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Methyl-β-cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and associated microbial activity in contaminated soil

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Abstract

The contamination of soils by polycyclic aromatic hydrocarbons (PAHs) is a widespread environmental problem and the remediation of PAHs from these areas has been a major concern. The effectiveness of many *in situ* bioremediation systems may be constrained by low contaminant bioavailability due to limited aqueous solubility or a large magnitude of sorption. The objective of this research was to evaluate the effect of methyl-β-cyclodextrin (MCD) on bioaugmentation by *Paracoccus* sp. strain HPD-2 of an aged PAH-contaminated soil. When 10% (*W*/*W*) MCD amendment was combined with bioaugmentation by the PAH-degrading bacterium *Paracoccus* sp. strain HPD-2, the percentage degradation of total PAHs was significantly enhanced up to 34.8%. Higher counts of culturable PAH-degrading bacteria and higher soil dehydrogenase and soil polyphenol oxidase activities were observed in 10% (*W*/*W*) MCD-assisted bioaugmentation soil. This MCD-assisted bioaugmentation strategy showed significant increases ($p < 0.05$) in the average well color development (AWCD) obtained by the BIOLOG Eco plate assay, Shannon-Weaver index (*H*) and Simpson index (λ) compared with the controls, implying that this strategy at least partially restored the microbiological functioning of the PAH-contaminated soil. The results suggest that MCD-aided bioaugmentation by *Paracoccus* sp. strain HPD-2 may be a promising practical bioremediation strategy for aged PAH-contaminated soils.

Key words: polycyclic aromatic hydrocarbons; methyl-β-cyclodextrin; biodegradation; *Paracoccus* sp. strain HPD-2; microbial activity
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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. Excessive inputs from anthropogenic activities have caused serious contamination and threaten to adversely affect human health (Wilcke, 2007; Gong et al., 2008; Sun et al., 2011). Proper remedial action to remove PAHs from contaminated soils is essential (Ding et al., 2008). Due to their high hydrophobicity and solid-water distribution ratio, PAHs in soil tend to interact with the non-aqueous phase and organic matter, and consequently become less available for further microbial degradation (Johnsen and Karlson, 2005). The utilization of PAHs in soil may result from the combined metabolism of microbial communities or from catabolism by individual strains (Juhasz et al., 1997).

Biodegradation is one of many techniques available for *in* or *ex situ* remediation of PAH-contaminated soils. However, the extent and rate of bioremediation of contaminated soils that can be achieved is often limited by PAH solubility and generally low aqueous PAH concentrations unable to sustain biodegradation (Talley et al., 2002; Yang et al., 2009, 2011). Biodegradation is therefore dependent on the ability of microorganisms to transform and degrade contaminants as well as the accessible concentrations of compounds capable of inducing or sustaining degradation (Allan et al., 2006).

While bioaugmentation with catabolically active microorganisms may be used to minimize microbial constraints, solubility-enhancing agents such as cyclodextrins have been the focus of numerous recent studies regarding their capacity to promote the release and microbial accessibility of contaminants in soils and solutions (Semple et al., 2007; Zhou and Zhu, 2007; Stroud et al., 2009). MCD is a cyclic, bucket-shaped macromolecule presenting a hydrophilic exterior rendering it water-soluble and a hydrophobic cavity enabling the formation of complexes with hydrophobic organic molecules, thereby increasing their aqueous solubility. It is also known to have low toxicity to bacterial cells and a very high solubility (solubility in water > 200 g in 100 mL at 25°C) (Petitgirard et al., 2009). Although the ability of cyclodextrins to enhance the biodegradation of pyrene in soil has been demonstrated, most studies of the impact of cyclodextrins on contaminant disappearance have been restricted to single and low molecular weight PAHs (Badr et al., 2004;

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Stroud et al., 2009) and the effects of MCD addition on the bioremediation of aged PAH-contaminated soils remain to be investigated.

The ultimate goals of any remediation approach are to remove the contaminants from the soil and to restore the capacity of the soil to function according to its potential (Epelde et al., 2009). Soil microorganisms are very sensitive to any shifts in ecosystem function because their activity and diversity are rapidly altered by perturbation (Margesin et al., 2000). Microbiological parameters such as soil enzyme activities and the functional diversity of soil microbial communities may serve as important indices of the impact of pollution on soil health (Shen et al., 2005; Epelde et al., 2008; Teng et al., 2010b).

In the present study several factors were investigated in order to accelerate and improve the bioremediation of an aged PAH-polluted soil, namely the degradative activity of indigenous soil microbial populations compared to that of an added PAH-degrading bacterial strain (*Paracoccus* sp. strain HPD-2), the effect of MCD addition on PAH degradation and on heterothropic microbial population dynamics, and the interaction between MCD addition and bioaugmentation by inoculation with *Paracoccus* sp. strain HPD-2. In addition, soil microcosms were set up to study MCD-assisted bioaugmentation by inoculation with *Paracoccus* sp. strain HPD-2 to evaluate the bioremediation potential of this strategy and examine associated changes in microbial activities in aged PAH-contaminated soil.

1 Materials and methods

1.1 Soil

The soil used for the experiment was collected from the top 15 cm of the soil profile of PAH-contaminated farmland in the Yangtze Delta, East China. The soil was contaminated approximately 30 years previously and the contaminants have therefore undergone a relatively long weathering process. Gravel and plant root residues in the sampled soil were discarded and the soil was air-dried, sieved through a 2-mm mesh, and stored at 4°C in the dark. Some physico-chemical characteristics of the experimental soil (Lu, 2000) show that the soil is a silt loam with 11.1 g/kg total organic carbon, a pH (in water) of 6.4, 1.0 g/kg total N, 14.7 g/kg total K, and 78.4 mg/kg hydrolysable N on a dry weight basis. The concentration of 16 individual PAHs was (9942 ± 91) μg/kg dry soil, with concentrations of the 3-, 4- and 5(+6)-ring PAHs of (823 ± 30), (5614 ± 119) and (3505 ± 181) μg/kg, respectively. According to the Canadian Environmental Quality Guidelines released by the Canadian Council of Ministers of the Environment (CCME, 2004), this soil would not be suitable for agricultural use because of the high concentration of PAHs, and especially of 4- and 5(+6)-ring PAHs.

1.2 Bacterial strain and culture conditions

Paracoccus sp. strain HPD-2 was isolated from a historically PAH-contaminated soil collected from Wuxi, Jiangsu Province, East China (Teng et al., 2010b). The strain was screened for its ability to degrade the high-molecular-weight (HMW) PAHs fluoranthene, pyrene and benzo[a]pyrene. After inoculation into MS medium containing B[a]P at 3.0 mg/L for 5 days, 89.7% of the B[a]P was degraded by the bacterium. When this strain was grown with pyrene and fluoranthene at 50 mg/L for 7 days, 47.2% and 84.5% of these was degraded, respectively. This strain may therefore have potential in improving HMW-PAH biodegradation. Strain HPD-2 was transferred onto a slant of nutrient agar medium. After 3 days of incubation at 28°C the slant was used to inoculate two 500-mL Erlenmeyer flasks each containing 100 mL of liquid medium from 3 g beef extract and 5 g peptone per liter of deionized water. The flasks were incubated for 48 hr at 28°C on a rotary shaker at 200 r/min and produced cell suspensions of 2.0×10^8 CFU/L. Cells were harvested by centrifugation at 5000 $\times g$ for 20 min. The supernatant was discarded and the cells were re-suspended in fresh autoclaved distilled water (repeated twice) for bioaugmentation studies.

1.3 Soil microcosms

The treatments comprised of: control (CK), with no added MCD, no bacterial inoculation, and soil moisture maintained at 60% of water holding capacity (WHC); MCD amendment M1, M2, M3, M4 at rates of 5%, 10%, 15%, and 20% (W/W), respectively, with the MCD added as solid particles mixed thoroughly into the soil; bioaugmentation (CKB), inoculation with 4% (V/W) *Paracoccus* sp. strain HPD-2 cell suspension; and MCD-assisted bioaugmentation (MB1, MB2, MB3, MB4), combining the same four rates of MCD amendment as above plus inoculation with 4% (V/W) *Paracoccus* sp. strain HPD-2 cell suspension. The experiment thus consisted of a total of 10 treatments in triplicate. Soil microcosms were set up using a series of beakers (upper diameter 11.5 cm, lower diameter 9 cm) covered with aluminum foil, each containing 1000 g of air-dried contaminated soil. After adjustment of the soil moisture, the microcosms were incubated in a controlled climate chamber for 16 weeks at (25 ± 0.5)°C. During incubation the soil moisture contents were adjusted by weighing the microcosms. After 1, 2, 4, 8, 12 and 16 weeks 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 analysis of microbial activity and the other freeze-dried and passed through a 60-mesh sieve prior to analysis for PAHs.

1.4 Extraction and analysis of soil PAHs

PAHs in bulk soil samples were extracted using Soxhlet extraction. In brief, 5 g of freeze-dried sample with filter paper was placed in a porous cellulose thimble (25 mm × 70 mm) and placed in a Soxhlet extractor. The extractor was then fitted to a 100 mL round bottom flask containing 60 mL dichloromethane and the extraction was performed for 24 hr. All the extracts in the round bottom flasks were dried by rotary evaporation. The residues were dissolved in 2 mL cyclohexane and 0.5 mL of the solute

was transferred, purified with a silica gel column (8 mm × 220 mm) and washed with a mixture of hexane and dichloromethane (1:1, *V*/*V*). The first 1-mL of eluate was discarded because it contained non-polar saturated hydrocarbons and was less retained than PAHs by silica gel. The second 2-mL aliquot of eluate was collected, dried by sparging with N_2 and then re-dissolved in 1 mL acetonitrile for HPLC determination. Determination of 16 EPA PAHs was carried out according to the method of Ni et al. (2008). Briefly, analysis was conducted on a Shimadzu Class-VP HPLC system (Shimadzu, Japan), with a fluorescence detector (RF-10AXL, Shimadzu, Japan). A reversed phase column C18 (VPODS 150_4.6 mm I.D., particle size 5 mm, Shimadzu, Japan), using a mobile phase of water and acetonitrile mixture (1:9, *V*/*V*) at a constant solvent flow rate of 0.5 mL/min, was used to separate the 16 PAHs. The excitation and emission wavelengths for individual PAHs were set separately.

An external standard mixture was used for quantification of the 16 PAHs. The detection limit of the HPLC method for the 16 PAHs was in the range of 0.12–1.57 μg/kg. Method blanks (solvent) and spiked blanks (standards of EPA610 PAH mixture, LA 96245, Supelco, USA, used to spike the soil) were extracted and analyzed by the methods described above. The recoveries and the relative standard deviations of this method for 16 PAHs were in the ranges of 74%–110% and 0.53%–3.57%, respectively. Results of blanks extracted under the same conditions were below detection limits and sample results are presented without recovery ratio correction. The percentage of PAH removal (*R*, %) was given by Eq. (1):

$$R = (1 - C_s/C_i) \times 100\% \quad (1)$$

where, C_s (μg/kg) is the concentration of PAHs in each treatment and C_i (μg/kg) is the initial PAH concentration present in the soil.

1.5 Enumeration of PAH-degrading bacteria in soil

After incubation, PAH-degrading bacteria in soil were counted using a miniaturized most probable number (MPN) method in 96-well microplates with five replicates per dilution (Wrenn and Venosa, 1996). Briefly, phenanthrene, anthracene, fluorene, and dibenzothiophene were added as sole carbon sources to support the proliferation of aromatics-degrading bacteria. Serially diluted samples were inoculated into the wells and the microplates were incubated at room temperature for 3 weeks. Wells turning yellow or brown owing to the accumulation of partial oxidation products of aromatic substrates were treated as positive. Published MPN tables were used to determine the MPN values.

1.6 Soil enzyme activities

Soil dehydrogenase activity (DHA) was assessed by a modification of the method described by Singh and Singh (2005). Weighed 5 g sub-samples of soil were placed in 50 mL polypropylene centrifuge tubes and mixed with 5 mL of 0.5% 1,3,5-triphenyltetrazolium chloride (TTC) solution. Tubes were incubated for 6 hr at 30°C in the dark. After incubation, triphenylformazan (TPF) formed by reduction of TTC was extracted with three batches of 100 mL methanol. Tubes were shaken in an orbital shaker at 300 r/min for 1 hr, centrifuged (1744 ×*g*, 5 min), and the supernatant was filtered through filter paper. Blanks without the addition of TTC were carried out in the same manner. The concentration of TPF was determined by spectrophotometry at 485 nm and the results were expressed as g TPF/(g soil·hr).

Polyphenol oxidase activity was performed as described by Clause and Filip (1990) measured by a UV-Vis spectrophotometer. Briefly, 1 g fresh soil was transferred into an 18 mm × 20 mm glass tube and mixed with 10 mL of 1% pyrogallol solution. After incubation at 30°C for 1 hr, 12.5 mL of 0.5 mol/L HCl was added into the mixture and shaken manually. The gallic acid produced was extracted with 10 mL of ether and measured spectrophotometrically at 430 nm. Activity was quantified by reference to a calibration curve constructed using gallic acid standard and expressed in mg gallnut/(g soil·hr).

1.7 Physiological profiles of the soil microbial community

Soil microbial community physiological profiles were performed as described by Yao et al. (2003). Briefly, 10 g fresh soil was added to 100 mL distilled water in a 250 mL flask and shaken for 10 min. Ten-fold serial dilutions were made and the 10^{-3} dilution was used to inoculate BIOLOG® Eco plates (BIOLOG, USA). The plates were incubated at 25°C, and color development in each well was recorded as optical density (OD) at 590 nm with a plate reader at regular 12 hr intervals. Microbial activity in each microplate, expressed as average well-color development (AWCD), was determined as Eq. (2):

$$\mathrm{AWCD} = \sum \mathrm{OD}_i/31 \quad (2)$$

where, OD_i (590 nm) is the optical density value from each well (*i*). The Shannon-Weaver index (*H*) was calculated using an OD of 0.25 as threshold for a positive response (Garland, 1996). The *H* was calculated by $H = -\sum p_i \ln p_i$, and Simpson's index (λ) of diversity (Simpson, 1949) was calculated by $\lambda = \sum {p_i}^2$, where p_i is the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum \mathrm{OD}_i$).

1.8 Statistical analysis

Statistical analysis was carried out using the SPSS 14.0 for Windows software package. Data were analyzed by two-way analysis of variance. Mean values were compared by least significant difference (LSD) at the 5% level using SPSS software.

2 Results and discussion

2.1 Soil PAH removal

Biodegradation has been proposed as a strategy to enhance the bioremediation of contaminated soil (Hamdi et al.,

2007; Teng et al., 2011b). In the present study all treatments showed a significant reduction in PAH concentration following 16 weeks of incubation ($p < 0.05$) (Fig. 1). However, compared to the MCD amendment and *Paracoccus* sp. strain HPD-2 inoculation combined treatments (MB1, MB2, MB3, and MB4), the PAH removal rate in the bioaugmentation treatment (CKB) was significantly lower ($p < 0.05$). The present data strongly suggest that microbial factors such as the presence of an adequate consortium of degraders are not the only explanation for the limited extent of biodegradation observed after 16 weeks. It is possible that other factors, such as limited nutrient availability (Teng et al., 2010a) and especially low contaminant bioaccessibility (Allan et al., 2007) may have influenced the degree of degradation.

It is generally accepted that a low level of bioavailability is one of the most important factors involved in the slow biodegradation of hydrophobic organic compounds in soils, as PAHs are very poorly soluble in the aqueous phase where microorganisms are active. It has already been demonstrated that MCD accelerates the degradation kinetics of PAH and PAH bioaccessibility in both the aqueous phase and the solid phase (Bardi et al., 2007). In treatments where MCD was added the extent of PAH degradation was greatly enhanced (Fig. 1) with residual concentrations significantly lower ($p < 0.05$) than in the control. Soil target PAH concentrations after amendment with MCD and inoculation with HPD-2 after 16 weeks are presented in Fig. 1. For each PAH group there were significant decreases ($p < 0.05$) in the PAH concentrations for treatments amended with MCD when compared with the initial concentrations. The total PAH concentration in soil with 10% MCD-assisted bioaugmentation (MB2) declined from (7679 ± 230) to (5293 ± 203) μg/kg after 16 weeks. Table 1 shows that MCD amendment, *Paracoccus* sp. strain HPD-2 inoculation and their combination treatments had significant effects on PAH removal (F_M = 1222.0, F_B = 802.8, $p < 0.01$). Moreover, the results also showed significant interaction between MCD amendment and *Paracoccus* sp. strain HPD-2 inoculation ($F_{M\times B}$ = 3.245, $p < 0.05$). This suggests that MCD contributes to contaminant desorption by increasing both the mass transfer from the soil particles to the aqueous phase and the solubility of the organic pollutant (Wang et al., 1998; Mayer et al., 2005), and microbial degradation likely acts as a sink for contaminant released into the aqueous/MCD phase by both mass transfer from soil particles to the aqueous phase and transfer from water to microorganisms, or by influencing the hydrophobicity of the surfaces of the microorganisms as shown for some biosurfactants.

Considering the influence that MCD had on specific PAHs, Fig. 2 shows the percentage of compound removal

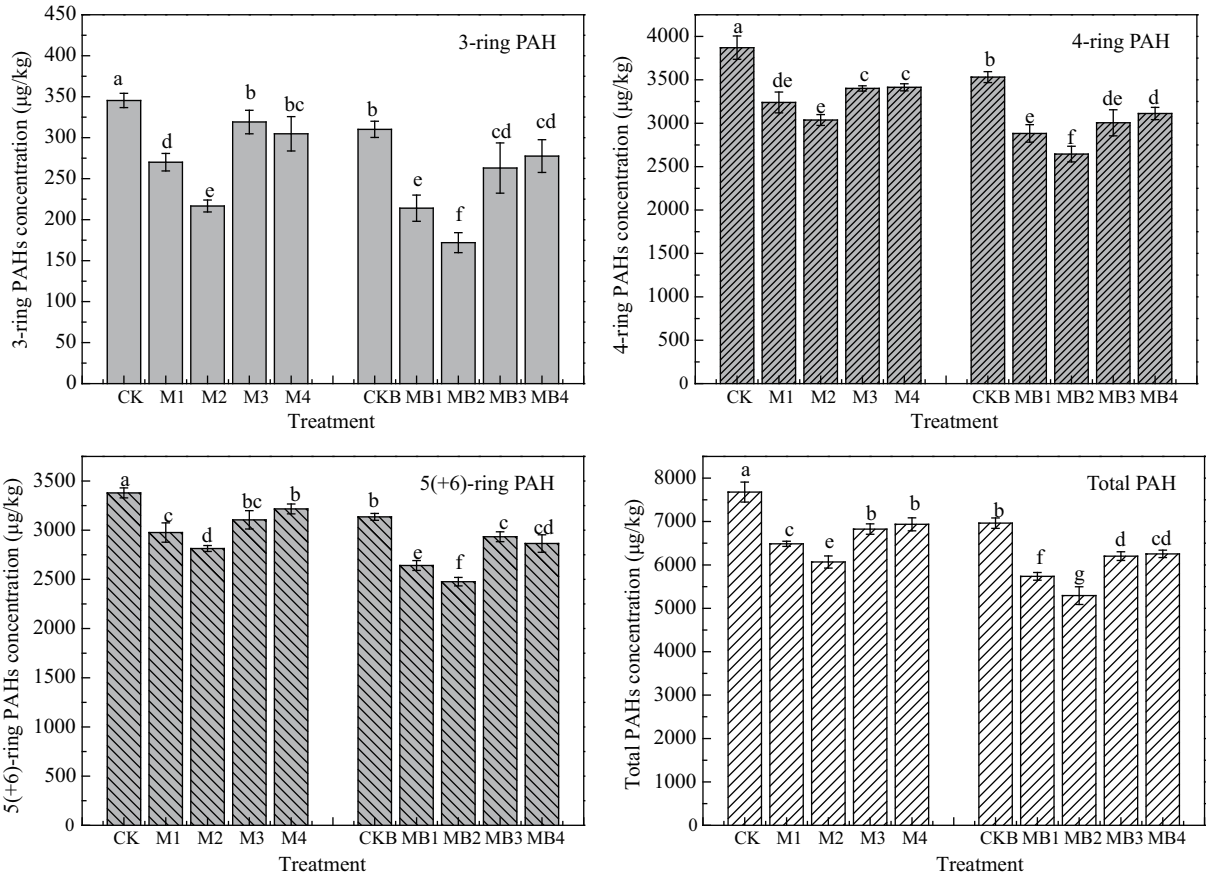

Fig. 1 Removal of 3-, 4-, 5-ring and total PAHs in soil before and after 16 weeks of biodegradation. PAH concentration values are means ± standard deviations of triplicate determinations. CK: control without MCD amendment or *Paracoccus* sp. strain HPD-2 inoculation; M1, M2, M3, M4: 5%, 10%, 15%, 20% (*W*/*W*) MCD amendment; CKB: 4% (*V*/*W*) *Paracoccus* sp. strain HPD-2 inoculation; MB1, MB2, MB3, MB4: 5%, 10%, 15%, 20% (*W*/*W*) MCD amendment + 4% (*V*/*W*) *Paracoccus* sp. strain HPD-2 inoculation. Mean values with the same letter are not significantly different among treatments by LSD at the 5% level.

Table 1 Effect of inoculation with *Paracoccus* sp. strain HPD-2 (B) and MCD amendment (M) on the total PAHs removal in the soils after 16 weeks of treatments

Inoculation (V/W)	MCD amendment (W/W)	Removal rate of total PAHs
0	0	5.38 ± 1.55 a
0	5%	20.09 ± 1.48 c
0	10%	25.24 ± 1.32 e
0	15%	15.89 ± 1.87 b
0	20%	14.54 ± 1.13 b
4%	0	14.05 ± 1.65 b
4%	5%	29.30 ± 1.37 f
4%	10%	34.78 ± 1.11 g
4%	15%	23.58 ± 1.46 d
4%	20%	22.93 ± 1.62 cd
	F_B	802.8**
Two-way ANOVA	F_M	1222.0**
	$F_{M\times B}$	3.245*

Values are means ± standard deviation of triplicate measurements. Mean values with the same letter are not significantly different among treatments by LSD at the 5% level.
** Significance at $p < 0.01$, * significance at $p < 0.05$.

following treatment with 10% MCD amendment and inoculation with HPD-2 (MB2) with respect to the control. MCD amendment enhanced the degradation rates of individual compounds, in the range of 15%–85% enhancement. No significant trends with logK_{ow} were observed for degradation enhancement by MCD in soil. However, MCD promoted a maximum biodegradation enhancement for 3-ring PAHs.

2.2 Soil PAH-degrading bacteria and microbial activities

In general, bioremediation of PAH-contaminated soils depends on the presence of degrading microorganisms with the desired catabolic capabilities (Margesin et al., 2000). Bioaugmentation has demonstrated an important role in bioremediation by promoting degrading microorganisms in soil contaminated with such compounds. In the present study a significant increase in counts of PAH-degrading bacteria was observed in bioaugmented microcosms after 16 weeks of incubation compared with

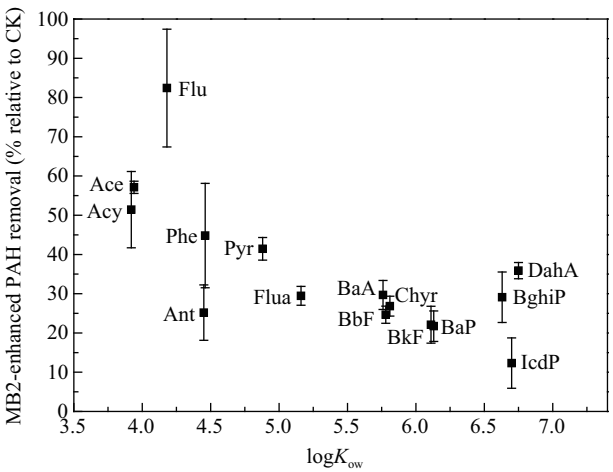

Fig. 2 The 10% MCD and *Paracoccus* sp. strain HPD-2 inoculation enhanced removal of PAHs in soil (relative percentage to the controls without MCD and inoculation) following biodegradation assays. Compounds have been ordered by K_{ow} value.

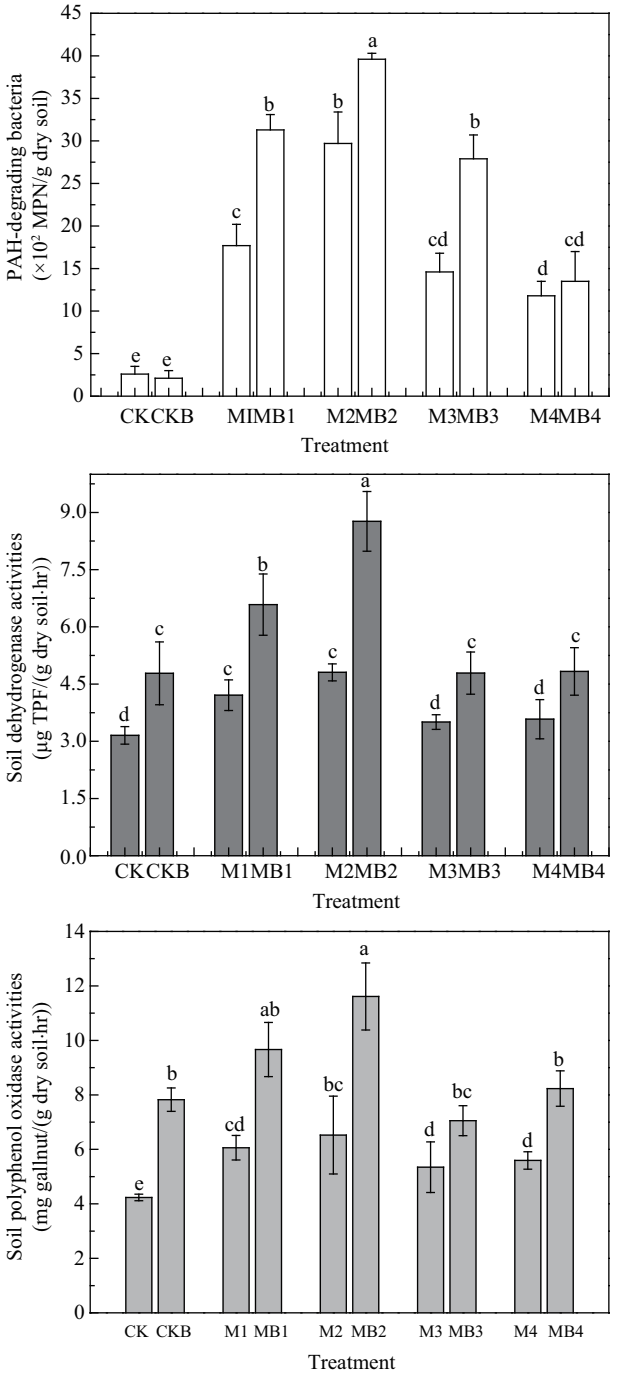

Fig. 3 Soil PAH-degrading bacteria, soil dehydrogenase activities and soil polyphenol oxidase activities under different treatments after 16 weeks of incubation.

the controls (Fig. 3). These results are similar to the results of Teng et al. (2010b) which showed higher proportions of PAH-degrading bacteria in bioaugmented soil treatments. Furthermore, Fig. 3 also shows that PAH-degrading bacteria counts in the MCD-assisted bioaugmented treatments were significantly higher ($p < 0.05$) than in non-MCD-assisted bioaugmented treatment, and this may have resulted in substantial degradation of 3-, 4- and 5(+6)-ring PAHs in the soil studied.

Soil enzyme activity is one way of describing the general condition of soil microbial populations (Margesin et al., 2000). Soil dehydrogenase activity (DHA), an intra-

cellular process that typically occurs in all intact and viable microbial cells, can be used to determine the presence of viable microorganisms and their oxidative capability (Andreoni et al., 2004). Soil polyphenol oxidase (PPO) is an oxygen-transferring enzyme and can act on specific recalcitrant pollutants by precipitation or transformation to other products, permitting a better final treatment of the waste (Durán and Esposito, 2000). Figure 3 shows that soil DHA and PPO activities increased significantly in the MCD-assisted bioaugmented microcosms. PAHs or their metabolites were likely used as substrates, thus increasing the activity of the enzymes. Changes in enzyme activities in the MCD-assisted bioaugmented microcosms also confirm that MCD-assisted bioaugmentation with *Paracoccus* sp. strain HPD-2 showed the highest microbiological activity.

2.3 Physiological profiles of the soil bacterial community

Soil is the microbial inhabitancy while soil microorganisms can be sensitive indicators, reflecting subtle changes of soil quality. Organic contaminants are generally considered to have adverse effects on soil microbial functional diversity influencing soil fertility and plant growth, which pose a serious threat to the sustainability of agricultural soils. Therefore, direct measurements of soil microbial community diversity are likely to provide more information for evaluating soil function. The BIOLOG Eco method was used to monitor community-level physiological profiling (CLPP) of soil microorganisms as carbon source utilization patterns and estimate soil bacterial taxonomic diversity (Choi and Dobbs, 1999). The AWCD reflects the sole-carbon-source utilization ability of the soil bacterial community and a measure of soil bacteria activity (Garland, 1996). The Shannon-Weaver index is a measure of the actual richness and evenness of the bacterial population, while the Simpson index is often used to quantify the number of species present, as well as the relative abundance of each species (Garland, 1996; Simpson, 1949). In the present study the community level physiological profiles indicate differences in soil microbial functional diversity among different treatments after 16 weeks of remediation (Fig. 4 and Table 2). The 10% MCD treatment showed a higher average well-color development, Shannon-Weaver index and Simpson index. This confirms the previous study of Teng et al. (2010b) which showed marked enhancement of microbial community diversity after 28 days of bioremediation with *Paracoccus* sp. strain HPD-2 inoculation and nutrient addition. It can be concluded that, as a result of bioremediation with *Paracoccus* sp. strain HPD-2 inoculation combined with MCD addition, the microbiological functioning of the PAH-contaminated soil has been at least partially restored.

Table 2 Sole-carbon-source utilization activity and functional diversity of the soil microbial community after 16 weeks of bioremediation

Treatment	AWCD	Shannon-Weaver index	Simpson index
CK	0.262 ± 0.045 g	2.747 ± 0.434 de	16.67 ± 0.01 f
M1	0.723 ± 0.084 f	2.932 ± 0.020 e	15.68 ± 0.47 g
M2	1.886 ± 0.029 b	3.319 ± 0.027 bc	26.55 ± 0.76 b
M3	1.631 ± 0.135 cd	3.271 ± 0.022 c	24.96 ± 0.80 c
M4	1.283 ± 0.037 e	3.123 ± 0.026 d	21.01 ± 0.47 d
CKB	0.599 ± 0.169 f	2.749 ± 0.184 e	12.78 ± 2.46 h
MB1	1.227 ± 0.039 e	3.079 ± 0.032 d	19.98 ± 0.79 e
MB2	2.012 ± 0.100 a	3.996 ± 0.010 a	28.51 ± 0.53 a
MB3	1.758 ± 0.063 c	3.310 ± 0.012 b	26.14 ± 0.39 bc
MB4	1.314 ± 0.197 d	3.121 ± 0.117 d	22.02 ± 1.25 d

Values are means ± standard deviation of triplicate measurements. Mean values with the same letter are not significantly different among treatments by LSD at the 5% level.

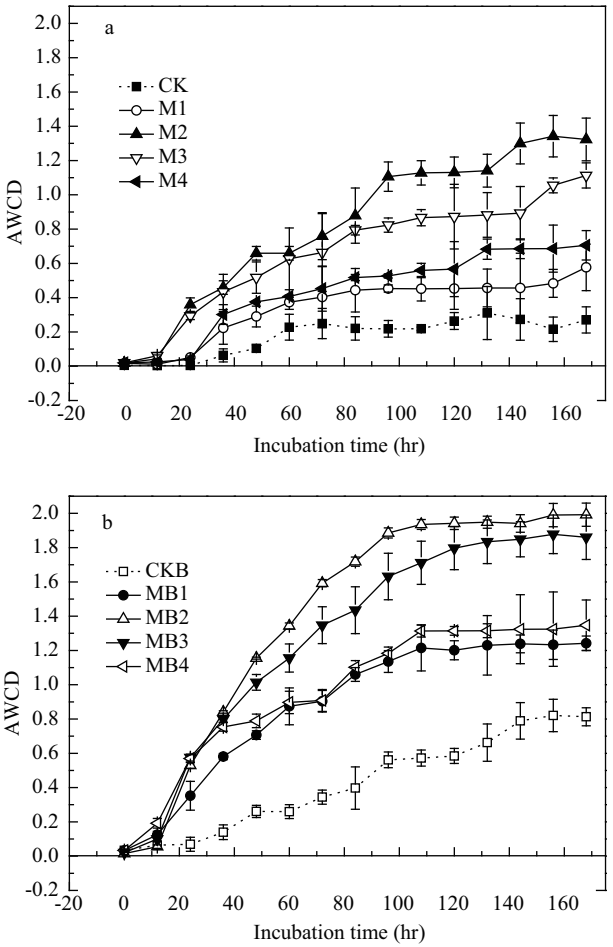

Fig. 4 Variation in average well color development (AWCD) for soil samples from the different treatments after 16 weeks of biodegradation. (a) treatments with only MCD amendment; (b) treatments with MCD amendment and *Paracoccus* sp. strain HPD-2 inoculation. Values are means ± standard deviations of triplicate measurements.

3 Conclusions

Bioremediation with *Paracoccus* sp. strain HPD-2 inoculation together with 10% MCD addition used in this microcosm study enhanced PAH dissipation in the soil, and increased PAH-degrading bacterial counts and microbial activities in soils, suggesting that this strain of *Paracoccus* and MCD can work together to increase soil microbiological activity, with some restoration of the microbiological functioning of the PAH-contaminated soil. Therefore, MCD-assisted bioaugmentation by *Paracoccus* sp. strain HPD-2 is a promising practical bioremediation strategy for aged PAH-contaminated soil. Elucidation of the potential

applicability of this strategy will require further studies involving different soil types and contaminants under field conditions.

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References

Allan I J, Semple K T, Hare R, Reid B J, 2006. Prediction of mono and polycyclic aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction. *Environmental Pollution*, 144(2): 562–571.

Allan I J, Semple K T, Hare R, Reid B J, 2007. Cyclodextrin enhanced biodegradation of polycyclic aromatic hydrocarbons and phenols in contaminated soil slurries. *Environmental Sciences and Technology*, 41(15): 5498–5504.

Andreoni V, Cavalca L, Rao M A, Nocerino G, Bernasconi S, Dell'Amico E et al., 2004. Bacterial communities and enzyme activities of PAHs polluted soils. *Chemosphere*, 57(5): 401–412.

Badr T, Hanna K, de Brauer C, 2004. Enhanced solubilization and removal of naphthalene and phenanthrene by cyclodextrins from two contaminated soils. *Journal of Hazardous Materials*, 112(3): 215–223.

Bardi L, Martini C, Opsi F, Bertolone E, Belviso S, Masoero G et al., 2007. Cyclodextrinenhanced in situ bioremediation of polyaromatic hydrocarbonscontaminated soils and plant uptake. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 57(1-4): 439–444.

CCME, 2004. Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health: Summary Tables. Updated. CCME, Winnipeg.

Choi K H, Dobbs F C, 1999. Comparison of two kinds of biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of Microbiological Methods*, 36(3): 203–213.

Claus H, Filip Z, 1990. Effects of clays and other solids on the activity of phenoloxidases produced by some fungi and actinomycetes. *Soil Biology and Biochemistry*, 22(4): 483–488.

Ding J, Cong J, Zhou J, Gao S X, 2008. Polycyclic aromatic hydrocarbon biodegradation and extracellular enzyme secretion in agitated and stationary cultures of *Phanerochaete chrysosporium*. *Journal of Environmental Sciences*, 20(1): 88–93.

Epelde L, Becerril J M, HernándezAllica J, Barrutia O, Garbisu C, 2008. Functional diversity as indicator of the recovery of soil health derived from *Thlaspi caerulescens* growth and metal phytoextraction. *Applied Soil Ecology*, 39(3): 299–310.

Epelde L, Mijangos I, Becerril J M, Garbisu C, 2009. Soil microbial community as bioindicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. *Soil Biology and Biochemistry*, 41(9): 1788–1794.

Garland J L, 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology and Biochemistry*, 28(2): 213–221.

Gong Z Q, Li P J, Wilke B M, Alef K, 2008. Effects of vegetable oil residue after soil extraction on physical-chemical properties of sandy soil and plant growth. *Journal of Environmental Sciences*, 20(12): 1458–1462.

Hamdi H, Benzarti S, Manusadžianas L, Aoyama I, Jedidi N, 2007. Bioaugmentation and biostimulation effects on PAH dissipation and soil ecotoxicity under controlled conditions. *Soil Biology and Biochemistry*, 39(8): 1926–1935.

Johnsen A R, Karlson U, 2005. PAH degradation capacity of soil microbial communities – does it depend on PAH exposure? *Microbial Ecology*, 50(4): 488–495.

Juhasz A L, Britz M L, Stanley G A, 1997. Degradation of fluoranthene, pyrene, benz[*a*]anthracene and dibenz[*a*, *h*]anthracene by *Burkholderia cepacia*. *Journal of Applied Microbiology*, 83(2): 189–198.

Lu R K, 2000. Analytical Methods of Soil and Agricultural Chemistry. China Agricultural Science and Technology Press, Beijing. 107–240.

Margesin R, Walder G, Schinner F, 2000. The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil. *Acta Biotechnologica*, 20(3-4): 313–333.

Mayer P, Karlson U, Christensen P S, Johnsen A R, Trapp S, 2005. Quantifying the effect of medium composition on the diffusive mass transfer of hydrophobic organic chemicals through unstirred boundary layers. *Environmental Sciences and Technology*, 39(16): 6123–6129.

Durán N, Esposito E, 2000. Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: A review. *Applied Catalysis B: Environmental*, 28(2): 83–99.

Ni J Z, Luo Y M, Wei R, Li X H, 2008. Distribution patterns of polycyclic aromatic hydrocarbons among different organic carbon fractions of polluted agricultural soils. *Geoderma*, 146(1-2): 277–282.

Petitgirard A, Djehiche M, Persello J, Fievet P, FatinRouge N, 2009. PAH contaminated soil remediation by reusing an aqueous solution of cyclodextrins. *Chemosphere*, 75(6): 714–718.

Semple K T, Doick K J, Wick L Y, Harms H, 2007. Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. *Environmental Pollution*, 150(1): 166–176.

Shen G Q, Lu Y T, Zhou Q X, Hong J B, 2005. Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. *Chemosphere*, 61(8): 1175–1182.

Simpson E H, 1949. Measurement of diversity. *Nature*, 163(4148): 688.

Singh J, Singh D K, 2005. Dehydrogenase and phosphomonoesterase activities in groundnut (*Arachis hypogaea* L) field after diazinon, imidacloprid and lindane treatments. *Chemosphere*, 60(1): 32–42.

Sun M M, Fu D Q, Teng Y, Shen Y Y, Luo Y M, Li Z G et al., 2011. In situ phytoremediation of PAH-contaminated soil by intercropping alfalfa (*Medicago sativa* L.) with tall fescue (*Festuca arundinacea* Schreb.) and associated soil microbial activity. *Journal of Soils and Sediments*, 11(6): 980–989.

Stroud J L, Tzima M, Paton G I, Semple K T, 2009. Influence of hydroxypropyl-β-cyclodextrin on the biodegradation of ^{14}C-phenanthrene and ^{14}C-hexadecane in soil. *Environmental Pollution*, 157(10): 2678–2683.

Talley J W, Ghosh U, Tucker S G, Furey J S, Luthy R G, 2002. Particle-scale understanding of the bioavailability of PAHs in sediment. *Environmental Sciences and Technology*, 36(3): 477–483.

Teng Y, Luo Y M, Ping L F, Zou D X, Li Z G, Christie P, 2010a. Effects of soil amendment with different carbon sources and other factors on the bioremediation of an aged PAH-contaminated soil. *Biodegradation*, 21(2): 167–178.

Teng Y, Luo Y M, Sun M M, Liu Z J, Li Z G, Christie P, 2010b. Effect of bioaugmentation by *Paracoccus* sp. strain HPD-2 on the soil microbial community and removal of polycyclic aromatic hydrocarbons from an aged contaminated soil. *Bioresource Technology*, 101(10): 3437–3443.

Wang J M, Marlowe E M, MillerMaier R M, Brussseau M L, 1998. Cyclodextrin-enhanced biodegradation of phenanthrene. *Environmental Sciences and Technology*, 32(13): 1907–1912.

Wilcke W, 2007. Global patterns of polycyclic aromatic hydrocarbons (PAHs) in soil. *Geoderma*, 141(3-4): 157–166.

Wrenn B A, Venosa A D, 1996. Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure. *Canadian Journal of Microbiology*, 42(3): 252–258.

Yang Y, Hunter W, Tao S, Gan J, 2009. Microbial availability of different forms of phenanthrene in soils. *Environmental Sciences and Technology*, 43(6): 1852–1857.

Yang Y, Zhang N, Xue M, Lu S T, Tao S, 2011. Effects of soil organic matter on the development of the microbial polycyclic aromatic hydrocarbons (PAHs) degradation potentials. *Envrionmental Pollution*, 159(2): 591–595.

Yao H Y, Xu J M, Huang C Y, 2003. Substrate utilization pattern, biomass and activity of microbial communities in a sequence of heavy metal-polluted paddy soils. *Geoderma*, 115(1-2): 139–148.

Zhou W J, Zhu L Z, 2007. Efficiency of surfactant-enhanced desorption for contaminated soils depending on the component characteristics of soil-surfactant-PAHs system. *Environmental Pollution*, 147(1): 66–73.

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