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Bioventing of diesel oil-contaminated soil: comparison of degradation rates in soil based on actual oil concentration and on respirometric data

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The effects of bioventing, nutrient addition and inoculation with an oil-degrading bacterium on biodegradation of diesel oil in unsaturated soil were investigated. A mesocosm system was constructed consisting of six soil compartments each containing 6 m^3 of naturally contaminated soil mixed 1 : 1 with silica sand, resulting in a diesel oil content of approximately 2000 mg kg^{-1}. Biodegradation was monitored over 112 days by determining the actual diesel oil content of the soil and by respirometric tests. The best agreement between calculations of degradation rates based upon the two methods was in July, when venting in combination with nutrient addition resulted in degradation rates of 23 mg kg^{-1} day^{-1} based on actual oil concentration in the soil and 33 mg kg^{-1} day^{-1} calculated from respirometric data. In September, these rates decreased to 9 and 1.4 mg kg^{-1} day^{-1}, and in October the degradation rates were 5 and 0.7 mg kg^{-1} day^{-1} based upon the two methods. The average ambient temperature during the respirometric tests was 14, 10 and 2°C in July, September and October, respectively. The combination of venting and nutrient addition resulted in an average residual oil content of the soil of 380 mg kg^{-1}. Neither venting alone nor inoculation enhanced oil degradation. The respiratory quotient averaged 0.40. The oil composition changed following degradation resulting in the unresolved complex mixture constituting up to 96% of the total oil content at the end of the experimental period.

Keywords: bioventing; biodegradation; respirometry; inoculation; nutrients; diesel oil

Introduction

In recent years, there has been increasing interest in developing well-documented and cost-effective *in situ* techniques for bioreclamation of oil-contaminated soil. The major problem of *in situ* clean-up using biological methods is the provision of the amount of oxygen necessary for biodegradation of the pollutant (a subsurface injection of 75 m^3 oxygen-saturated water is necessary to ensure complete mineralization of 1 kg of fuel hydrocarbons [9]). One method to address this problem is bioventing—ie forced aeration of the soil matrix to supply oxygen for biodegradation.

To optimize this process, knowledge of *in situ* oil degradation rates under different conditions is necessary. Quantification of the degradation rates from measurements of hydrocarbon concentration is often complicated by the heterogeneous distribution of the pollutant. Instead, measured *in situ* O_2-consumption rates are converted to biodegradation rates [16].

Several authors have described bioventing of hydrocarbon-polluted soil. van Eyk and Vreeken [4] calculated a zero-order diesel oil degradation rate from residual concentrations in the soil at the end of an experimental period. Gudehus *et al* [7] were unable to obtain degradation rates from measurements of hydrocarbon concentrations in the soil due to heterogeneous distribution of the pollutant; instead, degradation rates were estimated from respirometric data. Enhanced biodegradation of a jet fuel spill by bioventing was described by Hinchee *et al* [8] and Dupont *et al* [3]; degradation was documented by respirometric data and by measuring the isotopic ratios of carbon in the vent gas.

Besides oxygen availability, other factors may influence oil degradation rates. The availability of nitrogen and phosphorus in the soil may be limiting, but nutrient addition does not always result in increased degradation rates [12]. The oil-degrading potential of the microbial populations is also important and might be enhanced by inoculation of the soil. Gruiz [6] reported positive results combining bioventing and inoculation with oil-degrading bacteria to clean up a heating oil spill, but generally the beneficial effect of inoculation on oil spills is thought to be negligible [17].

Few data are available on oil degradation rates in connection with bioventing obtained from simultaneous determination of degradation rates by respirometry and measurements of actual diesel oil content in the soil. Furthermore, most investigations of the combined effect of bioventing and inoculation lack appropriate controls.

We therefore carried out the present bioventing study to provide data on O_2 consumption, CO_2 production and concentration of diesel oil in the soil during the venting process. Furthermore, the effects on degradation rates by nutrient addition and inoculation using an oil-degrading bacterium were investigated.

To overcome the problem of heterogeneous distribution of the pollutant, the experiment was conducted under simulated *in situ* conditions in a mesocosm system containing

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approximately 36 m^3 of diesel oil-polluted soil. In this way, we were able to exert a high degree of control over the experimental conditions, while generating data comparable to *in situ* investigations.

Materials and methods

Construction of mesocosm system

Two containers (inside measurements: L, 5.0 m; H, 2.15 m; W, 2.3 m) were placed inside a tent at the test site near Copenhagen, Denmark. Each container was divided into three compartments by steel plates welded to the sides and bottom. At the bottom, a grate allowed for water to drain off. The containers were equipped with 1-in and 1.5-in gas tight side ports for soil sampling.

Soil originating from a diesel oil-polluted site was mixed with oven dried quartz sand in the proportion 1 : 1 in an 8 m^3 concrete mixer, resulting in an average diesel oil concentration in the soil of approximately 2000 mg kg^{-1}. Each container compartment received 6 m^3 of soil. A bentonite membrane (2 cm thick) was placed on top of the soil to reduce air exchange through the surface. A polypropylene membrane (0.95 cm thick) inside each compartment was used to keep the soil in place. Soil characteristics were: average particle size, 0.31 mm; bulk density, 1.67 t m^{-3}; permeability coefficient, 1 m day^{-1}; pH, 6.8. Heavy metal concentrations were below detection levels.

A system of pipes was used for both nutrient addition and inoculation and consisted of two horizontal drain pipes in each container compartment. The pipes were surrounded by gravel and were buried 10 cm below the soil surface. The pipes were connected to two tanks used for mixing tap water with either nutrients or bacteria. The water was transported through the system by means of a centrifugal pump.

The venting system included one vertical injection well and one vertical evacuation well in each compartment (Figure 1). A well consisted of a slotted steel pipe surrounded by gravel; each well was approximately 2 m deep and corresponded to the height of the soil matrix. The slots (0.5 × 50 mm) covered the length of the pipes from 10 cm below the soil surface to 20 cm above the bottom of the soil matrix. Two compressors (Siemens, Bad Neustadt, Bavaria, Germany) provided air injection and evacuation. The venting cycle was divided into a 45-min period with air injection, followed by 195 min without air injection. This ensured an average air exchange of 0.36 pore volumes h^{-1}. Evacuated air was vented to the atmosphere through a chimney. On three consecutive days in July the evacuated air was passed through charcoal filters for 45 min and analysed for hydrocarbon content to estimate evaporation of diesel oil due to the venting process.

Oxygen and carbon dioxide concentrations in the soil gas were measured continuously by 24 probes (TEMAC Biogas Surveyor, Søborg, Denmark). Oxygen was measured electrochemically and carbon dioxide by IR absorption. Data from these measurements were collected on a computer.

Isolation and propagation of the bacterium used for inoculation

Diesel oil-degrading bacteria were isolated from enrichment cultures of bacteria from the diesel oil-polluted soil

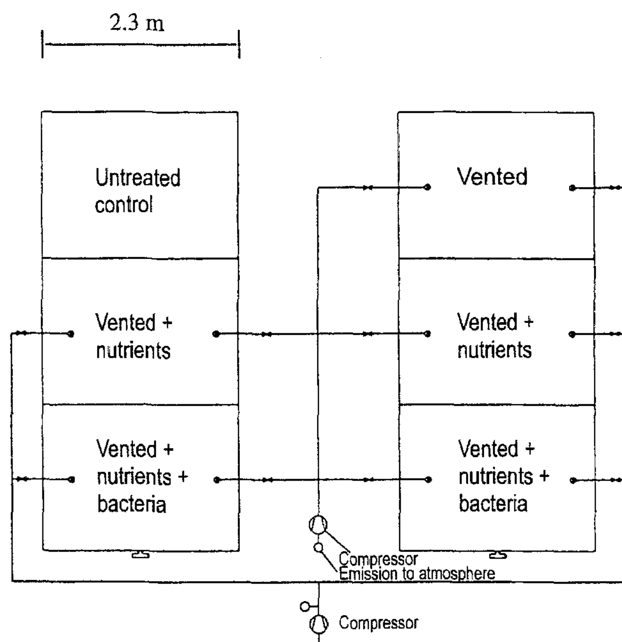

Figure 1 Venting system of the container compartments (the water system was omitted for clarity).

used in the containers. The isolates were tested in batch experiments with diesel oil-polluted soil and in liquid cultures supplemented with diesel oil. Based upon these experiments, an oil-degrading bacterium (rod-shaped, Gram-positive, oxidase and catalase-positive) capable of degrading 85% of the supplemented diesel oil when grown in liquid medium, was selected for inoculation. Pilot experiments in 2-L fermentors showed that the degradation capability of the oil-degrading bacterium was unaffected by propagation.

The culture was propagated in a 50-L fermentor (Biostat P, B Braun Melsungen, Melsungen, Hessen, Germany) using a mineral medium [15] supplemented with 20 g L^{-1} peptone, 10 g L^{-1} yeast extract and 20 g L^{-1} glucose. After three days of incubation at 25°C the culture density was 8.3×10^9 CFU ml^{-1}. The culture was cooled to 5°C overnight, concentrated to 2.2×10^{10} CFU ml^{-1} and washed with 40 L of 0.9% NaCl using a hydrophobic polysulphon hollow fibre membrane module (Romicon, Woburn, MA, USA).

Nutrient addition and inoculation

Experimental conditions for the six container compartments were as follows: one remained untreated, one was vented, two were vented and received nutrients and two were vented, received nutrients and oil-degrading bacteria.

Nutrients, N and P, were added as a mixture of $NaNO_3$, KNO_3 and Na_2HPO_4 dissolved in tap water to give a C : N : P-ratio of 120 : 10 : 1 [19] based upon the C-content of the oil. Four container compartments each received 2800 L of nutrient solution in two increments during the week prior to ventilation start-up. A second addition of nutrients containing twice the amount of nitrate was made in September, 69 days after the experiment was initiated. Two compartments received tap water without nutrients. The nutrient solution was pumped through the drain pipes and left to percolate through the soil matrix. After recircul-

ating the suspensions, the water level in each compartment was adjusted to cover the bottom 10–20 cm of the soil matrix. Two compartments additionally received a suspension of oil-degrading bacteria in 700 L of tap water. The suspension contained 1.4×10^8 CFU ml^{-1}. After inoculation, the water was recirculated for 5 h totalling four container volumes before draining it. Water samples from each compartment were diluted and spread on plate count agar plates to assess distribution of bacteria in the soil column. Water samples were also analyzed for content of hydrocarbons due to washout by water recirculation.

Respirometric tests

Diesel oil degradation rates were estimated from respirometric tests measuring O_2 consumption and CO_2 production in the soil gas. In July, 7 days after the ventilation start-up, rapid O_2 consumption and CO_2 production in the soil gas enabled calculations of degradation in the 195-min interval between ventilation cycles. Later in the study, ventilation was suspended for two periods: 4 days in September, 64 days after the venting was initiated and 7 days in October, 98 days after the venting was initiated, during which O_2 consumption and CO_2 production in the soil gas were monitored.

Analytical procedure

Three soil samples, each containing approximately 100 g, were taken from each container compartment at ten sampling dates. The diesel oil content was determined in soil subsamples of 50 g: a slurry was made by adding 100 ml of phosphate buffer solution, and 1 ml was removed for enumeration of bacteria (see below). The slurry was extracted with pentane containing *n*-triacontane as internal standard. The diesel oil content was determined by injecting 1-μl samples of the pentane phase into a Hewlett Packard (Hewlett-Packard Company, Avondale, PA, USA) 5890 gas-chromatograph equipped with a flame ionisation detector. A 30-m $\times$ 0.532-mm DB-1 (J & W Scientific, Folsom, CA, USA) fused silica capillary column was used with nitrogen as carrier gas. The injector and detector temperatures were 275°C and 300°C, respectively. Column temperature conditions were: 70°C for 5 min, 12°C min^{-1} to 275°C and 12 min at 275°C. Diesel oil concentrations were calculated by comparison with a standard curve derived from known amounts of diesel oil in pentane. Chromatograms were re-integrated using different base lines to estimate the relative amount of unresolved complex mixture (UCM) in the samples.

The total heterotrophic bacterial population was determined by spreading samples on plate count agar supplemented with 50 mg L^{-1} of cycloheximide. The plates were counted after 3 days of incubation at 25°C. The oil-degrading bacterial population was determined by spreading on minimal agar made from mineral medium [15] solidified with 20 g L^{-1} of Noble agar (Difco, Detroit, MI, USA), and supplemented with 50 mg L^{-1} of cycloheximide. Cooled (5°C) inoculated plates were sprayed with a 5% (vol/vol) solution of hexadecane in diethylether. The ether evaporated leaving an opaque hexadecane layer on the plate surface. Hexadecane was chosen as carbon source, since all diesel oil-degrading bacteria isolated from the soil were able to degrade this alkane. The plates were incubated at 10°C for 1 month before counting them. Colonies capable of plaque formation in the hexadecane layer were regarded as oil-degraders.

The concentration of nutrients in the soil was determined by extracting subsamples of 20 g of soil with 0.1 M KCl. The NO_3^--N and PO_4^{3-}-P content was then determined by flow-injection technique using an autoanalyzer (Tecator, Höganäs, Sweden). The moisture content of the soil was determined by re-weighing subsamples of 5 g of soil after 24 h at 90°C.

Results

Water draining off the container compartments contained hydrocarbons at concentrations only slightly above the detection levels, and only trace amounts of hydrocarbons were detected in the exhaust air (data not shown). Less than 4% of the decrease in diesel oil concentration was due to evaporation. Washout of hydrocarbons and evaporation were, therefore, considered of minor importance in the present study.

Figure 2 shows the diesel oil concentrations in the various container compartments from day 0 when venting of the soil was initiated to the end of the experimental period at day 112. Data from replicate compartments in the two containers were pooled. A modest amount of oil was degraded in the untreated soil (Figure 2a) resulting in an average diesel oil concentration of 1172 mg kg^{-1} after 112 days. Data from the vented soil (Figure 2b) were scattered and no general tendency was detected. In contrast, the combination of venting and nutrient addition (Figure 2c) and venting, nutrient addition and bacterial inoculation (Figure 2d) led to more extensive oil degradation, resulting in average diesel oil concentrations measured after 112 days of 380 and 384 mg kg^{-1}, respectively. Data were fit by exponential regression, that proved statistically significant at the 95% levels for the curves in Figure 2a, c and d. Regression carried out on the data in Figure 2b was not significant at the 95% level. The data were also fit by linear regression (not shown) which resulted in smaller regression coefficients for the data in Figure 2a, c and d than by exponential regression. Linear regression on the data in Figure 2b was not significant at the 95% level. Diesel oil degradation in the container compartments was thus better described by first-order than by zero-order kinetics. The degradation constants in Figure 2a, c and d corresponded to half-lives of 123, 46 and 48 days, respectively—the first being significantly longer than the two other half-lives.

Table 1 shows respirometric data of O_2 consumption and CO_2 production rates, and degradation rates calculated from actual diesel oil concentration in the soil and respirometric data, respectively. The O_2 consumption and CO_2 production rates declined more than one order of magnitude from July to October for all treatments of the soil. The respiratory quotient (the ratio of CO_2 production to O_2 consumption) averaged 0.40 based on data from the entire experimental period. Zero-order degradation rates based on actual diesel oil concentration of the soil were calculated from the differential coefficients of the first-order degradation curves in Figure 2 by inserting time values corresponding to the mid

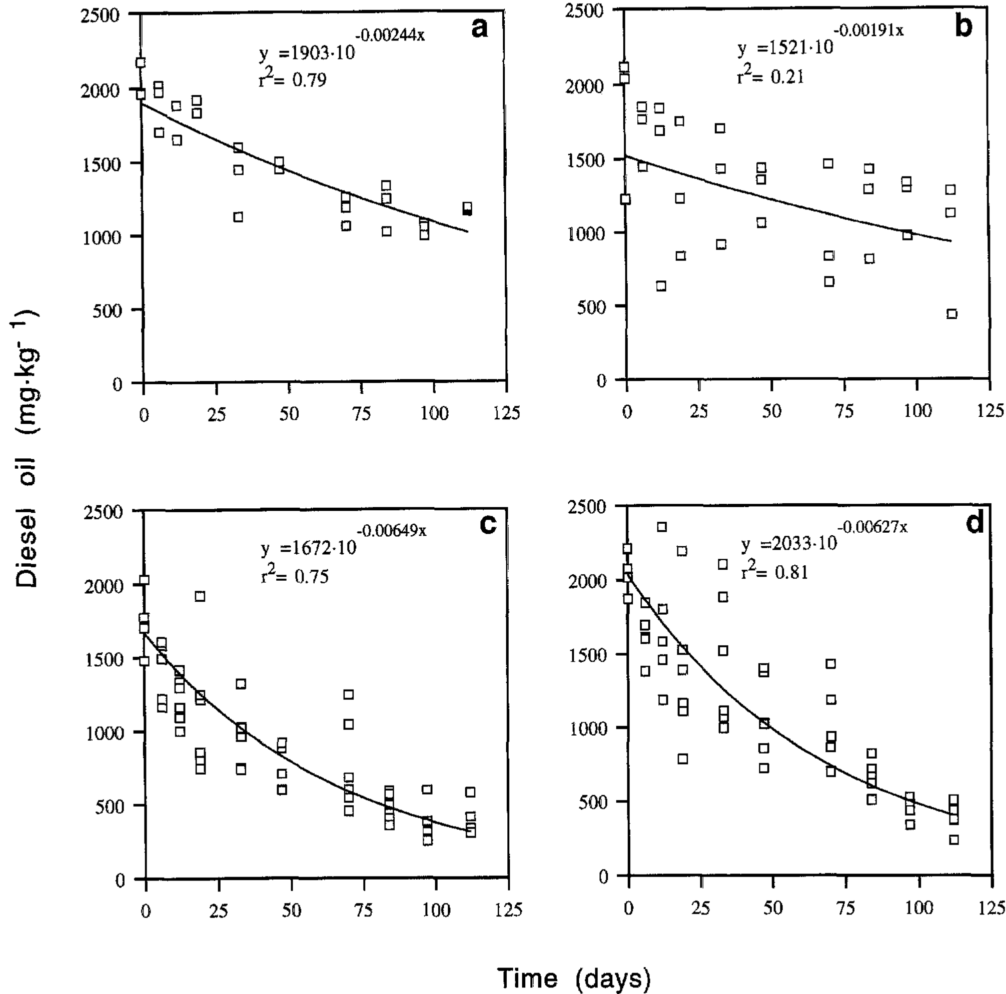

Figure 2 Diesel oil concentration in soil subjected to different treatments: untreated (a), vented (b), vented and supplemented with nutrients (c) and vented, supplemented with nutrients and inoculated with oil-degrading bacteria (d). Curves shown were fit by exponential regression. In (c) and (d) data from replicate container compartments were pooled.

points of the respirometric test periods. Respirometric data of O_2 consumption were converted to diesel oil degradation rates using the approximation formula of $CH_{2.16}$ for fuel oil [10]. The reaction of mineralization was assumed to be $CH_{2.16} + 1.54\ O_2 \Rightarrow CO_2 + 1.08\ H_2O$. The O_2 consumption rates were assumed to be zero-order [16]. Generally, the rates were highest in July as determined by both methods compared to the later determinations in September and October. The different treatments of the soil in the container compartments were reflected in the degradation rates determined by both methods: the rates in untreated soil were always slower than the rates in vented soil receiving nutrients and in the soil receiving bacteria as well. In September and October, the degradation rates based on respirometry were much smaller than the rates calculated from the first-order degradation curves. The average ambient temperature during the respirometric tests decreased from 14°C in July to 10°C in September ending at 2°C in October.

To describe oil degradation qualitatively, chromatograms were re-integrated allowing separation of the protruding peaks and the unresolved complex mixture (UCM) of the oil. In Figure 3 the relative amounts of UCM in the container compartments are shown. The composition of the oil changed during degradation leaving a higher relative proportion of UCM at the end of the experimental period. The portions of UCM in the untreated soil and the soil in the vented compartment were only slightly elevated from constituting 80% of the total oil content at the beginning of the experiment to 85% after 112 days, while the vented soil with nutrients added and the soil also receiving bacteria had residual UCM concentrations in excess of 95% of the total oil content of the soil.

The total culturable heterotrophic bacterial populations (Figure 4a) and the culturable oil-degrading populations (Figure 4b) followed the same general pattern: following an initial high density, the populations declined until day 47 paralleling the decline in oil concentration. After day 47, the population sizes began increasing again. On all sampling dates, the bacterial numbers were higher in both the vented soil with nutrients added and the soil receiving nutrients plus bacteria compared to the other two soil treatment conditions. On average, the population of oil-degrading bacteria constituted less than 10% of the total heterotrophic populations. The number of oil-degrading bacteria in the inoculated soil (Figure 4b) was higher than in the other compartments until day 70. Generally, populations sizes

Table 1 Respirometric data and diesel oil degradation rates in July, September and October in soil subjected to different treatments

Month	Soil treatment	Respirometric data			Diesel oil degradation rates ($mg\ kg^{-1}\ day^{-1}$)	
		O_2 consumption (vol % h^{-1})	CO_2 production (vol % h^{-1})	Respiratory quotient[a]	Calculated from actual oil concentration	Calculated from O_2 consumption
July	untreated	nd[b]	nd	nd	10	nd
	vented	0.41 (0.13)[c]	0.15 (0.35)	0.37	6[d]	8 (3)
	vented, nutrients	1.64 (0.56)	0.69 (0.35)	0.42	23	33 (11)
	vented, nutrients, bacteria	1.12 (0.42)	0.31 (0.15)	0.28	26	23 (8)
Sept	untreated	nd	nd	nd	7	nd
	vented	0.040 (0.010)	0.016 (0.004)	0.40	5[d]	0.8 (0.2)
	vented, nutrients	0.070 (0.023)	0.028 (0.010)	0.40	9	1.4 (0.5)
	vented, nutrients, bacteria	0.082 (0.018)	0.027 (0.005)	0.33	11	1.7 (0.4)
Oct	untreated	nd	nd	nd	6	nd
	vented	0.012 (0.013)	0.006 (0.006)	0.50	4[d]	0.2 (0.3)
	vented, nutrients	0.033 (0.019)	0.015 (0.008)	0.46	5	0.7 (0.4)
	vented, nutrients, bacteria	0.056 (0.013)	0.024 (0.006)	0.43	7	1.1 (0.3)

[a]Ratio of CO_2 production to O_2 consumption.
[b]Not determined: the probes did not work properly in this container compartment.
[c]Numbers in parentheses represent 1 standard deviation.
[d]Calculated from non-significant regression line.

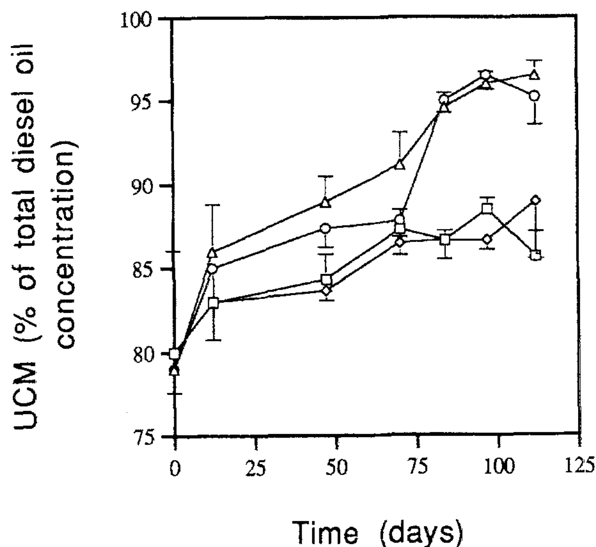

Figure 3 Change in oil composition. Unresolved complex mixture (UCM) in soil subjected to different treatments: untreated (□), vented (◇), vented and supplemented with nutrients (△) and vented, supplemented with nutrients and inoculated with oil-degrading bacteria (○). Data points represent the arithmetic average of three samples (□,◇) and six samples (△,○), respectively. Bars (one-sided) represent 1 standard deviation.

already differed at day 0. This could be explained by the fact that nutrients and bacteria were added during the week prior to start-up of the venting at day 0; in this manner an enrichment process could have taken place.

Figure 5 shows the concentrations of water-extractable nutrients in the soil. The first nutrient addition took place during the week prior to day 0, but after 19 days the NO_3^--N levels were indistinguishable from the background (Figure 5a). The PO_4^{3-}-P concentration of the soil remained stable for the first 33 days but had declined by day 47. A second nutrient addition took place at day 69 resulting in much higher concentrations of both nutrients in the soil. The moisture content of the soil averaged 6.9% (w/w) during the experimental period for all container compartments.

Discussion

Respirometric tests and data of actual diesel oil concentration in the soil combined with the fact that volatilization of the pollutant was insignificant, indicated that the disappearance of diesel oil from the unsaturated soil matrix was due to biodegradation. The failure in the present study to demonstrate enhanced biodegradation without nutrient addition is in contrast to Dupont *et al* [3] who found that nutrient addition did not significantly accelerate biodegradation of a fuel spill compared to moisture addition alone. Bulman and Newland [2] reported that venting and nutrient addition resulted in greater average removal rates than venting alone in a diesel oil remediation project. These findings illustrate the need for individual assessment of nutrient requirements in bioventing projects.

The effectiveness of inoculation using specific microbial degraders to enhance bioremediation of oil-polluted soil has been investigated by various authors: Jobson *et al* [11] and Lehtomäki and Niemelä [13] reported only marginal or no effect on degradation following inoculation. More recently, Mueller *et al* [14] tested the effect of inoculation on beach material polluted with crude oil and found no additional effects compared to nutrient addition. Gruiz [6] reported that in a pilot experiment the combination of venting, nutrient amendments and inoculation enhanced the degradation of a mixture of diesel oil and motor oil compared to venting alone. In the present study, no effects of inoculation were seen. Although the bacteria added were isolated from the same soil, the presence of a relatively large number of indigenous oil-degrading bacteria made the additional

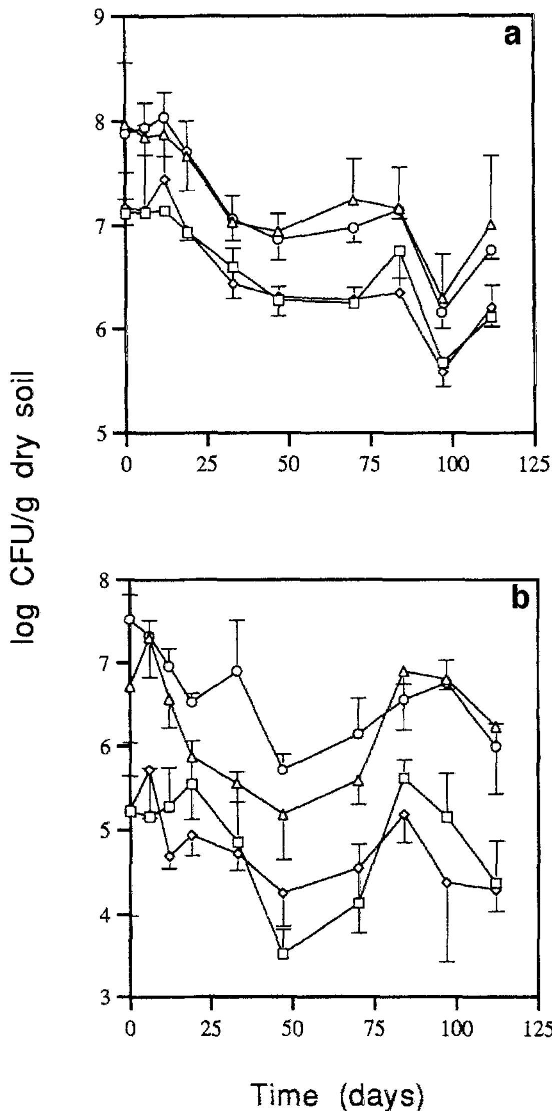

Figure 4 Total number of heterotrophic bacteria (a) and number of oil-degrading bacteria (b) in soil subjected to different treatments: untreated (□), vented (◇), vented and supplemented with nutrients (△) and vented, supplemented with nutrients and inoculated with oil-degrading bacteria (○). Data points represent the geometric average of three samples (□,◇) and six samples (□,○), respectively. Bars (one-sided) represent 1 standard deviation.

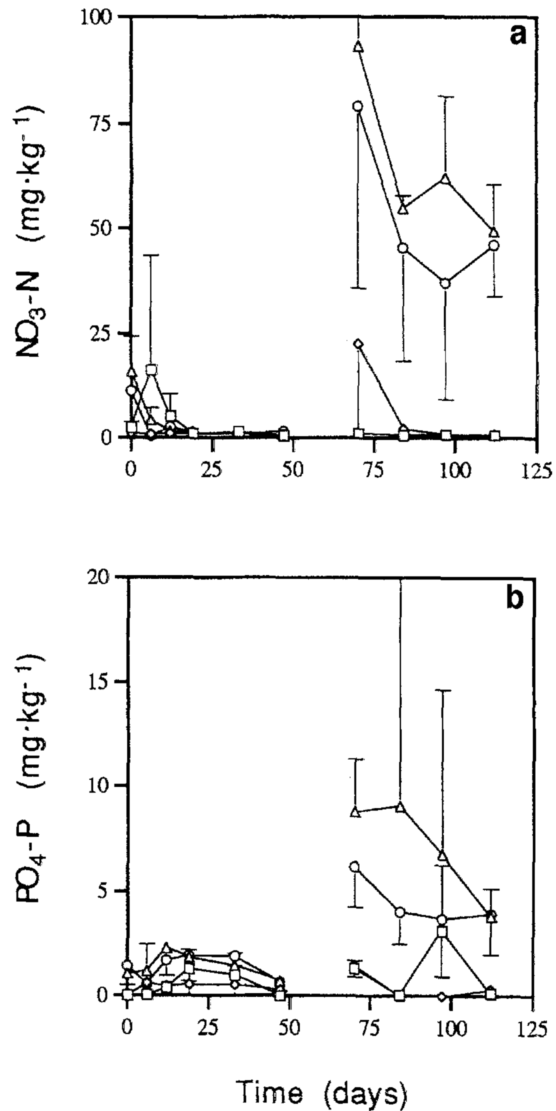

Figure 5 Concentration of NO_3-N (a) and PO_4-P (b) in soil subjected to different treatments: untreated (□), vented (◇), vented and supplemented with nutrients (△) and vented, supplemented with nutrients and inoculated with oil-degrading bacteria (○). A second nutrient addition was carried out at day 69. Data points represent the arithmetic average of three samples (□,◇) and six samples (△,○), respectively. Bars (one-sided) represent 1 standard deviation.

effects of the added bacteria marginal. Furthermore, bacteria introduced into soil may be more susceptible to predation than indigenous populations or may be unable to reach the contamination [5].

We based respirometric calculations of degradation rates upon O_2 consumption rather than CO_2 production [7,8]. The respiratory quotient will be between 0.6 and 0.85 if mainly hydrocarbons are mineralized and O_2 consumption is not associated with other biotic or abiotic processes in the soil [7]. In the present study, the respiratory quotients (Table 1) averaged 0.40 indicating that other processes than hydrocarbon mineralization were active in the soil. An explanation could be that biotic (eg nitrification) or abiotic processes (eg oxidation of iron) consumed O_2. Alternatively, CO_2 could have been lost by production of calcium carbonate.

In the bioremediation efforts, it is of interest to determine the kinetics of degradation to predict the time needed to clean up the contamination. According to Song *et al* [20], biodegradation of complex hydrocarbon mixtures in soil is intermediate between zero-order and first-order. The authors explained this by assuming that the oil undergoes a shift in composition during degradation leaving the more recalcitrant oil components, resulting in decreasing degradation rates. This phenomenon was demonstrated in the present study when the unresolved complex mixture (UCM) fraction of the oil increased to 95% at the end of the venting period. Nevertheless, in order to compare data sets, we calculated degradation rates from data of actual diesel oil concentration in the soil assuming first-order kinetics of degradation.

van Eyk and Vreeken [4] investigated removal of diesel oil by bioventing in a model experiment during a 12-month

period. They calculated an average zero-order degradation rate of 8 mg kg^{-1} day^{-1} from the residual diesel oil content of the soil. In the present study, this value was exceeded up to four times in the beginning of the venting period. Sayles *et al* [18] investigated bioventing of a JP-4 spill in a cold climate. By warming the soil to approximately 10°C, they achieved biodegradation rates of 2.9 mg kg^{-1} day^{-1} calculated from O_2 consumption rates. In an unheated control plot, biodegradation was 1.4 mg kg^{-1} day^{-1} although the temperature approached 0°C. Our data showed similarity with these rates: based upon O_2 consumption, maximum degradation rates were 1.7 mg kg^{-1} day^{-1} in September, decreasing to 1.1 mg kg^{-1} day^{-1} in October when the ambient temperature was 10°C and 2°C, respectively.

Comparison of zero-order degradation rates determined by degradation data (from the differential coefficients of the first-order curves) and respirometric data showed good correlation between the two methods in the beginning of the venting period in July, where the maximum difference was 43% (Table 1). In September and October, the deviation increased. The accumulation of degradation products could explain this difference to some extent: in the beginning of the study, easily degradable components of the diesel oil were mineralized at high rates resulting in good correlation between actual oil concentration and respirometric data. In the later part of the study, when the UCM portion of the oil increased, accumulation of degradation products could have taken place, resulting in an overestimation of mineralization rates from degradation data. Another factor was the drop in ambient temperature from 14°C in July to 2°C in October that undoubtedly resulted in decreased degradation rates. Consequently, use of the respirometric test in October to predict degradation rates at higher temperatures may be inappropriate unless a temperature/degradation rate relationship is established. The first-order degradation rates from actual diesel oil concentration in the soil were based on data from the entire venting period and may, therefore, be more accurate over a broader temperature range. The following example underlines the difference between the two approaches: using the first-order degradation constant (Figure 2c), it would require an additional 4 months of bioventing in order to comply with the 50 mg kg^{-1} limit for total hydrocarbon residue in soil stipulated by the Danish authorities [1]. In contrast, calculations based on the degradation rates of 0.7 mg kg^{-1} from the respirometric test in October points towards the need of a further 15.7 months of bioventing from day 112 to achieve this goal.

Additional work will have to be done to acquire a more precise knowledge of how degradation rates determined by respirometric tests vary with time and to establish the relationship between these rates and the actual change in oil concentration in the soil. Furthermore, the possibility of accumulation of degradation products will have to be investigated in order to correlate degradation rates based upon the two methods.

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