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Effect of surfactants on fluoranthene degradation by *Pseudomonas alcaligenes* PA-10

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Abstract Two surfactants, Tween 80 and JBR, were investigated for their effect on fluoranthene degradation by a Pseudomonad. Both surfactants enhanced fluoranthene degradation by *Pseudomonas alcaligenes* PA-10 in shake flask culture. This bacterium was capable of utilising the synthetic surfactant and the biosurfactant as growth substrates and the critical micelle concentration of neither compound inhibited bacterial growth. The biosurfactant JBR significantly increased polycyclic aromatic hydrocarbon (PAH) desorption from soil. Inoculation of fluoranthene-contaminated soil microcosms with *P. alcaligenes* PA-10 resulted in the removal of significant amounts (45± 5%) of the PAH after 28 days compared to an uninoculated control. Addition of the biosurfactant increased the initial rate of fluoranthene degradation in the inoculated microcosm. The presence of a lower molecular weight PAH, phenanthrene, had a similar effect on the rate of fluoranthene removal.

Keywords PAHs · Fluoranthene · Biodegradation · Surfactants

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are listed as priority pollutants by the US EPA (Keith and Telliard 1979). The hydrophobic nature of these compounds has resulted in their persistence and bioaccumulation in soil and sediment environments (Kanaly and Harayama 2000; Meador et al. 1995). A range of bacteria, fungi and algae capable of PAH degradation have been isolated and have been the subject of a number of reviews (Cerniglia 1997; Juhasz and Naidu 2000; Smith 1990; Smith et al. 1997; Wilson and Jones 1993). Failure of soil-bound PAHs to partition back into the aqueous phase can severely limit microbial degradation, and it is generally accepted that low bioavailability is one of the major factors affecting the persistence of these compounds in soil (Banat et al. 2000; Mueller et al. 1996).

Application of surfactants has been suggested as a possible way to increase bioavailability, and thus, biodegradation of these compounds. Although surfactants have been reported to facilitate biodegradation in some instances (Herman et al. 1997; Sobisch et al. 2000), they have been shown to retard or have no effect on contaminant removal in other cases (Deschênes et al. 1996; Laha and Luthy 1991, 1992). The failure of surfactant addition to promote biodegradation has been attributed to surfactant use as a preferential growth substrate by degrading microorganisms (Tiehm 1994; Tiehm et al. 1997), surfactant toxicity (Aronstein et al. 1991; Willumsen et al. 1998), substrate toxicity due to an increase in bioavailability brought about by surfactant solubilisation (Bramwell and Laha 2000) and a reduction in contaminant bioavailability due to its uptake into the surfactant micelle (Doong and Lei 2003; Laha and Luthy 1991). There is growing interest in the use of biosurfactants for environmental applications, as synthetic

surfactants are generally considered to be more toxic, less biodegradable and require higher concentrations than biosurfactants (Banat et al. 2000). However, further investigations are required to assess the potential of these compounds in bioremediation.

This study is concerned with investigating the effect of Tween 80, a synthetic surfactant, and JBR, a rhamnolipid biosurfactant, on fluoranthene degradation by *Pseudomonas alcaligenes* PA-10. This bacterium was recently isolated from a PAH-containing waste treatment bioreactor and found to co-metabolise fluoranthene in shake flask culture in the presence of yeast extract (Gordon and Dobson 2001). However, the performance of this isolate in soil has not been investigated.

Materials and methods

Soil, bacteria and reagents A soil which had no previous history of PAH contamination composed of 45.2% sand, 49.0% silt and 5.8% clay with an organic matter content of 5.6% was used. *P. alcaligenes* PA-10 was isolated from a PAH-containing waste treatment bioreactor (Gordon and Dobson 2001). JBR biosurfactant was provided by Jeneil Biosurfactant Company (Wisconsin, USA). Luria Bertani (LB) broth was purchased from Merck Biosciences (Nottingham, UK), tryptone from Difco (Unitech, Dublin, Ireland) and all other preprepared media from Oxoid (Fannin Healthcare, Dublin, Ireland). All gases used were obtained from BOC gases (Dublin, Ireland). All other reagents were purchased from Sigma-Aldrich (Dublin, Ireland). All reagents were analytical grade, except solvents, which were HPLC grade. The minimal salts growth medium (MSM) used was based on that of Lageveen et al. (1988). After autoclaving, $MgSO_4 \cdot 7H_2O$ (1.0 mM), autoclaved separately, and $FeSO_4 \cdot 7H_2O$ (0.04 mM), from a filter sterilised stock solution, were added. The initial pH was 7.2.

Bacterial growth P. alcaligenes PA-10 was grown in LB broth containing fluoranthene (5 mg l^{-1}) at 30°C and 200 rpm for 22 h. Of this culture, 0.5 ml was used to inoculate MSM amended with PAH, surfactants or additional carbon source. Cultures were incubated at 30°C and 200 rpm. Cell growth was determined by optical density (OD) at 650 nm using a Helios δ Spectrophotometer (Unicam, Leeds, UK) and by plate counts on nutrient agar. Surfactants did not interfere with OD readings at the concentrations used.

Effect of surfactants The effect of the surfactants on PAH degradation by *P. alcaligenes* PA-10 was determined by growing the bacterium in MSM containing yeast extract (0.5 g l^{-1}), tryptone (0.5 g l^{-1}) and PAHs in the presence/absence of two surfactants, Tween 80 (0.5 g l^{-1}) and JBR (0.5 g l^{-1}). The effect of surfactants on bacterial cell surface hydrophobicity was measured using the method of Rosenberg et al. (1980). The method used to measure the effect of surfactants on bacterial adhesion to PAHs was that of Stelmack et al. (1999).

Analysis of PAHs PAHs were analysed using a gas chromatograph equipped with a flame ionisation detector. Samples were injected onto a HP-5 MS column (Agilent, Dublin, Ireland). The mobile phase was helium (90 kPa). The temperature gradient used in analysis was: 50°C for 1 min, increased to 300°C at 8°C/min and lowered from 300 to 50°C at 40°C/min. The injection temperature was 270°C and the detector temperature was 300°C. The injection volume was 3 µl. PAH concentration was calculated based on peak area and comparison with authentic standard solutions.

Effect of surfactants on PAH desorption from soil The method used was a modification of Van Dyke et al. (1993). Soil was air-dried, passed through a 2-mm sieve and dried at 105°C for 24 h. One gram of soil was added to centrifuge tubes, selected PAHs (1 g kg^{-1}) were added, and the moisture content increased to 40%. Soil was incubated in an incubator shaker at room temperature for 1 h in the dark. Five milliliters of surfactant and 0.1 ml of NaOH (1.0 M) were then added to each tube. Tubes were shaken for 2 h at room temperature and 250 rpm in the dark before centrifugation at 3,000×*g* for 20 min to sediment out the soil. The PAH concentration in the supernatant was then determined by GC/FID. Controls containing no surfactants were also analysed.

Effect of surfactants on PAH degradation in soil Soil was air-dried for 2 days and sieved through a 2-mm sieve. Twenty grams of soil were added to 250-ml Erlenmeyer flasks, and the moisture content of the soil was adjusted to 30% by adding water, a bacterial inoculum (5.1×10^7 cfu g^{-1}), PAH (200 mg/kg) and either Tween 80 or JBR to a final concentration of 0.5 g kg^{-1}. Control flasks contained water instead of surfactants. Flasks were incubated for 28 days in the dark at room temperature. PAHs were extracted from a 10 g (dry weight) sample and analysed by GC/FID.

PAH extraction from soil PAHs were extracted from soil using a Soxtec Avanti 2055 Soxhlet extraction apparatus (Foss, Dublin, Ireland). The method used was based on US EPA Method 3541 (US EPA 1994). Ten grams (dry weight) of soil were removed from a flask, transferred to a cellulose thimble, and the moisture content adjusted to 30%. Eleven grams of anhydrous sodium sulphate were added to the

Table 1 Effect of surfactants on fluoranthene degradation by *P. alcaligenes* PA-10 in shake flask culture

Surfactant	Concentration (g l^{-1})	Fluoranthene degradation (%)
None	0	62.35±0.76[a]
Tween 80	0.5	79.63±2.53[b]
JBR	0.5	96.61±0.81[c]

P. alcaligenes PA-10 (initial cell density 5×10^7 cfu ml^{-1}) was grown in MSM containing yeast extract (0.5 g l^{-1}) and tryptone (0.5 g l^{-1}) and fluoranthene (5 mg l^{-1}) at 30°C and 200 rpm for 48 h. Values within a column with different superscripts are statistically different (Duncan, $n=3$, $p<0.05$).

cellulose thimble, which was inserted into the extraction apparatus. One hundred milliliters of HPLC grade acetone to hexane (1:1) were used as the extraction solvent. The thimbles were immersed in the solvent in the boiling position for 1 h, raised to the rinsing position for 2 h and finally placed in the recovery position, to evaporate the solvent, to a volume of 5 ml.

Soil toxicity testing Soil samples were withdrawn from the microcosm over time and stored at −18°C before analysis for toxicity against two test organisms, *Drosophila melanogaster* and *Bacillus megaterium* IMD 147. Soil toxicity towards *D. melanogaster* was measured using a method based on that developed by Grant (2001). Toxicity test kits (Bioassay Toxicity Testing, York, UK) containing five organisms each were used. PAHs and any metabolites were extracted from soil samples (10 g) and redissolved in ethanol, to a volume of 2 ml. Of each sample, 0.5 ml was then added to 9.5 ml tap water, and 1 ml of this preparation was added to a bioassay test kit, in duplicate, and the time taken for all five flies in the bioassay to die was recorded.

A modification of the method of McGrath and Singleton (2000) was used to measure soil toxicity towards *B. megaterium* IMD 147. PAHs and metabolites were extracted from soil samples (10 g) and redissolved in methanol to a volume of 10 ml. *B. megaterium* IMD 147 was inoculated into 50 ml of tryptone soy broth (TSB) and was grown for 18 h at 37°C and 160 rpm in the dark. The OD_{650nm} of this culture was adjusted to 0.3 with sterile Ringers solution; 1 ml of this culture and soil extract (1 ml) were added to TSB (10%). Cultures were incubated at 37°C and 160 rpm in the dark, and biomass levels were recorded over time. Control flasks contained 1 ml of methanol to allow for any growth inhibition by the solvent.

Results

PAH degradation P. alcaligenes PA-10 degraded 93±4% of phenanthrene (10 mg l^{-1}) and 68±5% of the four-ringed PAH, fluoranthene, after 120 h in shake flask culture. Although the bacterium could be induced (by inclusion of the appropriate PAH in the inoculum medium) to utilise the PAHs as sole carbon source, degradation was more extensive and cell densities were higher in the presence of yeast extract and tryptone. *P. alcaligenes* PA-10 was capable of degrading >50% of 40 mg l^{-1} fluoranthene but optimum

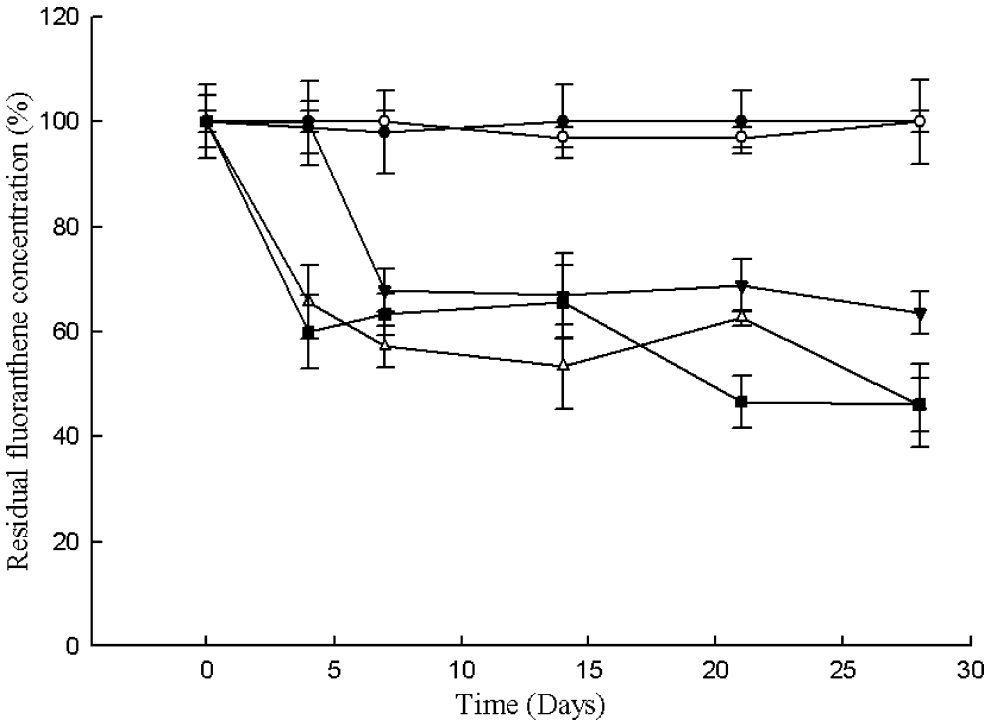

Fig. 1 Effect of *P. alcaligenes* PA-10, a biosurfactant (JBR) and phenanthrene on fluoranthene degradation in soil. Soil was spiked with 200 mg kg^{-1} of fluoranthene. *P. alcaligenes* PA-10 was added to the appropriate soil microcosms at a concentration of 5×10^7 cfu g^{-1}. Soil was then incubated at room temperature (*filled circles*) unamended (control), (*empty circles*) uninoculated and amended with JBR (0.5 g kg^{-1}), (*inverted filled triangles*) inoculated with *P. alcaligenes* PA-10, (*empty triangles*) amended with *P. alcaligenes* PA-10 and JBR (0.5 g kg^{-1}) and (*filled squares*) amended with *P. alcaligenes* PA-10 and phenanthrene (200 mg kg^{-1}). Error bars are the SED of $n=3$

degradation was observed with the lower concentration of 5 mg l^{-1}. Growth of the bacterium was significantly reduced by a concentration of 50–60 mg l^{-1} of either PAH.

Effects of surfactants on degradation and growth *P. alcaligenes* PA-10 was capable of using Tween 80, a chemical surfactant, and JBR, a rhamnolipid biosurfactant as sole carbon sources. Maximum cell densities of 1.43×10^7 cfu ml^{-1} were produced when the bacterium was grown on JBR (1.0 g l^{-1}) and similar levels (1.3×10^7 cfu ml^{-1}) were observed when Tween 80 was supplied as carbon source. The critical micelle concentration (0.5 g l^{-1}) of the two surfactants did not inhibit bacterial growth; however, the biosurfactant was toxic at a concentration tenfold higher than the cmc, reducing the cell density to 5% of that observed in the absence of surfactants (data not shown). Addition of Tween 80 significantly ($p<0.05$) increased fluoranthene degradation in shake flask culture by *P. alcaligenes* PA-10 from 62.5 to 79.6%, and the presence of the biosurfactant (JBR) resulted in almost complete removal of the four-ringed PAH by this organism (Table 1). *P. alcaligenes* PA-10 did not produce a surfactant under any conditions examined (data not shown).

Effect of surfactants on cell surface hydrophobicity and adhesion to PAHs Although neither Tween 80 nor JBR affected the cell surface hydrophobicity of *P. alcaligenes* PA-10, they did effect contact of the bacterium with solid PAHs (data not shown). In the absence of surfactant, 25% of *P. alcaligenes* PA-10 cells adhered to fluoranthene; addition of Tween 80 and JBR reduced adhesion to 8.5 and 4%, respectively. The surfactants had a similar effect on bacterial adhesion to the walls of test tubes.

Effect of surfactants on fluoranthene degradation in soil In the absence of surfactants, less than 1% of fluoranthene was transferred to an aqueous solution from soil. Although addition of Tween 80 did not increase the solubilisation of fluoranthene, the biosurfactant significantly ($p<0.05$) increased (11-fold) the amount of fluoranthene solubilised. A concentration of 200 mg kg^{-1} of fluoranthene was not removed from a soil which had no previous history of PAH contamination after 28 days (Fig. 1), nor was the PAH removed from soil when JBR (which had been shown to increase fluoranthene solubilisation) was added. Inoculation of the soil with *P. alcaligenes* PA-10 (5×10^7 cfu g^{-1}) resulted in 45±5% removal of fluoranthene after the same period, with most degradation occurring between days 4 and 7. Prolonged incubation of the inoculated soil did not result in any further degradation of the PAH (data not shown). Addition of JBR to the inoculated soil also significantly ($p<0.05$) reduced fluoranthene levels compared to the uninoculated soil. Furthermore, the surfactant increased the initial rate of degradation, on day 4 significantly ($p<0.05$); lower levels of the PAH remained in inoculated soil amended with JBR compared to those detected in the soil containing the *P. alcaligenes* PA-10 inoculum only. Interestingly, addition of phenanthrene (200 mg kg^{-1}) to the inoculated, fluoranthene-contaminated soil had a similar

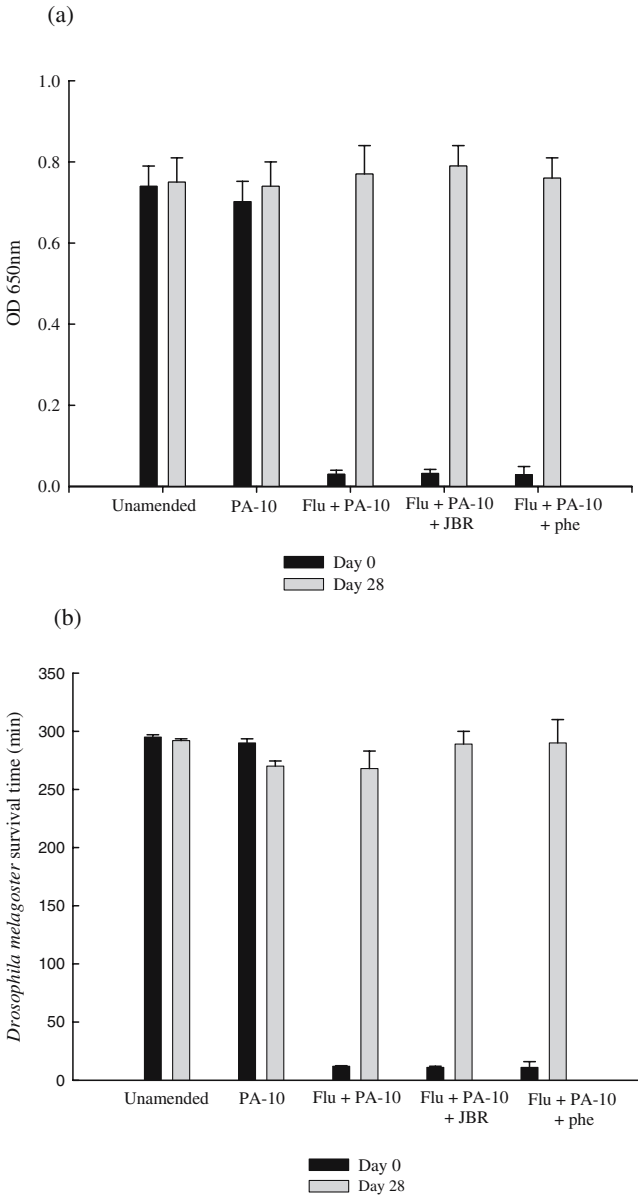

Fig. 2 Effect of amendment on toxicity of soil. **a** Effect of soil extracts on the growth of *Bacillus megaterium* IMD 147 in nutrient broth. Soil extracts from the various treatments (days 0 and 28) were added to nutrient broth inoculated with *B. megaterium* IMD 147. $OD_{650\ nm}$ was measured following incubation at 30°C for 8 h. **b** Toxicity of soil extracts towards *Drosophila melanogaster*. Five *Drosophila melanogaster* were exposed to soil extracts from days 0 and 28 of the various treatments, and the time taken for a lethal effect on all five *Drosophila melanogaster* flies in the test kit was recorded. *PA-10* Soil amended with *P. alcaligenes* PA-10 (5.1×10^7 cfu g^{-1}), *Flu* fluoranthene amended soil, *phe* phenanthrene. *Error bars* are the SED of $n=3$

effect on the initial rate of degradation (Fig. 1). Fifty percent of the added phenanthrene was removed from the soil by day 4, and only low levels (<10%) remained at the end of the incubation period (day 28) (results not shown).

Treated and untreated soils were extracted on day 0 and at the end of the incubation period (day 28), and extracts were analysed for toxicity towards two test organisms, *B. megaterium* IMD 147 and *D. melanogaster*. Analysis of soil from day 0 indicated that the uncontaminated soil and soil inoculated with *P. alcaligenes* PA-10 were not toxic to either *B. megaterium* IMD 147 (Fig. 2a) or *D. melanogaster* (Fig. 2b). However, soil extracts from any of the microcosms containing fluoranthene were toxic to both organisms. After 28 days incubation, the toxicity of the extracts from the contaminated soil inoculated with PA-10 alone or in the presence of JBR or phenanthrene was reduced to levels similar to those of the uncontaminated soil (Fig. 2).

Discussion

P. alcaligenes PA-10 has been reported to utilise naphthalene and phenanthrene as sole carbon and energy sources and to co-metabolise fluoranthene in liquid culture (Gordon and Dobson 2001). This study demonstrates that the bacterium can also utilise the four-ringed PAH as sole carbon source. The stimulation of fluoranthene degradation by *P. alcaligenes* PA-10 in shake flask culture by Tween 80 and the rhamnolipid JBR was probably due to increased solubilisation of the PAH by the surfactants and/or an increase in biomass levels in the presence of the surfactants, both of which were used as carbon sources by the bacterium.

Fluoranthene degradation by *P. alcaligenes* PA-10 in an artificially contaminated soil appeared to occur mainly between days 4 and 7. The absence of effective degradation beyond this point may have been due to poor survival of the organism in the soil environment (Briglia et al. 1990); however, similar levels of fluoranthene degradation were observed when *P. alcaligenes* PA-10 was added to a sterile artificially contaminated soil, even though cell numbers of the organism did not decrease over a 28-day period in this environment (data not shown). Although addition of the biosurfactant JBR did not increase the extent of fluoranthene degradation in soil by *P. alcaligenes* PA-10, it did significantly increase the initial rate of degradation. Addition of phenanthrene to the microcosms had a similar effect on the rate of degradation, indicating that the surfactant may have acted as a synergistic carbon source. However, a combined effect of increased availability and provision of a carbon source cannot be ruled out. Low molecular weight PAHs have been reported to stimulate degradation of high molecular weight PAHs. Chen and Aitken (2001), Juhasz and Naidu (2000) and Tiehm et al. (1997) reported that two surfactants, Arkopal N-300 and Sapogenat T-300, used as a carbon source by soil bacteria, succeeded in enhancing PAH biodegradation in soil columns containing manufactured gas-contaminated soil.

One of the problems associated with the degradation of xenobiotics in soil is that although degradation may be demonstrated, metabolites may remain which can be as toxic or more toxic than the parent PAH. The toxicity of fluoranthene-contaminated soil treated with *P. alcaligenes* PA-10 and either JBR or phenanthrene towards two test organisms, a eukaryote, *D. melanogaster* (Grant 2001), and a prokaryote, *B. megaterium* IMD 147 (McGrath and Singleton 2000), was reduced to levels similar to those of uncontaminated soil.

In conclusion, *P. alcaligenes* PA-10 is capable of removing significant levels of fluoranthene from both liquid culture and soil and the initial rate of degradation in soil is increased in the presence of JBR or phenanthrene, both of which are also metabolised by the organism. Therefore, although the use of surfactants as preferential growth substrates can inhibit degradation in soil, utilisation of these compounds by degrading microorganisms can also stimulate the rate of degradation.

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References

Aronstein BN, Calvillo YM, Alexander M (1991) Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. Environ Sci Technol 25:1728–1731

Banat IM, Makkar RS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53:495–508

Bramwell DA, Laha S (2000) Effects of surfactant addition on the biomineralization and microbial toxicity of phenanthrene. Biodegradation 11:263–277

Briglia M, Nurmiaho-Lassila E-L, Vallini G, Salkinoja-Salonen M (1990) The survival of the pentachlorophenol-degrading *Rhodococcus chlorophenolicus* PCP-1 and *Flavobacterium* sp. in natural soil. Biodegradation 1:273–281

Cerniglia CE (1997) Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J Ind Microbiol Biotechnol 19:324–333

Chen SH, Aitken MD (2001) Salicylate stimulates the degradation of high molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* P15. Environ Sci Technol 33:435–439

Deschênes L, Lafrance P, Villeneuve JP, Samson R (1996) Adding sodium dodecyl sulfate and *Pseudomonas aeruginosa* UG2 biosurfactants inhibits polycyclic aromatic hydrocarbon biodegradation in a weathered creosote-contaminated soil. Appl Microbiol Biotechnol 46:638–646

Doong RA, Lei WG (2003) Solubilization and mineralization of polycyclic aromatic hydrocarbons by *Pseudomonas putida* in the presence of surfactant. J Hazard Mater B96:15–27

Gordon L, Dobson ADW (2001) Fluoranthene degradation in *Pseudomonas alcaligenes* PA-10. Biodegradation 12:393–400

Grant RJ (2001) A bioassay for the measurement of insecticide concentration. Arch Environ Contam Toxicol 41:319–324

Herman DC, Zhang Y, Miller RM (1997) Rhamnolipid (biosurfactant) effects on cell aggregation and biodegradation of residual hexadecane under saturated flow conditions. Appl Environ Microbiol 63:3622–3627

Juhasz AL, Naidu R (2000) Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int Biodeterior Biodegrad 45:57–88

Kanaly RA, Harayama S (2000) Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J Bacteriol 182:2059–2067

Keith LH, Telliard WA (1979) Priority pollutants. I—A perspective view. Environ Sci Technol 13:416–423

Lageveen RG, Huisman HP, Ketelaar P, Eggink G, Witholt B (1988) Formation of polyesters by *Pseudomonas oleovorans*: effect of substrates on formation and composition of poly-(R)-3-hydroxyalkenoates. Appl Environ Microbiol 54:2924–2932

Laha S, Luthy RG (1991) Inhibition of phenanthrene mineralization by non-ionic surfactants in soil–water systems. Environ Sci Technol 25:1920–1930

Laha S, Luthy RG (1992) Effects of non-ionic surfactants on the solubilization and mineralization of phenanthrene in soil–water systems. Biotechnol Bioeng 40:1367–1380

McGrath R, Singleton I (2000) Pentachlorophenol transformation in soil: a toxicological assessment. Soil Biol Biochem 32:1311–1314

Meador JP, Stein JE, Reichert WL, Aranasi U (1995) Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev Environ Contam Toxicol 143:79–165

Mueller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. In: Crawford RL, Crawford DL (eds) Bioremediation: principles and applications. Cambridge University Press, Cambridge, UK, pp 125–194

Rosenberg M, Gutnick D, Rosenberg E (1980) Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol Lett 9:29–33

Smith MR (1990) The biodegradation of aromatic hydrocarbons by bacteria. Biodegradation 1:191–206

Smith MJ, Lethbridge G, Burns RG (1997) Bioavailability and biodegradation of polycyclic aromatic hydrocarbons in soils. FEMS Microbiol Lett 152:141–147

Sobisch T, Heá H, Niebelschutz H, Schmidt U (2000) Effect of additives on biodegradation of PAH in soils. Colloid Surf A 162:1–14

Stelmack PL, Gray MR, Pickard MA (1999) Bacterial adhesion to soil contaminants in the presence of surfactants. Appl Environ Microbiol 65:163–168

Tiehm A (1994) Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. Appl Environ Microbiol 60:258–263

Tiehm A, Stieber M, Werner P, Frimmel FH (1997) Surfactant-enhanced mobilization and biodegradation of polycyclic aromatic hydrocarbons in manufactured gas plant soil. Environ Sci Technol 31:2570–2576

US EPA (1994) Method 3541. Automated Soxhlet extraction. Revision 0

Van Dyke MI, Couture P, Brauer M, Lee H, Trevors JT (1993) Pseudomonas aeruginosa UG2 rhamnolipid biosurfactants: structural characterization and their use in removing hydrophobic compounds from soil. Can J Microbiol 39:1071–1078

Willumsen PA, Karlson U, Pritchard PH (1998) Response of fluoranthene-degrading bacteria to surfactants. Appl Microbiol Biotechnol 50:475–483

Wilson SC, Jones KC (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ Pollut 81:229–249