10°47'31" E), Denmark, at a former gasoline station. The site is contaminated with petroleum hydrocarbons due to leaching from underground storage tanks; the station was operating almost 30 yr until 2001, at which time the tanks were removed. At that time, around 200 m² of the polluted source area was excavated down to 2 to 6 m below ground surface (bgs); however, considerable amounts of hydrocarbons, of which benzene, toluene, ethylbenzene, and xylene isomers (BTEX) make up about 25%, still remain above the groundwater table, which is situated 15.5 to 16 m bgs.

The ground surface at the site has a slab or asphalt cover in the contaminated area, and water-phase leaching of contaminants and seasonal water content fluctuations in the vadose zone are limited. The site geology is heterogeneous, with a number of horizontal and different-textured soil layers, as exemplified in Fig. 1. The top 10 m are dominated by sandy loam glacial till enclosing a 2- to 3-m layer of water-bearing and unfractured limestone at depths of around 5 to 9 m bgs. From 10 to 13 m bgs, the soil type is fine sand, followed by various layers of silt and limestone just above the groundwater table. Seasonal water table fluctuations are <0.5 m. The highly layered geology has resulted in several unconnected light nonaqueous-phase liquid (LNAPL) hot spots, typically confined within thin sandy lenses on top of sediments of lower permeability. From these hot spots, petroleum vapors have generated a vapor plume covering most of the vadose zone below the source area. A

large but stable plume of hydrocarbons has been detected in the groundwater that flows in the southeastern direction.

A total of 26 soil-gas samples previously collected from eight well screens at depths ranging from 5 to 15 m bgs indicated that in the sandy loam (2–10 m bgs) and the silty capillary fringe (14–16 m bgs), O₂ concentrations were depleted to 0 to 1.5%, whereas the layer of fine sand (10–13 m bgs) was semiaerobic with an O₂ concentration of 4 to 5%. In screens located on the outer edge of the source area, O₂ concentrations were >9% in the sand layer. Carbon dioxide and total hydrocarbon vapor concentrations generally declined with increasing O₂ concentrations, as seen in Fig. 2, which shows data collected from a range of depths and locations at the site. This indicated that natural biodegradation of petroleum vapors probably occurs in the contaminated source area.

Sampling and Preparation of Soil Samples

Seven boreholes (B301–B307) situated as shown in Fig. 3 were drilled using a SonicSampDrill, Giesbeek, the Netherlands. Boreholes B301, B302, B306, and B307 were in the contaminated source area, while B303, B304, and B305 were in areas only slightly impacted at the time of the study. Intact soil cores (diameter = 50 mm) were extracted from below 2 m bgs throughout the boreholes. From these cores, a total of 100 samples of loose soil were collected every 0.5 m (with few exceptions) from 2 to 16 m bgs, as illustrated in Fig. 4. Of the soil samples collected, 80 samples could be classified as sandy loam, fine sand, or limestone. A total of 20 samples did not fit within this classification and are in the following referred to as “miscellaneous,” representing various textures.

All samples were stored in the absence of light and air at 7°C in gas-tight plastic bags. Before analysis of the physical, chemical, and biological soil properties, contaminated soil samples were left in the fume hood for 24 h while VOCs were evaporated. The loss of water during this period of time (<2% w/w) was subsequently replaced with distilled water. Representative subsamples were collected after mass reduction using a riffle splitter (10 chutes of 19.2 mm). Biological analyses (i.e., counts of total soil bacteria and soil slurry experiments) were performed typically within 1 mo, and maximum 2 mo, after soil sampling. Tests using loamy and sandy soil samples from another site indicated that the procedures for sample preparation and storage changed the results of the chemical and biological analyses <10%.

General Soil Characteristics

The gravimetric water content in 60-g subsamples was determined after oven drying for 48 h at 105°C. Additional basic soil characteristics were determined for air-dried, ground, and sieved subsamples (5 mm) of the 100 loose-soil samples. Soil pH was measured using a pH probe directly in a suspension of soil in 1 mol L⁻¹ KCl (soil/liquid ratio 1:5 w/w) after mixing for 1 h. Inorganic N (N_{inorg}) was extracted in a 1 mol L⁻¹ KCl solution (soil/liquid ratio 1:5 w/w) as described in Keeney and Nelson (1982). Soil total N contents (N_{total}) was determined for a suspended soil sample (soil/water ratio 1:20 w/w) after addition of a 10 g L⁻¹ K₂S₂O₈ solution (soil/oxidant ratio 1:10 w/w) and digestion at 120°C and 200 kPa for 30 min. Nitrogen concentrations were analyzed colorimetrically using an autoanalyzer (Technicon TRAACS 800, Bran+Luebbe GmbH, Norderstedt, Germany). Readily available P (P_{avail}) was extracted in a 0.5 mol L⁻¹ NaHCO₃ solution (soil/liquid ratio 1:20 w/w) as described in Olsen and Sommers (1982). Total P contents (P_{total}) was extracted in 1 mol L⁻¹ HCl (soil/liquid ratio 1:10 w/w) and digested at 120°C and 200 kPa for 30 min. Extractions of P_{total} were not performed for samples with a CaCO₃ content >40%. Phosphorous concentrations were analyzed colorimetrically using the molybdenum blue method (Murphy and Riley, 1962). All chemical analyses were performed in duplicate. Texture analysis was

performed using a combined hydrometer and wet-sieving method on eight representative soil samples, divided between sandy loam ($n = 4$), fine sand ($n = 2$), and limestone ($n = 2$). Detailed texture analysis could not be performed for samples containing $>40\%$ CaCO_3 .

Soil Core Measurements

The dry bulk density, water- and air-filled porosities, and the potential for soil gas diffusion in the main geologic layers of the field site were determined for 19 intact soil samples of 100 cm^3 collected from 3 to 15 m bgs during the borehole construction. The samples were divided between sandy loam ($n = 7$), fine sand ($n = 6$), limestone ($n = 3$), and miscellaneous ($n = 3$). The one-chamber method described in Rolston and Moldrup (2002) was used to determine the relative soil-gas diffusivity, D_p/D_0 , where D_p is the gas diffusion coefficient in soil ($\text{cm}^3 \text{ soil air cm}^{-1} \text{ soil s}^{-1}$) and D_0 is the gas diffusion coefficient in free air ($\text{cm}^2 \text{ air s}^{-1}$). Following the gas diffusion measurements, the water content of each core sample was measured gravimetrically and the dry bulk density and water- and air-filled porosities were calculated. In addition, the water content at -10 kPa , typically corresponding to the natural water-holding capacity (Al Majou et al., 2008), was determined for each soil core as described in Jury and Horton (2004).

Direct Counts of Soil Bacteria

Staining by 4',6-diamidino-2-phenylindole (DAPI) was used to determine the total numbers of soil bacteria in 11 samples divided between sandy loam ($n = 5$), fine sand ($n = 4$), and limestone ($n = 2$). Samples corresponding to 0.5 mg (dry weight) were fixed in 5 mL of 2% formaldehyde, whereupon the samples were diluted and homogenized using a filament piston. A volume of 950 μL of sample solution was stained with 50 μL of DAPI (1 mg L^{-1}). The bacteria were collected by filtration through a 0.22- μm black polycarbonate filter. The filters were mounted in paraffin oil and counts were determined using a fluorescence microscope.

Soil Slurry Experiments

Short-term slurry experiments on potential aerobic biodegradation of benzene were performed using subsamples with natural water content (corresponding to 10 g of dry matter) in 120-mL glass reactors. A volume of 30 mL of distilled water was added to each reactor, which was subsequently sealed with a gas-tight rubber stopper and acclimatized at 25°C for 3 d. Gaseous benzene was injected into the headspace, achieving a final dissolved concentration of about $3 \text{ mg benzene L}^{-1}$. The reactors were not amended with nutrients and it was calculated that the O_2 present in the reactor headspace was sufficient to maintain aerobic conditions throughout the experiment. This was confirmed by measurements of the O_2 concentration after biodegradation had finished. After benzene amendment, the reactors were placed on a shaking table and gas samples of 0.1 mL were extracted from the headspace (assuming equilibrium with the liquid phase) with 1-mL disposable plastic syringes every 4 to 12 h and for 75 h in total. Previous experiments suggested that adsorption of benzene to the plastic syringe and rubber stopper was negligible in the time frame considered. Gas sample analysis was performed using gas chromatography with flame ionization detection, with oven, injector, and detector temperatures of 80, 150, and 200°C , respectively. Gases were separated by a WCOT CP-select 624CB column (30 m by 0.53 mm) with N_2 as the carrier gas (5.2 mL min^{-1}). A first-order rate model including a lag phase was fitted to each set of data (dissolved benzene concentration vs. time). Abiotic controls of one sandy and one loamy sample were prepared by autoclaving for 1 h daily for 3 successive d. All soil slurry experiments were performed in duplicate.

Vadose Zone In Situ Respiration Tests

A test well was constructed at a distance of 0.5 m from Borehole B307 in the asphalt-covered source area as shown in Fig. 3. The soil stratigraphy in the borehole was: sandy fill material from 0 to 4 m bgs, sandy loam from 4 to 6.5 m bgs, water-bearing limestone from 6.5 to 9.5 m bgs, fine sand from 9.5 to 13.7 m bgs, and various layers of silt and sand from 13.7 m to the water table at 15.5 m bgs. Soil and gas characteristics were as given in Table 1. Two 22-mm internal diameter polyethylene screens were installed from 5 to 6 m (in sandy loam) and 11 to 12 m bgs (in fine sand). The screened intervals were packed with medium sand and sealed below and above with granular bentonite packing. A He tracer test showed that no leaking from the installations was taking place. In addition, the test showed that the 3-m layer of water-bearing limestone provided an effectively impermeable barrier between the two screens, allowing separate respiration tests to be performed simultaneously in the upper and lower screens. Atmospheric air was injected for 24 h using a flow of $2 \text{ m}^3 \text{ h}^{-1}$ in the sandy loam and $3 \text{ m}^3 \text{ h}^{-1}$ in the fine sand. The lower flow in the sandy loam was due to a lower permeability. After the aeration, O_2 and CO_2 concentrations were measured in the injection screens using a portable multigas analyzer (LMSx, Gas Data Ltd, Coventry, UK) after 1, 2, 3, 4, 6, 8, 16, 24, 32, 40, 48, and 72 h. The screens were prepumped before each measurement, corresponding to twice the volume of the 22-mm screen and tube.

Results and Discussion

Soil Characterization

Table 1 shows the results of the general characterization of the samples of sandy loam, fine sand, limestone, and “miscellaneous” soil. The sandy loam had a high gravel and coarse sand content, whereas the fine sand and limestone layers were relatively homogeneous. The soil pH was generally >8 , reflecting the calcareous conditions. Low contents of natural organic matter ($<0.2\%$ w/w) and N ($<20 \text{ mg total N kg}^{-1}$) illustrate typical subsurface conditions. In addition, $N_{\text{total}}/P_{\text{total}}$ ratios around 1:20 indicate possible N limitation of microbial growth in the contaminated soil zones where C is abundant. The total soil porosity generally varied from about $0.34 \text{ m}^3 \text{ m}^{-3}$ in the sandy loam to $0.40 \text{ m}^3 \text{ m}^{-3}$ in the fine sand. The water-filled porosity was generally in agreement with measurements of the natural water-holding capacity. This was expected, as temporal fluctuations of the soil water content are limited in the deep vadose zone beneath the paved source area. Since the moisture conditions are close to field capacity, biodegradation is not likely to be limited by low water content (Skopp et al., 1990; Stark and Firestone, 1995).

Potential for Gas Transport

Estimated air-filled porosities of $0.04 \text{ m}^3 \text{ m}^{-3}$ in the limestone and $0.21 \text{ m}^3 \text{ m}^{-3}$ in the fine sand illustrate significantly varying potential for gas transport across the different soil layers. In the sandy loam, the average air-filled porosity was $0.093 \text{ m}^3 \text{ m}^{-3}$, which is around the critical limit generally indicating an interconnected soil pore network (Troeh et al., 1982). Measurements of relative soil gas diffusivity (D_p/D_0) at in situ water contents showed results of 0.007 in the limestone, 0.021 in the sandy loam, and 0.031 in the fine sand. Early studies of Grable and Siemer (1968) and Stepniowski (1980, 1981) concluded that critical relative diffusivity limits for adequate soil aeration are in the range of 0.005 to 0.02. This suggests that uncontaminated soils with $D_p/D_0 > 0.02$ generally allow aerobic conditions to occur (Schjonning et al., 2007). Based on the airfilled porosity and gas diffusivity measurements, we inferred that gas diffusive fluxes of O_2 and petroleum vapors will be low in limestone layers, whereas gas diffusion to some extent is possible in sandy loam layers. High air-filled porosity and D_p/D_0 in the deep layer of fine sand demonstrate favorable conditions for gasphase diffusion. This is supported by O_2 gas concentrations of about 5% measured in the fine sand layer, as mentioned above. As the layer of limestone represents an

effective barrier for vertical gas diffusion of O₂ from the atmosphere, it is believed that O₂ diffuses horizontally from less contaminated soil zones where O₂ is abundant, through the sand layer, and into the contaminated soil zones where it will be available for aerobic biodegradation of petroleum hydrocarbons. Oxygen-limited biodegradation is in agreement with previous soil gas measurements in Fig. 2 suggesting that CO₂ and vapor TPH decline with increasing O₂ content.

Linking Slurry Data to Antecedent Soil Conditions

The soil slurry experiments generally proceeded as shown in Fig. 5. Abiotic controls of fine sand and sandy loam illustrated that sorption and abiotic degradation of benzene can be neglected in these low-organic subsoils, in which case decreasing benzene concentrations can be attributed to biodegradation. Biodegradation was typically observed after a lag phase of 5 to 20 h and followed first-order kinetics with rate constants (k_1) ranging up to 5 d⁻¹ with an arithmetic mean of 1.30 d⁻¹. For comparison, Table 2 shows a literature review on published k_1 values for benzene biodegradation. We note that the k_1 values found in this study are within the range of previous findings, despite the nutrient-poor conditions present in the soil.

Soil Stratigraphy and Texture

Figure 6 shows depth profiles of k_1 , water content, and soil stratigraphy in Boreholes B301 and B303, representing typical boreholes inside and on the outer edge of the contaminated source area. The water content was related to the varying geology, as layers of limestone were nearly saturated; the sandy loam retained water contents between 10 and 15% w/w; and the fine sand had slightly less moisture. The k_1 is highly variable throughout the profiles, which is probably related to a significant soil physical heterogeneity and the presence of minor lenses within the larger soil layers. A drastic decrease of k_1 was generally observed when the soil texture changed from sandy loam to fine sand at about 10 m bgs. This is in conflict with previous studies suggesting that the subsoil microbial density (and activity) follows a smooth curve with depth, mainly due to the natural variation of soil organic matter (Holden and Fierer, 2005). This would not be the case, however, at very low organic matter contents and when the soil physical properties vary as in this study.

To further illustrate the texture effect, average values of k_1 for the main soil types are shown in Fig. 7a. Despite standard deviations varying between 0.06 and 1.45 d⁻¹, the Mann–Whitney one-sided test (Mann and Whitney, 1947) showed that soil-type averages of k_1 were significantly different ($\alpha = 0.05$) in the order: sandy loam > fine sand > limestone. The results support the concept that fine-textured soils in general have a higher microbial activity than coarser soils as long as the air-filled porosity in the field allows sufficient aeration. If the antecedent air-filled porosity is low in the field, as was typically the case for the limestone, the microbial activity in the slurry experiments using limestone samples will be low as well.

Figure 7b shows empirical probability distributions for k_1 , which were found to be normally distributed. The remaining data were lognormally distributed, supporting the idea that biodegradation potentials are linked to more than one parameter. The high range of k_1 determined illustrates the difficult task of choosing input data for risk assessment modeling at the field scale. Thus, biodegradation kinetics should be selected based on site-specific conditions and should account for the significant natural variability (e.g., in terms of a probability distribution). In general, high geologic and soil physical heterogeneity result in high microbial heterogeneity as well (as illustrated in Fig. 6).

Total Bacterial Populations and Nutrient Availability

Direct counts of bacteria in selected soil samples were in the order of 10^8 cells g^{-1} and in agreement with previously reported counts from pristine subsoil samples collected below 2 m bgs that ranged from 10^7 to 10^9 cells g^{-1} (Konopka and Turco, 1991; Taylor et al., 2002; Kaufmann et al., 2004). The one-sided Mann–Whitney test showed that counts of total soil bacteria were significantly highest in the sandy loam ($\alpha = 0.05$) and lower but similar in samples of fine sand and limestone. The higher direct counts in soils of finer texture are consistent with the slurry biodegradation experiments and with the findings of Taylor et al. (2002) and Watwood et al. (1991), which showed a higher microbial abundance, enzymatic activity, and biodegradation rate in clayey soils than sandy soils. Although the petroleum pollution in this low-organic vadose zone represents the main C source for the soil bacteria and the nutrient availability is low, plots of the biodegradation potential in terms of k_1 against TPH concentration, direct counts of soil bacteria, and nutrient concentrations (N_{inorg} and P_{avail}) show no significant correlations ($R^2 < 0.1$) (data not shown). It should be noted that direct counts do not reflect active degrading bacteria, as was illustrated by relatively high cell numbers but low biodegradation potentials in the limestone samples. Furthermore, it is probable that biodegradation at this site is essentially due to metabolism of non-growing cells in soil zones where O_2 availability is sufficient for aerobic biodegradation to take place. This was also reported from a Danish field experiment on transport and biodegradation of an emplaced hydrocarbon mixture in a shallow nutrient-poor vadose zone (Kaufmann et al., 2004; Höhener et al., 2006). Furthermore, the conclusion that biodegradation can occur under nutrient-limiting conditions is supported by field experience from 135 test sites at more than 50 U.S. Air Force installations, which found that naturally occurring levels of N and P are generally sufficient to sustain adequate degradation rates in the field (Downey et al., 1999).

Water Saturation and Air-Filled Porosity

The sandy loam was the soil type displaying the highest range of k_1 . Figure 8a and 8b show k_1 in 45 samples of sandy loam as a function of the antecedent water saturation and air-filled porosity in the field. Except for eight samples displaying low microbial activity across a range of in situ water contents, the biodegradation potential generally declines with increasing in situ water saturation and decreasing air-filled porosity. The data were fitted with an empirical diffusion-based model of microbial activity suggested by Skopp et al. (1990), showing a relatively strong fit ($R^2 = 0.73$). This supports the concept that soil physical constraints in the undisturbed field environment had a prolonged impact on the size and activity of the indigenous microbial populations that could persist for some time (>1 – 2 mo) and influence the outcome of the slurry experiments. In addition, the results underline the fact that the potential for gas exchange of petroleum vapors and O_2 in the field is critical for significant microbial growth and natural biodegradation to occur.

The eight samples with low biodegradation potential have probably experienced inhibiting conditions other than high water content, possibly including toxic effects due to high contaminant concentrations or large-scale O_2 limitation related to impermeable geology. This demonstrates the idea that a range of different factors can be governing, but with the soil gas diffusivity creating an approximate upper boundary for the biodegradation potential, as proposed by Skopp et al. (1990).

Comparison with In Situ Respiration Data

Figure 9 shows that results of in situ respiration tests performed in layers of sandy loam and fine sand in the vadose zone. The O_2 utilization rate (k_{O_2}) was 4.5% O_2 d^{-1} in the sandy loam and 1.6% O_2 d^{-1} in the fine sand. Similarly, the CO_2 production rate was highest in the sandy loam, but was probably affected by the high alkalinity in the calcareous soils

dominating the field site (Hinchee et al., 1992). The O₂ utilization rates can be transformed to zero-order hydrocarbon degradation rates assuming a mean stoichiometric relationship of 3.5 kg O₂ kg⁻¹ TPH (based on hexane mineralization) for the average oxidation of petroleum hydrocarbons (Hinchee et al., 1992). The following equation was applied:

$$k_B = \frac{k_{O_2} \rho_{O_2} \varepsilon}{100 \rho_b B} \quad [1]$$

where k_B is the zero-order hydrocarbon degradation rate (kg TPH kg⁻¹ soil d⁻¹), ρ_{O_2} is the density of O₂ at ambient soil temperature (kg m⁻³), ε is the air-filled porosity (m³ air m⁻³ soil), B is the mass ratio of O₂ to hydrocarbon required for mineralization (kg O₂ kg⁻¹ TPH), and ρ_b is the dry bulk density (kg soil m⁻³ soil). At 10°C and 0.1 MPa, the ρ_{O_2} is calculated as 1.38 kg m⁻³, and the values of ε and ρ_b are given in Table 1. Consequently, the zero-order degradation rate is 0.94 mg TPH kg⁻¹ d⁻¹ in the sandy loam and 0.83 mg TPH kg⁻¹ d⁻¹ in the fine sand, corresponding to 1.6 and 1.3 g m⁻³ d⁻¹ in the field, respectively. The higher rates obtained in the sandy loam is in agreement with the slurry experiments, albeit the difference between sandy loam and fine sand appears to be less significant in the field. This suggests that diffusive limitation in the low-permeability sandy loam played a role during the in situ test, while this was not the case in the well-mixed slurries.

As for the slurry experiments, the in situ tests support the idea that aerobic degradation of hydrocarbons is possible in the unsaturated subsurface. The in situ rates obtained are, however, in the low range of what is commonly observed during wellfunctioning bioventing (Downey et al., 1999). Since BTEXs were dominating the petroleum vapors at the site and were probably degraded first after O₂ injection, it is reasonable to compare field data with slurry experiments on benzene biodegradation. The mean estimated zero-order degradation rates from the slurry experiments at 10°C were 1.67 mg benzene kg⁻¹ d⁻¹ in sandy loam and 0.96 mg benzene kg⁻¹ d⁻¹ in fine sand, which are less than a factor of 2 higher than the rates estimated from the in situ test.

Since the physical environment surrounding the microbes was drastically changed in the transition from field to slurry conditions, the k_1 were assumed to represent the relative size of active aerobic bacterial populations degrading benzene and other nonpersistent hydrocarbons, rather than rates under field conditions (Balba et al., 1998). Thus, the agreement between field and laboratory data was unexpected and in contrast to a number of studies suggesting that biodegradation rates increase with the decreasing scale of the experiments due to heterogeneity effects and diffusion limitation on a larger scale (Davis et al., 2003; Aichberger et al., 2005). Our study demonstrates that this relation is probably site and contaminant specific, as also illustrated by the results of Hohener et al. (2006).

Conclusions

The potential for biodegradation of petroleum vapors in a 16-m-deep vadose zone was evaluated. The site geology was dominated by different-textured horizontal soil layers, causing a heterogeneous distribution of pollutants. The subsurface was low in natural organic matter and the pollution probably represented the essential C source to the indigenous soil microbial populations.

Laboratory and field results confirmed aerobic intrinsic biodegradation of petroleum vapors as a potential attenuation process in the deep subsurface. Benzene biodegradation rates obtained from aerobic and well-mixed slurry experiments were generally consistent with the

range of rates reported in the literature and also with hydrocarbon degradation rates determined from two in situ respiration tests performed at the site.

Slurry experiments using different-textured soil samples further indicated that the first-order rate constant (k_1) was related to the soil type (rather than the sample depth) in the order: sandy loam > fine sand > limestone. Similarly, the in situ respiration tests suggested slightly faster O₂ consumption in the sandy loam than in the fine sand.

In slurries of the sandy loam, k_1 declined with decreasing airfilled porosity in the field. This supports the idea that soil physical constraints in the undisturbed soil environment had a prolonged impact on the size and activity of the microbial populations that influenced the outcome of the slurry experiments. In addition, the results emphasize that in situ potential for gas-phase transport of both petroleum vapors and O₂ is essential to support significant intrinsic biodegradation of petroleum vapors.

Lastly, this study demonstrates that the soil physical environment, including the texture and air-filled porosity, generally selects for population sizes, and thus probably biodegradation rates in petroleum-contaminated vadose zones. Hence, soil layering and variable total soil porosity and moisture conditions, as observed at the field site, demand careful attention when evaluating natural vadose zone movement and attenuation of petroleum hydrocarbons.

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Abbreviations

bgs	below ground surface
BTEX	benzene, toluene, ethylbenzene, and isomers of xylene
LNAPL	light nonaqueous-phase liquid
TPH	total petroleum hydrocarbons
VOC	volatile organic compound

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Fig. 1. Soil stratigraphy from Borehole B301 representing a typical profile at the field site. The profile includes field observations and concentration of total petroleum hydrocarbons (TPH). The TPH was measured using gas chromatography with flame ionization detection (n/a, not analyzed; bgs, below ground surface; LNAPL, light nonaqueous- phase liquid).

Fig. 2. Soil air measurements of (a) CO_2 and (b) vapor concentrations of total petroleum hydrocarbons (TPH) related to measurements of O_2 concentrations in boreholes at the field site (note the logarithmic y axis). The TPH was measured using gas chromatography with flame ionization detection after adsorptive sampling on activated carbon.

Fig. 3.
Outline of the field site and locations of Boreholes B301 to B307 and a test well for in situ respiration testing. The gray area represents the estimated contaminated vadose zone area.

Fig. 4.

Transect (marked in Fig. 3) showing study boreholes. Soil sampling from intact drill cores from each borehole was performed approximately every 0.5 m as marked by dots. Missing samples were due to technical difficulties during sample collection.

Fig. 5.

Examples of typical benzene degradation curves from slurry reactor experiments using soil samples from Borehole B301. Data shown represent averages of duplicates and error bars show standard deviations. Data from non-sterile experiments are fitted with a first-order degradation model.

Fig. 6. Profiles from (a) Borehole B301 and (b) Borehole B303 of benzene biodegradation rates, gravimetric water content, and soil stratigraphy. The groundwater table was in both cases located about 15.5 m below ground surface (bgs). Samples are missing in both profiles due to technical difficulties during sampling of intact soil cores.

Fig. 7.

(a) Soil type averages of first-order rate constants, k_1 , for benzene degradation in samples consisting of sandy loam ($n = 45$), fine sand ($n = 17$), limestone ($n = 18$), and miscellaneous soil samples ($n = 20$) with error bars representing the standard deviation; and (b) the empirical probability distribution of k_1 in the four main soil types.

Fig. 8.

First-order rate constant (k_1) in samples of sandy loam as a function of (a) water saturation, θ/Φ (θ is volumetric water content, Φ is total soil porosity) and (b) air-filled porosity. The values of θ and Φ were based on measurements of the total soil porosity in 19 intact soil samples from the site. The dotted line represents the best fit of a model presented by Skopp et al. (1990). The fit does not include eight samples with $k_1 < 0.6 \text{ d}^{-1}$.

Fig. 9. Concentrations of O_2 and CO_2 vs. time in the test well during in situ respiration tests in layers of sandy loam and fine sand (bgs, below ground surface). It should be noted that the detection limit for the CO_2 measurements was 0.1%, causing an uncertainty at low concentrations.

Table 1

Soil characteristics of four main soil types from the vadose zone of the test site. Numbers in parentheses are standard deviations.

Soil type	Sandy loam	Fine sand	Limestone	Miscellaneous
General information				
Soil samples, no.	45	17	18	20
Depth, m below ground surface	2–10	10–13	5–9 and 13–15	2–16
Max. vapor TPH [†] conc., mg m ⁻³	5700	1700	NA [‡]	NA
Max. soil TPH conc., mg kg ⁻¹	4500	6400	450	81
Physical soil characteristics				
Clay (<2 mm), kg kg ⁻¹	0.176 (0.0042)	0.05 (0.0056)	NA	NA
Silt (2–20 mm), kg kg ⁻¹	0.154 (0.034)	0.025 (0.0049)	NA	NA
Fine sand (20–200 mm), kg kg ⁻¹	0.372 (0.109)	0.868 (0.081)	NA	NA
Coarse sand (200–2000 mm), kg kg ⁻¹	0.297 (0.071)	0.057 (0.079)	NA	NA
Gravel (>2000 mm), [§] kg kg ⁻¹	~0.100	~0.020	~0	NA
CaCO ₃ , [§] kg kg ⁻¹	0.249 (0.05)	0.130 (0.029)	>0.800	NA
Soil organic matter, [§] kg kg ⁻¹	0.0019 (0.0021)	0.0012 (0.00093)	NA	NA
Dry bulk density, [¶] kg L ⁻¹	1.75	1.59	1.71	1.65
Total soil porosity, [¶] L L ⁻¹	0.34	0.40	0.35	0.36
Water-filled porosity, [#] L L ⁻¹	0.24 (0.052)	0.19 (0.055)	0.31 (0.068)	0.21 (0.11)
Air-filled porosity, [#] LL ⁻¹	0.093 (0.046)	0.21 (0.055)	0.04 (0.068)	0.15 (0.10)
Water saturation ^{††}	0.71 (0.12)	0.45 (0.15)	0.88 (0.20)	0.65 (0.19)
Gas diffusivity(<i>D_p/D₀</i>)	0.021 (0.025)	0.031 (0.071)	0.007 (0.017)	0.009 (0.035)
Geochemical and biological soil characteristics				
pH (in KCl)	8.2 (0.3)	8.8 (0.3)	9.2 (0.6)	8.1 (0.5)
Inorganic N, mg kg ⁻¹	1.1 (0.4)	1.1 (0.4)	1.2 (0.3)	1.1 (0.6)
Total N, mg kg ⁻¹	19.8 (9.9)	10.2 (4.7)	9.3 (6.6)	17.6 (12.0)
Available P, mg kg ⁻¹	3.6 (2.0)	2.1 (0.9)	6.4 (1.5)	5.9 (9.2)
Total P, mg kg ⁻¹	363 (88)	279 (37)	NA	342 (56)
Inorganic N/available P	0.31	0.52	0.18	0.19
Total N/total P	0.05	0.04	NA	0.05
Direct counts of soil bacteria, cells g ⁻¹	9.0 × 10 ⁸ (1.2 × 10 ⁸)	2.3 × 10 ⁸ (6.4 × 10 ⁷)	3.3 × 10 ⁸ (7 × 10 ⁷)	NA

[†]TPH, total petroleum hydrocarbons.

[‡]NA, not analyzed.

[§]Contents of gravel, CaCO₃, and organic matter is per kilogram of total dry matter.

[¶]Dry bulk density and total soil porosity are estimated based on 19 intact 100-cm³ soil cores.

[#]Water- and air-filled porosity are based on the total soil porosity of the soil type and measurements of the gravimetric water content.

^{††}Water saturation is the water-filled porosity divided by the total soil porosity.

Table 2

Studies of benzene biodegradation in various experimental systems. The summary is based on reviewed references. First-order kinetics is assumed. The literature data yield an arithmetic mean of the first-order rate constant $k_1 = 0.76 \text{ d}^{-1}$ ($n = 14$).

k_1 d^{-1}	Reference	Sample description	Method description
<u>Microcosms</u>			
0.004–0.06	Franzmann et al. (1999)	shallow sandy unsaturated soil	aerobic unsaturated microcosms, 22°C
0.0057	Watwood et al. (1991)	surface soil; sandy, calcareous Aridisol	aerobic unsaturated microcosms, 25°C, water added to reach half of the water-holding capacity
0.023	Watwood et al. (1991)	surface soil; calcareous, clay-rich riparian soil	aerobic unsaturated microcosms, 25°C, water added to reach half of the water-holding capacity
0.31	Christensen et al. (2007)	unsaturated subsurface soil from contaminated site	aerobic slurry microcosms
3.35	Christensen et al. (2007)	unsaturated subsurface soil from contaminated site	aerobic slurry microcosms
2.8	Kristensen (2006)	unsaturated fine sand from 4.5 m below ground surface	aerobic slurry microcosms, 25°C
0.12	Lee and Ryan (1979)	river sediment	aerobic slurry microcosms, 22°C
0.20	Nielsen and Christensen (1994)	contaminated sandy aquifer material	aerobic slurry microcosms, 10°C, natural groundwater used
1.2	Karlson and Frankenberger (1989)	contaminated groundwater	aerobic slurry microcosms
<u>Soil columns</u>			
0.21	Hohener et al. (2006)	unsaturated sand from shallow sandy vadose zone	aerobic soil column experiment on VOC [†] gas diffusion and biodegradation, 22°C
<u>In situ studies</u>			
1.95	Hohener et al. (2006)	shallow sandy vadose zone with emplaced VOC source	gas profile analysis and modeling
0.07–0.31	Lahvis et al. (1999)	capillary fringe at petroleum-contaminated site	gas profile analysis and modeling

[†]VOC, volatile organic compound.