Research Articles: Soil Sciences

Aliphatic Hydrocarbons in an Oil-Contaminated Soil

Carbon Economy During Microbiological Decontamination

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Abstract

Microbial decontamination of hydrocarbon-polluted soil was paralleled with soil respiration measurements. About 1,500 tons of a loamy top soil were found to be contaminated with approximately 2000 mg/kg of aliphatic hydrocarbons, mainly oleic (C18:1) and linoleic acid (C18:2) found in the vicinity of a linoleum manufacturing and then a car dewaxing plant. The contaminated soil was analysed for dry matter, pH, dehydrogenase activity, electrical conductivity and nutrient content viz. nitrate, phosphorus and potassium, as well as a number of indigenous microbes. The soil was low in salt and nutrients. This paper describes the procedure and measures to decontaminate this bulk soil on site from approx. 2,000 to 500 mg of aliphatic hydrocarbons/kg dry matter by use of a nutrient emulsion, indigenous micro-organisms and aeration over 13 months. This 75% reduction in aliphatic hydrocarbons resulted in a concomitant carbon efflux, measured as soil respiration, and was used to calculate carbon fluxes.

Keywords: Biodegradation; (de-)contamination; hydrocarbons; micro-organisms; PAHs; PCBs; pollution; polychlorinated biphenyls (PCBs); polycyclic aromatic hydrocarbons (PAHs); respiration; soil

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) as components of petroleum and derived products are environmental pollutants because of their carcinogenicity (AKHLAQ, 1997). Soil contamination by such xenobiotic chemicals has become a serious global problem, not only because it reduces the value of land for recreational use and habitation, but also because these chemicals are potential sources of water pollution. The effects of environmental disasters, such as those in Alaska, Kuwait or Russia have produced a worldwide increase in the awareness of land hydrocarbon pollution and subsequent requirements for large-scale soil decontamination.

Xenobiotic compounds such as hydrocarbons may persist in soil because the physical or chemical environment is unsuitable for degradation. These constraints can include inappropriate soil pH, low levels of mineral nutrients and oxygen deprivation. In soils where the appropriate indigenous bacterial strains are present, bioremediation may consist of optimising soil conditions such as modifying pH, nutrient amendment and aeration, a process sometimes referred to as biostimulation (ELLIS, 1992). However, the biological activity of the soil microbes during decontamination is not completely understood. There is no indication how the microbial breakdown of hydrocarbons affects soil activity *viz.* respiration, carbon turnover and carbon balance and whether these factors could be used for *in situ* monitoring of the sigmoidal rate of degradation (LOTTER, HEERENKLAGE and STEGMANN, 1993). Monitoring of environmental conditions and soil respiration could be used to determine those situations which favour indigenous microbes. Hence, the objective of the present work was to monitor soil respiration as a measure of microbial activity during hydrocarbon degradation. Therefore, a time course study related to hydrocarbon concentration was established to examine whether soil respiration and the resulting carbon balance could be used as a bio-indicator for microbial decontamination. The work was conducted as part of an industrial project in Germany on a soil affected by the demolition of a linoleum manufacturing and the subsequent establishment of a car dewaxing plant.

2 Experimental

2.1 Soil and treatment

The topsoil requiring decontamination comprised a pleistocene loess and loess-loam with a predominant silt fraction. The soil was from the environs of an old linoleum manufacturing plant in Bedburg, near Cologne, Germany (Rheinische Linoleum Betriebe) which had partially been converted into a car de-waxing plant. It was heavily mono-contaminated with an average of 2,000 mg hydrocarbons per kg soil dry matter. The hydrocarbons consisted of polar and non-polar aliphatic molecules and mainly oleic (C18:1) and linoleic acid (C18:2). There was no polycyclic aromatic hydrocarbon (PAH) or polychlorinated biphenyl (PCB) contamination.

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The aim was to reduce the contamination from 2,000 to 500 mg hydrocarbon/kg dry matter under the supervision of Claytex Consulting GmbH, Bergheim, Germany who also performed the chemical analysis. About 1,450 t of contaminated soil was removed for microbial treatment. It was sorted, i.e. rocks and building debris removed, mixed and loosened 3 times by a rotating cultivator in March and during the beginning and at the end of April. The soil was covered on-site with two tents of approx. 16 m*26 m each (416 m^2) over 60 cm of soil height.

A nutrient (NPK) emulsion containing natural plant oils (palm kernel oil, coconut oil and soya oil) was added at a rate of 300 g per t of disturbed soil at the start of the decontamination and after six months to favour the selection of indigenous oil-degrading microbes under ambient temperature using the endogenous winter soil moisture. Soil aeration was achieved by repeated mechanical perturbation.

2.2 Chemical and biochemical soil analysis

Hydrocarbons were determined following the German DIN 38 409 H 18 protocol: 50 g air-dried soil was extracted with 20 ml of 1,1,2-trichlorotrifluoroethane (TTF), soil and solvent mixed for 15 min in an ultrasonic bath and left to settle. The supernatant, i.e. the organic fraction including the hydrocarbons, was eluted on a 20 x 250 mm column chromatograph using 8 g aluminium oxide. The optical transmission of the eluate was measured at 3.38 µm for CH_3 and at 3.42 µm for CH_2 groups in an IR spectrophotometer.

Nitrate was determined after extraction of 25 g fresh soil with 100 ml of 1% K_2SO_4 + 0.05 M $CaCl_2$, shaken for 1 h, filtered and nitrate in the supernatant was reduced by cadmium to nitrite in a segmented flow analysis system. The formation of the red colour complex was then measured photometrically at 540 nm (Skalar Autoanalyser).

Potassium and phosphorus were extracted from 5 g of air-dried and sieved (2 mm) soil with 100 ml calcium lactate, pH 4.1 after 2 h of shaking and subsequent filtration. For the determination of the phosphorus concentration, 1 ml of concentrated HNO_3 was added to 25 ml of this filtrate, 5 ml dye solution (containing HNO_3, ammonium vanadate and ammonium heptamolybdate) were added, mixed, left for 1 h and the absorbance was measured photometrically at 436 nm. Potassium was measured in the filtrate by atomic absorption spectrometry (AAS) at 880 nm. The water-holding capacity was determined gravimetrically using 100 g of dry soil which was then saturated with water.

Soil moisture was determined by drying samples at 105°C until their weight was constant. Dehydrogenase activity (DHA) was measured at 546 nm in a VIS spectrophotometer using the reduction of triphenyltetrazolium-chloride (TTC) to triphenylformazan (TPF) by soil microbes (ALEF and NANNIPIERI, 1996). Fresh soil was sieved and 5 g extracted with 5 ml of 1% [w/v]) TTC in Tris-HCl buffer adjusted to pH 7.6, incubated at 30°C for 24 h. The incubation was stopped by the addition of 30 ml acetone (with 10 ml dest. water), shaken for 2 h in the dark and values expressed as µg TPF/g soil dry matter. Each soil sample was assayed three times for DHA.

CFU were determined following the method of ALEF and NANNIPIERI (1996). Ten grams of contaminated soil were suspended under aseptic conditions in 100 ml tetrasodium pyrophosphate solution, shaken for 30 min, left 2-5 min to settle and the clear supernatant was used for a dilution series in NaCl, KCl and NaH_2PO_4. Aliquots of 0.1 ml of each dilution step were spread on agar plates and incubated at 20°C for 10 days.

The electrical conductance was determined using a measuring cell type LTA/F from WTW Weilheim, Germany and attached data-logger type LF3000, and a soil:water ratio of 1.5 following the manufacturer's instructions.

2.3 Measurement of CO_2 evolution

Soil respiration was measured using a portable, battery-driven infrared CO_2 analyser type EGM-1 and a 1.2 litre respiration chamber type SRC-1 (both from PP Systems, Hitchin, UK). The technology became commercially available at the end of 1993 and a first prototype was made available from the Institut für Obstbau, Bonn, Germany. The measuring principle is based on a closed air circuit between a ventilated respiration chamber and the CO_2 analyser (BLANKE, 1996). The system integrates the increases in CO_2 concentration in this closed air circuit due to soil CO_2 efflux over time (PARKINSON, 1981). The analyser features automatic zero-calibration every 60 secs., and for ambient CO_2 before each recording. Records were stored in the EGM datalogger and transferred to a computer. Soil temperature was assessed by a dedicated soil temperature probe (PP Systems) in 10 cm soil depth.

2.4 Carbon balance

Respiration values were compared with carbon balance data and calculated on the basis of degraded hydrocarbons, to verify soil respiration measurements as an indicator of the degree of biodegradation.

3 Results

3.1 Soil characteristics

To characterise the soil and identify potential treatments, chemical and biological soil properties were investigated before decisions on biodegradation practices. Four samples of the contaminated soil were examined for dry matter, pH,

dehydrogenase activity, number of indigenous microbes as well as electrical conductivity and nutrient content, viz. nitrate, phosphorus and potassium, to demonstrate the soil properties (→ *Tables 1* and *2*). The soil was characterised as neutral and base-saturated with a large ion exchange capacity (data not shown).

The soil contained a relatively large number of indigenous microbes (→ *Table 1*), a condition typical of many agricultural soils rather than for a soil from an industrial area. The measurements of electrical conductivity (→ *Table 2*) showed that the soil under examination exhibited a low salt concentration. The soil under investigation was also characterised by a relatively low nutrient level in terms of nitrogen, phosphorus and potassium (→ *Table 2*), a condition typical for a soil from an industrial site. Hence, to ascertain ample nutrient supply for the microbes, a NPK nutrient emulsion was applied to the soil beds at a rate of 300 g/t soil in November 1993 and the treatment was repeated in May 1994.

3.2 Laboratory batch trial

To test the appropriate decontamination procedure, a laboratory batch test trial was started using approx. 10 kg of soil which was additionally contaminated with approx. 20 ml of diesel (gasoil) in August 1993. The batch trial comprised two replicates, either without or with NPK emulsion to cope with the low nutrient level (→ *Table 2*). Overall, the microbial numbers increased 13 or 26-fold over two months from 2.8-8.4 x 10^6/g soil in the beginning to 72-110 x 10^6/g soil at the end (→*Fig. 1*). The NPK emulsion induced a vigorous microbial propagation which peaked after six weeks followed by a sharp decline and smaller further increase, while the untreated soil showed a slow and steady increase in microbial numbers with a final peak (→ *Fig. 1*). In the same period, the aliphatic hydrocarbon (hc) content was measured weekly and decreased sigmoidally from 3,600 mg hc/kg dry matter at the beginning to 200 mg hc/kg dm after the two months (→ *Fig. 2*), a curve shape typical of most microbial degradations. Unexpectedly, the differences in hydrocarbon biodegradation between the NPK treatment and control were small relative to the large differences between bacterial numbers by the NPK application and possibly also due to simultaneous stimulation of non-degrading microbes.

3.3 On-site decontamination

The on-site treatment in two tents comprised the NPK emulsion which was shown in the batch trial to cope with the low endogenous nutrient level in the original soil (→ *Table 2*). The NPK emulsion containing natural plant oils was added in November 1993 and in the beginning of May 1994 at 300g/t soil to favour the selection of endogenous oil-degrading microbes. Soil aeration was achieved by mechanical perturb-

Table 1: Dry matter, pH, dehydrogenase activity, and number of indigenous microbes of the contaminated soil before biodegradation (means, standard error and deviation)

Sample stats	Dry matter [% dm]	Soil PH	Dehydrogenase [Xg TPF/g dm]	ColoniesFU [x10^6 g^{-1} dm]
1	79	8.1	2.5	11.5
2	82	6.8	82.9	34.5
3	80	6.7	375	6
4	82	7.3	32.9	3.6
∅	80.7	7.23	123.3	13.9
sd	1.5	0.64	171	14.1
se	0.75	0.32	85.51	7

Table 2: Nitrate, phosphorus and potassium contents, electrical conductivity and water holding capacity (WHC) of the contaminated soil before biodegradation (means, standard error and deviation)

Sample stats	Water hc[%]	E.cond. [mS/m]	Nitrate [mg/kg dm]	Phosphorus [mg/kg dm]	Potassium [mg/kg dm]
1	46	18.9	9.5	25	135
2	27	52.9	11	50	190
3	32	13.5	44	45	100
4	23	27.6	43	200	650
∅	32	28.23	26.88	80	268.75
sd		17.45	19.21	80.73	256.85
se		8.72	9.61	40.36	128.43

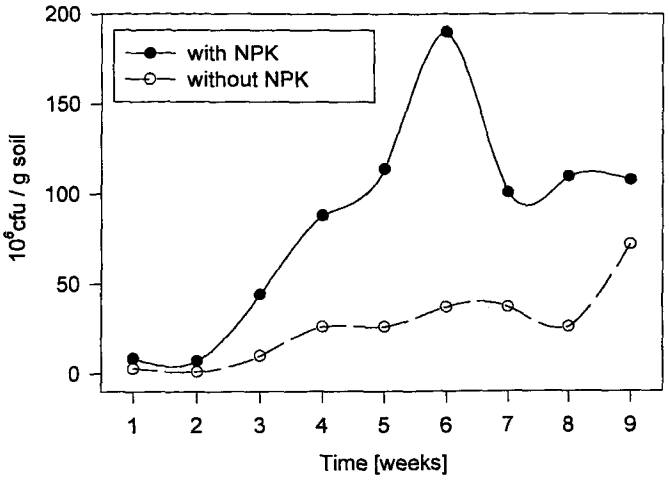

Fig. 1: Increase in microbial numbers (cfu per g soil) of the batch trial over two months

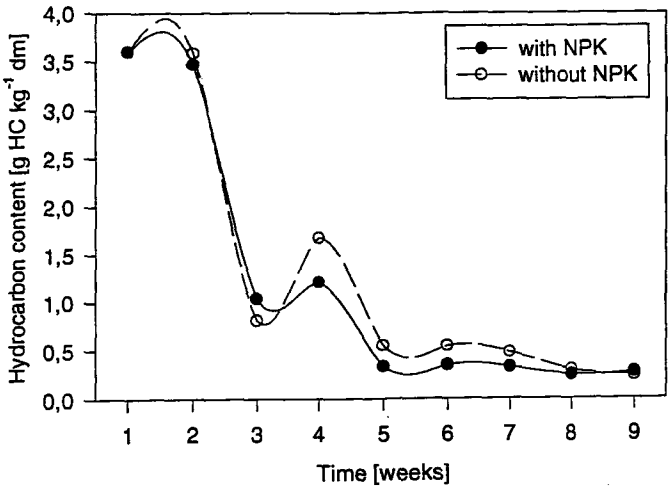

Fig. 2: Microbial degradation of the hydrocarbon content of the batch trial treated with or without an NPK emulsion which contained NPK plus natural plant oils

Fig. 3: Soil respiration measurement during soil decontamination showing the portable instrument and respiration chamber

ance. Based on the data from the batch trial, hydrocarbons should be degraded in the on-site trial by indigenous microbes from approx. 2,000 to 500 mg hc/kg dm during the decontamination.

3.4 Soil respiration and carbon balance

Soil respiration was measured before (5 April 94) and after soil aeration (10 May 1994) and the second NPK emulsion application was performed during 3-5 May 1994 (→ *Fig. 3*). Over this period, soil respiration increased from 0.5 g CO_2 m^{-2} h^{-1} to 2.2 g CO_2 m^{-2} h^{-1}. Parallel measurements showed that the CO_2 concentration in the tent had risen to 405-412 ppm CO_2, compared to 320 ppm CO_2 outside the tent.

The rate of soil respiration paralleled that of the microbial hydrocarbon degradation. The carbon balance was calculated based on soil hydrocarbon contents before and after breakdown, soil mass and soil respiration rate (→ *Table 3*).

4 Discussion

4.1 Soil decontamination

The contaminated top soil was low in salt and nutrients. Hence, the nutrient (NPK)-containing emulsion with plant oils added at the beginning and after half a year favoured the selection of indigenous bacteria (→ *Fig. 1*). Previous work has shown that indigenous *Pseudomonas* bacteria are

Table 3: Carbon economy of hydrocarbon biodegradation

Hydrocarbon content per kg dry soil	
before biodegradation	2,000 mg hc/kg soil
after biodegradation	500 mg hc/kg soil
to degrade:	1,500 mg hc/kg soil
For 1,450 t soil (1,160 t dry mass)	1,740 t hc /1,160 t dry soil
For 832 m^2 soil bed (tent) surface	2.09 kg hc/m^2
On a molar basis (1 mol oleic acid=282g)	7.41 mol/m^2
1 Mol oleic acid produces 18 moles CO_2	133.4 mol CO_2/m^2
To degrade in 2 months	25.7 µmol CO_2 m^{-2} s^{-1}
Soil respiration measured	20-30 µmol CO_2 m^{-2} s^{-1}

frequently responsible for such a breakdown (VERHAGEN et al. 1995); the bacteria are renowned for a complete breakdown of compounds without leaving residual metabolites. The sigmoidal decrease during bacterial breakdown in aliphatic hydrocarbon content from 3,600 mg hc/kg dm at the beginning to 200 mg hc/kg dm after the two months of the batch trial is typical of many microbial degradations. The successful decontamination to 200 mg hc/kg produced a soil suitable for non-food horticulture or agriculture.

4.2 Soil respiration and carbon balance

Soil respiration, measured before and after soil aeration and the second NPK emulsion application, increased from 0.5 g CO_2 m^{-2} h^{-1} to 2.2 g CO_2 m^{-2} h^{-1} (→ *Table 3*). Parallel measurements showed that the CO_2 concentration in the tent had risen to 405-412 ppm CO_2, compared to 320 ppm CO_2 outside the tent. The fast soil respiration rates of 20-30 µmol CO_2 m^{-2} s^{-1} during biodegradation compare with 0.2 - 24 µmol CO_2 m^{-2} s^{-1} in horticultural soils (BLANKE, 1996). The data in **Table 3** showed excellent correspondence between the measured (soil respiration) and calculated hydrocarbon content. The soil respiration measurements confirmed the carbon balance calculations based on inherent hydrocarbon contents before and after microbial breakdown, soil mass and time (→ *Table 3*) and suggest soil respiration as a good indicator.

5 Conclusions

The results may indicate that a carbon budget calculation aided by soil respiration measurement in situ is one indicator to monitor the state of such biodegradation.

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