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Potential of preliminary test methods to predict biodegradation performance of petroleum hydrocarbons in soil

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Abstract

Preliminary tests at different scales such as degradation experiments (laboratory) in shaking flasks, soil columns and lysimeters as well as *in situ* respiration tests (field) were performed with soil from two hydrocarbon contaminated sites. Tests have been evaluated in terms of their potential to provide information on feasibility, degradation rates and residual concentration of bioremediation in the vadose zone. Sample size, costs and duration increased with experimental scale in the order shaking flasks – soil columns – lysimeter – *in situ* respiration tests, only time demand of respiration tests was relatively low. First-order rate constants observed in degradation experiments exhibited significant differences between both, different experimental sizes and different soils. Rates were in line with type and history of contamination at the sites, but somewhat overestimated field rates particularly in small scale experiments. All laboratory experiments allowed an estimation of residual concentrations after remediation. *In situ* respiration tests were found to be an appropriate pre-testing and monitoring tool for bioventing although residual concentrations cannot be predicted from *in situ* respiration tests. Moreover, this method does not account for potential limitations that might hamper biodegradation in the longer term but only reflects the actual degradation potential when the test is performed.

Introduction

Biological technologies are a widely used, cost efficient and sustainable approach for the remediation of hydrocarbon contaminated sites (Leeson & Hinchee 1997). Amongst the known technologies, bioventing is a frequently applied method for the remediation of the vadose zone involving the injection or extraction of air to supply oxygen for aerobic biodegradation of fuel residuals (Downey et al. 1999). Notwithstanding the widespread application, *in situ* bioremediation is a site-specific process and feasibility studies are required before full-scale remediation can be applied successfully (Balba et al. 1998).

Preliminary tests to assess feasibility of biotreatment of hydrocarbon contaminations are typically performed in the laboratory (for example degradation experiments) or in the field (chiefly *in situ* respiration tests). In general, the quantity of soil used in laboratory experiments is relatively small compared to the quantity of soil that has to be treated on-site and thus spatial heterogeneity is rarely considered in laboratory experiments. This might in particular be true for small scale experiments and/or for inhomogeneous sites. Furthermore, samples may be disturbed during sampling, storage and pre-treatment. Also, most laboratory experiments impose steady environmental conditions while in an outdoor climate these conditions show dynamic behaviour (Frijer et al. 1996). Moreover, diffusion rates in laboratory experiments might be improved compared to field conditions thus favouring higher degradation rates (Davis et al. 2003). Nevertheless, degradation rates and in particular residual concentrations derived from laboratory experiments can be used to predict achievable rates and concentrations in the field (Höhener et al. 2003). *In situ* respiration tests determine biodegradability potential indirectly *via* O_2 consumption or CO_2 production in the field (Balba et al. 1998). Rates then have to be corrected by background respiration result from biodegradation of organic substances and other oxygen consuming processes (Baker et al. 2000). Field tests consider site conditions comprising local heterogeneity and changing environmental conditions. Permeability in the subsoil is rarely uniform but typically reveals significant local differences that can only be assessed *in situ* (Davis et al. 2003). By the application of preliminary field tests, relevant information can be deducted on the definite system design including actual flow rates, radii of influence, blower and well layout (Leeson & Hinchee 1997; Baker et al. 2000; Höhener et al. 2003).

Preliminary assessments account for three critical prerequisites for bioremediation namely for (i) biodegradability of predominating compounds, (ii) bioavailability of contaminants to indigenous microbial populations and (iii) environmental conditions present at a site.

At given environmental conditions, the degree of hydrocarbon biodegradation is mainly affected by the type of hydrocarbons in the contaminant matrix (Huesemann 1995). Of the various petroleum fractions, n-alkanes and branched alkanes of intermediate length (C10–C20) are the preferred substrates to microorganisms and tend to be most readily degradable. Longer chain alkanes (> C20) are hydrophobic solids and are difficult to degrade due to their inherent recalcitrance and their poor water solubility (Chaineau et al. 1995). Cycloalkanes are degraded more slowly than corresponding n- and branched alkanes.

Second, preliminary tests have to consider bioavailability of the compounds. Strong interactions between soil matrix and hydrophobic pollutants can evolve causing pollutant retention or even irreversible binding to sorbents. This phenomenon known as ageing increases with time and significantly reduces bioavailability of hydrophobic contaminants in soil (Hatzinger & Alexander 1995). Pollutant retention over time is governed by physical–chemical characteristics of the pollutant and by soil characteristics. Strong or even irreversible sorption onto soil is in general attributed to the soil organic matter (e.g., Luthy et al. 1997; Huang et al. 2003). However, several studies reported that the degree of hydrocarbon degradation was mainly affected by the type of hydrocarbons in the contaminant matrix and only to a less extent to soil characteristics (e.g., Huesemann 1995; Nocentini et al. 2000; Breedveld & Sparrevik 2001). This might be true in particular for soils derived from further depths in the subsoil, where relatively low amounts of soil organic matter are present (Nierop & Verstraten 2003).

Third, environmental factors have to be regarded comprising availability and type of electron acceptors, temperature, pH, moisture content, availability of mineral nutrients and contaminant concentration. Several studies have shown that microorganisms can degrade hydrocarbons even under extreme environmental conditions even if rates might be lower and/or degradation might be less complete (Margesin et al. 1997; Mohn & Stewart 2000). Most petroleum-related hydrocarbons are readily degraded via aerobic microorganisms although a number of studies has shown that in absence of oxygen with alternate electron acceptors such as nitrate, manganese (IV), iron (III), sulphate and carbon dioxide (Holliger & Zehnder 1996; Heider et al. 1999; Grishchenkov et al. 2000; Boopathy 2002; Massias et al. 2003) hydrocarbons can be biodegraded, whereby degradation rates are substantially lower than under aerobic conditions. Addition of nutrients was reported to have a beneficiary effect on hydrocarbon degradation in soil (Dott et al. 1995; Breedveld & Sparrevik 2001; Chaineau et al. 2003), whereby a carbon:nitrogen:phosphorous (C:N:P) ratio of 100:10:1 is commonly proposed (Oudot & Dutrieux 1989; Atagana et al. 2003). Microbial activity proceeds optimally in the presence of water at between 50% and 70% field capacity (Margesin et al. 2000).

The objective of this study was to evaluate preliminary tests at varying scales in terms of their potential to predict remediation performance including information on degradability of hydrocarbons in soil comprising recalcitrance, bioavailability and environmental conditions. The experimental work involved degradation experiments at three laboratory scales (shaking flask, soil column and lysimeter) and at field scale (*in situ* respiration test). Test systems have been evaluated regarding their potential to provide information on degradation rates, limitations present in soil

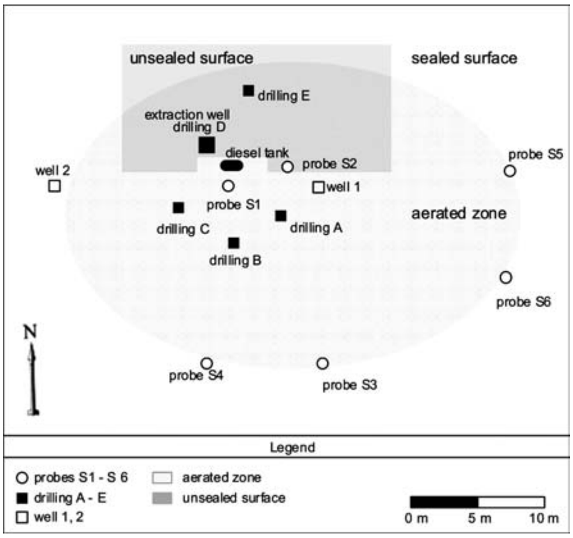

Figure 1. Schematic representation of site Feuerwache showing bioventing design and sampling locations. Also shown is the former location of the underground diesel tank south to the unsealed area. Aerated zone covers complete contaminated area.

and achievable residual concentrations after biodegradation.

Materials and methods

Site background

Study sites (site Feuerwache and site Tuttendorf) are located in and around Vienna, respectively. Site Feuerwache is the location of a former fire brigade covering approximately 300 m^2, whereby parts of the surface are sealed with concrete (Figure 1). Leaking of the underground storage tank was discovered in the mid 1990s, when inventory resulted in a loss of mineral oil of approximately 3,000–4,000 L diesel. Exploration of the site revealed a diesel layer on groundwater and hydrocarbon contamination of the vadose zone with maximum Total Petrol Hydrocarbon (TPH) concentrations of 7,000 mg kg^{-1} soil.

The area of the former mineral oil refinery Tuttendorf is situated in the Korneuburg basin close to the river Danube (Figure 2). During operating time of the refinery (1927–1960) considerable amounts of crude oil and mineral oil products infiltrated the soil and consequently polluted the groundwater heavily. The brownfield which covered an area of 400 m × 300 m was soon reused industrially and today the area hosts storage halls. The surface is discontinuously covered with asphalt, buildings and green space. Hydrogeology and summaries of investigations performed at the site are detailed elsewhere (Nahold et al. 2003). At present, the mineral oil contaminated zone comprises depths between 3 and 7 m below surface. The contamination is mainly composed of aliphatic mineral oil hydrocarbons (TPH 2,000–20,000 mg kg^{-1}) with negligible concentrations of mono- and poly-aromatic compounds.

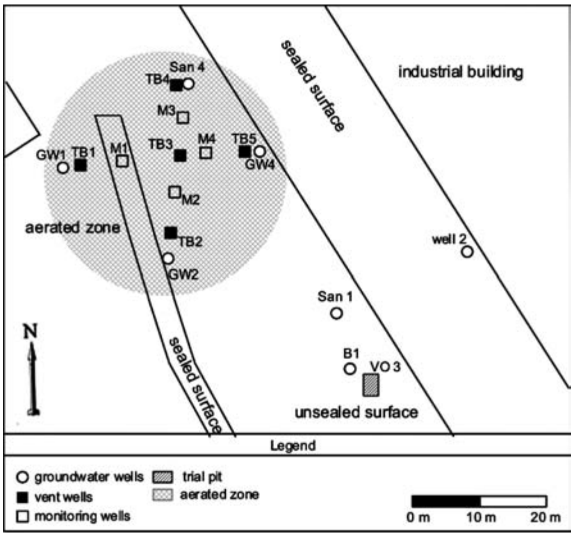

Figure 2. Schematic representation of site Tuttendorf showing the system design and sampling locations.

Soil samples

Samples for laboratory experiments were collected at both sites in 1996 and again in 2002 at site Tuttendorf, when another soil column experiment was performed. Sampling locations are displayed in Figure 1 for site Feuerwache and in Figure 2 for site Tuttendorf.

At Feuerwache, shaking flask experiments were performed with soil from three drillings (designated B, D, E), material for soil columns and lysimeters was derived from a trial pit near well 1. All 1996 samples from site Tuttendorf originated from a trial pit in the vicinity of well B1 at a depth of 5–6 m. Soil for the 2002 soil column was collected from a trial pit (VO 3) from above ground water level. Relevant physical and chemical

Table 1. Characteristics of soils used in laboratory experiments

	FW96 B[a]	FW96 D[a]	FW96 E[a]	FW96[b]	TB96[c]	TB02[d]
Depth (m)	5–6	4–6	5–6	2–4	5–6	3–4
Sand $> 63\ \mu m$ (%)	54	49	85	74	91	96
Silt 2–63 μm (%)	34	36	9	19	5	4[e]
Clay $< 2\ \mu m$ (%)	10	13	4	9	4	n.a.[f]
Dry mass dm (%)	93	93	99	93	94	97
pH (0.01 M $CaCl_2$)	7.7	7.6	7.9	7.4	7.9	7.2
Total organic carbon TOC (%)	1.9	3.0	1.3	1.0	0.3	0.1
Σ TPH[g] (mg kg^{-1})	2,360	7,010	2,710	2,060	1,810	3,330

[a] Shaking flasks Feuerwache (1996), drillings B, D, E.
[b] Soil column and lysimeter Feuerwache (1996).
[c] Soil column and lysimeter Tuttendorf (1996).
[d] Soil column Tuttendorf (2002).
[e] Silt and clay.
[f] Not analysed.
[g] Total petrol hydrocarbons (FTIR)

properties of the experimental soils are presented in Table 1.

Laboratory experiments

In the 1996 experimental series, rates of aerobic biodegradation were measured with soil from both sites in laboratory experiments at three scales namely suspension flasks, soil columns and lysimeters. In 2002, another soil column using soil from site Tuttendorf was operated. Several studies have shown that the use of pre-cultivated microorganisms had no benefit on the degradation of hydrocarbon-polluted soil in particular in field application (Dott et al. 1995) and that in general biodegradation works well without inoculation (Margesin et al. 2000; Atagana et al. 2003). All experiments were performed with indigenous microorganisms.

Experimental conditions for all laboratory experiments are summarised in Table 2.

Suspension flask experiments were performed with 10 g contaminated soil that has been homogenized and sieved at 2 mm prior to use. To overcome nutrient depletion and to establish similar experimental conditions, a mineral medium was added as previously described by Szolar et al. (2003). Suspension flasks were incubated at 20 °C in the dark and analysed for residual hydrocarbons at day 0, 5, 9, 16, 23, 40 and 61.

Medium laboratory scale experiments were conducted in soil columns. Stones exceeding 5 cm

Table 2. Conditions of laboratory experiments

Label	Mixing conditions	Soil fraction	Appr. soil mass	Nutrient addition	Water content
Feuerwache & Tuttendorf (1996)					
Suspension flask	Well mixed	< 2 mm	0.01 kg	Day 0: mineral medium	Slurry
Soil column	Homogenized prior to filling	< 50 mm	90 kg	Day 0: N, P	55% WHC
Lysimeter	Homogenized prior to filling	< 50 mm	1,500 kg	Day 0: N, P	55% WHC
Tuttendorf (2002)					
Soil column	Homogenized prior to filling	< 50 mm	90 kg	Day 69: N, P	Day 45: 55% WHC

in diameter were removed for homogeneous filling. To avoid lack of nutrients, NH_4NO_3 and K_2HPO_4 (both Fluka, Buchs, Switzerland) were added in a proportion C:N:P = 100:10:1 on day 0 (C calculated based on TPH concentration in soil). Water content was kept at 55% of maximum water holding capacity during the whole experiment. Compressed air was moistened by streaming through two water-filled bottles and thereafter was injected from the bottom of the column with a constant flow of 100 ml/min. Exhaust air was cleaned using activated carbon. At sampling dates, air was collected in gas bags (Linde, Höllriegelskreuth, Germany) and analysed for O_2, CO_2 and TPH. Soil samples were collected from the upper 10 cm of the column and only the final sample was taken as a mixed sample from the remaining homogenised soil. The experiment was conducted over a period of 391 days with soil Feuerwache and 381 days with soil Tuttendorf.

In 2002, another soil column was taken into operation with soil from site Tuttendorf. Nutrient analysis revealed low levels in available mineral nutrients being 0.2 mg kg^{-1} of NO_3–N, 0.7 mg kg^{-1} of NH_4-N and < 0.6 mg kg^{-1} of PO_4–P. Other than at the 1996 experiment, nutrients were not added before, but during experimental time (day 69) by pouring water charged with dissolved nutrients C:N:P in proportion 100:2.5:0.25 from the top of the column.

Lysimeters were filled with 1 m^3 contaminated soil whereby stones with diameters exceeding 5 cm were removed. Prior to the experiment nutrients were added in a ratio C:N:P = 100:10:1. Water content was kept at 55% of maximum water holding capacity during the whole experiment. Soil was aerated using constant flow of 5,000 ml/min previously moistened air. Exhaust air was cleaned over activated carbon. Samples were collected and analysed periodically during the experimental time of 296 days.

Field application

At site Feuerwache, first clean-up measures started in 1996 with the mechanical removal of the diesel phase from groundwater and start of bioventing operation. The remediation design is presented in Figure 1 consisting of one central vent well and surrounding monitoring wells also used as aeration inlets. Background respiration was measured in the uncontaminated well S6. *In situ* respiration tests involved soil aeration until O_2 saturation was measured in all wells (injected air volume: 45 m^3; h^{-1}; 24 h) and thereafter, aeration was stopped. Decrease of oxygen concentrations in monitoring points was measured with hand-held field instrumentation over approximately one week. *In situ* respiration tests were performed in 1998, 2000, 2001 and 2002.

Field application at site Tuttendorf started in 2002, when a pilot bioventing system was installed comprising five vent wells surrounded by four multilevel soil gas monitoring probes (Figure 2). Oxygen was introduced to the subsurface *via* well TB3 (injected air volume: 15 m^3 h^{-1} over first 15 h followed by 145 m^3 h^{-1} over 2.3 h.) until O_2 saturation was measured in every monitoring well. Thereafter, O_2 consumption in the probes TB1, TB2, TB3, TB4 and TB5 as well as the monitoring wells M1S1, M1S2, M2S1, M2S2, M2S3, M3S2, M4S1 and M4S2 (Figure 2) was determined over 8 days. Three respiration tests were performed at intervals of 2 and 3 months, respectively.

Analytical methods

Treated and untreated samples were analysed for total petrol hydrocarbons using Fourier Transform Infrared Spectroscopy (FTIR) according to the German standard method DIN 38 409-H18 (1980). After extraction of 20 g chemically dried (Na_2SO_4 (Fluka, Buchs, Switzerland)) soil with 1,1,2-trichloro-trifluoro-ethane (JT. Baker, Deventer, Netherlands), the hydrocarbon content was quantified by FTIR. Selected samples were analysed using gas chromatography and flame ionisation detection (GC-FID) according to the international standard method DIN ISO DIS 16 703 (2002). This method involves extraction with acetone/n-heptane (2 + 1) (both JT. Baker, Deventer, the Netherlands), analysis and detection using GC-FID and integration of the area confined by n-decane and n-tetracontane (Sigma–Aldrich, Vienna, Austria).

Results

Soils used in the experiments were classified as sandy soils (49–98% sand) with low to medium contents of clay (< 13%) and silt (< 19%). Only

Table 3. Parameter values and coefficients of determination for first-order degradation in laboratory experiments

Experimental system	Substrate	Coefficient of determination R^2	First-order rate constant k (day^{-1})	Ratio k/k (Lysimeter Tuttendorf (1996)
Suspension flask B	Feuerwache (1996)	0.784	0.0376	6.6
Suspension flask D	Feuerwache (1996)	0.875	0.0204	3.6
Suspension flask E	Feuerwache (1996)	0.770	0.0587	10.3
Soil column	Feuerwache (1996)	0.808	0.0168	2.9
Lysimeter	Feuerwache (1996)	0.782	0.0101	1.8
Suspension flask	Tuttendorf (1996)	0.904	0.0120	2.1
Soil column	Tuttendorf (1996)	0.875	0.0058	1.0
Lysimeter	Tuttendorf (1996)	0.891	0.0057	1.0

soils used in suspension flask experiments derived from drillings B and D (Feuerwache) had a medium silt content with 34 and 36%, respectively (Table 1).

Hydrocarbon degradation

Degradation of hydrocarbons was studied in laboratory experiments at different scales. The overall trend of hydrocarbon reduction follows first-order kinetics as observed before by other authors (e.g., Van Gestel et al. 2003; Li et al. 2004). Table 3 contains first-order rate constants (k) for all laboratory experiments. As can be seen from the determination coefficients (R^2) the first-order kinetic model describes well hydrocarbon degradation in all laboratory experiments.

Three sets of suspension flask experiments (drilling B, D, E) were performed with soil from site Feuerwache with initial TPH concentrations of 2,360, 2,710 and 7,010 mg kg^{-1}, respectively. In the course of the full experimental time of 61 days the initial concentration could be reduced by 79, 81 and 66% for drillings B, D and E, respectively. At suspension flask experiments using soil from Tuttendorf, the concentration of TPH was reduced by 44% of the initial value. The kinetic parameters based on the first-order degradation model, indicate that the highest rate of reduction of hydrocarbons occurred in suspension flask E followed by B and D (Feuerwache). Hydrocarbon degradation in soil Tuttendorf occurs at a slower rate of 0.012 day^{-1} (Table 3).

The 1996 soil column experiment involved two unsaturated columns, one with soil from site Feuerwache with an initial hydrocarbon concentration of 2,060 mg kg^{-1} the other one from site Tuttendorf with an initial concentration of 1,810 mg kg^{-1}. TPH reduction after experimental time was 93% of the initial concentration for Feuerwache (391 days) and 81% for Tuttendorf (381 days) (Figure 3).

Another soil column experiment was conducted in 2002 with the objective to assess whether any

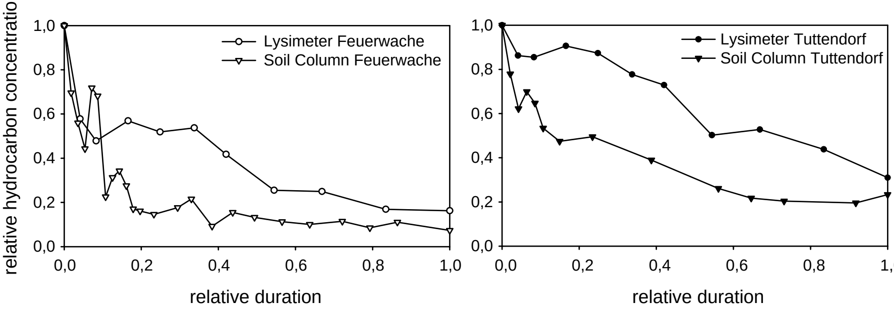

Figure 3. Hydrocarbon degradation in Lysimeters and soil columns with soil from sites. (Feuerwache & Tuttendorf 1996). Duration is expressed relatively to the total experimental time. Hydrocarbon concentration is shown relatively to the concentration on day 0 of the experiment.

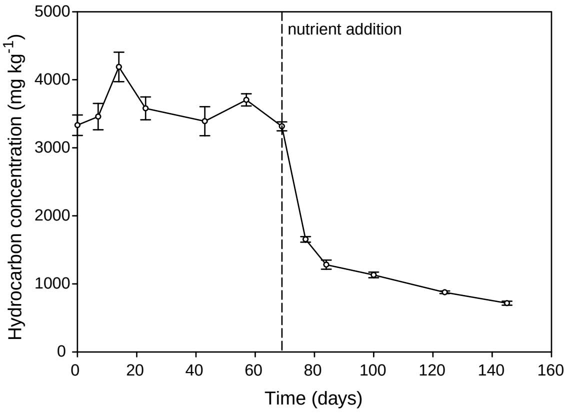

Figure 4. Degradation of hydrocarbons in 2002 soil column with soil from site Tuttendorf during 145 days. Error bars indicate standard deviation ($n=3$). Nutrient addition is indicated as vertical line on day 69.

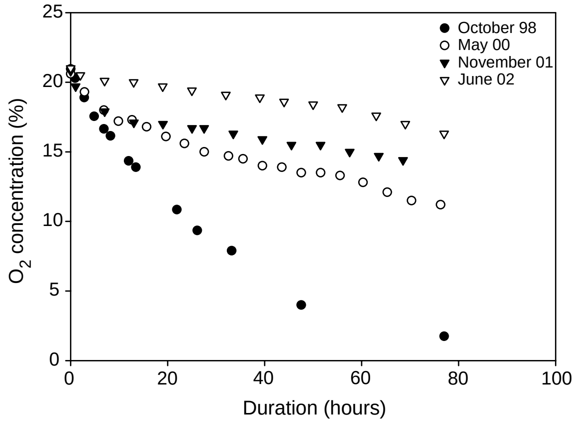

Figure 5. Oxygen concentration measured in respiration tests (1998, 2000, 2001, 2002) at site Feuerwache.

other limitations beside oxygen depletion would hamper biodegradation in the soil. Therefore, neither water nor nutrients were added at the beginning of the experiment but only after 45 and 69 days, respectively. Addition of water did not initiate degradation in the unsaturated column, but so did nutrient addition in a ratio C:N:P 100:2.5:0.25. Degradation boosted from a TPH value of 3,320 mg kg^{-1} before nutrient addition (day 69) to 1,650 mg kg^{-1} on day 77. Degradation curve levelled out on day 145 at a residual concentration of approximately 700 mg kg^{-1}. Figure 4 depicts the degradation of hydrocarbons in the soil column.

Lysimeter experiments delivered lowest daily degradation rates of all laboratory experiments (Table 3). Within 296 days a decrease of TPH concentration of 87% in soil Feuerwache and 70% in soil Tuttendorf was observed (Figure 3). Leachate analysis showed a significant decline of leachable hydrocarbons from 0.10 to 0.02 mg l^{-1} (Feuerwache) and from 0.32 to 0.03 mg l^{-1} (Tuttendorf).

Respiration rates

At site Feuerwache *in situ* respiration tests have been performed during the bioventing period to monitor the remaining biodegradation potential. O_2 consumption was measured in well D. When the first test was performed after a one-year full-scale bioventing operation period, oxygen consumption rates of 9.9% per diem were measured. Further tests in 2000, 2001 and 2002 showed decreasing respiration rates as depicted in Figure 5. In 2002, TPH concentration in soil has been reduced below 500 mg kg^{-1}. The site was then closed after successful remediation.

At site Tuttendorf pilot-scale bioventing operation was commenced in 2002 with an initial *in situ* respiration test. Oxygen consumption rates ranged from 13.1 to 2.2% per day. After 2 months of bioventing operation, another respiration test was performed using the same 13 sampling points revealing rates between 2.2% and 0.2%. Bioventing was terminated when rates in the third and final test have decreased further, ranging from 1.2 to 0.4% in the five probes that have been utilising best in the second test.

Discussion

Preliminary tests performed within this study involve degradation experiments in the laboratory in scales ranging from several grams (jar-size) to tons (Lysimeter) as well as *in situ* respiration tests. Table 4 summarises characteristics of the tests including their advantages and drawbacks. Sample size, costs and duration increase from the left to the right of the table, only time demand of respiration tests is relatively low. Quality of results in terms of representativeness increases from left to right.

First-order rate constants observed in laboratory experiments exhibited significant differences in terms of both, different experimental sizes and

Table 4. Characteristics of preliminary tests used in this study

	Suspension flasks	Soil column	Lysimeter	Respiration tests
Sample size	small (10 g)	medium (100 kg)	high (1,500 kg)	–
Costs	low costs, standard equipment	medium costs, non-standard equipment	medium - high costs, non-standard equipment	high costs (installations on site), field equipment
Duration	fast (2–4 weeks)	long (~ 100 days)	long (~ 100days)	fast (~ 1 week)
Representative-ness	Bad	medium	medium – high	high
Other factors	(–) insufficiently represents conditions in soil	(+) experimental conditions partly reflect field conditions	(+) experimental conditions close to field conditions	(–) subsequent tests necessary to account for limitations
	(–) overestimation of degradation rates	(+) information on flow rates	(+) information on flow rates and characteristics	(–) no prediction on residual concentration possible
			(+) measurement of leachate possible	(+) performed at field conditions

different sites. Table 3 specifies first-order rate constants and their ratio to the lowest observed constant, which was the one calculated for the lysimeter experiment with soil Tuttendorf 1996. Ratios obtained for preliminary tests with soil Feuerwache are 1.8- to 4.9-fold higher compared to those with soil Tuttendorf. As shown in Figure 3 for soil columns and lysimeters, hydrocarbon degradation in soil Tuttendorf is at every scale slower and residual concentrations are higher than in soil Feuerwache reflecting well contamination characteristics at both sites: hydrocarbons in soil Feuerwache originate from diesel and have been in contact with the soil for less than 5 years. Several studies have shown that diesel consisting mostly of linear and branched alkanes with different chain lengths and a variety of aromatic compounds easily biodegrades under laboratory conditions (Taylor & Viraraghavan 1999; Marquez-Rocha et al. 2001). The contamination at site Tuttendorf is somehow different: the dominant hydrocarbons originate from crude oil and are represented by branched and cyclic compounds of high molecular weight. Such compounds are known as being relatively persistent to biodegradation (Chaineau et al. 1995). Also the contaminant has been submitted to weathering and ageing processes such as solubilisation, evaporation, sorption and biodegradation. Chromatographic analysis revealed that more persistent high molecular compounds were most abundant in soil Tuttendorf (data not shown here). In addition, a significant hump of unresolved complex mixture indicates the presence of compounds that are more resistant to microbial degradation than n-alkanes (Chaineau et al. 1995). Beside alterations of the contaminant at site Tuttendorf, sorption on and diffusion into the soil matrix are likely to have occurred in the 60 years contact time leading to binding of the contaminant and thus to restricted access for degrading microorganisms (Luthy et al. 1997). In principle residual concentrations in soil after biodegradation are supposed to differ dependent on the hydrocarbon composition (type of hydrocarbons present in the mixture) and the period of ageing. Straight chain or branched alkanes and mono-cyclic saturates are supposed to be degraded most complete (90%) followed by di-cyclic (75%) and tri-cyclic (50%) saturates (Huesemann 1995). In laboratory experiments with diesel residual concentrations of 5–25% of the initial concentration have been suggested to remain after biodegradation (Taylor & Viraraghavan 1999; Nocentini et al. 2000; Marquez-Rocha et al. 2001). For crude oil contaminated soils degradation up to 75% of the initial TPH concentration has been reported (De Jonge et al. 1997; Chaineau 2003). In our study residual concentrations between 7 and 20% for Feuerwache and 20 and 45% for Tuttendorf were observed thus, strongly supporting the influence of the contaminant type and history on residual concentrations.

Generally, the transfer of biodegradation rates obtained in the laboratory to the biodegradation rates under field conditions is sound, as long as differences in environmental conditions have been considered (Frijer et al. 1996). However, the estimated first-order rate constant for full scale bioventing at site Feuerwache (operating time 45 months) amounts to approximately 0.002 (day^{-1}) and is more than a fivefold lower than the lowest rate obtained in the laboratory for this soil. In particular rates obtained in suspension flask experiments highly overestimated field rates. Rates decreased with the scale of the experiment for both sites. Improved rates in small scale experiments are on one hand believed to be due to microbial and structural heterogeneity at test sites (Davis et al. 2003) and on the other hand could be addressed to enhanced degradation as a result of optimum environmental conditions provided all over the soil that was subject to biodegradation.

Other than degradation tests in the laboratory, which were employed to predict degradation performance and to identify degradation limitations present in soil, *in situ* respiration tests were used to estimate biodegradation potential and to consider site conditions. Besides, observed respiration rates – corrected by values for background respiration – can be used for the calculation of hydrocarbon biodegradation (Baker et al. 2000; Miles & Doucette 2001). Oxygen utilisation rates greater that 1% day^{-1} are a good indicator that bioventing may be feasible at a site (Leeson & Hinchee 1997). At site Feuerwache *in situ* respiration rates from initially 9.9% per day (max TPH ~7,000 mg kg^{-1}) to below 1% per day (max TPH ~500 mg kg^{-1}) in 2002 were observed. The decrease of respiration rates (Figure 5) reflects the calculated degradation of hydrocarbons in the vadose zone. However, rates can only give an indication of the actual degradation potential at the time when the *in situ* respiration test is performed and do not necessarily correspond to the long-term degradation behaviour. A measured oxygen consumption rate corresponds only to one single point on the hydrocarbon degradation curve and is subject to change with the hydrocarbon concentration and/or other limitations. At site Tuttendorf initial rates between 13.1 and 2.2% day^{-1} indicated ongoing biodegradation and thus feasibility of bioventing. However, oxygen consumption rates measured at the second and the third test (only 2 and 3 months after initial test, respectively) were one order of magnitude lower than at the first test. The soil column experiment 2002 revealed that nutrient depletion obviously has hampered biodegradation in the field. Our results strongly suggest that rates obtained at an initial respiration test might not necessarily be representative for full-scale bioventing.

Conclusion

The focus of this study was to evaluate the potential of preliminary tests at different scales to predict biodegradation performance and to provide information on potential limitations at reasonable costs and time.

All laboratory experiments delivered a good indication of hydrocarbon degradability in soils and allowed the estimation of residual concentrations after remediation. Results corresponded well with different contamination types and site histories, although rates somewhat overestimated field rates particularly in small scale laboratory experiments. Elaborate experimental design allowed to deduct useful information on potential limitations from laboratory experiments. Establishing optimum conditions in laboratory tests might not always be the method of choice to characterise remediation behaviour at a site as potential limitations might be overlooked.

As earlier suggested (Leeson & Hinchee 1997) *in situ* respiration tests were found to be an appropriate pre-testing and monitoring tool for bioventing. However, residual concentrations cannot be predicted from preliminary *in situ* respiration tests. Moreover, this method does not account for potential limitations that might hamper biodegradation in the longer term but only reflects the actual degradation potential when the test is performed. This potential might change due to different residual hydrocarbon concentrations and/or to other limitations. Consequently, we recommend extended soil venting before performing an initial *in situ* respiration test.

Preliminary tests are strongly recommended in order to identify limitations to biodegradation and to predict remediation performance and thereby ruling out technologies being inappropriate for the clean-up of the site of concern. If not performing preliminary tests at both, laboratory and field scale, the selection of the proper test scale should be governed by considerations on conditions prevailing at the site and on the information that should be derived. However, based on our results we suggest to combine cost-efficient small scale laboratory tests with *in situ* respiration testing.

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