The effect of soil: water ratios on the mineralisation of phenanthrene: LNAPL mixtures in soil

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Abstract

Contamination of soil by polycyclic aromatic hydrocarbons is frequently associated with non-aqueous-phase liquids. Measurement of the catabolic potential of a soil or determination of the biodegradable fraction of a contaminant can be done using a slurried soil respirometric system. This work assessed the impact of increasing the concentration of transformer oil and soil:water ratio on the microbial catabolism of [14C]phenanthrene to 14CO2 by a phenanthrene-degrading inoculum. Slurrying (1:1, 1:2, 1:3 and 1:5 soil:water ratios) consistently resulted in statistically higher rates and extents of mineralisation than the non-slurried system (2:1 soil:water ratio; \( P < 0.01 \)). The maximum extents of mineralisation observed occurred in the 1:2–1:5 soil:water ratio microcosms irrespective of transformer oil concentration. Transformer oil concentrations investigated displayed no statistically significant effect on total mineralisation (\( P > 0.05 \)). Soil slurries 1:2 or greater, but less than 1:5 (soil:water), are recommended for bioassay determinations of total contaminant bioavailability due to greater overall mineralisation and improved reproducibility.

Keywords: Microbial degradation; PAH NAPL mixtures; Soil-water ratios

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent an important class of hydrophobic organic contaminants because of their ubiquity and persistence in soils and sediments [1]. PAH contamination is often associated with non-aqueous-phase liquids (NAPLs), for example creosote or diesel, which may enter the soil through spillage [2]. Once in the soil, these chemicals are subject to various chemical and physical loss processes; however, one of the key PAH loss processes from soil is through microbial degradation [1,3]. Biodegradation may not always progress as rapidly, or to the same extent, as observed in laboratory studies due to various biological, chemical and/or physical limitations, including low microbial activity [4] and enhanced soil–contaminant interaction(s) (ageing/sequestration) that result in limited/reduced bioavailability of the contaminant [5,6]. Furthermore, the presence of a NAPL has been shown to influence rates and extents of PAH biodegradation by indigenous micro-organisms and enrichment inocula due to suppression of essential nutrient supply rates [7] or preferential degradation of the NAPL [8].

Various studies have shown reduced biodegradation rates of organic compounds present in NAPLs such as creosote [9] and pure organic solvents [7]. The degree to which biodegradation is affected is related to contaminant bioavailability, and is dependent on the nature of both the NAPL [10] and the target chemical [11]. The reduction in biodegradation rate was initially postulated to be a consequence of the (potentially) limiting mass transfer rate (i.e. from the NAPL to the aqueous phase) as it was assumed that microflora could only utilise substrates dissolved in the aqueous phase. The presence of a NAPL, however, does not always reduce biodegradation. Several bacterial species have been found to grow at the NAPL–water interface, allowing direct access to the C source [12]. Other bacterial species have been found to produce surface-active chemicals (biosurfactants, e.g. rhamnolipids).
that increase the rate of mass transfer and contaminant solubility into the aqueous phase when present at concentrations above the critical micelle concentration [13]. Furthermore, the presence of a NAPL has been shown to result in the biodegradation of microbially recalcitrant compounds including benzo[a]pyrene, as a result of co-metabolism [14].

Studies have shown that the type of NAPL present may markedly affect both the rate and extent of organic substrate mineralisation [7], and that ‘slurrying’ and/or the addition of an acclimated bacterial inoculum may enhance mineralisation of several organics, including phenanthrene, initially dissolved in various NAPLs [11,15]. These studies only compared non-slurried systems with 1:1 soil:water slurries. The effects caused by the extent of slurrying, however, as well as possible interactions between extent of slurrying and NAPL concentration, have not been specifically investigated.

In this work, transformer oil, a PAH-containing, naphthenic-based light non-aqueous phase liquid (LNAPL) mineral oil, was used. The oil acts as a heat and electrical insulator in the electrical transformers that form part of the high-voltage electricity transmission system of England and Wales. The aims of this work were (i) to quantify the effects of transformer oil on phenanthrene mineralisation by a phenanthrene-degrading inoculum and (ii) to investigate the effect that soil slurrying has on the initial rate and overall extent of phenanthrene mineralisation.

2. Materials and methods

2.1. Soils and spiking procedure

A brown-earth soil (clay loam, 2.7% organic matter, pH 6.5) was collected from the top 20 cm of a pasture field that had been organically managed for approximately 9 years. Prior to spiking, the soil was partially air-dried and passed through a 2-mm sieve. Following rehydration to 35% (w/w) (with dH2O) in a large glass bowl, the phenanthrene–transformer oil mixture was added and blended using a stainless steel spoon, giving good homogeneity (relative standard deviation (RSD) ≤ 10%). Phenanthrene (> 96%, HPLC-grade, Sigma-Aldrich, Germany) and [9-14C]phenanthrene (Sigma-Aldrich) were mixed with transformer oil (National Grid Transco, UK) and added to the soil to give 10 mg kg⁻¹ and 1 kBq g⁻¹. Total 14C activity and spike homogeneity per treatment (i.e. transformer oil concentration) was determined via combustion (1-g samples, n = 6, Sample Oxidiser, model 307, Packard). Quantification was by liquid scintillation counting (LSC, Canberra Packard Tri-Carb 2000TR).

The transformer oil concentrations investigated were 0%, 0.01% and 0.05%, which are representative of the low concentrations associated with the periphery of a migrating NAPL plume. Where transformer oil could not be used as a carrier (i.e. oil concentration = 0%), phenanthrene was dissolved in acetone prior to spiking (based on the guidelines of Northcott and Jones [16]), and blended as above.

2.2. Mineralisation experiments

A bacterial inoculum (isolated from PAH-contaminated soil and identified as a Pseudomonas sp. [17]), able to utilise phenanthrene as a sole carbon source for growth, was cultured on 0.1 g phenanthrene l⁻¹ in 600 ml of a minimum basal salts (MBS) solution [18] at 22 ± 2°C, on a rotary shaker operating at 100 rpm. After 4 days incubation (late exponential phase) the culture was centrifugation at 10000 × g for 30 min (Beckman J-6M, Beckman Coulter, USA). The supernatant was poured off, to remove residual phenanthrene, while retaining the cells as a concentrated suspension; this procedure was repeated twice to ensure thorough washing of the cells. The final inoculum, suspended in 250 ml of fresh MBS, contained ~10⁹ cells ml⁻¹ (colony-forming units were counted following serial dilutions of the inoculum broth plated out, in triplicate, on plate count agar, and incubated at 21°C for 3 days).

Mineralisation assays were performed in 250-ml Schott bottles using the method developed by Reid et al. [18]. In short, 10 ± 0.2 g of soil was added to the microcosm and 5 ml of enriched inoculum added (c. 10⁷ cells g⁻¹ soil). Autoclaved MBS solution was then added to each microcosm to give soil:liquid ratios of 2:1, 1:1, 1:2, 1:3 or 1:5. Mineralisation of 14C-labelled phenanthrene was determined after 4, 8 and 12 h by measuring 14CO₂ trapped in 1 M NaOH, then every 12 h until day 4 and then every 24 h until day 10, at 20 ± 3°C, in triplicate. Quantification was by LSC using Ultima Gold scintillation fluid (Canberra Packard, UK). Mean rates and overall extents of mineralisation were compared statistically via ANOVA testing (SigmaStat 2.0, Tukey test).

3. Results

Mineralisation of [14C]phenanthrene by a phenanthrene-degrading Pseudomonas sp., in soil amended with 0, 0.01 or 0.05% transformer oil, was monitored over a 10-day period in microcosms containing a soil:water slurry ratio of either 2:1, 1:1, 1:2, 1:3 or 1:5. Degradation was determined by the mineralisation of [14C]phenanthrene to 14CO₂ (Fig. 1).

3.1. Effect of soil:water ratio

The effect of soil:water ratio on the mineralisation of phenanthrene is displayed graphically in Fig. 1. Slurrying (1:1, 1:2, 1:3 and 1:5 soil:water ratios) consistently resulted in statistically higher rates and extents of mineralisation than the (effectively) non-slurried system (2:1 soil:water slurry).
The mean extents of mineralisation after 1 day increased in the order 2:1 < 1:5 ≈ 1:3 < 1:2 < 1:1; there were no significant differences (P > 0.05) between the 1:1, 1:2, 1:3 and 1:5 soil:water ratios.

The maximum extents of mineralisation observed (i.e., 10-day totals) occurred in the 1:2–1:5 soil:water ratio microcosms, irrespective of transformer oil concentration, and were not statistically different from each other (P > 0.05).

The highest initial rates of mineralisation were observed under the 1:1 and 1:2 soil:water ratios (Table 1), with 55–65% of the [14C]phenanthrene mineralised in the first 24 h, irrespective of transformer oil concentration. Mineralisation measured for 1:3 and 1:5 ratios remained lower than the 1:1 and 1:2 soil:water ratio microcosms over the course of the first 3 days, for all transformer oil concentrations, as a result of the initially slower rates of degradation. In all of the microcosm experiments, i.e., all ratios and oil concentrations investigated, hourly mineralisation rates increased rapidly during the first 12 h to their maximum rate (ranging 0.7–5.8% h⁻¹; Table 1) before plateauing over the course of the next 24 h, and subsequently declining exponentially until the experiment was terminated. Maximum mineralisation rates were significantly lower for the non-slurried system (2:1 soil:water ratio) compared to all of the slurried systems (Student’s t-tests, P < 0.001).

Soil slurrying improved reproducibility of results at each transformer oil concentration investigated. The 1:1–1:5 soil:water ratio microcosms displayed generally very low variability between triplicates (RSD < 5%). This is in comparison to the much larger variability displayed in the effectively non-slurried (2:1 soil:water ratio) system (RSDs 10.8, 33.7 and 18.1% for 0, 0.01 and 0.05% oil concentrations, respectively).

3.2. Effect of transformer oil concentration

Mineralisation curves for each soil:water ratio enabled direct comparison of [14C]phenanthrene mineralisation between the various transformer oil concentrations investigated (graphs not shown). The mineralisation curves for the 0.01 and 0% transformer oil-amended soils are virtually indistinguishable from each other across the 10-day period, and the total mineralisation values are consistently higher than the 0.05%–amended soil (Table 1). With respect to initial rates of mineralisation, the trends were not statistically significant (P > 0.05; Table 1). In terms of the overall extents of mineralisation, the decrease in the 0.05%–amended soil observed for each of the soil slurry conditions investigated was statistically different between the 0.01 and 0.05% transformer oil concentrations only (P < 0.1). However, in microcosms containing the 2:1 soil:water ratio, a significantly lower extent of mineralisation was observed in 0.05%–amended soil compared to soils amended with lower transformer oil concentrations (P < 0.05). Statistical analysis of the data (ANOVA, P > 0.05) displayed no significant interactions between the transformer oil concentrations and soil:water ratios investigated.
Alexander [11] observed an increase in the extent of mineralisation in non-slurried systems. For example, Labare and Alexander [11] observed an increase in the extent of mineralisation of phenanthrene in dibutyl phthalate from 4.4 to 36.9% in a 34-day test period. In this study [11], and in the case of Ortega et al. [15], soil slurries were 1:1 ratios, with either water or MBS solution. Enhanced mineralisation in slurried systems is thought to be a result of stimulated microbial activity related to increased surface area at the NAPL–water interface [11]. This phenomenon may be further enhanced by mixing, putatively facilitating both enhanced rates of substrate partitioning to the water phase, or perhaps increased colonisation at the NAPL interface [15]. However, it remains unclear whether the latter is achieved only in static slurries or can be maintained or even enhanced, due to increased microbial distribution, through agitation.

In this study, the employed methodology required inoculation of soil slurries with a concentrated inoculum of a catabolically active degrading pseudomonad. The addition of the 5-ml bacterial inoculum added to each respirometer meant that the minimum soil:water ratio that could be investigated was constrained to 2:1. At this ratio, the soil is (effectively) not slurried and mineralisation was notably reduced compared to higher soil:water ratios, e.g. 61% and 86% in 2:1 and 1:1 ratio microcosms, respectively; supporting the findings of previous studies that compared non-slurried to 1:1-slurried systems [11,15]. An additional effect of slurrying was also observed: in the 2:1 soil:water ratio microcosms, a statistically lower mean extent of mineralisation was observed between the 0.05%- and 0%-amended soils, but not between the 0.01%- and 0%-amended soils. These statistical differences in mean extents of mineralisation suggested a decrease in microbe–PAH interaction with increasing oil concentration; however, the pattern was not observed in any of the slurried systems.

Limited rates and extents of mineralisation observed in ‘non-slurried’ soils can be explained in terms of limited contaminant and/or microbial inoculum mobility, or a lack of physical mixing. Further evidence to support this statement arises from the observation that after 5 days, when mineralisation had stabilised to a very slow but steady rate, substantial mineralisation (an additional 25%) was induced, and maintained for some 24 h, as a result of stirring the soil with a stainless steel spatula (data not shown).

Increasing the soil:water ratio to 1:1 and 1:2 resulted in the fastest mineralisation rates per transformer oil concentration observed in this experiment. The observed rapid increase in mineralisation, in comparison to a 2:1 ratio, supports the contaminant–microbe mobility constraints on mineralisation discussed above. Soil slurring results in: (i) an increase in exposed soil surface area as the soil particles separate and move into suspension, which facilitates rapid partitioning of the substrate into the aqueous phase; (ii) dissociation of the NAPL into the water phase either as small surface droplets or as micelles, facilitating phenanthrene dissociation into the aqueous phase; and (iii) increased microbe mobility.

Previous studies have shown the presence of a NAPL to increase the biodegradation of PAHs in soil [12]. However, in this study, the transformer oil did not affect the rate or the extent of phenanthrene biodegradation. However, the physical act of slurring the soils did influence the rates and extents of phenanthrene mineralisation. These results imply that a more slurry-like environment is preferable for the degradation of phenanthrene in soil. It is, however, postulated that the observed increase in mineralisation is a consequence of enhanced mixing only (as discussed above) and that further increases in soil:water ratio will result in decreased mineralisation rates and extents due to dilution effects. This postulation is supported in this study by the observation that slightly lower rates and extents of mineralisation were observed in 1:5 soil:water slurries than in 1:3 slurries.

Mineralisation assays are frequently employed to assess the maximum biodegradable fraction of various contaminants in soils [17,18]. To this end, it is imperative that experimental conditions are optimised with respect to the test organism(s), e.g. supply of essential nutrients, incubation conditions, etc.
tion at optimum temperature, or use of a degrading inoculum. With regard to bioremediation of contaminated land, wherein success is strongly dependent on contaminant biodegradation, only an accurate assessment of the true size of the potentially biodegradable fraction will illustrate the extent to which bioremediation is possible. Paramount to the success of bioremediation is the catabolic potential of the indigenous soil microbes and to what extent bioavailability can be increased through chemical and physical manipulation of the soil [5]. This research displays the importance of a comprehensive pre-study, both for laboratory and for industrial assessments of bioavailability (and, hence, biodegradability), simply because the degree to which a soil sample is slurried has been demonstrated to significantly influence mineralisation and hence the quantified putatively bioavailable fraction.

5. Conclusions

This study further supports claims that the slurrying of contaminated soil with either water or MBS solution significantly enhances both the initial rates and overall extents of phenanthrene mineralisation, irrespective of the presence of a NAPL (up to the 0.05% concentration investigated). Soil slurries greater than 1:2, but less than 1:5, are recommended for the reproducible determination of the total fraction of contaminant available for biodegradation.

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References