Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 143 (2007) 65–72
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Degradation characteristics of waste lubricants under different nutrient conditions

Sang-Hwan Lee [a,*], Seokho Lee [b], Dae-Yeon Kim [c], Jeong-gyu Kim [c]

[a] *Office of Environmental Geology, Korea Rural Community & Agricultural Corporation, Uiwang 430-600, Republic of Korea*
[b] *Graduate School of Environmental Studies, Kwangwoon University, Seoul 139-701, Republic of Korea*
[c] *Division of Environmental Science and Ecoligical Engineering, Korea University, Seoul 136-713, Republic of Korea*

Received 29 November 2005; received in revised form 19 August 2006; accepted 29 August 2006
Available online 1 September 2006

Abstract

We investigated the limits and extent of lubricants biodegradation at different nutrient conditions and evaluated several soil biological activities with regard to their usefulness for monitoring the bioremediation process in a soil contaminated with lubricants. To examine the effects of nutrient addition on lubricants biodegradation, a bench-scale investigation was conducted under different nutrient conditions for over 105 days testing period. When nutrients were added to contaminated soil with aged lubricant, great stimulation was occurred in fertilized soil for hydrocarbon degradation activity compared to non-fertilized soil. At the end of the experiment (105 days after), the initial level of contamination (9320 ± 343 mg/kg) was reduced by 42–51% in the fertilized soil, whereas, only 18% of the hydrocarbon was eliminated in the non-fertilized soil. The effect of biostimulation of indigenous soil microorganisms declined with time, apparently 42% of the initial concentration of hydrocarbon remained at the end of experiment. Lubricants biodegradation process could be monitored well by soil biological parameters. In fertilized soil, biological parameters (number of HUB, soil respiration, dehydrogenase and catalase activities) were significantly enhanced and correlated with each other, as well as the residual lubricant concentration.

Keywords: Bioremediation; Biostimulation; Lubricant oil; Microbial activity

1. Introduction

Bioremediation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contamination, has been established as an efficient, economic, versatile and environmentally sound treatment [1]. Bioremediation techniques accelerate the naturally occurring biodegradation by optimizing conditions for biodegradation through aeration, addition of nutrients and control of pH and temperature [2,3].

About 1% of total mineral oil consumption is used to formulate lubricants and the total consumption of lubricating mineral oils in 1995 exceeded 36 million tonnes worldwide and significant amounts of lubricants were lost into environment [4]. Most of these lubricants are used in loss lubrication frictional contacts and in circulation systems, which are disposed improperly. Additionally, lubricants are emitted from leaks and significant amounts remaining in filters and containers have to be taken into account. For these reasons, the distribution, biodegradability and toxicity of lubricants are important factors with respect to environmental management. In spite of the large number of works on petroleum, petroleum derivatives and hydrocarbon biodegradation, little information is available on lubricant oils biodegradation [5,6].

The most widely used bioremediation procedure is the biostimulation of indigenous microorganisms by the addition of nutrients because the input of large quantities of carbon sources tends to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P. Levels of N and P added to stimulate biodegradation at contaminated sites are often estimated from C/N ratios. Unfortunately recommended C/N ratios for hydrocarbon degradation in soils vary widely. Optimum C/N ratios of between 9/1 and 200/1 have been reported (reviewed by Morgan and Watkinson [3]). The wide range of values found in the literature has been suggested to be affected by differences

* Corresponding author. Tel.: +82 2 3290 3474; fax: +82 2 3290 3474.
E-mail address: soillsf@korea.ac.kr (S.-H. Lee).

0304-3894/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2006.08.059

in the contaminants, biodegradation kinetics, unamended soil nutrient levels, and experimental differences [7]. Therefore, for a successful bioremediation the understanding of nutrient effects at a specific site is essential [8]. Usually most studies to date have used chemical measurements to assess reclamation and the end point of the target compound remaining in soil [9]. These chemical analyses alone do not take into account the bioavailability of the contaminants; furthermore, many compounds are transformed to metabolites of unknown persistence and toxicity [10,11]. So there has been an increased interest in the use of biological assays, such as enzyme activities, as diagnostic and monitoring tools in soil remediation [12–14]. So far, soil biological parameters have been mainly used as monitoring tool in the soil of freshly or experimentally contaminated with defined petroleum hydrocarbon such as gasoline, diesel. There is little information on the potential of soil biological parameters as indicators of heavy mineral oil degradation processes, despite the widespread environmental contamination.

The objective of this study was to determine the feasibility of bioremediation as a treatment option at an abandoned repairing workshop of the army contaminated with lubricants and the evaluation of several soil biological methods with regard to their usefulness for monitoring the bioremediation process. Laboratory microcosms were used to measure the changes in hydrocarbon concentration and the accompanying changes in microbial activities with time.

2. Material and methods

2.1. Contaminated soil

The material selected for this study was from the site used as a repairing yard for army vehicles during 35 years. During this time, hydrocarbon-containing materials were used extensively including diesel, lubricant oils, and other hydrocarbon products. Initial oil contamination level of the soil was $9320 \pm 343\ mg\ kg^{-1}$ of TEM (total extractable matters) soil with carbon number range of 20 to over 40.

2.2. Microcosms and their set-up

The feasibility of bioremediation for contaminated soils with aged lubricants at different nutrient condition was examined using laboratory microcosms. To study the effects of nutrient levels for the biodegradation of aged lubricants, C/N/P ratio was adjusted to 100/10/1, 200/10/1, and 500/10/1 by adding $(NH_4)_2SO_4/K_2HPO_4$ at the rate of 4300/810, 2150/405, and $1075/162\ mg\ kg^{-1}$ soil, respectively. There was a control without any nutrient addition in order to evaluate lubricant degradation rate in its own condition (natural attenuation). For each microcosm, approximately 300 g of soil was added to a $1000\ cm^3$ glass jar and incubated at the room temperature (20–25 °C). During the experiment, soil samples were collected and analyzed periodically (0, 15, 30, 45, 60, 75, 90, and 105 days) for the determination of lubricants contents and microbial activities. The water content was checked and adjusted regularly with sterilized water to maintain about 70% of the water holding capacity. To achieve sufficient aeration, the contents of jars were mixed thoroughly twice a week.

2.3. Analytical methods

2.3.1. Soil properties

The soil was analyzed for various chemical and physical properties: pH by glass electrode method (1:5 water suspension); available P by the Bray method [15]; organic matter by the Turyn method [16]; cation exchange capacity (CEC) by the NH_4^+ saturation and distillation method [17]; texture by the pippeting method [18]; NH_4^+ and NO_3^- by the KCl extraction and spectrometric method [19]; total N by the Kjeldhal method [19].

2.3.2. Hydrocarbon analyses

The TEM and concentration of each fraction were determined by column chromatography (flash chromatography) [20]. The lubricant was extracted by soxhlet using chloroform as a solvent. The extract that contained total oil compounds and some biogenic lipids was estimated by weighing the dry residue after evaporation of the solvent. The dry extracts were suspended in 60 ml of hexane. The hexane-insoluble fraction containing the asphaltenes of the soils (and some polar lipids) were determined after filtration of the hexane solution on the solvent-washed pre-weighed Whatman GF/A glass—microfiber filters. Filters retaining the asphaltenes were dried and weighed. The hexane-soluble fraction (maltenes) was separated by solid–liquid chromatography on a 15 cm length × 1 cm diameter activated (overnight, at 120 °C) silica-gel (100–200 mesh) column. Successive elution was performed with 60 ml of hexane, 60 ml of a 60:40 (v/v) mixture of hexane–dichloromethane, and 60 ml of methanol. The fractions eluted with these solvents are named saturates, aromatics, and polars fraction of the oil, respectively. After evaporation of the solvents, the residual hydrocarbon was determined by gravimetrically.

2.3.3. Microbiological activities

A five-tube microbial numbers assay was used to estimate the number of hydrocarbon utilizing bacteria (HUB) [21]. A representative 10 g of soil was weighed and transferred to a bottle containing phosphate buffer. After stirring at 500 rpm for 30 min, the suspension was left to stand for 5 min, and then it was serially diluted 10-fold. Inoculation assay was conducted with Bushnell-Hass medium (250 μl) contained in 96-well ELIZA tray. A lube oil aliquot of 5 μl was used as a sole carbon source, and inocula (10 μl) were added to each well and incubated at 25 °C for 20 days. Then 10 μl of resazurin ($0.1\ g\ l^{-1}$) was added to each well and left to incubate for an additional 12 h. Resazurin was used as a redox indicator, which changed color of the medium from blue to pink, occurrence of a color change was counted as positive.

Soil samples for the respiration test were incubated in a closed vessel at 25 °C. The CO_2 produced was absorbed in 0.05 M sodium hydroxide and quantified by titration with 0.1 M hydrochloric acid. Before the titration, barium chloride was added to precipitate the absorbed CO_2 as barium carbonate [22].

Catalase activity was determined by measuring the H_2O_2 disappearance rate [23]. Forty milliliters of distilled water and 5 ml

of 30% H_2O_2 were added to 0.5 g of soil. The mixture was shaken for 10 min and then 5 ml of 3N H_2SO_4 were added. Twenty-five milliliters of the filtered solution was titrated with 0.05N $KMnO_4$. Controls were made in the same way, but with out adding H_2O_2.

Dehydrogenase activity was determined by the reduction of 2,3,5-triphenylterazolium chloride (TTC) to triphenyl formazane (TPF) [24]. Ten milliliters of TTC solution (1%) and 1 ml of distilled water were added to a 10 g of soil sample mixed with 0.1 g of $CaCO_3$. After 24-h incubation at 25 °C, the reaction product, TPF was extracted with methanol, and the absorbance measured at 485 nm.

3. Results and discussion

3.1. Soil properties

The soil had an optimal range of soil reaction, pH 7.6 for bioremediation, thus no further amendment was needed to ameliorate the soil reaction (Table 1). The contents of sand, silt, and clay were 60.3, 29.8, and 9.9%, respectively. Soil texture analysis result indicated that this soil has moderate air permeability and water holding capacity.

The level of heavy metals for the sample soil was very low. They were 3.52, 5.95, 3.31, and 0.08 mg kg^{-1} soil for Cu, Pb, Zn and Cd, respectively. This is in the range of the background level in Korea and suggested that there would be no heavy metal toxicity to microorganisms during bioremediation.

The level of available N (NH_4^+ and NO_3^-) and P (Bray P) were not sufficient enough to sustain microbial activity. Initial C/N/P ratio was calculated at 1160/4/1; this was calculated based on the carbon content of the TEM (85% of C) and only NH_4^+, NO_3^- and Bray P were readily available forms of N and P for microorganisms.

Examined soil contained approximately 8.58 log MPN g^{-1} soil hydrocarbon utilizing bacteria (HUB). Relatively high number of HUB contained in this soil can be explained by the long-lasting (30–40 years) contamination of the soil. Microorganisms had enough time to adapt themselves and their metabolism to the contamination.

3.2. Lubricants degradation

Bioremediation treatment by adding inorganic nutrients significantly enhanced lubricants degradation rate by the indigenous soil microorganisms compared to natural attenuation (non-fertilized). After 105 days, the initial lubricants contamination level, 9320 ± 343 mg kg^{-1} soil, was reduced to 4576–5393 mg kg^{-1} soil in fertilized soil, corresponding to the decontamination percentages of 42–51%, whereas, only 18% of the initial lubricant was eliminated in non-fertilized soil (Fig. 1(a)). Rhykerd et al. [5] reported similar results that 35–45% of the initial loading (50,000 mg kg^{-1}) lubricants degraded during 128 days. They adjusted the soil C/N/P ratio to 250/5/1, corresponding to 500/10 of C/N ratio in this experiment. These results confirmed that biostimulation of the indigenous soil microorganisms by nutrients addition resulted in an accelerated biodegradation of lubricating mineral oils. The hydrocarbon degrading activity was stimulated to a greater extent for the microcosm with the lowest nutrient level (C/N/P = 500/10/1), rather than that of with the highest nutrient level (C/N/P = 100/10/1).

There were some adverse effects for treated soil with high levels of nutrients, especially for the soil of C/N/P ratio of 100/10/1. Inhibition of degrading activity by excessive nutrients in highly contaminated soil applying enough nutrients to establish a narrow C/N/P ratio has been reported. Braddock et al. [8] found the rates of N application of 200–300 mg kg^{-1} to soil gasoline contaminated soil (max. 3500 mg kg^{-1} soil) reduced microbial degradation activity. Previously mentioned, to establish examined soil C/N/P ratio of 100/10/1, 4300 mg $(NH_4)_2SO_4$ kg^{-1} soil (equivalent 890 mg N kg^{-1} soil) was added in this experiment.

The decline of lubricants contents could be characterized by rapid degradation (i.e., pseudo- or first order decay) at initial stage and then reduced ('level off') to a much slower rate. For the fertilized soil, more than 80–90% of the overall lubricants disappeared within the 75 days of the incubation for the fertilized soil, and minimal amount of lubricants were disappeared thereafter. The remaining are known to be less available for biodegradation due to their recalcitrance and very limited bioavailability. This 'hockey stick' degradation curve is commonly observed regard-

Table 1
Chemical and physical properties of the soil used in this study

Property	Value	Property	Value
pH	7.6	Heavy metal[a]	
TEM[b] (mg/kg)	9320 ± 343	Cu (mg/kg)	3.52
Bray P (mg/kg)	17	Pb (mg/kg)	5.95
NH_4–N (mg/kg)	25	Zn (mg/kg)	3.31
NO_3–N (mg/kg)	4	Cd (mg/kg)	0.08
TKN (mg/kg)	230	Texture	Sandy loam
OM (%)	1.69	Sand (%)	60.3
CEC (cmol/kg)	10.53	Silt (%)	29.8
WHC[c] (%)	18	Clay (%)	9.9
Initial C/N/ratio[d]	1160/4/1	HUB (log MPN g^{-1} soil)	8.58

[a] 0.1N HCl extractable heavy metal.
[b] TEM: total extractable matter.
[c] Water holding capacity.
[d] Calculated value: based on 85% of carbon in TEM (total extractable matter), NH_4–N and NO_3–N, and Bray P content.

less of the initial hydrocarbon concentration or soil type in many experiments [21,25].

3.3. Degradation of each hydrocarbon fraction in lubricants

The degradation of saturates in lubricants exhibited the same pattern as the TEM degradation with a substantial drop in saturates removal rate after the 75 days (Fig. 1(b)). The effect of nutrient addition on hydrocarbon degradation was more obvious than that of the TEM. After the 105 days, the initial saturates hydrocarbon contamination of $5378 \pm 290\ mg\ kg^{-1}$ soil was reduced to 1671–1932 $mg\ kg^{-1}$ soil for the fertilized soils, corresponding to decontamination percentages of 66–71%, whereas, only 26% of the hydrocarbon was eliminated for the non-fertilized soil. The remaining saturates are known to be mainly composed of polycyclic alkylated saturate hydrocarbon [26,27] and branched molecules [28].

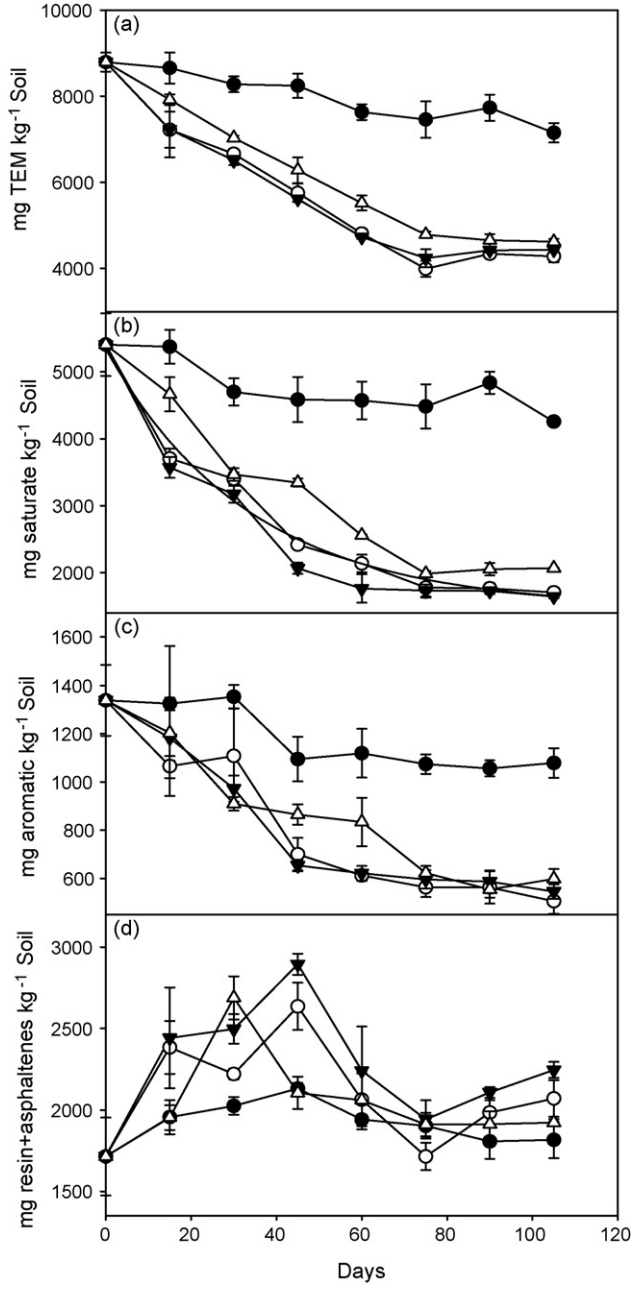

Fig. 1. Effect of nutrient levels on the degradation of lubricants and each fraction in soil microcosm (●) non-fertilized, (○) C/N/P (500/10/1), (▼) C/N/P (200/10/1), and (□) C/N/P (100/10/1)). Error bars show the standard deviation ($n = 3$).

Though great stimulation of aromatics degradation activity was also seen in fertilized soils but aromatics degraded much slower than that of saturates degradation rate (Fig. 1(c)). After 105 days, the initial aromatic hydrocarbon contamination of $1272 \pm 42\ mg\ kg^{-1}$ soil was reduced to 472–564 $mg\ kg^{-1}$ soil in fertilized soils, corresponding to decontamination percentage of 56–63%. In non-fertilized soil, only 15% of the hydrocarbon was eliminated. Among fertilized soils, similar extent of aromatics degraded in C/N/P ratio of 500/10/1 and 200/10/1 adjusted soil but in the soil of C/N/P ratio of 100/10/1, lower aromatics degradation activity was observed. The remaining aromatics are known to be polyaromatics and alkyl-substituted benzoid structure [26,27].

Fluctuating decomposition patterns were observed for polar fractions (Fig. 1(d)). These results strongly indicate that polars resulting from the biotransformation of poorly degradable hydrocarbons were pooled with polar fractions. It is confirmed by the fact that fertilized soils showed high degradation activity with more polar fractions detected than non-fertilized soils. Production of polar hydrocarbons during biodegradation of complex mixture was also suggested in other hydrocarbon degradation experiments [20,28–31]. Huesemann and Moore [30] ascribed this increase of polar fraction to the results of biomass formation associated with the biodegradation of hydrocarbons. Chaîneau et al. [31] also reported that polar metabolic compounds were released by hydrocarbon degraders during the biodegradation. Polar fraction is mainly composed of resins, nitrogen- and oxygen-heterocyclics, the toxicity of this residue is very low, which is fortunate because polar fractions are very stable [32,33].

3.4. Change in relative distribution of each fraction during biodegradation

Relative distribution of saturates, aromatics, and polars changed during biodegradation progresses. Initially, lubricants oil in this experiment composed of 65% of saturates 14% of aromatics and 20% of polar fraction.

Biodegradation produced a progressive decrease in the relative amounts of saturates and an increase in the polar compounds, apart from these two fractions aromatic was almost unchanged in fertilized soil, in which vigorous degradation activities occurred (Fig. 2). Saturates were significantly decreased from 60% to 38–41%, but polar fractions were increased from 5% to 20–30% in the fertilized soil. In non-fertilized soil, each fraction was almost unchanged during the whole experiment period.

These results were in agreement with other studies that demonstrated that saturates and the aromatics as a group were more susceptible to biodegradation than polars [28]. Moreover, the heterogeneous polar fraction could include biodegradation

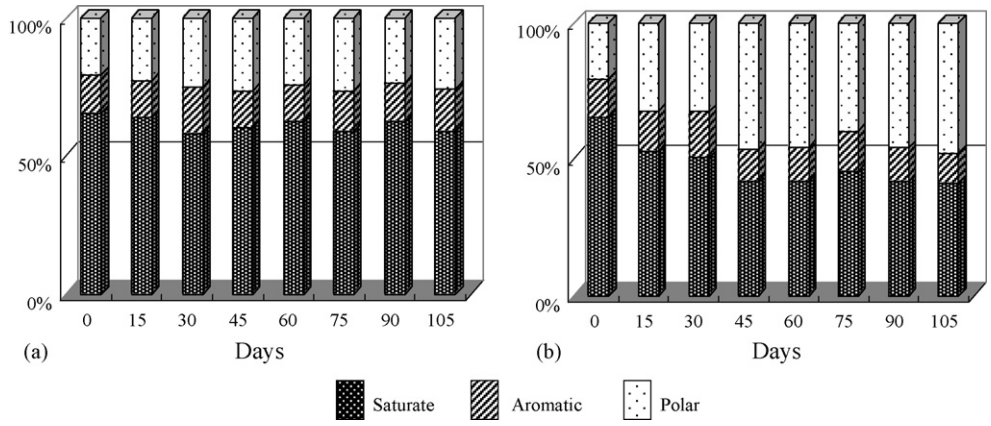

Fig. 2. The change of relative distribution of each fraction during bioremediation for various nutrient levels ((a) non-fertilized and (b) C/N/P = 500/10/1).

metabolites that could contribute to an increase in their mass following the biodegradation processes.

The decrease of degradation rates at later time (75 days or thereafter) were resulted from the increase of recalcitrant polar component. At this time, nutrient availability would no longer be the limiting factor once the content of polar component of the oil residue reaches some degree (60–70%) of the total mass [32].

3.5. Hydrocarbon degradation rates at different nutrient levels

Biodegradation of hydrocarbon-contaminated soils has been generally modeled by assuming simple first-order kinetics because it is suitable to describe the degradation pattern over time. First order kinetic implies an exponential decay of substrate concentration with an asymptote to zero. But, the soil hydrocarbon cannot be reduced to zero by using biological decontamination, even after a prolonged treatment. Because of the remaining hydrocarbons became less available for biodegradation due to their recalcitrance and very limited bioavailability [6,31,34,35].

For these reasons, it is important to avoid using kinetic data from early stages of bioremediation to predict parameters on later hydrocarbon losses, such as the time required to attain a cleanup standard [36]. When the biodegradation reached the residual concentration, a further intensive treatment is rarely useful and generally represents a waste of resources. It is noteworthy that the reduction of this residual concentration may often be the critical step to determine the time and limitation for the remediation, and the possibility to attain the regulatory objectives [6].

In this study, an asymptotic concentration was significantly higher than zero, even for the easily biodegradable saturates and/or the aromatics. From these results, it can be said that lubricants are consisted of two parts same with fuel oil, one is readily biodegradable and the other is refractory [34,35].

To reflect the existence of the remaining or refractory fraction of hydrocarbons, a term representing the refractory fraction was added to a first-order reaction, representing the degradation of hydrocarbon:

$$C_t = C_0\, e^{-kt} + b$$

where C_0 is the initial biodegradable contaminant concentration, C_t the total concentration of fraction at time t from the start of the test, and b is the estimated refractory fraction determined from the curve fitting.

The pseudo-first order degradation rate constants were calculated in accordance with the above equation summarized in Table 2.

Biodegradation rates of lubricants calculated from the new equation showed that even in the optimal condition, i.e., optimal nutrient conditions, significant amount of refractory fractions were remained. This fraction did not degrade even after prolonged treatment (see Table 2). For example, 1477 mg kg^{-1} soil of saturates, 316 mg kg^{-1} soil of aromatics, and 3348 mg kg^{-1} soil of TEM would be remained in the microcosm of C/N/P ratio of 500/10/1 adjusted soil. These refractory or hardly degradable fractions correspond to 25, 24 and 36% of the initial concentration, respectively. Many bioremediation experiments for soil have similarly demonstrated that a large fraction of applied or weathered crude oil or fuel was retained and sequestered and not further biodegraded [6,34,35].

3.6. Microbial activity during biodegradation

Results of the number of HUB are given in Fig. 3(a). Hydrocarbon degrading bacterial populations increased rapidly during the first 30 days of treatment in all soils, which corresponds with the rapid depletion of hydrocarbons during these periods. This early rapid increase of the hydrocarbon degrading population has been proposed as an indicator of the feasibility of bioremediation [21]. However, the absence or presence of nutrients did not influence significantly the number of HUB.

Fig. 3(b) shows the soil basal respiration during the bioremediation, resulting in substantial increases in soil respiration after 15 days from the incubation. Thereafter it gradually decreased to background level in the nutrient level of 200/10/1 and 500/10/1. For the highest level of nutrient treatment, further activity increase was observed after 60 days. Soil respiration is

Table 2
Biodegradation rates and estimated refractory fractions

Treatment	Loss rate constant (k[a]) (day^{-1})			Residual concentration ($mg\,kg^{-1}$ soil)		
	TEM	Saturates	Aromatic	TEM	Saturates	Aromatic
Non-fertilized	0.0046	0.0042	0.0074	7.668	4.233	891
C:N:P (500:10:1)	0.0236	0.0296	0.0206	3.348	1.477	316
C:N:P (200:10:1)	0.0241	0.0266	0.0202	3.777	1.519	358
C:N:P (100:10:1)	0.0136	0.0194	0.0155	3.955	1.628	431

[a] k = degradation rate constant of easily degradable.

a measure of the total biological activity and results from degradation of organic matter; the formation of CO_2 is the last step of carbon mineralization [14]. An initial increase in soil respiration in fertilized soil is interpreted as an indication of the successful hydrocarbon mineralization [13,14]. The trends matched well with that of the soil enzyme activities (Fig. 3(c) and (d)) (catalase and dehydrogenase).

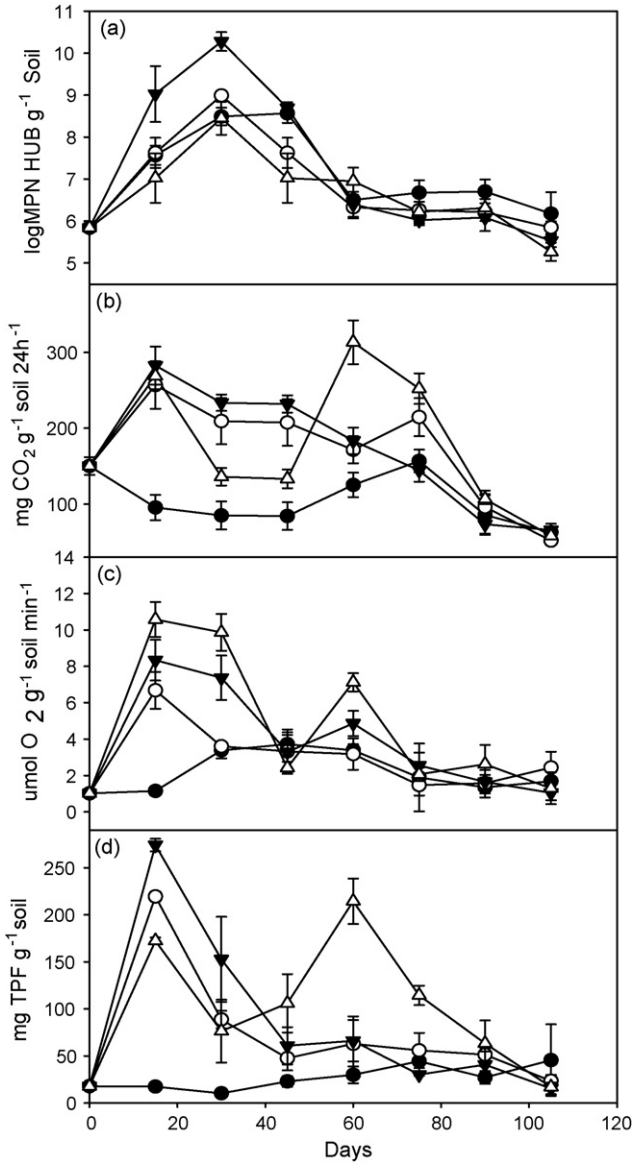

Fig. 3. Changes in the number of hydrocarbon degrading microorganisms (a), respiration in soil microcosms (b), catalase activity (c), and dehydrogenase activity (d) during bioremediation at four different nutrient levels: (●) non-fertilized, (○) C/N/P (500/10/1), (▼) C/N/P (200/10/1), and (□) C/N/P (100/10/1). Error bars show standard deviations (n = 3).

Catalase activities were significantly increased by nutrient addition (Fig. 3(c)). Substantial activity increase was observed after 15 days from the incubation; further activity peaks were observed after 60 days. In early periods, there were significantly higher catalase activities in fertilized soils than that of the non-fertilized soil. But at the end of the experiment, there was no significant difference. Catalase activity in soil has been considered as an indicator of an aerobic microbial activity (the obligate anaerobic microorganisms do not have catalase) and it has been related to both the number of aerobic microorganisms and soil fertility [37]. Catalase activity is a sensitive indicator because its substrate, H_2O_2 is highly toxic to microorganisms. As a result, the rate of the degradation of the hydrogen peroxide, catalase activity, reflects the relative activity and mass of aerobic microorganisms [37].

The time course of soil dehydrogenase activity in this study (Fig. 3(d)) was similar to that of the catalase activity (Fig. 3(c)). Dehydrogenase activities were substantially increased after 15 days; further activity increase was observed after 60 days in the 100/10/1 nutrient treatment. There was no significant change of dehydrogenase activity during the whole study in the non-fertilized soil. The nutrient treatments resulted in an increased dehydrogenase activity by 10-fold with a maximum value after the 15 days and then gradually decreased to background levels in the 200/10/1 and the 500/10/1 treatment. Dehydrogenase activity has also been correlated with the rate of oil removal and respiration [13,38]. The dehydrogenase activity in the 100/10/1 treatment was lower than that of the other nutrient treatment at early stage, while the intensities of the activities were reversed for further incubation. This result indicated that there were some adverse effects of high level of NH_4^+ on the microbial oxidative activity and hydrocarbon degradation activity. Frankenberger and Bingham [39] also reported that dehydrogenase was inhibited by high concentrations (>150 $mg\,ml^{-1}$) of $(NH_4)_2SO_4$.

Correlations between parameters measured during biodegradation experiment are present in Table 3. In the fertilized soil, all biological parameters were significantly correlated with each other, as well as with the amount of hydrocarbon degradation. Saturates concentration correlated strongly positively ($p < 0.01$) with dehydrogenase and catalase activities, respiration and the number of HUB. Aromatics concentration correlated positively ($p < 0.05$) with respiration and the number of HUB. There were also several significant correlations between the other measure soil parameters in fertilized soil. Activities of dehydrogenase,

Table 3
Correlation matrix for the investigated parameters during the bioremediation

Treatment	Parameter	Correlation coefficient					
		Aromaticc	Polar	Dedydrogenase	Catalase	Respiration	HUB
Non-fertilized	Saturates	−0.191	0.013	−0.050	0.335	−0.411*	0.211
	Aromatic		−0.314	−0.014	0.146	−0.143	0.369
	Polar			0.218	−0.165	0.370	−0.502*
	Dehydrogenase				−0.184	0.090	−0.297
	Catalase					−0.256	0.290
	Respiration						−0.424*
Biostimulation (C/N/P = 500/10/1)	Saturates	0.644**	−0.646**	0.667**	0.663**	0.643**	0.704**
	Aromatic		−0.415*	0.229	0.381	0.452*	0.504*
	Polar			−0.375	−0.357	−0.064	−0.458*
	Dehydrogenase				0.823**	0.566**	0.598**
	Catalase					0.450*	0.683**
	Respiration						0.583**

Twenty-one samples in each treatment were used in the analysis (three replicates were used for each treatment and sampling date). Significant at $^{*}p < 0.05$, $^{**}p < 0.01$.

catalase and soil respiration correlated with each other. It was suggested that the correlation between the soil biological parameters and with amount of hydrocarbon degradation or residues could help in elucidating microbial contributions to hydrocarbon elimination from soil [21].

4. Conclusions

Bioremediation by nutrient addition (biostimulation) is a viable choice for the remediation of aged lubricants. A significant reduction in the lubricating mineral oil could be achieved by nutrients addition. The carbon input (i.e., petroleum contaminants) may result in the depletion of the available pools of major inorganic nutrients such as N and P. When nutrients were added to soil at three levels (C/N/P = 500/10/1, 200/10/1, and 100/10/1) in this experiment, the greatest stimulation in microbial degrading activity occurred at the lowest nutrient treatment, rather than the highest one. However, providing nutrients for bioremediation did not result in the complete degradation of oil. A minimum of 40% of initial oil remained even in the most active bioremediation treatment. Kinetics on further hydrocarbon degradation would be much slower because the remaining hydrocarbons are more resistant to biodegradation. Biodegradation process of aged heavy mineral oil (lubricants) could be monitored well by soil biological parameters, such as soil respiration, number of HUB, and soil dehydrogenase and catalase activity. Soil biological methods should complement chemical methods when evaluating and assessing the success of decontamination process of lubricants.

References

[1] R.D. Norris, Handbook of Bioremediation, CRC Press, Boca Raton, FL, 1994.
[2] R.M. Atlas, R. Bartha, Hydrocarbon biodegradation and oil spill bioremediation, Adv. Microb. Ecol. 12 (1992) 287–338.
[3] P. Morgan, R.J. Watkinson, Hydrocarbon degradation in soils and methods for soil biotreatment, Crit. Rev. Biotechnol. 8 (1989) 305–333.
[4] W.J. Bartz, Lubricants and the environment, Tribiol. Int. 31 (1998) 35–47.
[5] R.L. Rhykerd, R.W. Weaver, K.J. McInnes, Influence of salinity on bioremediation of oil in soil, Environ. Pollut. 90 (1995) 127–130.
[6] M. Nocentini, D. Pinelli, F. Fava, Bioremediation of a soil contaminated by hydrocarbon mixtures: the residual concentration problem, Chemosphere 41 (2000) 1115–1123.
[7] M.H. Huesemann, Guidelines for land-treating petroleum hydrocarbon-contaminated soils, J. Soil Contamin. 3 (1994) 299–318.
[8] J.F. Braddock, M.L. Ruth, P.H. Catterall, Enhancement and inhibition of microbial activity in hydrocarbon-contaminated arctic soils: implications for nutrient-amended bioremediation, Environ. Sci. Technol. 31 (1997) 2078–2084.
[9] A. Saterback, R.J. Toy, B.J. McMain, M.P. Williams, P.B. Dorn, Ecotoxicological and analytical assessment of effects of bioremediation on hydrocarbon-containing soils, Environ. Toxicol. Chem. 19 (2000) 2154–2160.
[10] C.A.M. van Gestel, J.J. van der Waarde, J.G.M. Derksen, E.E. van der Hoek, M.F.X.W. Veul, S. Bouwens, B. Rusch, R. Kronenburg, G.N.M. Stokman, The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils, Environ. Toxicol. Chem. 20 (2001) 1438–1449.
[11] W.F. Ritter, R.W. Scarborough, A review of bioremediation of contaminated soils and groundwater, J. Environ. Health 30 (1995) 323–330.
[12] J.G. Xu, R.L. Johnson, Root growth, microbial activity and phosphatase activity in oil-contaminated, remediated and uncontaminated soils planted to barely and field pea, Plant Soil 173 (1995) 3–10.
[13] J.J. van der Waarde, E.D. Dijkhuis, M.J.C. Henssen, S. Keuning, Enzyme assays as indicators for bioremediation, in: R.E. Hinchee, G.S. Douglas, S.K. Ong (Eds.), Monitoring and Verification of Bioremediation, Battelle Press, Columbus, 1995, pp. 59–63.
[14] R. Margesin, G. Walder, F. Schinner, The Impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activity and microbial properties of soil, Acta Biotechnol. 20 (2000) 313–333.
[15] R.H. Bray, L.T. Kurtz, Determination of total organic and available forms of phosphorus in soils, Soil Sci. 59 (1947) 39–45.
[16] D.W. Nelson, L.E. Sommers, Total carbon, organic carbon, and organic matter, in: D.L. Sparks (Ed.), Method of Soil Analysis: Part III-Chemical Methods, American Society of Agronomy, Madison, WI, 1996, pp. 961–1010.
[17] M.E. Sumner, W.P. Miller, Cation exchange capacity and exchange coefficients, in: D.L. Sparks (Ed.), Method of Soil Analysis: Part III-Chemical Methods, American Society of Agronomy, Madison, WI, 1996, pp. 1201–1229.
[18] G.W. Gee, J.W. Bauder, Particle-size analysis, in: A. Klute (Ed.), Method of Soil Analysis: Part I-Physical and Mineralogical Methods, American Society of Agronomy, Madison, WI, 1996, pp. 384–411.

[19] J.M. Mremner, Nitrogen-total, in: D.L. Sparks (Ed.), Method of Soil Analysis: Chemical methods, Part III. Agronomy Monograph No. 5, American Society of Agronomy, Madison, WI, 1996, pp. 1085–1121.
[20] J. Oudot, F.X. Merlin, P. Pinvidic, Weathering rates of oil components in a biodegradation experiment in estuarine sediments, Marine Environ. Res. 45 (1998) 113–125.
[21] H.G. Song, R. Bartha, Effects of jet fuel spills on the microbial community of soil, Appl. Environ. Microbiol. 56 (1990) 646–651.
[22] F. Schinner, R. Öhlinger, E. Kandler, R. Margesin (Eds.), Methods in Soil Biology, Springer, 1996, pp. 93–110.
[23] R. Rodriguez-Kabana, B. Truelove, Effects of crop rotation and fertilization on the catalase activity in a soil of the south-eastern United States, Plant Soil 69 (1982) 97–104.
[24] L.E. Casida, D.A. Klein, T. Santoro, Soil dehydrogenase activity, Soil Sci. Soc. Am. J. 47 (1964) 599–603.
[25] L.N. Chaîneau, J.L. Morel, J. Oudot, Microbial degradation in soil microorganisms of fuel oil hydrocarbon from drilling cutting, Environ. Sci. Technol. 29 (1995) 1615–1621.
[26] J. Oudot, Rates of microbial degradation of petroleum components as determined by computerized capillary gas chromatography and computerized mass spectrometry, Marine Environ. Res. 13 (1984) 277–302.
[27] S.D. Killops, M. Al-Juboori, Characterization of the unresolved complex mixture (UCM) in the gas chromatograms of biodegraded petroleum, Organic Geochem. 15 (1990) 147–160.
[28] M.A. Gough, S.J. Rowland, Characterization of the unresolved complex mixtures of hydrocarbons in petroleum, Nature 344 (1990) 648–650.
[29] Y.S. Oh, W.Y. Choi, Y.H. Lee, S.C. Choi, S.J. Kim, Biological treatment of oil-contaminated sand: comparison of oil degradation based on thin-layer chromatography/flame ionization detector and respirometric analysis, Biotechnol. Lett. 22 (2000) 595–598.
[30] M.H. Huesemann, K.O. Moore, Compositional changes during landfarming of weathered Michigan crude oil-contaminated soil, J. Soil Contamin. 2 (1993) 245–264.
[31] C.H. Chaîneau, J. Morel, J. Dupont, E. Bury, J. Oudot, Comparison of the fuel oil biodegradation potential of hydrocarbon-assimilating microorganisms isolated from a temperatural soil, Sci. Total Environ. 227 (1999) 237–247.
[32] J.R. Bragg, R.C. Prince, E.J. Harner, R.M. Atlas, Effectiveness of bioremediation for the Exxon Valdez oil spill, Nature 368 (1994) 413–418.
[33] A.D. Venosa, M.T. Suisan, D. King, B.A. Wrenn, Use of hopane as a conservative biomarker for monitoring the bioremediation effectiveness of crude oil contaminating sand beach, J. Indust. Microbiol. Biotechnol. 18 (1997) 131–139.
[34] K.W. Brown, K.C. Donnelly, Influence of soil environment on biodegradation of refinery and a petrochemical sludge, Environ. Pollut. (Ser. B) 6 (1983) 119–132.
[35] D.E. Demque, K.W. Bigger, J.A. Heroux, Land treatment of diesel contaminated sand, Can. Geotech. J. 34 (1997) 421–431.
[36] J.M. Brown, J. Syslo, Y.H. Lin, S. Getty, R. Vemuri, R. Nadeau, On-site treatment of contamination soils: an approach to bioremediation of weathered petroleum compounds, J. Soil Contamin. 7 (1998) 773–800.
[37] F. Schinner, R. Öhlinger, E. Kandler, R. Margesin (Eds.), Methods in Soil Biology, Springer, 1996, pp. 235–245.
[38] R. Margesin, F. Schinner, Bioremediation of diesel-oil-contaminated alpine soils at low temperatures, Appl. Microbiol. Biotechnol. 47 (1997) 462–468.
[39] W.T. Frankenberger, F.T. Bingham, Influence of salinity on soil enzyme activities, Soil Sci. Soc. Am. J. 46 (1982) 1173–1177.