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Bioremediation of coal tar PAH in soils using biodiesel

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Abstract

The addition of biodiesel together with nitrate and phosphate to soil containing coal tar, in laboratory and field experiments, resulted in degradation of coal tar polycyclic aromatic hydrocarbons (PAH) that was not apparent when the nutrients alone were added. The addition of motor diesel fuel instead of biodiesel was also tested. Over the 55 days of the field and laboratory experiments, the biodiesel resulted in an increased degradation of naphthalene in the coal tar by 52% and 85%, respectively, and motor diesel resulted in increased depletions of 85% and 96%, respectively. Other PAH containing up to four rings were depleted to lesser extents. The increases in PAH biodegradation by the diesel treatments were ascribed to tar solubilisation and dispersion thereby increasing the PAH bioavailability. The ready biodegradability and low phytotoxicity of biodiesel suggest that it may be suitable as a novel treatment for the bioremediation of coal tar contaminated soils. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Coal tars are by-products of the coal carbonization process and are often present as contaminants at the sites of coke production and manufactured gas plants (e.g., Erickson et al., 1993; Luthy et al., 1994). Coal tar is a denser than water non-aqueous phase liquid (DNAPL) composed of a complex mixture of organic compounds, including abundant polycyclic aromatic hydrocarbons (PAH) as well as more volatile components such as benzene and alkylated benzenes (Ghoshal et al., 1996).

Many of the constituent compounds that make up coal tar cannot be identified or quantified through chromatographic methods and thus it is difficult to accurately detail a tar composition (Peters and Luthy, 1993). Elemental analysis of coal tars shows that they not only comprise hydrocarbons but contain significant and variable amounts of compounds containing other elements and values of 58–79% carbon, 5–7 % hydrogen, 13–29% oxygen, 0.8–1.2% nitrogen and 0.5% sulphur have been reported by Haeseler et al. (1999). Analysis of a coal tar composition by Haeseler et al. (1999) found that 17% by weight of the coal tar comprised the 16 PAH in the EPA list of priority pollutants, and 21% was other PAH.

A number of treatment strategies, including bioremediation, exist for the in situ clean-up of NAPL contaminated sites but these are usually constrained by mass transfer limitations and for PAH with low aqueous solubilities this results in low bioavailability and thus low biodegradation rates (Ramaswami et al., 1997). Increasing the surface area of NAPL by dispersion will increase mass transfer and thus may enhance their biodegradation (Ramaswami et al., 1997). Attempts to increase the contact between degrading bacteria and recalcitrant organic contaminants have also been made using solvents and surfactants which directly break up and disperse otherwise viscous and poorly water soluble

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contaminants. Previous bioremediation strategies focusing on this principle of enhancing bioavailability of PAH contaminants have included the use of water-miscible solvents (Bonten et al., 1999) and non-ionic surfactants (Madsen and Kristensen, 1997; Tiehm et al., 1997).

A recent study by Miller and Mudge (1998) investigated the effect of using biodiesel on the mobility and degradation of crude oil within artificial sand columns. The biodiesel used acted in two ways, firstly, the oil dissolved within the biodiesel, facilitating dispersion and prevented incorporation into the sediment and secondly, the surface area of the oil was increased which subsequently accelerated microbial degradation. These reported properties of biodiesel together with its low phytotoxicity (Birchall et al., 1995) and readily biodegradable nature (Zhang et al., 1998) prompted this study which investigates the use of biodiesel as a dispersant to enhance the bioremediation of coal tars and compares its effectiveness with motor diesel.

2. Materials and methods

Coal tar samples and soil samples were obtained from the site of the former Bowden Close colliery and cokeworks, Helmington Row, County Durham, UK. Samples of tar dripping from the side of a site investigation trial pit were collected from approximately 1 m below the soil surface, while soil samples uncontaminated by tar were collected approximately 500 m away from the trench.

2.1. Laboratory biodegradation experiments

The samples for the laboratory biodegradation experiments were prepared in duplicate as follows: Aliquots of tar (200 mg) and aliquots of tar dissolved in biodiesel (1 ml) or motor diesel fuel (1 ml) were added to dry soil (6 g) and transferred to 100 ml conical flasks containing wet soil (5 g) and distilled water (50 ml). The biodiesel used was a rapeseed oil methyl ester (RME) supplied by Raine and the motor diesel fuel was from a BP car filling station. A slow release fertiliser pellet (50 mg) was added as a source of nutrients to each flask except one set. Control flasks containing mercury(II)chloride (20 mM) were also prepared to assess any abiotic degradation of the tar. The soil slurry flasks were incubated in the dark at room temperature (ca. 20°C) on an orbital shaker at 60 rpm.

2.2. Field bioremediation experiments

Using the soil and tar collected from the site, experiments were set up to determine the extent of degradation of PAH in the tar with time under various conditions. Extraction and analysis of samples were carried out at the start of the experiment (day 0) and again after 55 days. The samples were prepared in duplicate as follows: Aliquots (ca. 40 g) of wet soil were weighed and transferred to a nylon mesh square (approximately 25×25 cm^2, mesh size 0.5 mm). An aliquot of tar (500 mg) was dissolved in either biodiesel (2 ml) or motor diesel (2 ml) and then transferred onto an aliquot of dry soil (10 g). A similar aliquot of tar was added to the dry soil without diesel. The dry soil with tar (plus biodiesel/motor diesel where appropriate) was transferred to the centre of the wet soil on the mesh square. Approximately 0.2 g of a slow release fertiliser (Fisons) comprising 14% each of nitrogen, phosphorous and potassium, were added to the designated samples. The mesh squares were formed into bags which were buried approximately 5 cm below the surface of the soil at the site and left for 55 days.

2.3. Analysis of tar composition

Aliquots ($\sim$30 mg) of the tar samples were spiked with extraction (surrogate) standards (squalane, BDH Chemicals; and 1,1′-binaphthyl, Kodak Chemicals) and total hydrocarbon fractions were obtained using silica gel/alumina column chromatography by elution with 70 ml of light petroleum spirit (Bp 40–60°C) and 70 ml of 50% dichloromethane (DCM) in light petroleum spirit (both Distol grade solvents, Fisher UK). After addition of internal standards (*p*-terphenyl, Fluka, and the Supleco, deuterated PAH internal standard mix comprising Acenaphthene-D10, Chrysene-D12, Naphthalene-D8, Perylene-D12, Phenanthrene-D10 and 1,4,-Dichlorobenzene-D4) for quantitation, the PAH – containing hydrocarbon fractions were analysed by GC–MS. The standard deviations relative to the means of the measurements, determined by triplicate analysis (duplicate for naphthalene) of individual PAH compounds in a tar sample, were better than 3.5%.

2.4. Soil extraction method

After addition of surrogate standard (1,1′-binaphthyl), the soil plus tar samples from the laboratory and field bioremediation experiments were extracted firstly using ultrasonic extraction with successive 40 ml portions of methanol and DCM and secondly using soxhlet extraction with DCM and methanol (93:7) as the solvent mixture. The PAH in the solvent extracts were separated and analysed in the same way as the tar samples.

2.5. Analytical equipment

The GC–MS instrument used was a Hewlett Packard 5890-5972 MSD. Splitless injection (at 280°C) via a HP 7673 autosampler onto a fused silica capillary column

(30 m × 0.25 mm i.d) coated with 0.25 μm 5% phenyl methyl silicone (HP-5) was used and the oven was programmed from 40–300°C at 4°C min. Data were acquired in both SIM and scan mode.

3. Results

3.1. Tar solubility in diesel

An aliquot (0.5 g) of the tar added to biodiesel (25 ml) in the laboratory, slowly dispersed into the biodiesel and on agitation it almost completely dissolved with only some asphaltene-like particles remaining in suspension. The addition of tar to motor diesel did not result in the same efficient dissolution even after agitation, and more and larger black particles remained undissolved compared with those in the biodiesel. Filtration and weighing of the hexane washed and then dried undissolved material indicated that the tar solubility was approximately 95% in the biodiesel and 87% in motor diesel. However, these solubility results are only semi-quantitative due to the presence of variable quantities of small coaly particles in the tar.

3.2. Laboratory experiments

The quantity and composition of PAH in the laboratory incubations were determined at the start of the experiment and after 55 days incubation. In the samples treated with nutrients but not diesel, the PAH depletion was limited to the most volatile components. For example, after 55 days of incubation naphthalene was depleted in both the nutrient treated samples and the abiotic control samples, indicating abiotic removal, probably due to volatilisation. Fig. 1 shows small decreases in the amounts of some other higher molecular weight PAH compounds in the day 55 nutrient treated experiment relative to the equivalent untreated experiment but they were not significant ($P = 0.05$). The effect of adding nutrients to the tar-in-soil aqueous slurry on the degradation of PAH in the tar was therefore negligible in these experiments. However, it was clear that two- and three-ring aromatic compounds in the tar had been substantially degraded in slurries treated with both biodiesel and motor diesel in addition to nutrients (Fig. 1). For example, naphthalene had been reduced by as much as 85% and 96% in the biodiesel and motor diesel, treated experiments, respectively, compared with the slurries treated with nutrients alone. Three-ring aromatics such as phenanthrene were also noticeably depleted in the biodiesel and motor diesel treated experiments (by up to 26% and 70%, respectively). However, no depletion of four- and five-ring compounds was detected (Fig. 1).

3.3. Field experiments

Although the absolute PAH concentrations in field tar degradation experiments examined after 55 days showed greater losses of some, especially lower molecular weight PAH components in the biodiesel and motor diesel treated experiments compared with the nutrient treated and untreated experiments (see Fig. 2), the data showed relatively large differences between replicate samples. This was attributed to heterogeneity of the tar distribution in the soil. Consequently, interpretation of data based on absolute concentrations is difficult. Nevertheless, in addition to the clear depletion of naphthalene in the biodiesel and motor diesel treated soil in absolute terms (Fig. 2), comparison of the ratios of three- and four-ring aromatic compounds to a relatively

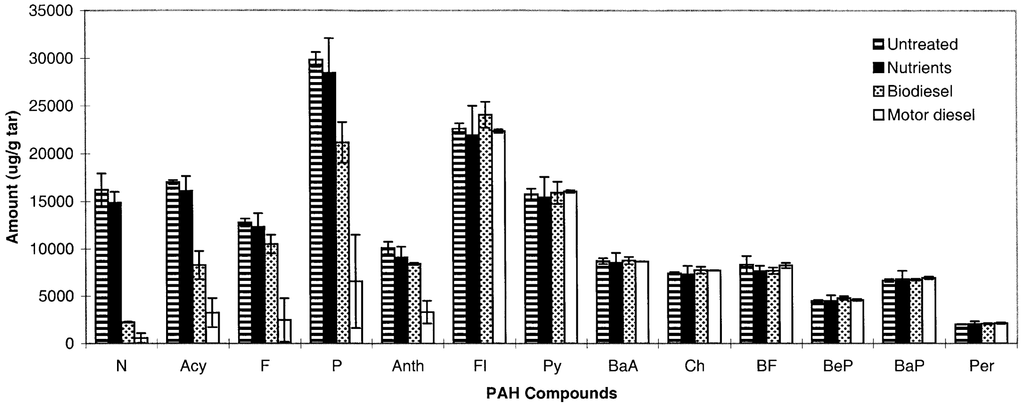

Fig. 1. Effects of the treatments on tar PAH concentrations after 55 days under laboratory conditions. Error bars indicate one standard deviation. PAH assignments are: N – naphthalene; ACY – acenaphthylene; F – fluorene; P – phenanthrene; Anth – anthracene; FL – fluoranthene; PY – pyrene; B(a)A – benzo(a)anthracene; Ch – chrysene; BF – benzo(j + k)fluoranthene; BeP – benzo(e)pyrene; BaP – benzo(a)pyrene; Per – perylene.

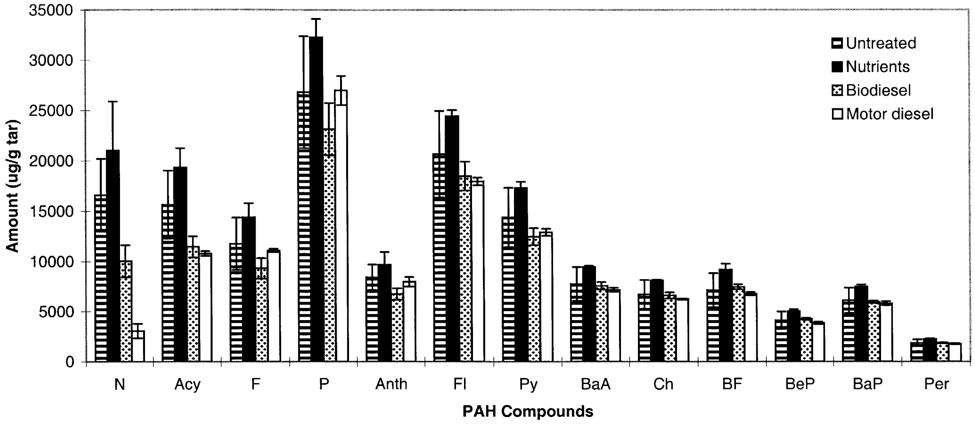

Fig. 2. Effects of the treatments on tar PAH concentrations after 55 days under field conditions. Error bars indicate one standard deviation. PAH assignments are as in Fig. 1.

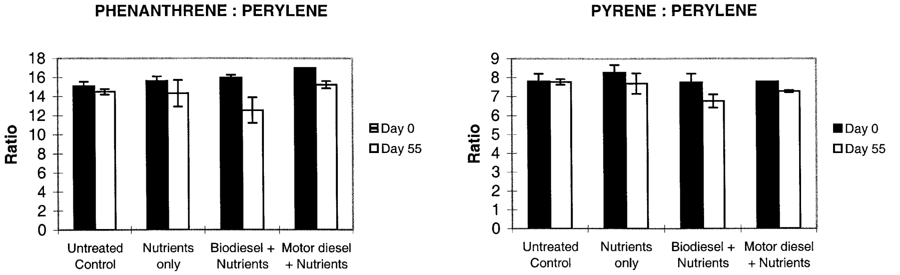

Fig. 3. Ratios of phenanthrene and pyrene relative to perylene at day 0 and day 55 for the different treatments in field conditions. Error bars indicate one standard deviation.

recalcitrant five-ring compound (perylene) indicates that there has been up to 21% depletion of phenanthrene in the biodiesel treated soil samples (Fig. 3). Some relative depletions shown by these ratios also appear to have occurred of four-ring components in the biodiesel and motor diesel experiments, but the depletions are close to the standard deviations of the experiments and no replicates were available for the day 0 motor diesel treated samples.

4. Discussion

The provision of limiting nutrients has been shown to be effective in stimulating hydrocarbon degradation after oil spills (e.g., Pritchard et al., 1992; Swannell et al., 1999). In this present work, however, simple nutrient amendment was not sufficient to enhance the degradation of PAH in coal tars. Since no significant degradation of the tar PAH occurred in the nutrient treated samples in this work and assuming the presence of indigenous hydrocarbon-degrading microorganisms, it may be reasonable to conclude that the degradation is not nutrient limited and that the low bioavailability of the PAH compounds accounts for the lack of degradation. This is consistent with the findings of Erickson et al. (1993) who noted that there were no significant losses of PAH from coal tar-in-soil microcosms and who concluded that the failure to observe losses was due to the PAHs being unavailable to microorganisms, since freshly added PAH were readily biodegraded. Weissenfels et al. (1992) also noted that PAH in contaminated soil from a coke works site were unavailable to degrading microorganisms. However, there have also been reports of successful efforts to enhance the biodegradation of coal tar by adding nutrients (and a mixed bacterial consortium) in laboratory and field conditions (e.g., Jack et al., 1996).

The enhanced degradation of the majority of two- and three-ring PAHs in the biodiesel and motor diesel treated samples probably resulted from increased mass transfer of the PAH from the solid tar into solution by solubilisation and dispersion thus increasing their bioavailability. The added diesels may also induce increased microbial activity by themselves acting as a source of bioavailable carbon. Thus the action of the diesels on the solubilisation of PAH in coal tar is comparable with the effects of surfactants which have been used to enhance the bioremediation of coal tar in contaminated soil (e.g., Tiehm et al., 1997; Madsen and Kristensen, 1997). It is also consistent with recent observations on PAH mass transfer constraints from organic phases of varying viscosities, where organic phase resistance to naphthalene dissolution was noted to be dominant in high viscosity lubricating oils but then to disappear in low viscosity oils (Ortiz et al., 1999).

The degradation of lower molecular weight two- and three-ring PAH compounds is known to occur much more rapidly than degradation of four-, five- and six-ring PAH (e.g., Wilson and Jones, 1993; Shuttleworth and Cerniglia, 1995; Jack et al., 1996). Although there was no evidence of degradation of four-ring PAH from the tar in the present laboratory studies, there were some indications of enhanced depletion of these compounds relative to perylene in the field samples treated with biodiesel and motor diesel experiments. However, the data are equivocal due to the relatively high standard deviations of the measurements.

While it is clear is that the addition of motor diesel resulted in the most degradation of the naphthalene in the field experiments and of the two- and three-ring PAH in the laboratory experiments, this is unlikely to be wholly due to increased bioavailability since the solubility of coal tar in motor diesel was lower than that in the biodiesel. One possible explanation may be that the presence of motor diesel stimulated the activity of indigenous bacterial populations sufficiently to enhance the degradation of the PAH compounds. The addition of a large supply of aliphatic hydrocarbons together with the smaller proportions of aromatic compounds in the form of readily bioavailable motor diesel would be likely to stimulate microbial activity and increase the specific microbial population able to degrade both the motor diesel hydrocarbons and the PAHs from the coal tar (cf. Scotsky et al., 1994). However, these experiments were not designed to assess the suitability of motor diesel as a treatment for the bioremediation of tar polluted soil since motor diesel is itself a hydrocarbon pollutant. Thus, despite motor diesel having a more pronounced effect on the degradation of many PAH in the soil experiments, biodiesel with its low phytotoxicity (Birchall et al., 1995) and ready biodegradability (Zhang et al., 1998), may represent a more environmentally benign treatment to enhance PAH degradation.

Comparison of the biodiesel and motor diesel treatments on PAH degradation demonstrated that the biodiesel was less effective at stimulating PAH degradation in the laboratory experiments. There may be several reasons for this observation. The ability of the biodiesel to dissolve nearly all of the tar may be initially detrimental because it may increase the availability of substances which are toxic to microorganisms. Also, microorganisms may have the ability to hydrolyse fatty acid methyl esters to produce methanol, which at high concentrations can be an inhibitor to microbial growth. Thus, the volume of biodiesel added together with the conditions of degradation could be of importance. In the 55-day field experiments, the biodiesel treatment apparently resulted in greater degradation of some three- and four-ring aromatic components than the motor diesel treatment, but the variability of the data and insufficient replicate samples mean that further field experiments are needed to confirm this.

5. Conclusions

The effect of nutrient addition alone to soils containing coal tar in both laboratory and field experiments failed to significantly enhance biodegradation of coal tar PAH. Similar findings have been noted in some other studies (Weissenfels et al., 1992; Erickson et al., 1993) and it is widely believed that restricted PAH mass transfer and bioavailability are the main factors limiting the degradation of PAH from coal tars and coal tar contaminated soils (Luthy et al., 1994; Ramaswami et al., 1997; Tiehm et al., 1997; Bonten et al., 1999).

The addition of both biodiesel and motor diesel with inorganic nutrients resulted in enhanced degradation of a number of PAH components over the untreated and nutrient-only treated samples in both the laboratory and field experiments, with the greatest depletions occurring of the two- and three-ring components. There also appeared to be depletion of some four-ring PAH in the field experiments though the variability of the measurements prevented definitive conclusions being drawn. Longer term (> 55 days) experiments with a greater number of replicates are needed to confirm the enhanced degradation of the larger PAH. Nevertheless, these preliminary results indicate that biodiesel is capable of solubilising and increasing the bioavailability of coal tar PAHs in soils. The low phytotoxicity of biodiesel and its readily biodegradable nature means that it may have potential use as a novel treatment in the bioremediation of coal tar contaminated soils.

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