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ORIGINAL PAPER

Effects of soil amendment with different carbon sources and other factors on the bioremediation of an aged PAH-contaminated soil

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Abstract Carbon supplementation, soil moisture and soil aeration are believed to enhance in situ bioremediation of PAH-contaminated soils by stimulating the growth of indigenous microorganisms. However, the effects of added carbon and nitrogen together with soil moisture and soil aeration on the dissipation of PAHs and on associated microbial counts have yet to be fully assessed. In this study the effects on bioremediation of carbon source, carbon-to-nitrogen ratio, soil moisture and aeration on an aged PAH-contaminated agricultural soil were studied in microcosms over a 90-day period. Additions of starch, glucose and sodium succinate increased soil bacterial and fungal counts and accelerated the dissipation of phenanthrene and benzo(a)pyrene in soil. Decreases in phenanthrene and benzo(a)pyrene concentrations were effective in soil supplemented with glucose and sodium succinate (both 0.2 g C kg^{-1} dry soil) and starch (1.0 g C kg^{-1} dry soil). The bioremediation effect at a C/N ratio of 10:1 was significantly higher ($P < 0.05$) than at a C/N of either 25:1 or 40:1. Soil microbial counts and PAH dissipation were lower in the submerged soil but soil aeration increased bacterial and fungal counts, enhanced indigenous microbial metabolic activities, and accelerated the natural degradation of phenanthrene and benzo(a)pyrene. The results suggest that optimizing carbon source, C/N ratio, soil moisture and aeration conditions may be a feasible remediation strategy in certain PAH contaminated soils with large active microbial populations.

Keywords Phenanthrene · Benzo(a)pyrene · Indigenous microorganisms · Carbon · Soil moisture · Soil aeration

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Introduction

It is well known that soils, including those contaminated with PAHs, usually contain large numbers of PAH-degrading microorganisms. However, soil microbial degradation capacity is often limited by the recalcitrance of PAHs to microbial attack and environmental factors such as availability of carbon substrates, nitrogen or other nutrients, aeration conditions, pH and temperature (Morris 1994; Morgan and Watkinson 1989; Eggen 1999; Sun et al. 1998; Coulon et al. 2005; Chaîneau et al. 2005; Wu et al. 2008). Furthermore, soil characteristics such as organic carbon and moisture contents can affect the activities of soil enzymes (Bogan and Sullivan 2003; Baran et al. 2004; Atagana et al. 2003; Núñez et al. 2009).

Carbon substrates are necessary for the survival of most heterotrophic microorganisms and numerous studies have demonstrated that the carbon present in co-metabolic substrates leads to enhanced biodegradation of PAHs in the soil (Kanaly and Bartha 1999; Lee et al. 2003; Sarkar et al. 2005; Lladó et al. 2009). Co-metabolic substrates that have been studied include salicylic acid, phthalic acid, biphenyls and mineral oils (Tittle et al. 1995; Mahaffey 1988; Song et al. 2001; Calstrom and Tuovinen 2003; Gong et al. 2005). Johnson and Scow (1999) examined the effects of nitrogen and phosphorus addition on phenanthrene biodegradation in four soils and found that phenanthrene mineralization rates were related to the initial nitrogen and phosphorus concentrations in the soils. Adding carbon and nitrogen can change the C:N ratio in soil and thereby influence PAH degradation. Zitrides (1983) found that the biodegradation of hydrocarbons has an optimal C:N:P ratio of 33:5:1. Zhou and Crawford (1995) pointed out that under low nutrient conditions microorganisms did not have enough nutrients for optimal growth (C:N 300:1) and under higher nutrient conditions a low C:N ratio likely favored microbial growth (C:N 50:1, 18:1 or 15:1). Excessive addition of nitrogen (C:N 1.8:1) almost stopped biodegradation, possibly due to ammonia toxicity that inhibited soil microbial growth. Antizar-Ladislao et al. (2005) investigated that the influence of soil/green waste ratio (S:GW 0.6:1, 0.7:1, 0.8:1, and 0.9:1) on composting and bioremediation of an aged coal tar soil and greater removal of PAHs was observed at S:GW of 0.8:1 compared to other amendment ratios. Godoy-Faúndez et al. (2008) also found that a soil-to-sawdust ratio (S:SD) of 1:3 and a correct nutrient balance were required to achieve a maximum overall hydrocarbon removal from weathered and aged wastes contaminated with fuel oil. It is therefore very important to make appropriate adjustment to the proper C/N ratio for any particular contaminated soil.

Moisture content is an important factor influencing soil conditions and thus water amendments may often be necessary during bioremediation processes of PAH contaminated soils. Antizar-Ladislao et al. (2006) reported that for small, medium and large PAHs maximum achievable degradation in an aged coal tar contaminated soil during composting optimal moisture content was in the range of 60–80% at 38°C. Adding water to the soil helps in PAH degradation (Lewis 1992) and increasing the ratio of water to soil to a certain extent can increase the mineralization rate of phenanthrene, with the maximum extent of mineralization observed occurring at a range of moisture contents of 20–50%. Soil slurries with moisture contents of 20% or greater, but less than 50%, were recommended for bioassay determinations of total contaminant bioavailability due to greater overall mineralization and improved reproducibility (Doick and Semple 2003). Li et al. (2006) also found that submerged conditions can inhibit the degradation of B[a] P in soil.

A recent study found that total concentrations of 15 PAHs in topsoils in the Yangtze River Delta region, east China ranged from 8.6 to 3,881 μg kg^{-1} with an average of 397 μg kg^{-1}. Half of the soil samples were considered to be contaminated with PAHs (>200 μg kg^{-1}) (Ping et al. 2007). Bioremediation of PAH-contaminated agricultural soils is therefore a major concern in this region. In the present study we evaluated the effects of carbon and nitrogen supplementation and of soil moisture content and soil aeration on the biodegradation of PAHs and associated microbial counts during the bioremediation of an aged PAH-contaminated soil in microcosms over a 90-day period. The study was designed to provide the knowledge base for enhancing the natural attenuation of PAH-contaminated soil.

Materials and methods

Experimental materials

Soil samples were taken from the top 15 cm of the soil profile of PAH-contaminated agricultural land in the Yangtze Delta area, east China. The soil was contaminated approximately 30 years previously and the contaminants have therefore undergone a long weathering process. Gravel and plant root residues in the soil samples were discarded and the soil was air-dried to a moisture content of 20% at room temperature and passed through a 2-mm sieve. Soil physico-chemical analysis (Lu 2000) showed that the soil is a silt loam with 11.1 g kg^{-1} total organic carbon, a pH (in water) of 6.4, 1.0 g kg^{-1} total nitrogen, 14.7 g kg^{-1} total potassium, and 78.4 mg kg^{-1} hydrolysable nitrogen on a dry weight basis. The concentrations of phenanthrene and benzo(a)pyrene were 72.8 and 77.9 μg kg^{-1} dry weight, respectively.

The carbon sources used were starch, glucose and sodium succinate and were provided by Shanghai Sangon Biological Engineering Co. Ltd. The analytical grade reagents urea, potassium dihydrogen phosphate and potassium hydrogen phosphate were used as fertilizers.

Experimental design

The treatments comprised (1) control (CK), with no added carbon source, no change in soil aeration, and soil moisture maintained at 60% of water holding capacity (WHC); (2) three carbon sources (starch, glucose and sodium succinate) at rates of 0.2, 1.0, and 5.0 g C kg^{-1} dry soil, respectively. The carbon substrates were added as solid particles mixed thoroughly into the soil; (3) three soil C:N ratios of 40:1, 25:1 and 10:1 set up by adding urea at three different rates (Table 1); (4) soil aeration (stirring); when the glucose was added at 0.2 and 5.0 g C kg^{-1} dry soil stirring and non-stirring treatments were set up, the former with manual stirring with a stainless steel spatula every three days; (5) submerged treatment; when glucose was added at 0.2 and 5.0 g C kg^{-1} dry soil soil moisture content of 70% WHC and submerged treatments (water depth of 1 cm) were set up. The experiment thus consisted of a total of 17 treatments in triplicate (Table 1). Soil microcosms were set up using a series of beakers (upper diameter 11.5 cm, lower diameter 9 cm) covered with tin foil, each containing 600 g of air-dried contaminated soil. After adjustment of soil moisture, the microcosms were incubated in a controlled climate chamber for 90 days at 25 ± 0.5°C. During incubation the soil moisture contents were adjusted by weighing the microcosms. After 10, 30, 60 and 90 days soil samples were collected from each soil microcosm using a mini stainless steel soil drill. Each soil sample was divided into two parts, one of which was placed in a small plastic bag at 4°C for subsequent microbial counts, and the other was placed on tin foil, air-dried, and passed through a 1-mm sieve prior to analysis for PAHs.

Soil PAH analysis and microbial counts

Five grams of air-dried soil were extracted with 20 ml distilled dichloromethane in an ultrasonic bath at 40°C for 2 h and 10 ml of each extract were transferred to a round bottom flask and dried with a rotary evaporator. The residue was dissolved in 2 ml of cyclohexane and 0.5 ml of the solute was extracted and purified by a silica gel column (8 × 220 mm) and washed with a

Table 1 Key to the experimental treatments

Treatment	Amendment	C or N (g kg^{-1})	C:N ratio	Soil moisture	Soil aeration
CK	None	0.0	No adjustment	60% WHC	No stirring
ST1	Starch	0.2	No adjustment	60% WHC	No stirring
ST2	Starch	1.0	No adjustment	60% WHC	No stirring
ST3	Starch	5.0	No adjustment	60% WHC	No stirring
G1	Glucose	0.2	No adjustment	60% WHC	No stirring
G2	Glucose	1.0	No adjustment	60% WHC	No stirring
G3	Glucose	5.0	No adjustment	60% WHC	No stirring
SU1	Na succinate	0.2	No adjustment	60% WHC	No stirring
SU2	Na succinate	1.0	No adjustment	60% WHC	No stirring
SU3	Na succinate	5.0	No adjustment	60% WHC	No stirring
GCN1	Urea	0.6	10:1	60% WHC	No stirring
GCN2	Urea	7.8	25:1	60% WHC	No stirring
GCN3	Urea	18.6	40:1	60% WHC	No stirring
G1W	Glucose	0.2	No adjustment	Submerged	No stirring
G3W	Glucose	5.0	No adjustment	Submerged	No stirring
G1S	Glucose	0.2	No adjustment	60% WHC	Stirring
G3S	Glucose	5.0	No adjustment	60% WHC	Stirring

mixture of hexane and dichloromethane (1:1) (Ding et al. 2004a, b). The first 1 ml of eluate was discarded because it contained non-polar saturated hydrocarbons and was less retained than PAHs by the silica gel. The second 2-ml aliquot of eluate was collected, dried by sparging with N_2 and dissolved in 1 ml acetonitrile. Analysis of phenanthrene (PA) and benzo(a)pyrene (B[a]P) was conducted using a Shimadzu Class-VP HPLC system with a fluorescence detector (RF-10AXL). Separation was carried out on a reversed phase column C18 (VPODS150 × 4.6 mm I.D., particle size 5 mm) using a mixture of water and acetonitrile (1:9, v/v) as the mobile phase at a constant solvent flow rate of 0.5 ml min^{-1}. The chromatograms were monitored using an excitation wavelength of 270 nm and an emission wavelength of 350 nm for PA, with corresponding wavelengths for BaP of 296 and 404 nm. All data were subject to strict quality control procedures (Ping et al. 2007).

Counts of soil cultivable bacteria, fungi and actinomycetes were conducted using standard dilution-plating procedures on various agar media (Larkin 2003). About 10 grams of soil were suspended in 90 ml of sterile water and vigorously stirred for 5 min. Then ten-fold serial dilutions were made and used to inoculate different selective media. Bacterial and actinomycete plates were incubated at 28°C for 3 and 10 days, respectively, and fungal plates at 25°C for 7 days prior to enumeration of viable colonies. Data are expressed as number of colony forming units (CFU) per gram of dry soil.

Statistical analysis

Statistical analysis was carried out using the SPSS 13.0 for Windows software package. Data were analysed by one-way analysis of variance. Mean values were compared by least significant difference (LSD) at the 5% level using SPSS software.

Results

Effects of different carbon sources on concentrations of phenanthrene and benzopyrene and microbial counts

Figure 1 presents the changes in soil PA and B[a]P concentrations after adding different carbon sources to the soil. After 10 days starch addition had no effect on the PA concentration in the soil. After 30 days the PA concentration in soil which received starch at a rate of 1.0 g C kg^{-1} dry soil (ST2) was significantly higher ($P < 0.05$) than that of starch added at 5.0 g C kg^{-1} dry soil (ST3) and the PA concentration in the ST3 treatment was clearly higher than starch at 0.2 g C kg^{-1} (ST1) (Fig. 1a). After 60 and 90 days, the PA concentrations in soil with added starch were significantly lower ($P < 0.05$) than the control (CK), and the PA concentration in soil which received starch at 1.0 g C kg^{-1} dry soil (ST2) was lower than both ST1 and ST3 ($P < 0.05$). The influence of starch addition on the B[a]P concentration was less pronounced than on PA (Fig. 1b). No significant differences in B[a]P concentration were found in soil which received starch as carbon source at 10 and 30 days but after 60 and 90 days the B[a]P concentration in soil amended with starch at 1.0 g C kg^{-1}(ST2) was much lower than in ST1 or ST3.

During the incubation stage glucose addition significantly affected the concentrations of PA and B[a]P in the soil (Fig. 1c, d). PA concentrations in soil amended with glucose at both 1.0 (G2) and 5.0 g C kg^{-1}(G3) were lower ($P < 0.05$) than that in the control (CK). The PA concentration in soil amended with glucose at 0.2 g C kg^{-1} dry soil (G1) was also much lower ($P < 0.05$) than the control (CK) except after 10 days, but the PA concentrations in soil amended with all three levels of glucose showed no significant differences at 60 and 90 days. The B[a]P concentration in soil amended with glucose at 0.2 g C kg^{-1} dry soil (G1) was also much lower ($P < 0.05$) than glucose at 1.0 (G2) or 5.0 g C kg^{-1} dry soil (G3).

Figure 1 also shows that the PA concentration in soil which received sodium succinate at a rate equivalent to 5.0 g C kg^{-1} dry soil (SU1) was lower ($P < 0.05$) than in soil amended with sodium succinate at 1.0 (SU2) or 5.0 g C kg^{-1} dry soil (SU3) after 10 days (Fig. 1e, f). After 90 days the PA concentration in soil amended with sodium succinate at 1.0 g C kg^{-1} (SU2) was much higher than the other two levels. The B[a]P concentrations in soils amended with sodium succinate were lower ($P < 0.05$) than the control (CK) at 60 and 90 days. The B[a]P concentration in soil amended with sodium succinate at 1.0 g C kg^{-1} (SU2) was lower than the other two levels after 10 days. After 30 days soil B[a]P

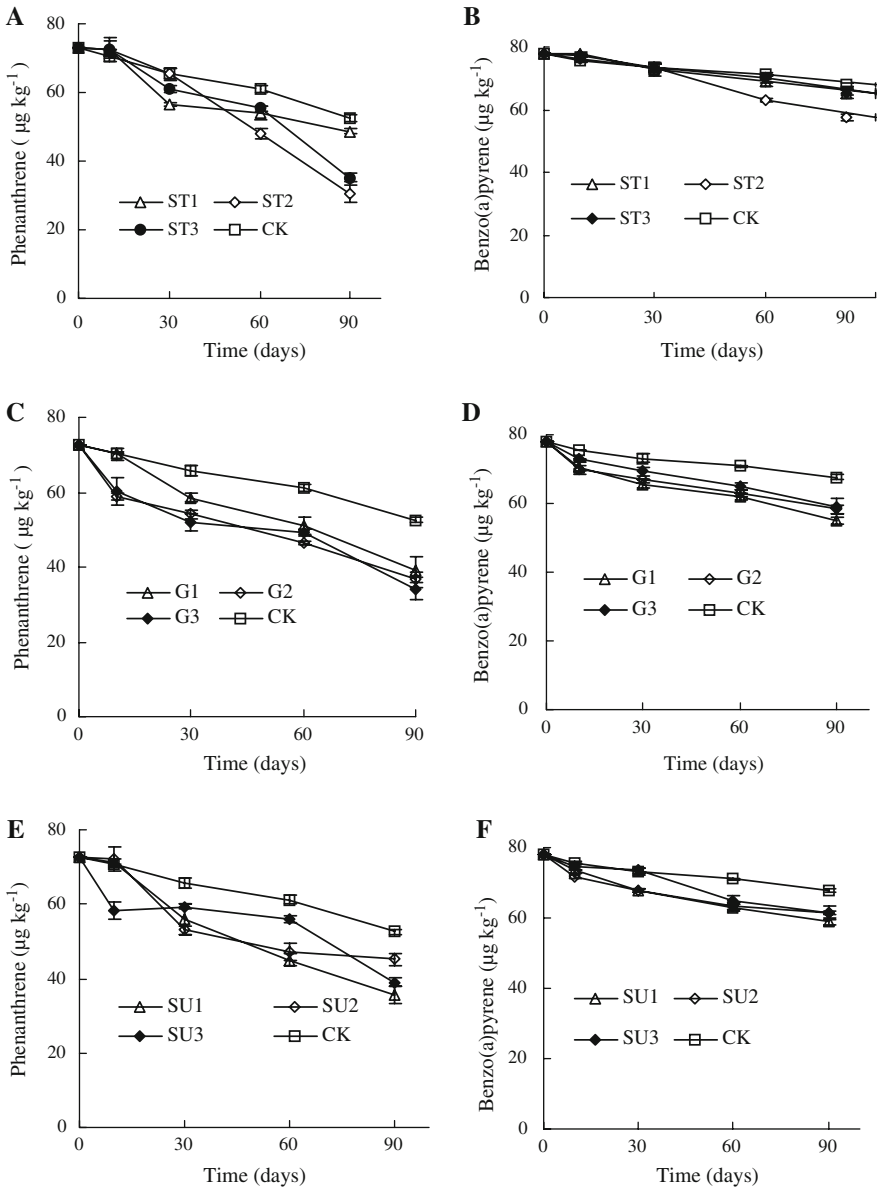

Fig. 1 Effects of different carbon amendments on soil phenanthrene and benzo(a)pyrene concentrations

concentrations showed no significant difference between SU3 and CK but the B[a]P concentration in soil which received sodium succinate at 5.0 g C kg^{-1} dry soil (SU3) was higher ($P < 0.05$) than sodium succinate at either 0.2 (SU1) or 2.0 g C kg^{-1} dry soil (SU2).

After 90 days the PA concentrations in soil amended with carbon sources were lower than the control (CK). The PA concentrations in soils amended with starch (ST2 and ST3) and glucose (G3) were all significantly lower ($P < 0.05$) than those in SU2, G1 or ST1. The B[a]P concentrations in soil amended with glucose at 0.2 (G1) and 2.0 g C kg^{-1} dry soil (G2) were lower ($P < 0.05$) than those of ST1 and ST3 treatments.

Figure 2 shows the changes in microbial counts in the soil with added carbon sources. After 10 days the bacterial and fungal counts increased largely in the soils supplemented with carbon sources, especially the bacterial counts which increased by 1–2 orders of magnitude, but there was no discernible increase in actinomycete counts. The different carbon sources

had different effects ($P < 0.05$) on the soil microbial counts. Sodium succinate and glucose, which are readily available to microorganisms, had a greater influence than starch. Sodium succinate at 1.0 and 5.0 g C kg^{-1} dry soil and glucose at 1.0 g C kg^{-1} dry soil led to higher bacterial counts and sodium succinate at 5.0 g kg^{-1} dry soil led to substantially higher bacterial and actinomycete counts. After 30 days the soil microbial counts delined in all treated soils and may have been related to the consumption of the added carbon sources.

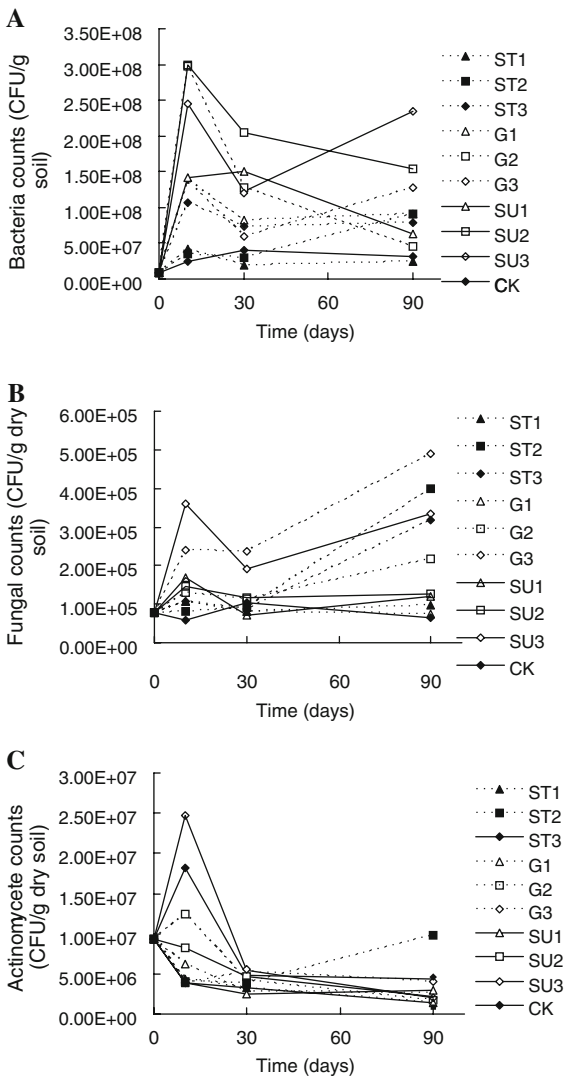

Fig. 2 Dynamic changes in soil microbial counts with different carbon amendments

Effects of different C/N ratios on phenanthrene and benzopyrene concentrations in soil

Figure 3 shows the changes in soil phenanthrene and benzo(a)pyrene concentrations under different C/N ratios. The concentrations of PA and B[a]P in all treated soils showed a gradual decrease trend which was more pronounced in PA. Except for the PA concentration in CCN3 treated soil showing no difference from the control soil (CK) at 90 days, the PA concentrations in other soil C/N ratios were significantly higher ($P < 0.05$) than in the control soil (CK). The phenanthrene concentration showed the lowest value at soil C/N 10 and the highest at C/N 40. After 10 days the B[a]P concentration in the C:N 10:1 treated soil was higher ($P < 0.05$) than in either C:N 25:1 (CN2) or C:N 40:1 (CN3), but the B[a]P concentration at C:N 40:1 (CN3) was higher ($P < 0.05$) than in either CN1 or CN2 after 90 days.

Effects of soil moisture and stirring on the changes in phenanthrene and benzopyrene contents and microbial counts in soil

Figure 4 shows the effects of soil moisture and stirring on phenanthrene and benzo(a)pyrene concentrations in soil amended with glucose at 0.2 g and 5.0 g C kg^{-1} dry soil. Significant decreases in soil PA and B[a]P concentrations over time were observed. Addition of glucose at 0.2 g C kg^{-1} dry soil produced no significant difference in PA between the submerged (G1W) and non-submerged soil (G1) but the PA concentrations in both submerged and non-submerged soil decreased ($P < 0.05$) compared with the control at 60 and 90 days. Glucose at 5.0 g C kg^{-1} dry soil led to higher ($P < 0.05$) PA and B[a]P concentrations in submerged soil (G1W) compared to non-submerged soil (G1) and the control.

With glucose added at 0.2 g C kg^{-1} dry soil (Fig. 4a, b) the PA concentrations in stirred and non-stirred soils did not differ significantly from the control (CK) at 10 and 30 days but the PA concentration in the stirred soil (G1S) was higher than in non-stirred soil (G1). The PA concentrations in both stirred and non-stirred soils were much lower than the control after 60 and 90 days. Moreover the stirred soil had lower PA than non-stirred soil after 90 days. The B[a]P concentration in non-stirred soil was lower than in stirred soil and the control ($P < 0.05$), and the

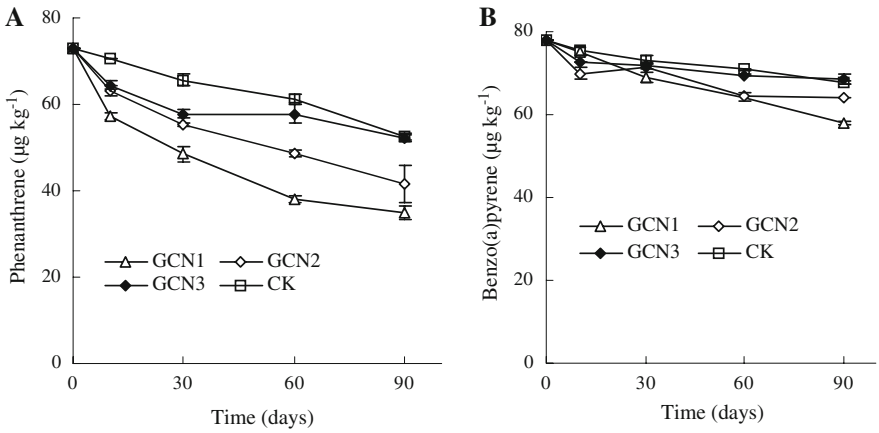

Fig. 3 Effects of different soil C/N ratios on soil phenanthrene and benzo(a)pyrene concentrations

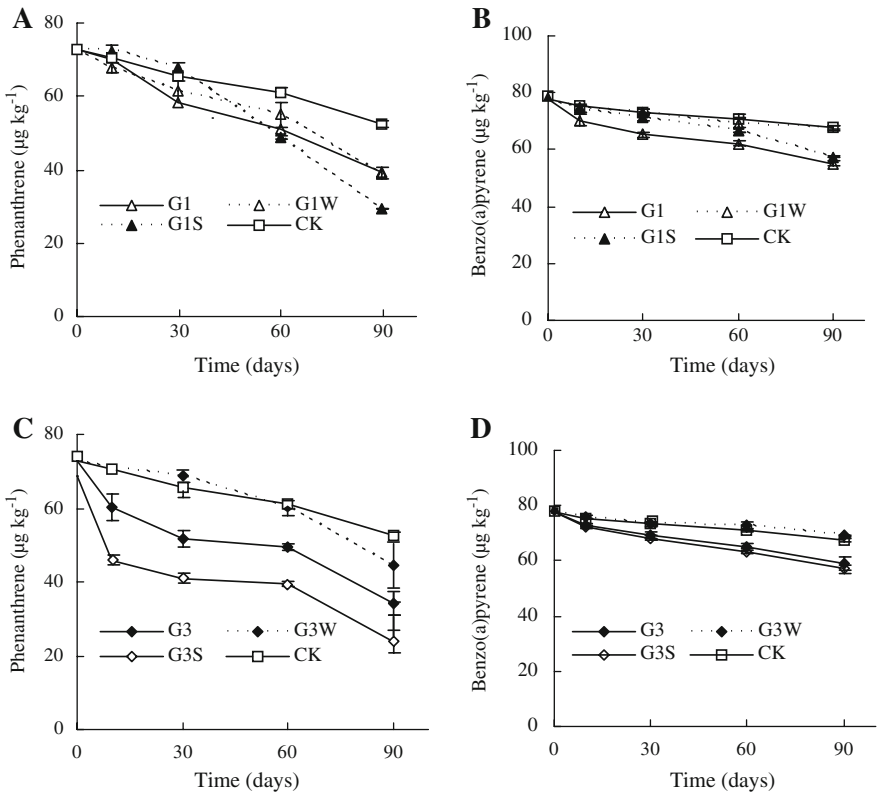

Fig. 4 Effects of soil moisture and aeration on soil phenanthrene and benzo(a)pyrene concentrations

stirring aeration treatment had a higher concentration than the control after 30 and 90 days. When glucose was added at 5.0 g C kg^{-1} (Fig. 4c, d), the PA concentration in the control soil was clearly higher than in non-stirred soil, but the non-stirred soil had higher PA than stirred aerated soil ($P < 0.05$). No significant difference was found between stirred and non-stirred treatments but both treatments were substantially lower in PA than the control ($P < 0.05$).

After 90 days the PA concentration in control soil was higher than in stirred and submerged soil and the stirring treatment (G1S) gave lower values than the submerged (G1W) or non-stirring treatments (G1), but the difference between G1W and G1 was not significant. The B[a]P concentrations in soils showed different trends from the PA concentrations. The B[a]P concentration in non-stirred (G1) soil was much lower than submerged (G1W) and stirred (G1S)

or CK treatments during incubation. Moreover, the PA and B[a]P concentrations in submerged (G3W) soil were much higher than either non-stirred (G3) with 70% WHC or stirred soil (G3S) ($P < 0.05$).

Figure 5 shows that soil stirring and submerging both affected the soil microbial counts to some extent. Stirring promoted higher bacterial and fungal counts but submerging decreased them, especially the fungal counts at 5.0 g glucose C kg^{-1} dry soil. There were significant declines in actinomycete counts following stirring and submerging of the soil, with a more pronounced effect of stirring.

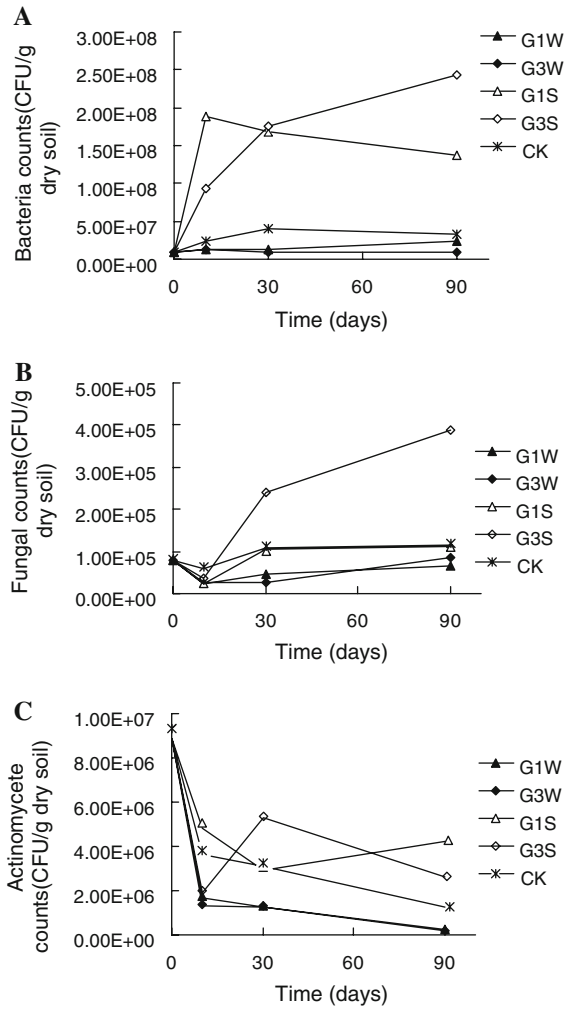

Fig. 5 Dynamic changes in soil microbial counts under different moisture and aeration conditions

Discussion

The most widely used mechanism in bioremediation procedures is stimulation of the indigenous microorganisms by addition of carbon substrates and nutrients because the rate of natural attenuation is generally slow and measures are often adopted to speed up the removal of the pollutants (Margesin and Schinner 2001). Indigenous soil microorganisms have some capacity to degrade PAHs but the effectiveness of degradation is limited and long periods of time are required. Carbon sources are usually added as co-metabolic substrates or soil enzymatic activity is accelerated to enhance the bioremediation of PAH-contaminated soil.

In the present study addition of sodium succinate and glucose increased the rate of degradation of phenanthrene and benzo(a)pyrene in soil maintained at a moisture content of 70% of water holding capacity. Starch addition decreased soil PA concentration but only starch addition at 1.0 g C kg^{-1} dry soil promoted a decline in soil B[a]P. The carbon substrate amendments increased soil bacterial and fungal counts (Fig. 2). Numerous previous studies have shown that most of the PAH-degrading microorganisms in soil are bacteria and fungi (Juhasz and Naidu 2000). Adding carbon sources might therefore be expected to increase the number and activity of PAH-degrading microorganisms and may also enhance soil enzymatic activity, resulting in lower soil PA and B[a]P concentrations. Furthermore, B[a]P has a high molecular weight and is not readily available to microorganisms. It can therefore be degraded only through co-metabolism and with the addition of other co-metabolic substrates. Therefore, the influence of starch, glucose and sodium succinate addition on the soil B[a]P contents may by their direct function as co-metabolic substrates or by their decomposition to form co-metabolic substrates. Mahaffey (1988) observed that a *Beijerinckia* strain designated strain Bl could oxidize benz[a]anthracene after induction with biphenyl, m-xylene, and salicylate, and the presence of salicylic acid improved the capacity of *Pseudomonas putida* NCIB9816 to degrade fluoranthene and benzo(a)pyrene. Gong et al. (2002) reported that when soil is pre-treated with salicylic acid or phthalic acid, benzo(a)pyrene might be gradually degraded in the soil environment. Thus, lower PA contents in soils may result from

added carbon sources increasing the soil microbial biomass and soil enzymatic activity and lowering of B[a]P content may mainly due to the co-metabolic substrate role of the carbon source. After 90 days the addition of starch and sodium succinate (1.0 g C kg^{-1} dry soil) was most effective in reducing the PA concentration in the soil. Sodium succinate addition had a slight influence on the decline in soil B[a]P concentration at different carbon addition rates but adding glucose (0.2 g C kg^{-1} dry soil) and starch (1.0 g C kg^{-1} dry soil) greatly assisted the degradation of B[a]P in the soil. Previous studies have shown that B[a]P, one of the high molecular weight (HMW) PAHs, does not serve as a carbon or energy source for microbial populations during degradation (Juhasz and Naidu 2000; Rentz et al. 2005). Kanaly et al. (2000) also found that the biodegradation process of B[a]P by a cometabolic mechanism required the combined efforts of different soil microbial populations. The initial ring attack with mono- or di-oxygenase enzymes is accomplished by microorganisms that may or may not directly benefit from the reactions. Once hydroxylated, however, PAHs become increasingly soluble and can be attacked by enzymes from additional members of the bacterial community (Cerniglia 1992; Kanaly and Bartha 1999; Kanaly et al. 2000, 2002). This suggests that initial ring hydroxylating microorganisms may utilize glucose and/or starch as a sole carbon and energy source for co-metabolism of HMW PAHs during biostimulation. These results also indicate that different types of carbon source have different roles in the biostimulation of PAH degradation in the soil, and their biostimulation effects are likely to be related to the concentrations and properties of the carbon sources present. On one hand, soil microbial counts and microbial activity are different in the presence of different carbon sources and levels. On the other hand, there are significant differences in the bioavailability of different carbon sources. Starch, for example, is degraded slowly and is not utilized directly by most soil microorganisms, while glucose can be directly utilized by and remain in the soil for only a short time. Thus, the appropriate substrates and addition rates must be determined to optimize pollutant degradation.

Nitrogen is the other major nutrient commonly added in bioremediation projects in order to adjust the carbon-to-nitrogen ratio of the soil. Nitrogen is primarily used for cellular growth (NH_4^+ or NO_3^-) and as an alternative electron acceptor (NO_3^-). It is commonly added as urea or ammonium chloride but may also be supplied as any ammonium salt or ammonium nitrate. All of these forms are readily assimilated in bacterial metabolism (Liebeg and Cutright 1999). In the present study the different soil C:N ratios influenced the degradation of soil phenanthrene and benzo(a)pyrene. The effectivemess of PAH degradation in soil adjusted to a C:N ratio of 10:1 was superior to both C:N 25:1 and C:N 40:1. Our findings are in agreement with the previous reports by Zitrides (1983) and Riser-Roberts (1998) who concluded that a C:N:P ratio of 33:5:1 was optimal for the biodegradation of hydrocarbons. Thompson et al. (2008) also reported that with no plants present and pyrene-C:urea-N (C:N) ratios of 4.5:1, 9:1, 18:1, and 36:1, the mean biodegradation rates of pyrene were 31, 52, 77, and 88%, respectively, indicating that increased inorganic-N concentrations in the soil lowered pyrene degradation in treatments without plants. At the highest N rate, pyrene degradation in the unvegetated treatments was reduced with added N. Therefore, nutrient addition is important to achieve the optimal carbon/nitrogen balance and successful biodegradation of PAH contaminants.

Biodegradation of PAHs has been observed under both aerobic and anaerobic conditions (Haritash and Kaushik 2009). Nutritional and physical factors (e.g., aeration, oxygen concentration) affect the biodegradation of PAHs by the soil microbial populations and the presence of oxygen is a main factor affecting microbial oxidation of hydrocarbons (Zhou and Crawford 1995; Gallizia et al. 2004). It is understood that the initial step in the aerobic degradation of PAHs by bacteria occurs via oxidation of the PAH to a dihydrodiol by a multicomponent enzyme system (Kanaly and Harayama 2000) and this is regarded as the most difficult step for bacterial degradation of PAHs. In the present study we found that the combination of glucose addition with soil waterlogging had a negative impact on the degradation of benzo(a)pyrene. Because good aeration can help in the natural degradation of PAHs (benzo(a)pyrene) aeration may also be useful to accelerate PAH degradation by the soil microbial community. However, the degradation of phenanthrene in the soil with glucose amendment and waterlogging was higher than in the control and this

may be explained by glucose addition favouring the anaerobic degradation of phenanthrene in soil (Coates et al. 1996; Bregnard et al. 1996; Ambrosoli et al. 2005). Soil aeration (stirring) helped glucose to enhance the degradation of PA and B[a]P, which may further emphasize the inhibitory effect of soil moisture. We also found that soil aeration by stirring accelerated the growth of aerophilic fungi and bacteria (Fig. 5) and may have increased the number of PAH-degrading microorganisms in the soil. This accords with the results of Zhang et al. (1999) and Ding et al. (2004a, b) who found that increasing of air flow accelerated the degradation of phenanthrene and pyrene. A recent study by Newcombe and Crawford (2007) found that mineralization rates of glucose were significantly higher in aerated soil slurries and aerobic organisms have been shown to play a role in establishing conditions that are most conducive to the dissipation of TNT.

Conclusions

Different types of added carbon sources had different roles in the biostimulation of PAH degradation in the soil, and their bioremediation effects were related to the quality and quantity of the carbon sources present. Adding starch, glucose and sodium succinate stimulated an increase in the numbers of bacteria and fungi and enhanced the degradation of phenanthrene and benzo(a)pyrene in the soil. The bioremediation effect with a C/N ratio of 10:1 was superior to that with C/Nratios of 25:1 and 40:1. Excessive soil moisture can decrease the stimulatory effect of glucose on PAH degradation. Stirring increased the capacity of glucose to enhance the degradation of PA and B[a]P. This indicates that soil moisture content and stirring change soil aeration and further affect the numbers and types of soil microorganisms present. Hence, it is important to optimize factors carbon source, C/N ratio, soil moisture and aeration conditions affecting the metabolic activities of soil microorganisms to enhance and accelerate the natural attenuation of PAH-contaminated soils.

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