

MINERALISATION OF TARGET HYDROCARBONS IN THREE
CONTAMINATED SOILS FROM FORMER REFINERY FACILITIES

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Abstract

This study investigated the microbial degradation of ^{14}C -labelled hexadecane, octacosane, phenanthrene and pyrene and considered how degradation might be optimised in three genuinely hydrocarbon contaminated soils from former petroleum refinery sites. Hydrocarbon mineralisation by the indigenous microbial community was monitored over 23 d. Hydrocarbon mineralisation enhancement by nutrient amendment (biostimulation), hydrocarbon degrader addition (bioaugmentation) and combined nutrient and degrader amendment, was also explored. The ability of indigenous soil microflora to mineralise ^{14}C -target hydrocarbons was appreciable; $\geq 16\%$ mineralised in all soils. Generally, addition of nutrients or degraders increased the rates and extents of mineralisation of ^{14}C -hydrocarbons. However, the addition of nutrients and degraders in combination had a negative effect upon ^{14}C -octacosane mineralisation and resulted in lower extents of mineralisation in the three soils. In general, the rates and extents of mineralisation will be dependent upon treatment type, nature of the contamination and adaptation of the indigenous microbial community.

Capsule: Bioremediation strategy, native hydrocarbon concentrations and prior exposure histories of the microbial community influence hydrocarbon degradation in soil.

Keywords: Mineralisation, hydrocarbons, catabolic activity, nutrient addition and degrader amendment

1. Introduction

Anthropogenic hydrocarbon contamination of soil is a global issue throughout the industrialised world (Macleod et al., 2001; Brassington et al., 2007). In England and Wales alone, 12% of all serious contamination incidents in 2007 were hydrocarbon related (Environment Agency, 2005). Soil acts as a repository for many hydrocarbons, which is a concern due to their adverse impact on human health and their environmental persistence (Jones et al., 1996; Semple et al., 2001). Consequently, UK legislation has been introduced enforcing that if risk is posed to human, water or ecological receptors, contaminated land must be remediated to a level suitable for use (Paton et al., 2005). Sustainable remediation has therefore become a desirable option for the treatment of hydrocarbon-contaminated soils (Semple et al., 2001).

Predominately, hydrocarbon soil contamination is associated with the accidental spillage and leakage of refined petroleum derived products (Pollard et al., 1994). Consequently, potentially toxic and persistent contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and mid to long-chained aliphatic hydrocarbons, are often dispersed ubiquitously in the environment (Wild and Jones, 1995). Polycyclic aromatic and heavier aliphatic hydrocarbons, which have a stable recalcitrant molecular structure, exhibit high hydrophobicity and low aqueous solubility, are not readily removed from soil through leaching and volatilisation. In addition, as these hydrocarbons ‘weather’ in soil, their bioavailability, phase partitioning, toxicity and degradability change (Brassington et al., 2007).

The degradation of petroleum-derived hydrocarbons has been widely studied and it has been established that microbial degradation is a key removal pathway of

76 hydrocarbons from the soil matrix (Atlas, 1981; Leahy and Colwell, 1990; Cerniglia,
77 1992; Budzinski et al., 1998; Loser et al., 1999; Bogane et al., 2003). Primarily,
78 contaminant bioavailability plays a pivotal role in hydrocarbon degradation in soil.
79 Additional factors that influence the degradation process included soil pH, moisture
80 and organic matter content and hydrocarbon aqueous solubility, octanol-water
81 partitioning coefficient and structure (Leahy and Colwell, 1990; Ramírez et al., 2008).
82 Effective biodegradation is dependent upon optimal biological (microbial
83 functionality and biomass size), chemical (bioavailability and nutrients) and physical
84 (water holding capacity) parameters.

85

86 It is acknowledged that with ‘weathering’, the hydrocarbon bioavailability changes.
87 This impacts upon the relative toxicity of the hydrocarbons and their susceptibility to
88 biodegradation, assessed for bioremediation purposes (Brassington et al., 2007;
89 Ramírez, et al., 2008). Bioassays may be used to evaluate the potential of indigenous
90 soil microbial populations to degrade representative petroleum hydrocarbons, the
91 impact these hydrocarbons have upon microbial activity and provide information on
92 the bioavailability of contaminants in soils (Chaineau et al., 1995; Reid et al., 2000;
93 Stokes et al., 2005). Through the measurement of lag phases (time prior to
94 mineralisation reaching 5 %), and maximum rates and extents of a ^{14}C -labelled target
95 hydrocarbon, in laboratory based mineralisation assays, biodegradation performance
96 and justifiable end points can be accurately assessed (Macleod et al., 2001).

97

98 The hypotheses for this study were (i) soils contaminated with high concentrations of
99 weather petroleum hydrocarbons have active indigenous microbial degrader
100 populations able to degrade aliphatic and aromatic hydrocarbons and (ii) the addition

of nutrients and/or hydrocarbon degraders will increase catabolic activity in these soils, and enhance hydrocarbon degradation. To address these hypotheses, the following aims were considered: (i) to describe the microbial degradation of ^{14}C -labelled hexadecane, octacosane, phenanthrene and pyrene; (ii) to consider how degradation might be optimised in three genuinely hydrocarbon contaminated soils from former petroleum refinery sites, and (iii) to investigate enhancing microbial mineralisation of the target hydrocarbons by increasing microbial activity through (a) nutrient amendment, (b) addition of hydrocarbon degraders and (c) combined nutrient and degrader amendment.

2. Materials and methods:

2.1 Materials

Non-labelled and ^{14}C -labelled aliphatic and aromatic hydrocarbons; [$1\text{-}^{14}\text{C}$] hexadecane-n (specific activity = $12.0 \text{ mCi mmol}^{-1}$), [$14, 15\text{-}^{14}\text{C}$] octacosane (specific activity = $20.5 \text{ mCi mmol}^{-1}$), [$9\text{-}^{14}\text{C}$] phenanthrene (specific activity = $55.7 \text{ mCi mmol}^{-1}$) and [$4, 5, 9, 10\text{-}^{14}\text{C}$] pyrene specific activity = $48.5 \text{ mCi mmol}^{-1}$), were all supplied by Sigma Aldrich Co, UK. Ultima Gold liquid scintillation cocktail was obtained from Canberra Packard, UK. Merck, UK supplied the NaOH. The K_2HPO_4 , KH_2PO_4 and salicylic acid were obtained from Fisher Scientific, UK. BDH Laboratory Supplies, UK supplied the NH_4NO_3 . The hydrocarbon degrading inoculum and Bushnell Hass was obtained from Remedios Limited, UK. Oxoid Ltd, UK supplied the plate count agar (Tryptone Glucose Yeast Agar), minimal agar and Ringer solution. Internal standards utilised in the TPH extractions; Nonadecane- d_{40} , Triacontane- d_{62} , Naphthalene d_8 , Phenanthracene- d_{10} , Chrysene- d_{12} and Perylene d_{12} , were all purchased from Sigma Aldrich, UK.

2.2 Soil preparation and characterisation

Three different soils collected at a depth of 5 – 20 cm from former oil refinery facilities were labelled A, B and C (to maintain owner anonymity). Soil A and C were untreated, whilst soil B had been previously biopiled. The soils were air-dried for 24 h to enable them to be sieved through 2 mm, in order to remove stones, plant material and facilitate mixing. Prior to air drying the field moisture content was determined in triplicate by oven drying at 105°C for 24 h. Soils were then stored at 4°C in the dark before use.

The standard physical and chemical properties of each soil were analysed in triplicate (Table 1). Following particle size analysis, determined using 40 ± 0.1 g soil (dry wt) as per the hydrometer method (Klute, 1986), and based upon the USDA texture classification system, soils A and B were categorised as clay and soil C as sandy clay loam. The organic matter content (LOI) of each soil was measured by combustion at 450°C in a furnace for 24 h, according to ASTM Method D297487. Soils A and B had organic matter content of $\leq 15\%$, whilst soil C contained the highest amount of organic matter ($26.47 \pm 1.45\%$) and organic carbon ($15.39 \pm 0.84\%$; Table 1). All soils were determined to be slightly acidic with a pH range of 6.1 to 6.6, measured with a PHM 220 lab pH meter (Model 657R-00) in a 1:5 soil (dry wt) to liquid suspension, using 0.01 M calcium chloride (CaCl_2) solution and then separately with deionised water.

Determination of the total petroleum hydrocarbon (TPH) concentration in the soil was performed by sequential ultrasonic solvent extraction as described by Risdon et al

(2008). Total hydrocarbon petroleum (TPH), aliphatic and aromatic fractions were identified and quantified by gas chromatography-mass spectrometry using a Perkin Elmer AutoSystem XL gas chromatograph coupled to a Turbomass Gold mass spectrometer operated at 70 eV in positive ion mode. The column used was a Restek fused silica capillary column (30 * 0.25 mm internal diameter) coated with RTX®-5MS (0.25 µm film thickness). Splitless injection with a sample volume of 1µl was applied. The oven temperature was increased from 60°C to 220°C at 20°C min⁻¹ then to 310°C at 6°C min⁻¹ and held at this temperature for 15 min. The mass spectrometer was operated using the full scan mode (range *m/z* 50-500) for quantitative analysis of target alkanes and PAHs. For each compound, quantification was performed by integrating the peak at specific *m/z*. External multilevel calibrations were carried out for both oil fractions, quantification ranging from 0.5 to 2500 µg ml⁻¹ and from 1 to 5 µg ml⁻¹, respectively. Internal standards for the alkanes were nonadecane-d₄₀ and Triacontane-d₆₂ and Napthalene d₈, Phenanthracene-d₁₀, Chrysene-d₁₂ and Perylene d₁₂. For quality control, a 500 µg ml⁻¹ diesel standard and mineral oil were analysed every 10 samples. In addition, duplicate reagent control and reference material were systematically used. The reagent control was treated in exactly the same manner as the samples but contained no soil. The reference material was an uncontaminated soil of known characteristics, and was spiked with a diesel and mineral oil standard at a concentration equivalent to 16000 mg kg⁻¹. High concentrations of TPH (≥1.8%) were measured in all soils (despite soil B having undergone an active remedial treatment). Soil C contained the largest fraction of total aromatic hydrocarbons, and soil B aliphatic hydrocarbons.

Available ammonium and nitrogen was determined by extraction with 1 M KCl solution following the method by Stockdale and Rees (1994). Acetic acid-extractable phosphorus was determined by shaking 0.5 ± 0.1 g soil with 40ml of 2.5% acetic acid. All extracts were then filtered through Whatman 44 filter paper and analysed on a flow injection analyzer (FIAstar). No significant levels of ammonium, nitrate and phosphorus were detected in any of the three soils

Enumeration of colony forming units (CFUs g^{-1} soil) for total heterotrophic and hydrocarbon degrading microbes were estimated using the spread plate technique on plate count agar and minimal agar supplemented with 0.1% diesel or enriched with four separate hydrocarbons (hexadecane, octacosane, phenanthrene and pyrene). Soil was extracted in a 1:10 ratio with quarter strength Ringer's solution, and then 1 ml of the extract serially diluted with Ringer's solution. The resultant solutions (0.1 ml) were then spread evenly over agar plates and incubated at $25 \pm 1^\circ\text{C}$, with plate counts performed at 4, 7, 10 and 15 days. Soil B had the largest indigenous heterotrophic microbial community, and number of diesel and PAH degraders. The greatest number of aliphatic hydrocarbon degraders (hexadecane and octacosane) was in soil C (Table 1).

2.3 Soil Spiking with Target Hydrocarbons

Prior to spiking, soils A, B and C were rehydrated with deionised water to 70% soil water holding capacity (32, 31 and 26 mg per 100 g soil dry wt, respectively). Samples of each soil, (120 g wet wt) were then spiked with $^{12/14}\text{C}$ -labelled hydrocarbons: hexadecane, octacosane, phenanthrene or pyrene using acetone as the carrier solvent to give a final ^{12}C -hydrocarbon concentration of 50 mg kg^{-1} (dry wt)

with a related ^{14}C - activity of approximately 83 Bq g^{-1} soil (dry wt). Each soil-contaminant mixture was then blended following the method developed by Doick et al (2003). Controls consisting of rehydrated soil (40 g wet wt) only were produced as analytical blanks.

2.4 Mineralisation Assays

Biodegradation of the four ^{14}C -labelled petroleum hydrocarbons in each of the three soils was measured (in triplicate) through the evolution of $^{14}\text{CO}_2$ produced using the method developed by this group (Semple et al., 2006). The mineralisation assay was performed in a 'respirometer': a modified 250 ml Schott bottle into which $10 \pm 0.1 \text{ g}$ (wet wt) soil along with 30 ml of mineral salts medium (MBS) was placed. Glass vials (7 ml) containing 1 ml of 1 M NaOH were attached to the respirometer Teflon-lined lids to trap the $^{14}\text{CO}_2$ formed during the mineralisation assay.

Non-amended respirometers were prepared as outlined above, with $10 \pm 0.1 \text{ g}$ hydrocarbon spiked soil (wet wt) and 30 ml of autoclaved MBS solution. To investigate techniques to optimise the biodegradation of the target hydrocarbons, respirometers were also prepared as above but with the following treatments: nutrient amendment, hydrocarbon degrader amendment and combined nutrient and degrader amendment. The carbon content of the soils was calculated (measured TPH + $50 \text{ mg kg}^{-1} \text{ }^{12}\text{C}$ -PAH added) to be 23.2 mg, 32mg and 20.2 mg g^{-1} (dry wt) soil for soils A, B and C, respectively; and nutrients were added to respirometers, to give a C: N: P soil ratio of 100:10:1, (Leys et al., 2004). Nitrogen was given in the form of a 0.2 M ammonium nitrate solution (prepared using autoclaved deionised water), added to respirometers at 0.007 ml, 0.009 ml and 0.006 ml g^{-1} soil (wet wt), for soils A, B and

C, respectively. Potassium was added to respirometers (0.007 ml, 0.010 ml and 0.007 ml g⁻¹ soil wet wt, for soils A, B and C respectively) via a 1 M potassium phosphate buffer (pH 7) prepared using anhydrous potassium orthophosphate and anhydrous dipotassium orthophosphate.. The degrader amendment comprised of a commercial mixed hydrocarbon degrader inoculum (identification unknown) able to utilise hexadecane octacosane, phenanthrene and pyrene as a carbon source for growth. The inoculum was cultured in autoclaved 3.27g l⁻¹ Bushnell-Haas medium (prepared using deionised water, supplemented with 1000 mg l⁻¹ salicylic acid and 1 % ethanol) at 20 ± 1°C on an orbital shaker at 100 rpm. After 2 days incubation (stationary phase), the hydrocarbon degrader inoculum was added to the respirometric flasks (0.1ml) such that numbers of cells rose to 10⁶ cells g⁻¹ dry wt soil. In respirometers amended with nutrients and/or degraders, MBS was added at 30 ml – amendment volume.

Respirometers containing only rehydrated soil were also prepared as analytical blanks. The respirometers were placed on an orbital shaker at 100 rpm and incubated in the dark at 20 ± 1°C. At regular intervals (24 h) over an incubation period of 23 d the ¹⁴CO₂ traps were replaced and 5ml Ultima Gold scintillation cocktail added to the sampled vial. Following overnight storage to normalize the effects of chemiluminescence, the samples were counted by liquid scintillation counting LSC (Tri-Carb 2250CA) and quantified using standard counting protocols and automatic quench correction (Macleod and Semple, 2002).

2.5 Statistical analysis

Statistical analysis of the results after blank correction was performed in SigmaStat for Windows (Version 2.03 SPSS). All graphs were presented using SigmaPlot 2000

for Windows (Version 6.10, SPSS). The respirometric data for the four treatments (non amended, nutrient amended, inoculum amended and nutrient/inoculum amended) for each soil were tested for significance by analysis of variance (ANOVA), using Tukey and/or Student t-tests to investigate significant differences between lag phases, mineralisation rates and overall mineralisation extents, between different soil treatments, where $P \leq 0.05$.

3. Results

3.1. Mineralisation of target hydrocarbons in soils.

Hydrocarbon mineralisation in the three soils followed a standard 3-stage mineralisation curve. Firstly, there was a lag phase where the indigenous microbial community adapted to their freshly amended hydrocarbon and mineralisation was minimal (below 5%). Following this, there were increases in the rates of mineralisation, as displayed by an 'exponential' phase; after which catabolic activity reached a plateau (Figures 1-3).

The indigenous microbial communities in the three soils were able to mineralise each of the ^{14}C -target hydrocarbons (Figures 1-3; Tables 2-4). Rapid and extensive mineralisation ($\geq 40\%$) and high catabolic activity was observed in each of the soils spiked with ^{14}C -phenanthrene (Figures 1-3). This initial high catabolic activity resulted in significantly shorter ($P \leq 0.05$) lag phases of 21.72 ± 0.28 h in soil A, 9.03 ± 0.68 h soil B and soil C 4.23 ± 0.15 h, when compared to other ^{14}C -hydrocarbons in the same soil. Furthermore, maximum rates of ^{14}C -phenanthrene mineralisation were statistically quicker ($P \leq 0.05$) in the three soils, than those of ^{14}C -hexadecane, ^{14}C -octacosane and ^{14}C -pyrene (Tables 2-4). Conversely, there was no statistical

275 difference in the overall extents of mineralisation for ^{14}C -phenanthrene between soils.
 276 Although, differences in maximum ^{14}C -phenanthrene mineralisation rates were
 277 evident, with a significantly faster ($P \leq 0.05$) rate of $2.37 \pm 0.08 \text{ \% h}^{-1}$ occurring in soil
 278 C, in comparison to $1.11 \pm 0.04 \text{ \% h}^{-1}$ for soil A and $1.12 \pm 0.09 \text{ \% h}^{-1}$ for soil B.
 279 Similar observations were also observed for ^{14}C -phenanthrene lag phases in each of
 280 the soils; it took less time for extents of mineralisation to reach 5% in soil C followed
 281 by soil B, with soil A exhibiting the longest ^{14}C -phenanthrene lag phase (Tables 2-4).
 282 The higher levels of phenanthrene degradation in soils B and C were also reflected by
 283 a significantly larger ($P \leq 0.05$) number of phenanthrene degraders present in both
 284 soils (B, $4.1 \times 10^5 \pm 1.3 \times 10^5 \text{ CFU g}^{-1}$) and (C, $4.3 \times 10^5 \pm 2.9 \times 10^5 \text{ CFU g}^{-1}$),
 285 compared to soil A ($1.8 \times 10^4 \pm 2.9 \times 10^3 \text{ CFU g}^{-1}$).
 286
 287 In general, the ingenious microbial communities in the three soils exhibited less
 288 ability to mineralise ^{14}C -pyrene, compared to other ^{14}C -hydrocarbon amendments
 289 (Figures 1-3). This reduced response to pyrene resulted in significantly higher (P
 290 ≤ 0.05) lag phases in soils A, B and C, in comparison to other ^{14}C -hydrocarbon lag
 291 phases (Table 2-4). This response correlated with lower numbers of pyrene degraders,
 292 in relation to hexadecane, octacosane and phenanthrene degraders, being present in
 293 soils A and C (Table 1). Significant differences in lag phases, maximum rates and
 294 overall extents of mineralisation for ^{14}C -pyrene were also apparent between the three
 295 soils. In soil B there were greater levels of catabolic ability with regard to the
 296 mineralisation of ^{14}C -pyrene than in soils A and C; the overall extent of ^{14}C -pyrene
 297 mineralisation was 1.72 and 1.43 times higher than in soils A and C, respectively
 298 (Figures 1-3). This higher catabolic ability for pyrene also resulted in a significantly
 299 faster ($P \leq 0.05$) maximum mineralisation rate of $0.29 \pm 0.01 \text{ \% h}^{-1}$, compared to 0.10

300 $\pm 0.00 \text{ \% h}^{-1}$ in soil A and $0.19 \pm 0.00 \text{ \% h}^{-1}$ in soil C. Furthermore, significantly more
301 ($P \leq 0.05$) pyrene degraders were determined to be present in soil B, than in A and C
302 (Table 1).

303

304 High levels of ^{14}C -hexadecane mineralisation were apparent in soils A and C with
305 overall extents of mineralisation reaching $36.94 \pm 0.53\%$ and $50.48 \pm 2.00\%$,
306 respectively. Between soils, hexadecane associated catabolic activity was higher in
307 soil C, which resulted in a significantly shorter ($P \leq 0.05$) ^{14}C -hexadecane lag phase
308 and significantly faster ($P \leq 0.05$) maximum mineralisation rate than measured for
309 other soils (Tables 2-4). However, there was no significant difference ($P \geq 0.05$)
310 between the number of hexadecane degraders in soils A and C (Table 1). Conversely,
311 the microbial community in soil B demonstrated significantly lower ($P \leq 0.05$) levels
312 of mineralisation towards this hydrocarbon, with only $20.00 \pm 0.66\%$ ^{14}C -hexadecane
313 mineralised over 23 d. This represents a decrease of 1.84 and 2.52 times on overall
314 extents of mineralisation for ^{14}C -hexadecane in soils A and C, respectively. There was
315 also significantly less ($P \leq 0.05$) ^{14}C -hexadecane mineralised in soil B in comparison
316 to the overall extents mineralised for other ^{14}C -hydrocarbons in this soil (Figure 2,
317 Table 3). Lower levels of catabolic activity towards hexadecane in soil B further
318 correlated with the statistically significantly small ($P \leq 0.05$) number of hexadecane
319 CFUs of $1.3 \times 10^4 \pm 4.1 \times 10^4$ measured in this soil.

320

321 The indigenous microbial communities of all three soils exhibited high levels of
322 degradation for octacosane, with rapid and extensive ^{14}C -octacosane mineralisation
323 observed over 23 d (Figures 1-3). In soils A and C, the microbial communities
324 exhibited greater ability to mineralise ^{14}C -octacosane than ^{14}C -hexadecane, ^{14}C -

phenanthrene and ^{14}C -pyrene; overall extents of mineralisation for ^{14}C -octacosane were higher than for other ^{14}C -hydrocarbons in the same soil (Table 2 and 4). Between soils, there was lower octacosane degradative activity in soil B ($45.84 \pm 0.68\%$), with overall extents of mineralisation for ^{14}C -octacosane significantly higher ($P \leq 0.05$) at $54.93 \pm 1.30\%$, and $54.32 \pm 0.25\%$ in soils A and C, respectively. No significant difference ($P \geq 0.05$) in the overall extents of mineralisation was observed between soils A and C. ^{14}C -octacosane was mineralised at a faster maximum rate of $0.91 \pm 0.01 \text{ \% h}^{-1}$ in soil C, compared to $0.54 \pm 0.04 \text{ \% h}^{-1}$ for soil A and $0.76 \pm 0.00 \text{ \% h}^{-1}$ for soil B. Furthermore, the ^{14}C -octacosane lag phase for soil C was significantly shorter ($P \leq 0.05$) by 122.34 h and 36.7 h compared to soils A and B, respectively (Tables 2-4). This high level of octacosane degradation was also reflected by the presence of a significantly larger ($P \leq 0.05$) culturable octacosane degrader population compared to the other soils; 3.23 and 1.64 times more CFUs g^{-1} than in A and B, respectively (Table 1).

3.2 Enhancement of ^{14}C -target hydrocarbon mineralisation in soils

With nutrient amendment the microbial communities in soils B and C exhibited greater ability to mineralise ^{14}C -phenanthrene and ^{14}C -pyrene (Figures 2 and 3). In comparison to non-amended soils (to which no N and P were added) overall extents of mineralisation were significantly higher ($P \leq 0.05$) in soil B by 13.55% and 30.52%, and soil C by 40.41% and 17.33% for ^{14}C -phenanthrene and ^{14}C -pyrene, respectively. Nutrient addition also significantly enhanced ($P \leq 0.05$) maximum rates of mineralisation for ^{14}C -pyrene in both these soils (Tables 3 and 4). For example, the maximum rate of mineralisation for ^{14}C -pyrene was $0.26 \pm 0.02 \text{ \% h}^{-1}$, following nutrient addition in soil C, and $0.19 \pm 0.00 \text{ \% h}^{-1}$ for the non-amended control.

Furthermore, nutrient amendment to soil B resulted in the significantly highest ($P \leq 0.05$) overall extent of mineralisation for ^{14}C -pyrene (61.32%) and the shortest lag phase of 104.84 ± 0.65 h. However, nutrient treatment did not result in (i) an increase in maximum rates of mineralisation and (ii) decrease in lag phases for ^{14}C -phenanthrene in soils B and C (Tables 3 and 4). This treatment also had no significant effect ($P \geq 0.05$) on catabolic activity and the overall extent of mineralisation for ^{14}C -phenanthrene or ^{14}C -pyrene in soil A (Figure 1).

Conversely, in nutrient treated soil A, overall extents of mineralisation for ^{14}C -hexadecane and ^{14}C -octacosane were 2.19 and 1.45 times higher, respectively, than overall extents measured for non-treated soils (Figure 1). This increase in catabolic activity was further reflected by a significant decrease (compared to non-amended soils) ($P \leq 0.05$) in lag phases to (Table 2). Furthermore, with nutrient amendment maximum rates of mineralisation for ^{14}C -hexadecane increased by $0.35 \% \text{ h}^{-1}$ in soil A (Table 2). The overall extent of mineralisation for ^{14}C -octacosane also increased by $\geq 40\%$ following nutrient amendment to $81.17 \pm 0.93\%$ in soil C. This was not significantly different to the overall extent mineralised in soil A (Figures 1 and 3). In soil B, overall extents of mineralisation for ^{14}C -hexadecane were also significantly higher ($P \leq 0.05$) with nutrient amendment ($27.65 \pm 0.70\%$), compared to non-amended soil ($20.00 \pm 0.66\%$), but was notably less than extents mineralised in soils A and C (Figures 1-3).

Hydrocarbon degrader treatment also significantly increased ($P \leq 0.05$) catabolic ability and overall extents of mineralisation for ^{14}C -hexadecane and ^{14}C -pyrene in all soils (Figures 1-3). This was the only treatment to increase the extent of

375 mineralisation for ^{14}C -hexadecane in soil C (Table 4). In comparison to non-amended
 376 soils, extents of mineralisation for ^{14}C -hexadecane increased by 42% (A), 9% (B) and
 377 23% (C); and by 4% (A), 26% (B) and 24% (C) for ^{14}C -pyrene. However, this
 378 treatment did not enhance maximum rates of mineralisation for ^{14}C -hexadecane or
 379 reduce the lag phases for ^{14}C -hexadecane in soils B and C (Tables 3 and 4).
 380 Conversely, increases in pyrene associated catabolic activity for all degrader -
 381 amended soils were reflected by faster maximum mineralisation rates and
 382 significantly shorter ($P \leq 0.05$) lag phases, in comparison to non-amended soils
 383 (Tables 2-4). Compared to other treatments, degrader amendments also yielded the
 384 most extensive ^{14}C -pyrene mineralisation over 23 d for soils A and C (Figures 1 and
 385 3). However, in soils A and B extents of mineralisation for ^{14}C -hexadecane were not
 386 statistically different ($P \geq 0.05$) for degrader and nutrient treatments (Figures 1 and 2).
 387
 388 With bioaugmentation the percentage of ^{14}C -phenanthrene mineralised in soil A
 389 decreased from $43.68 \pm 1.80\%$ (non-amended soil) to $27.37 \pm 0.72\%$. This reduction
 390 in catabolic activity resulted in a significant increase in ^{14}C -phenanthrene ($P \leq 0.05$)
 391 lag phase (Table 2). When compared to non-amendment and other treatments in soil
 392 A, there was also a significant decrease ($P \leq 0.05$) in the maximum ^{14}C -phenanthrene
 393 mineralisation rate of $\geq 0.25\% \text{ h}^{-1}$. Conversely, in soil C the overall extent of
 394 mineralisation for ^{14}C -phenanthrene was 29.35% higher following degrader treatment
 395 in comparison to non-amended soil (Table 4). The overall extent of mineralisation for
 396 ^{14}C -phenanthrene was also 2.55 and 1.61 times higher than measured for degrader
 397 treated soils A and B, respectively (Figures 1-3). Enhanced octacosane related
 398 catabolic activity, was also observed in soils A and C following degrader addition.
 399 Compared to non-amended systems, extents of mineralisation increased from $54.93 \pm$

1.30% to $64.18 \pm 0.65\%$ in soil A; and $54.32 \pm 0.25\%$ to $84.82 \pm 1.31\%$ in soil C.

Although, overall extents of mineralisation for ^{14}C -octacosane and lag phases were similar for nutrient and degrader treatments in soil C (Table 4). In comparison to non-amended soil, the overall extent and maximum rate of mineralisation for ^{14}C -octacosane significantly decreased by $\geq 10\%$ and $0.61\% \text{ h}^{-1}$ following degrader treatment in soil B (Figure 2, Table 3).

Following combined nutrient + degrader treatment, an increase in catabolic activity and ability to mineralise ^{14}C -phenanthrene was observed in all soils. Significantly higher ($P \leq 0.05$) extents of ^{14}C -phenanthrene were mineralised in soils A, B and C, in comparison to non-amended, nutrient and degrader amended soils (Figures 1-3).

Furthermore, in relation to nutrient and degrader treatments, ^{14}C -phenanthrene was mineralised at significantly faster maximum rates ($P \leq 0.05$) with shorter lag phases in all soils treated with nutrient + degrader (Tables 2-4). Between soils, there was no significant difference ($P \geq 0.05$) in extents of mineralisation for nutrient + degrader treatments. Although, a faster rate of mineralisation for ^{14}C -phenanthrene of $1.22 \pm 0.02\% \text{ h}^{-1}$ was observed for soil C, compared to $1.22 \pm 0.02\% \text{ h}^{-1}$ for soil A and $0.93 \pm 0.01\% \text{ h}^{-1}$ for soil B. However, in respect to non-amended soils, nutrient + degrader amendment did not reduce lag phases or increase maximum rates of mineralisation for all soils (Tables 2-4). Nutrient + degrader treatment also increased the overall extent of mineralisation for ^{14}C -pyrene to $41.10 \pm 0.36\%$ in soil B and $30.90 \pm 0.19\%$ in C.

When compared to nutrient and degrader treatments, extents of mineralisation were significantly lower ($P \leq 0.05$) in both soils for this treatment (Figures 2 and 3).

Furthermore, nutrient + degrader treatment was observed to have a negative effect upon octacosane related catabolic activity in all soils (Figures 1-3). Compared to non-

amended soils, extent of mineralisation for ^{14}C -octacosane was 6.59, 6.21 and 6.57 times lower in soils A, B and C, respectively (Tables 2-4). This reduction in catabolic activity was reflected by statistically significant increases ($P \leq 0.05$) in ^{14}C -octacosane lag phases to 305.26 ± 3.02 h, 273.65 ± 9.52 h and 338.13 ± 8.79 h for soils A, B and C, respectively. Nutrient + degrader treatment also did not cause increases in the rate or extent of mineralisation for ^{14}C -hexadecane in all soils (Figures 1-3). However, a significant decrease ($P \leq 0.05$) in ^{14}C -hexadecane lag phases by 148 h and 4 h was observed for soils A and C, respectively, compared to non-amended soils (Tables 2-4). In respect to non-amended soils, this treatment also increased the overall extent of mineralisation for ^{14}C -pyrene in soils B and C by $\geq 9\%$ (Figures 2 and 3).

4. Discussion

4.1 Catabolic activity of the indigenous microflora in soils A, B and C.

Many studies have documented the biological remediation of petroleum hydrocarbon contaminated sites (Trindade et al., 2005; Mancera-López et al., 2008; Liu et al., 2009) and it is widely acknowledged that for bioremediation strategies to be successfully applied, the potential for hydrocarbon biodegradation needs to be accurately assessed (Head, 1998). In this present study, the microbial degradation of ^{14}C -hexadecane, ^{14}C -octacosane, ^{14}C -phenanthrene and ^{14}C -pyrene was assessed in three genuinely hydrocarbon contaminated soils from former refinery facilities, which were all contaminated with high concentrations of TPH ($\geq 1.8\%$ w/w) and contained only trace concentrations of phosphorus and no ammonium or nitrate. Optimising the degradation of the ^{14}C -target hydrocarbons through increasing microbial activity by nutrient amendment (nitrogen and phosphorus addition), addition of hydrocarbon degraders and combined nutrient and degrader amendment was also investigated. The

results reveal that prior to treatments, the indigenous microbial communities in the three soils had the capability to degrade the representative ^{14}C -petroleum hydrocarbons, and significant microbial activity was observed in all of the contaminated soils. This is unsurprising due to the contamination histories of the soils, which would result in extended exposure of the indigenous microbial communities to selective hydrocarbons, and was further reflected by the large number of aliphatic and aromatic degraders in each of the soils (Table 1).

Exposure of microbial populations to hydrocarbons in contaminated environments has been observed to result in a selective enrichment in the numbers of indigenous degrading organisms (Spain et al., 1980; Leahy and Colwell, 1990; Song et al., 1990; Atlas, 1995) Furthermore, pre-exposure of microbial communities to hydrocarbons can effect degradation, with biodegradation rates influenced by contaminant concentration and prior exposure history (Spain and VanVeld, 1983; Aeolin et al., 1989; Leahy and Colwell, 1990). This may be linked to hydrocarbon concentrations in each of the soils. For example Grosser et al (1995) found that the mineralisation of 8 different ^{14}C -PAHs was faster and more extensive in genuinely hydrocarbon contaminated soils containing 8 to 15 times higher concentrations of total hydrocarbons(% g^{-1} dry wt soil). In this current study, levels of indigenous microbial activity and mineralisation of ^{14}C -pyrene were significantly higher ($P \leq 0.05$) in soil B, which contained the highest concentrations (mg kg^{-1}) of pyrene and total TPH. Furthermore, the largest diesel and pyrene degrader populations and their ratio in the heterotrophic microbial community were enumerated in this soil. In contrast, more extensive ^{14}C -hexadecane and octacosane mineralisation occurred in soil C, which in terms of aliphatic hydrocarbon burden contained the largest proportion of

hexadecane/octacosane (31.06% of total aliphatic hydrocarbon burden) in comparison to soils A (26.27%) and B (21.42%), and the highest ratio of aliphatic hydrocarbon degraders to total heterotrophic microbial population numbers. Comparable findings were observed for ^{14}C -hexadecane mineralisation in a study by Caparello and LaRock (1975), who reported an increase in ^{14}C -hexadecane mineralisation with increasing hydrocarbon burden. It was concluded that hydrocarbon concentrations indicate catabolic ability of indigenous microbes and their oxidising potential towards hydrocarbons (Caparello and LaRock, 1975).

In terms of soil physicochemical parameters, no definitive relationship was apparent between ^{14}C -hydrocarbon mineralisation parameters (lag phases, rates and extents of mineralisation) in the control soils and soils pH, organic matter content and particle composition. SOM is known to be predominant in orchestrating contaminant sequestration, and reducing bioavailability. High levels of SOM were measured in soil C, which contained $\geq 11\%$ more soil organic matter (SOM) than soil A and B. However, differences in hydrocarbon degradability (between soils) as a result of declining bioavailability through contaminant sorption is suggested not to be a key factor in this study as all ^{14}C -hydrocarbons were freshly spiked in to the soils (Hatzinger and Alexander, 1997; Xing and Pignatello, 1997; Nam et al., 1998).

Furthermore, studies have reported that freshly added PAHs can desorb from soil faster than they are degraded (Calvillo and Alexander, 1996; Cornelissen et al., 1998).

4.2 The role of treatments on microbial activity and implications for hydrocarbon bioremediation.

Depletion of essential nutrients can limit hydrocarbon biodegradation, especially in soils with high organic carbon contents (Breedveld and Sparrevik, 2000; Gallego et al., 2001; Joo et al., 2001; Bento et al., 2005; Horel and Schiewer, 2009). For example, Horel and Schiewer (2009) demonstrated the addition of N and P releasing fertilisers increased respiration (by 76% and 119%) over a 17 week period in contaminated Alaskan soil samples. In this current study, nutrient treatment in the form of nitrogen and phosphorus (added to soils at a C:N:P ratio of 100:10:1) had a stimulatory effect on catabolic activity, as in all soils extents of mineralisation, overall, significantly increased for the target ^{14}C -hydrocarbons. However, this increase in catabolic activity generally did not result in a decrease in ^{14}C -hydrocarbon lag phases, suggesting the added nutrients were being assimilated for microbial growth (Bamforth and Singleton, 2005). In certain instances, the addition of nutrients had no significant effect on the overall extents of ^{14}C -hydrocarbon mineralisation, when compared to extents of mineralisation in non-amended soils to which no nitrogen and phosphorus were added. Several studies have reported similar observations (Lehtomaki and Niemela, 1975; Seklemova et al., 2001; Chaîneau et al., 2005; Chaillan et al., 2006; Ramírez et al., 2008). This has been attributed to the heterogeneity of soils, nutrient availability, toxicity of nutrient intermediaries and the presence of nitrogen fixing bacteria (Bossert and Bartha, 1984; Mills and Frankenberger, 1994; Seklemova et al., 2001; Sarkar et al., 2005). In this study there was no definitive evidence as to which of the factor/s may have contributed to non-increase in mineralisation parameters, following nutrient addition.

There have been a number of findings on the use of commercially available microbial inocula to remediate hydrocarbon contaminated soil, with some studies reporting

increased degradation and others negative or no effect on hydrocarbon degradation (Jobsen et al., 1974; Goldstein et al., 1985; Capelli et al., 2001; Bento et al., 2005). For example, Bento et al (2005) investigated the effect bioaugmentation had on diesel degradation in soil samples from Long Beach and Hong Kong over a 12 week period. Optimum biodegradation performance for Long Beach soil was achieved following the addition of a pre-selected microbial degrading consortium; whereas, this treatment had no significant effect on diesel degradation in the Hong Kong soils. In this current study, bioaugmentation of the three soils with a mixed hydrocarbon degrader consortium enhanced hexadecane, phenanthrene and pyrene degradation. However, degrader treatment was deemed to be less successful than the nutrient only treatment, which generally exhibited greater ^{14}C -mineralisation. Furthermore, the use of both nutrients and degrader inoculum as a combined soil treatment resulted in a decrease in extent of mineralisation for ^{14}C -octacosane, which reduced to $\leq 10\%$ in all three soils. This reduction in octacosane related catabolic activity may be the result of competition between indigenous and introduced degrader populations in the soil (Goldstein et al., 1985).

5. Conclusion

This study shows that the indigenous microbial communities in genuinely hydrocarbon-contaminated soils have the potential to actively and extensively degrade target aliphatic and aromatic hydrocarbons. Furthermore, by enriching the microbial activity and number of degrading microbes in hydrocarbon-contaminated soils, through biostimulation and bioaugmentation techniques, hydrocarbon degradation can be enhanced. However, the rate and extent of degradation will be dependent upon treatment type, contaminant structure, native hydrocarbon concentrations and

microbial community. In this current study, as the indigenous microbial populations of all three soils had an established and capable degrader population, the addition of degraders in this instance was not an effective solution to enhance the degradation of the ¹⁴C-contaminants in the soils. Similar observations have been noted and has been suggested the advantages of a bioaugmentation remediation approach rarely outweigh the costs involved. However, the successful bioremediation of hydrocarbon-contaminated soils is dependent upon many soil, contaminant and microbial parameters and should therefore be assessed on an individual site basis.

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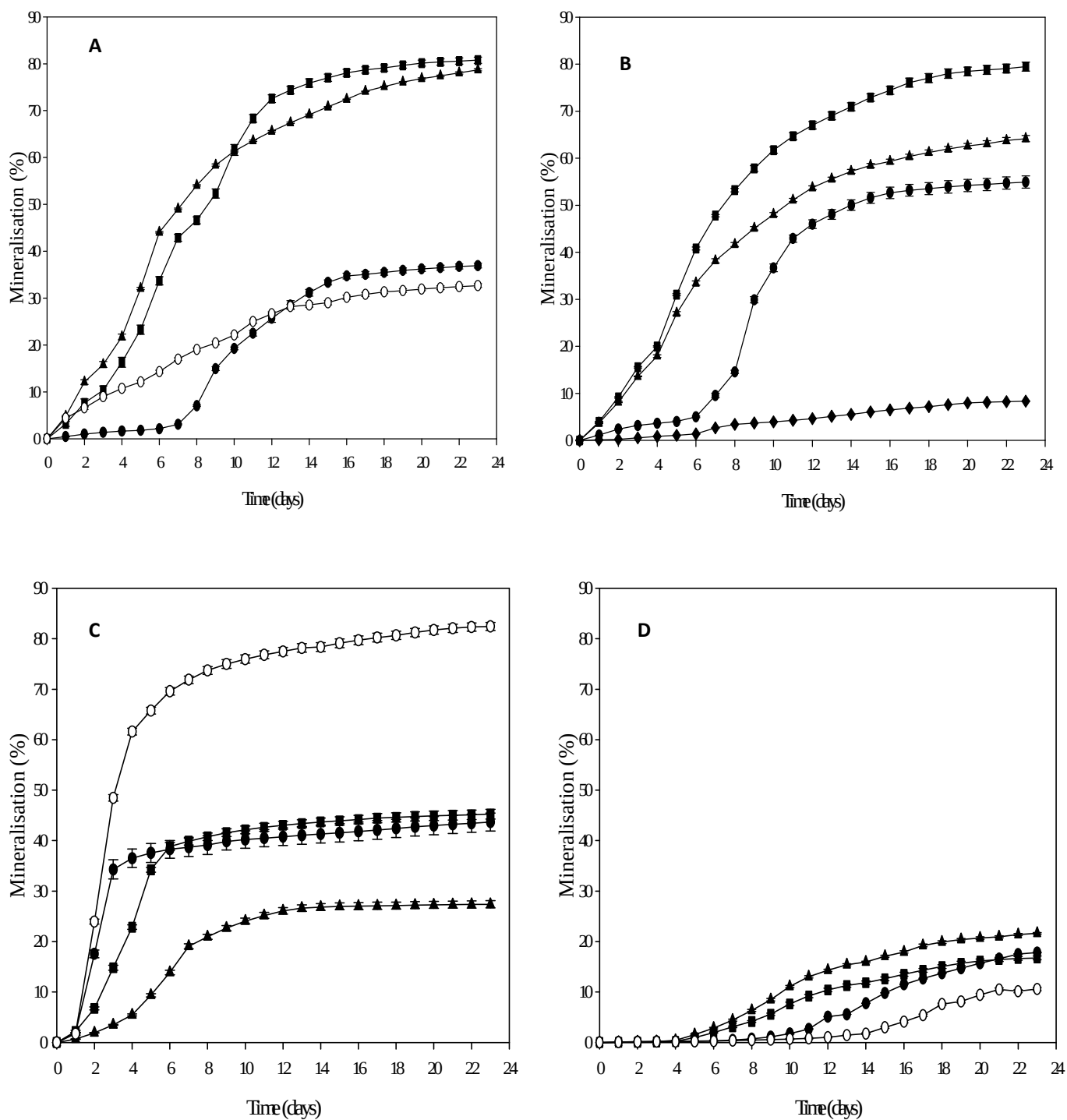
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Figure legends

Figure 1. Mineralisation of (A), ^{14}C -hexadecane (B) ^{14}C -octacosane (C) ^{14}C -phenanthrene (D) ^{14}C -pyrene occurring over 23 days in soil A – an industrial surface soil obtained from a disused oil refinery. The soil was amended with different treatments, no treatment (●) nutrient treatment (■), hydrocarbon degrader treatment (▲) and nutrient + degrader (○). Error bars where visible are 1 SEM based on n=3.

Figure 2. Mineralisation of (A), ^{14}C -hexadecane (B) ^{14}C -octacosane (C) ^{14}C -phenanthrene (D) ^{14}C -pyrene occurring over 23 days in soil B – an industrial surface soil remediated with biopiling. The soil was amended with different treatments: no treatment (●), nutrient treatment (■), hydrocarbon degrader treatment (▲) and nutrient + degrader treatment (○). Error bars where visible are 1 SEM based on n=3.

Figure 3. Mineralisation of (A), ^{14}C -hexadecane (B) ^{14}C -octacosane (C) ^{14}C -phenanthrene (D) ^{14}C -pyrene occurring over 23 days in soil C – an industrial surface soil from an old oil refinery site. The soil was amended with different treatments: no treatment (●), nutrient treatment (■), hydrocarbon degrader treatment (▲) and nutrient + degrader treatment (○). Error bars where visible are 1 SEM based on n=3.



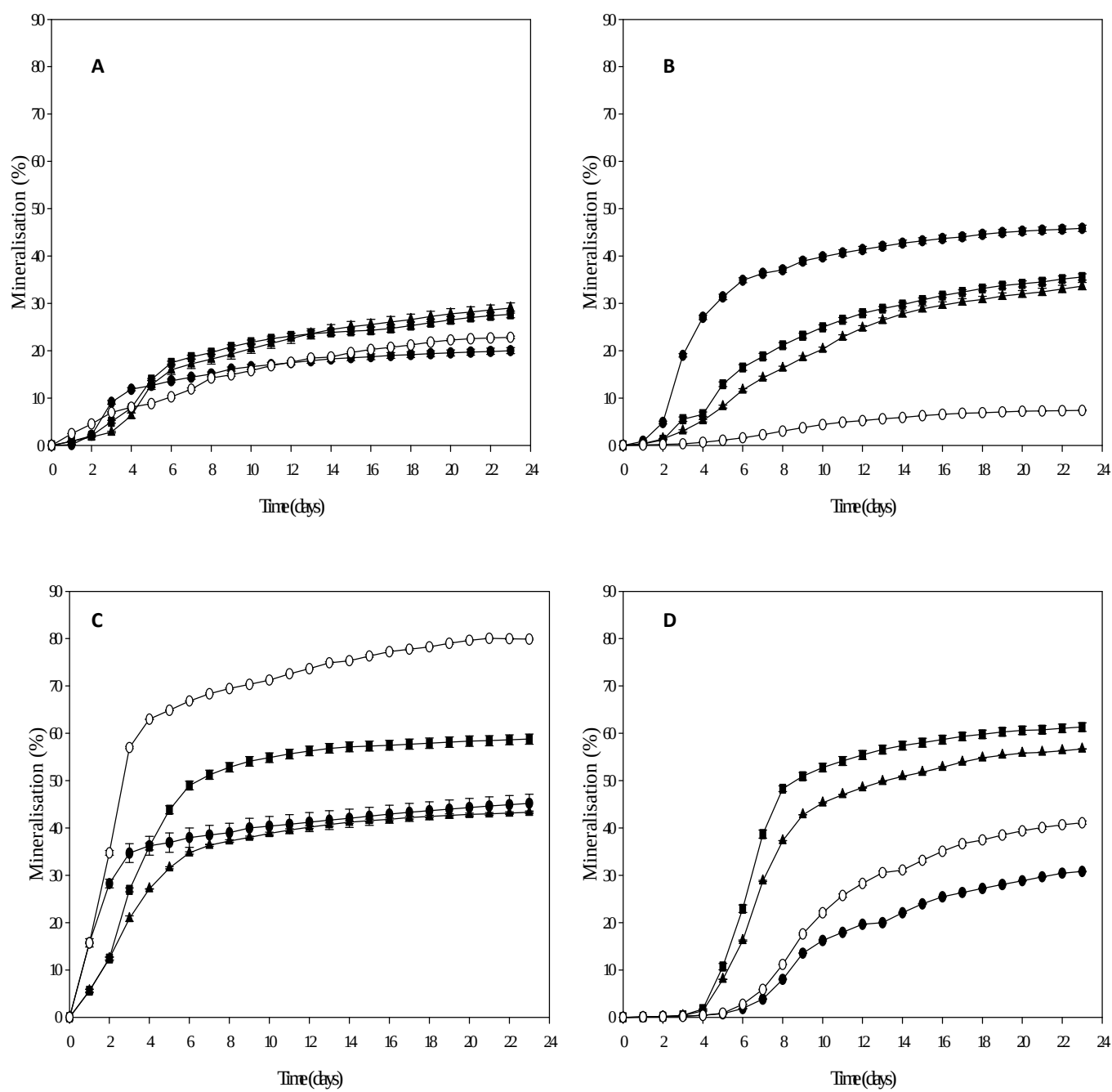


Figure 3

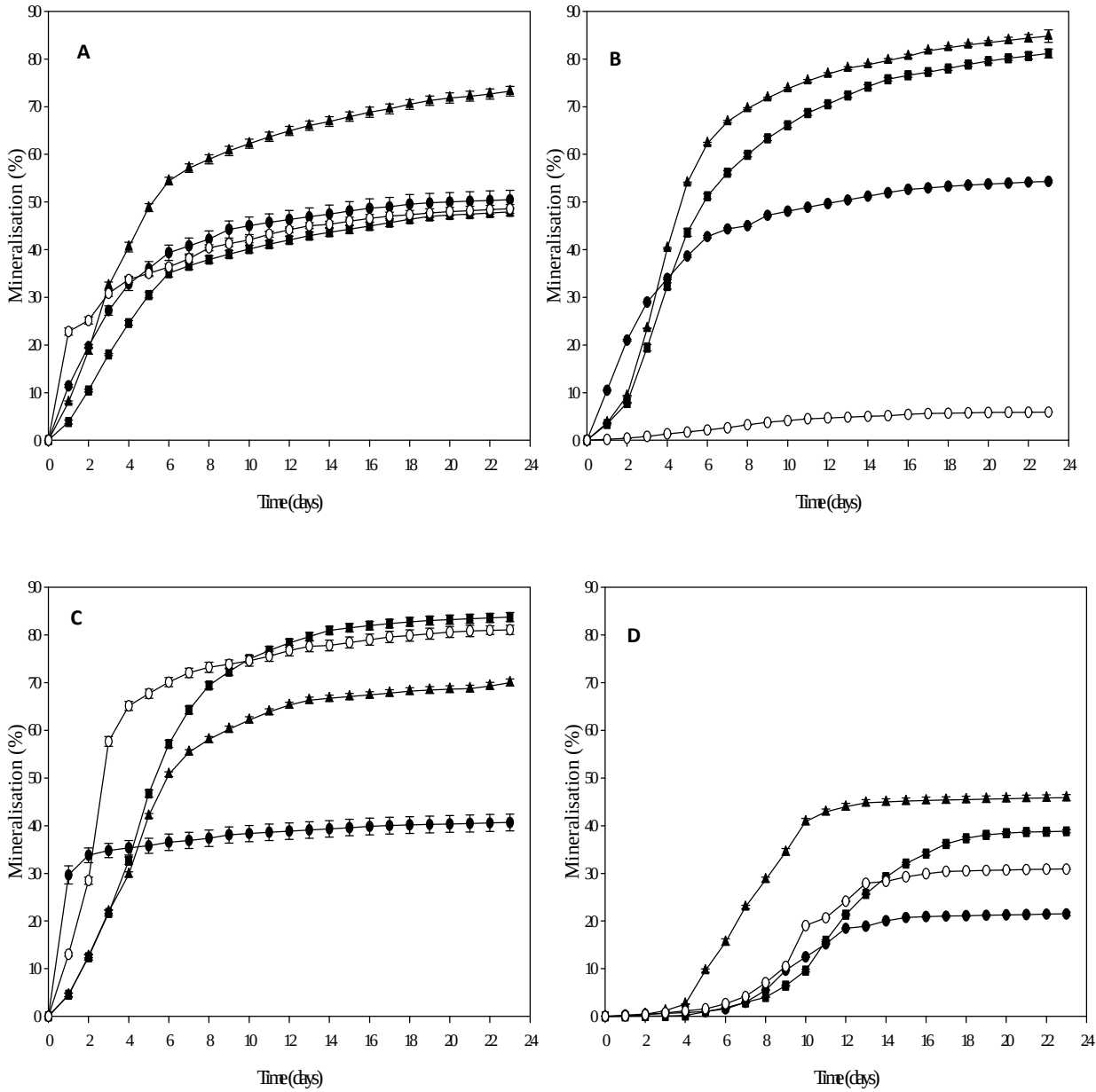


Table 1: Characteristics and properties of soil A, B and C. Errors are shown as 1 SEM (n=3).

Properties	Soil A	Soil B	Soil C
Moisture content (%)	15.60 ± 0.41	22.13 ± 1.56	33.12 ± 0.22
Bulk density (kg l ⁻¹)	0.97	0.82	0.58
pH in water	6.80 ± 0.23	7.47 ± 0.03	6.77 ± 0.09
pH in 0.01 M CaCl ₂	6.50 ± 0.00	6.60 ± 0.00	6.10 ± 0.06
Organic carbon (%)	6.75 ± 0.20	8.50 ± 2.24	15.39 ± 0.84
Organic matter (LOI %)	11.60 ± 0.34	14.62 ± 3.85	26.47 ± 1.45
DOC (µg ml ⁻¹)	75.33 ± 12.17	151.67 ± 34.71	133.67 ± 22.45
TOC (µg g ⁻¹)	167.67 ± 7.62	280.33 ± 67.16	230.33 ± 23.21
Carbon content (%)	8.91 ± 0.48	7.80 ± 0.85	17.50 ± 1.61
Extractable nitrogen content (%)	0	0	0
Extractable phosphorus (%)	0	0	0
Hydrocarbon Fraction (mg/kg)			
Total Aliphatic	15091	19869	7271
EC ≥ 10 – 12	115	915	4379
EC ≥ 12 – 16	11000	14608	2259
EC ≥ 16 – 35	3965	4256	
			11014
Total Aromatic	5756	9686.1	58
EC ≥ 10 – 12	26	86	1801
EC ≥ 12 – 16	968	1599	3797
EC ≥ 16 – 21	2645	4275	
TPH (mg/kg)	20848	29555	18285
Particle size Analysis:			
Clay (%)	50.61 ± 0.14	43.60 ± 0.23	30.67 ± 1.10
Sand (%)	24.66 ± 0.99	32.12 ± 0.14	49.62 ± 0.24
Silt (%)	24.73 ± 1.07	24.28 ± 0.36	19.67 ± 0.89
Total Heterotrophs (CFU g ⁻¹)	5.9E ⁰⁵ ± 7.6E ⁰⁴	7.1E ⁰⁷ ± 1.8E ⁰⁷	9.8E ⁰⁶ ± 1.3E ⁰⁶
Total degraders (CFU g ⁻¹):			
- Diesel	1.1E ⁰³ ± 5.8E ⁰³	9.9 E ⁰⁶ ± 2.6E ⁰⁶	3.3E ⁰⁵ ± 1.0E ⁰⁵
- Hexadecane	1.0E ⁰⁵ ± 1.0E ⁰³	1.3E ⁰⁴ ± 4.1E ⁰⁴	1.0E ⁰⁵ ± 1.0E ⁰⁴
- Octacosane	3.4E ⁰⁴ ± 1.5E ⁰³	6.7E ⁰⁴ ± 5.8E ⁰⁴	1.1E ⁰⁵ ± 4.7E ⁰⁴
- Phenanthrene	1.8E ⁰⁴ ± 2.9E ⁰³	4.8E ⁰⁵ ± 1.3E ⁰⁵	8.3E ⁰⁴ ± 2.9E ⁰⁴
- Pyrene	1.1E ⁰⁴ ± 3.2E ⁰³	4.8E ⁰⁵ ± 1.3E ⁰⁵	1.3E ⁰⁴ ± 2.9E ⁰³

Table 2. Lag phase, rate and overall extent of ^{14}C -hydrocarbons mineralised over 23 d in non-treated and treated soil A. Errors are 1 SEM (n=3).

Soil Treatment	Lag phase (h)	Maximum rate of ^{14}C mineralisation (% h ⁻¹)	Overall extent of ^{14}C mineralisation (%)
Hexadecane			
None	179.12 ± 0.60	0.21 ± 0.01	36.94 ± 0.53
Nutrient	24.67 ± 0.46	0.56 ± 0.02	80.77 ± 0.67
Degrader	34.42 ± 3.73	0.46 ± 0.02	78.73 ± 0.25
Nutrient + degrader	31.03 ± 2.70	0.18 ± 0.01	32.65 ± 0.40
Octacosane			
None	133.38 ± 7.50	0.54 ± 0.04	54.93 ± 1.30
Nutrient	28.84 ± 0.21	0.46 ± 0.01	79.47 ± 0.94
Degrader	31.01 ± 0.54	0.38 ± 0.01	64.18 ± 0.65
Nutrient + degrader	305.26 ± 3.02	0.05 ± 0.00	8.34 ± 0.08
Phenanthrene			
None	21.72 ± 0.28	1.11 ± 0.04	43.68 ± 1.80
Nutrient	38.90 ± 1.42	0.47 ± 0.01	45.25 ± 0.95
Degrader	90.49 ± 2.05	0.22 ± 0.00	27.37 ± 0.72
Nutrient + degrader	27.92 ± 0.24	1.02 ± 0.01	82.45 ± 0.80
Pyrene			
None	294.94 ± 0.32	0.10 ± 0.00	17.81 ± 0.05
Nutrient	195.79 ± 0.86	0.08 ± 0.00	16.76 ± 0.36
Degrader	175.14 ± 2.61	0.11 ± 0.00	21.64 ± 0.16
Nutrient + degrader	400.72 ± 4.47	0.05 ± 0.00	10.59 ± 0.32

Table 3. Lag phase, rate and overall extent of ¹⁴C-hydrocarbons mineralised over 23 d in non-treated and treated soil B. Errors are 1 SEM (n=3).

Soil Treatment	Lag phase (h)	Maximum rate of ¹⁴ C mineralisation (% h ⁻¹)	Overall extent of ¹⁴ C mineralisation (%)
Hexadecane			
None	40.07 ± 0.15	0.35 ± 0.02	20.00 ± 0.66
Nutrient	72.50 ± 2.25	0.25 ± 0.01	27.65 ± 0.70
Degrader	87.96 ± 2.88	0.27 ± 0.02	29.01 ± 1.05
Nutrient + degrader	52.18 ± 0.78	0.10 ± 0.00	22.82 ± 0.29
Octacosane			
None	47.74 ± 0.73	0.76 ± 0.00	45.84 ± 0.68
Nutrient	70.48 ± 2.83	0.26 ± 0.02	35.56 ± 0.66
Degrader	93.56 ± 2.58	0.15 ± 0.00	33.65 ± 0.79
Nutrient + degrader	273.65 ± 9.52	0.03 ± 0.00	7.38 ± 0.14
Phenanthrene			
None	9.03 ± 0.68	1.12 ± 0.09	45.23 ± 1.92
Nutrient	21.61 ± 1.23	0.60 ± 0.01	58.78 ± 1.06
Degrader	21.67 ± 1.17	0.35 ± 0.02	43.31 ± 0.27
Nutrient + degrader	7.64 ± 0.09	0.93 ± 0.01	79.92 ± 0.16
Pyrene			
None	162.18 ± 0.65	0.29 ± 0.01	30.80 ± 0.07
Nutrient	104.84 ± 0.65	0.66 ± 0.01	61.32 ± 0.92
Degrader	109.21 ± 0.16	0.52 ± 0.00	56.67 ± 0.06
Nutrient + degrader	161.40 ± 0.58	0.27 ± 0.01	41.10 ± 0.36

813 Table 4. Lag phase, rate and overall extent of ¹⁴C-hydrocarbons mineralised over 23 d in non-treated
814 and treated soil C. Errors are 1 SEM (n=3).

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Soil Treatment	Lag phase (h)	Maximum rate of ¹⁴ C mineralisation (% h ⁻¹)	Overall extent of ¹⁴ C mineralisation (%)
Hexadecane			
None	9.96 ± 0.28	1.00 ± 0.03	50.48 ± 2.00
Nutrient	28.18 ± 1.42	0.32 ± 0.00	48.60 ± 0.82
Degrader	15.08 ± 0.45	0.57 ± 0.01	73.26 ± 1.01
Nutrient + degrader	5.27 ± 0.17	0.85 ± 0.03	48.55 ± 0.93
Octacosane			
None	11.04 ± 0.07	0.91 ± 0.01	54.32 ± 0.25
Nutrient	32.67 ± 0.90	0.54 ± 0.00	81.17 ± 0.93
Degrader	29.71 ± 0.79	0.70 ± 0.01	84.82 ± 1.31
Nutrient + degrader	338.13 ± 8.79	0.03 ± 0.00	5.91 ± 0.07
Phenanthrene			
None	4.23 ± 0.15	2.37 ± 0.08	40.67 ± 1.77
Nutrient	25.43 ± 1.3	0.59 ± 0.01	83.76 ± 1.19
Degrader	25.44 ± 1.18	0.51 ± 0.01	70.02 ± 0.75
Nutrient + degrader	9.24 ± 0.25	1.22 ± 0.02	81.08 ± 0.94
Pyrene			
None	170.92 ± 0.39	0.19 ± 0.00	21.48 ± 0.41
Nutrient	195.66 ± 0.22	0.26 ± 0.02	38.81 ± .31
Degrader	104.31 ± 0.36	0.31 ± 0.02	45.89 ± 0.68
Nutrient + degrader	175.01 ± 0.19	0.36 ± 0.00	30.90 ± 0.19

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Mineralisation of target hydrocarbons in three contaminated soils from former refinery facilities

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