Two-Liquid-Phase Slurry Bioreactors To Enhance the Degradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons in Soil

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High-molecular-weight (HMW) polycyclic aromatic hydrocarbons (PAHs) are pollutants that persist in the environment due to their low solubility in water and their sequestration by soil and sediments. The addition of a water-immiscible, nonbiodegradable, and biocompatible liquid, silicone oil, to a soil slurry was studied to promote the desorption of PAHs from soil and to increase their bioavailability. First, the transfer into silicone oil of phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene added to a sterilized soil (sandy soil with 0.65% total volatile solids) was measured for 4 days in three two-liquid-phase (TLP) slurry systems each containing 30% (w/v) soil but different volumes of silicone oil (2.5%, 7.5%, and 15% [v/v]). Except for chrysene, a high percentage of these PAHs was transferred from soil to silicone oil in the TLP slurry system containing 15% silicone oil. Rapid PAH transfer occurred during the first 8 h, probably resulting from the extraction of nonsolubilized and of poorly sorbed PAHs. This was followed by a period in which a slower but constant transfer occurred, suggesting extraction of more tightly bound PAHs. Second, a HMW PAH-degrading consortium was enriched in a TLP slurry system with a microbial population isolated from a creosote-contaminated soil. This consortium was then added to three other TLP slurry systems each containing 30% (w/v) sterilized soil that had been artificially contaminated with pyrene, chrysene, and benzo[*a*]pyrene, but different volumes of silicone oil (10%, 20%, and 30% [v/v]). The resulting TLP slurry bioreactors were much more efficient than the control slurry bioreactor containing the same contaminated soil but no oil phase. In the TLP slurry bioreactor containing 30% silicone oil, the rate of pyrene degradation was 19 mg L^{-1} day^{-1} and no pyrene was detected after 4 days. The degradation rates of chrysene and benzo[*a*]pyrene in the 30% TLP slurry bioreactor were, respectively, 3.5 and 0.94 mg L^{-1} day^{-1}. Low degradation of pyrene and no significant degradation of chrysene and benzo[*a*]pyrene occurred in the slurry bioreactor. This is the first report in which a TLP system was combined with a slurry system to improve the biodegradation of PAHs in soil.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread hydrophobic organic pollutants occurring mostly in the environment as a result of fossil fuel combustion and as byproducts of industrial processes. Since many of the high-molecular-weight (HMW) PAHs are potentially genotoxic and carcinogenic, exposure to PAHs represents a public health risk and raises environmental concerns (*1*). Microbial transformation is the principal natural process responsible for the removal of PAHs from contaminated sites but HMW molecules often persist in the environment. Many microorganisms degrading lower-molecular-weight PAHs have been described and their mechanisms of action studied (*2, 3*). The biodegradation of HMW PAHs is much less documented (*4, 5*).

Biological treatment of environmental pollutants offers economical and ecological alternatives to traditional physicochemical and thermal technologies (*6, 7*). The major factor limiting the bioremediation of soils and sediments contaminated with PAHs is the poor availability of these hydrophobic contaminants to microorganisms (*8, 9*). The bioavailability of a molecule may be characterized by its mass transfer rate relative to its uptake and degradation rates by the microorganisms (*10*). Even if the capacity to degrade is present and environmental conditions are adequate, the inability of microorganisms to have access to these pollutant molecules restricts degradation. Limited bioavailability may be due to low aqueous solubility and a strong sorption to the soil or sediment matrix (*11*). It is usually assumed that the water-dissolved fraction of chemicals is the only one available to microorganisms (*12*−*14*). Therefore, degradation rates are dependent on the mass transfer rates of PAHs from the solid or soil-bound phase to the aqueous phase (*15*).

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10.1021/bp000118j CCC: $19.00
Published on Web 10/03/2000

The principal approach to increase the mass transfer to the aqueous phase is based on enhancing the solubilization or dissolution rates. This can be achieved by increasing the total surface area between the substrate and the aqueous phase and is often accomplished by the addition of surfactants (*16*). Surface-active agents have extensively been used to help solubilize and sometimes increase the biodegradation of hydrophobic contaminants (*17, 18*). However, results about their effectiveness have been contradictory (*19, 20*). Many difficulties related to the use of synthetic surfactants are recognized. They can prevent the biodegradation of contaminants due to their toxicity to microorganisms (*21*) or because of sequestration of target compounds within surfactant micelles (*22, 23*). Moreover, they are often costly, poorly specific toward contaminants, difficult to biodegrade, and may adsorb onto soil particles. Microbially produced surface-active compounds represent a promising alternative to chemical surfactants (*24*). Their use as additives to enhance the biodegradation of many hydrophobic contaminants has been demonstrated (*25, 26*), but they exhibit many of the limitations encountered with synthetic surfactants. The isolation of microorganisms producing biosurfactants when grown on PAHs has been reported (*27*), but although biosurfactants production is often suggested as an important attribute of cells growing on insoluble substrates, the purpose of this feature and the mechanisms involved are still insufficiently understood.

Alternative methods to improve the bioavailability and biodegradation of hydrophobic compounds are required. Two-liquid-phase (TLP) bioreactors are used for the bioconversion of hydrophobic/toxic substrates into products of commercial interest (*28, 29*). In this type of system, a water-immiscible liquid is added to enhance its efficiency by increasing the substrate bioavailability, or by decreasing substrate toxicity (*30*). This liquid acts as a nonbiodegradable and biocompatible liquid phase "reservoir" in which hydrophobic organic substrates are dissolved and then released to the microorganisms. There is increasing interest in the application of TLP bioreactors to enhance the biodegradation of organic pollutants, such as PAHs and halogenated aromatics (*31*−*35*; reviewed in *30*). For example, Jimenez and Bartha (*35*) used different hydrophobic solvents such as Parafilm oil in TLP bioreactors to enhance mineralization of pyrene by a *Mycobacterium* sp. strain. Vanneck et al. (*34*) used silicone oil to improve biodegradation of several PAHs such as pyrene and benzo[*a*]pyrene in a mixed culture.

In this paper, we describe an efficient TLP slurry biosystem for the degradation of HMW PAHs in soil. We used silicone oil as a nonbiodegradable and biocompatible solvent to increase the bioavailability of PAHs by promoting PAH desorption from soil and PAH transfer to microorganisms, thus allowing the development of a HMW PAH-degrading consortium. Biodegradation of HMW PAHs in soil was studied by combining the soil slurry concept with a TLP biosystem. This is the first report on the use of a TLP slurry biosystem to degrade HMW PAHs in soil.

Materials and Methods

Chemicals and Soil. The PAHs used were phenanthrene, chrysene, benzo[*a*]pyrene, perylene (Aldrich Chemical, Oakville, ON, Canada), and pyrene (Sigma, Oakville, ON, Canada). Stock solutions were prepared in acetone or dichloromethane (Anachemia Science, Lachine, Canada). Silicone oil (poly(dimethylsiloxane), Sigma) was 20 cS and had a density of 0.95 g/mL. The MC soil used in TLP slurry bioreactors was a fine-to-medium sandy noncontaminated soil containing 0.65% total volatile solids as determined by the standard combustion method (550 °C for 16 h).

Determination of PAH Concentrations in Soil and in Silicone Oil. PAH concentrations in slurry systems were determined by gas chromatography−mass spectrometry (GC-MS) or by high-performance liquid chromatography (HPLC). In TLP slurry systems, PAH concentrations in the soil and silicone oil was determined by HPLC. The extraction efficiency of PAHs from soil was more than 90% and from silicone oil, more than 95%.

GC-MS. PAHs were extracted from the slurry systems using the whole content of the Erlenmeyer flask after addition of 100 mL of water, 1 mL of concentrated HCl, and 40 mL of ethyl acetate (Sigma). The mixture was shaken 10 min and centrifuged 5 min at 11 000*g*. The slurry was extracted three more times with 20 mL of ethyl acetate. The solvent fractions were pooled, dried with anhydrous sodium sulfate, and analyzed by GC (Varian 3500) equipped with a DB5 capillary column (30 m × 0.25 μm) and connected to a Finnigan Mat 800 mass spectrometer. The injection volume was 1 μL, the carrier gas was helium, and the flow rate was 2.4 mL/min. The oven temperature was initially set at 40 °C for 3 min, then reached 120 °C at 10 °C/min, then 180 °C at 3 °C/min, then 290 °C at 6 °C/min, and finally 310 °C at 12 °C/min, and stayed 8 min at that temperature. The detection limits of phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene were, respectively, 0.5, 0.5, 0.5, and 1 ppm.

HPLC. For the TLP slurry cultures, PAHs were extracted from samples consisting of 1 g of soil or 0.5 mL of silicone oil. Silicone oil samples were vortexed with 1 mL of *N*,*N*-dimethyl formamide (DMF, EM Science, Toronto, ON, Canada) for 2 min and centrifuged at 1150*g* for 1 min to separate the two phases. A volume of 0.5 mL of the upper phase (DMF) was then mixed with 0.5 mL of acetonitrile (Anachemia) containing 0.1% acetic acid and analyzed by HPLC (Waters) with a reverse-phase Nova Pack C18 column (3.9 × 150 mm). Elution was performed using a linear gradient ranging from 65 to 85% acetonitrile in 0.1% acetic acid in 8 min at a flow rate of 2 mL/min. PAHs were detected at 254 nm (Water 486 tunable absorbance detector) and 2-vinyl naphthalene was used as an internal standard. Soil samples were mixed vigorously for 1 min with 2.5 mL of ethyl acetate and centrifuged for 10 min at 1150*g*. This was repeated three more times. The solvent fractions were pooled and evaporated. The extracted PAHs were then redissolved in 0.5 mL of DMF and 0.5 mL of acetonitrile (containing 0.1% acetic acid), and a 20 μL-volume was injected.

Determination of PAH Solubility in Silicone Oil. Saturating amounts of phenanthrene, chrysene, pyrene, and benzo[*a*]pyrene were added separately or together into 10 mL of silicone oil in a covered 50-mL beaker and mixed with a magnetic stirrer for 4 days at room temperature (22−25 °C). The PAH-saturated oil was then filtered onto a Whatman #41 paper filter to remove undissolved particles. PAH concentrations were determined by HPLC. The assay was done nine times for individual PAH solutions and six times for the mixed PAH solution.

Abiotic Transfer of PAHs from Soil to Silicone Oil. The MC soil was screened (<2 mm), oven-dried, and sterilized by autoclaving three times for 60 min at 1-day intervals. PAHs dissolved in acetone were carefully mixed with this soil, and the solvent was allowed to evaporate. Seven days later, three TLP slurry systems were generated each containing 120 g of soil mixed with mineral

salt medium (Bushnell-Haas [BH], Difco Laboratories, Detroit, Mich.) but different volumes of silicone oil (10 mL [2.5% volume silicone oil/total volume TLP slurry system], 30 mL [7.5% v/v] or 60 mL, [15% v/v]). The volume of the TLP slurry systems (including the silicone oil) was adjusted to 400 mL with BH medium to get a 30% weight of soil/total volume TLP slurry system. The slurry was agitated with a magnetic stirrer at maximum speed and room temperature in a 1 L-Erlenmeyer flask. For sampling, agitation was stopped to allow the silicone oil to separate at the top of the water phase and the soil to sediment at the bottom. Soil (1 g) and oil (0.5 mL) samples were taken periodically (in duplicate for soil samples) for determination of PAH concentrations. Each oil sample was divided in two subsamples for duplicate analyses. The two TLP slurry systems with, respectively, 2.5 and 7.5% silicone oil contained soil contaminated initially with 237 mg/kg (of soil) [±22; SD] phenanthrene, 231 mg/kg [±31] pyrene, 281 mg/kg [±16] chrysene and 256 mg/kg [±21] benzo[*a*]pyrene. The 15% silicone oil TLP slurry system contained soil contaminated initially with 305 mg/kg (of soil) phenanthrene, 228 mg/kg pyrene, 138 mg/kg chrysene, and 151 mg/kg benzo[*a*]pyrene. PAH concentrations for this system were the mean of two measurements and variation was between 4 and 7%.

Enrichment of a HMW PAH-Degrading Microbial Consortium in TLP Slurry Bioreactors. A creosote-contaminated soil (50 g) from a wood-treatment facility (Tracy, Québec, Canada) was screened (<2 mm) and mixed with 23 mL of BH medium and 20 mg/kg each of pyrene, chrysene, benzo[*a*]pyrene, and perylene. This slurry was agitated at 150 rpm in a 500 mL Erlenmeyer flask at 30 °C for 4 months. The slurry was then hand-shaken vigorously and centrifuged at low speed (1150*g*, 5 min). The microbial fraction was recovered in the supernatant which was transferred into a soil slurry containing 10 g of the screened (<2 mm) MC soil, 90 mL of BH medium, the same four PAHs each at 50 mg/L, and 20 mL of silicone oil. This TLP slurry culture was agitated (25 °C, 150 rpm) in a 500 mL Erlenmeyer flask for 30 days. Enrichment was performed by consecutive 10% (v/v of the liquid phase) transfers at 30 day intervals.

PAHs Mineralization in TLP Slurry Bioreactors. Mineralization assays were performed with 50 mL serological bottles with Teflon-lined stoppers. Each TLP slurry bottle contained 9 mL of BH medium, 1 g of the MC soil, 1 mL of the PAH-degrading consortium, 0.5 mg each of pyrene, chrysene, benzo[*a*]pyrene, and perylene, and 2 mL of silicone oil. [4,5,9,10-^{14}C]-Pyrene [Sigma] (150 000 dpm) was added in six bottles and [5,6,11,12-^{14}C]-chrysene [Chemsyn, Lenexa, KS] (110 000 dpm [once] and 950 000 dpm [twice]) was added in three other bottles. One milliliter of 1 N KOH in an inserted test tube acted as a CO_2 trap. The cultures were incubated at room temperature in a gyratory shaker (150 rpm). Periodically, the KOH was replaced by fresh KOH and mixed with 5 mL of scintillation cocktail (Ultima gold XR, Packard, Meriden, CT). The solution radioactivity was measured with a liquid scintillation counter (model LS 1701, Beckman Instruments, Inc., Irvine, CA). The composition of the abiotic controls was identical except for the addition of 1% (w/v) sodium azide.

PAH Degradation in Slurry and TLP Slurry Bioreactors. Pyrene, chrysene, and benzo[*a*]pyrene were added to screened and sterilized MC soil as described previously. Fourteen days later, three TLP slurry systems were made, each containing 240 g of soil (30% weight of soil/total volume including silicone oil) and 80 mL of the PAH-degrading consortium, but different volume of silicone oil (80 [10% v/v)], 160 [20% v/v], or 240 mL [30% v/v]). The final volume of the TLP slurry bioreactors was then adjusted to 800 mL with BH medium. The slurry bioreactor contained 240 g of MC soil (30% w/v) and 80 mL of the consortium, and was adjusted to 800 mL with BH medium. The abiotic control was identical to the slurry system except that no microbial culture was added. These were agitated with a magnetic stirrer at maximum speed and room temperature in 2 L Erlenmeyer flasks. Periodically, soil (1 g) and oil (0.5 mL) samples were taken (in duplicate for soil samples), and PAH concentrations were determined. Each oil sample was divided into two subsamples for duplicate analyses. The TLP slurry bioreactors and the abiotic control contained the same soil preparation. The initial PAH concentrations of this preparation were 358 mg/kg (of soil) pyrene [±11; SD], 255 mg/kg [±10] chrysene, and 250 mg/kg [±7] benzo[*a*]-pyrene. A second soil preparation was made for the slurry bioreactor with 196 mg/kg pyrene, 192 mg/kg chrysene, and 163 mg/kg benzo[*a*]pyrene. PAH concentrations for the second soil preparation were the mean of two measurements, and variation was between 1 and 3%.

Table 1. Solubility of PAHs in Silicone Oil

		silicone oil mg/L [SD]	
	water[a] mg/L	individually	together[b]
phenanthrene	1.29	5172 [±224]	5273 [±185]
pyrene	0.14	2188 [±265]	2055 [±250]
chrysene	0.0020	55 [±13]	73 [±3]
benzo[*a*]pyrene	0.0038	240 [±12]	431 [±25]

[a] From Cerniglia (*46*). [b] The four PAHs were dissolved together in silicone oil.

Results

PAHs Solubility in Silicone Oil. The solubility of phenanthrene, chrysene, pyrene, and benzo[*a*]pyrene in silicone oil was determined separately or together in order to evaluate the solvent capacity to preferentially partition these PAHs from soil. Table 1 shows that the four PAHs are from 10^3 to 10^5 times more soluble in silicone oil than in water. The solubility of phenanthrene and pyrene was not influenced by the presence of the other three PAHs. In contrast, the solubility of benzo[*a*]-pyrene in silicone oil almost doubled in the presence of the other PAHs, whereas there was a small increase of chrysene solubility.

Abiotic Transfer of PAHs from Soil to Silicone Oil. The rate and extent of PAH transfer from soil to silicone oil were investigated to estimate the potential of silicone oil to desorb PAHs from soil. Four PAHs were added to a sterilized soil. This artificially contaminated soil was then used to generate three TLP slurry systems containing 2.5, 7.5, or 15% (v/v) silicone oil. Figure 1 shows that the highest extent of transfer, for all PAHs, was achieved in the TLP slurry system containing 15% silicone oil. In this system, 88% phenanthrene, 74% pyrene, and 65% benzo[*a*]pyrene were transferred from soil to silicone oil in 4 days (Table 2). Figure 1 also shows that PAH transfer was still occurring after 4 days for these three PAHs. For chrysene, approximately 13% was transferred into the oil in the 15% TLP system after 2 h, and then no significant additional transfer was observed. Silicone oil was not saturated by chrysene since its concentration corresponded to 50% of the saturating value (Table 2).

Enrichment of a HMW PAH-Degrading Consortium. A HMW PAH-degrading microbial consortium was enriched from a creosote-contaminated soil by successive

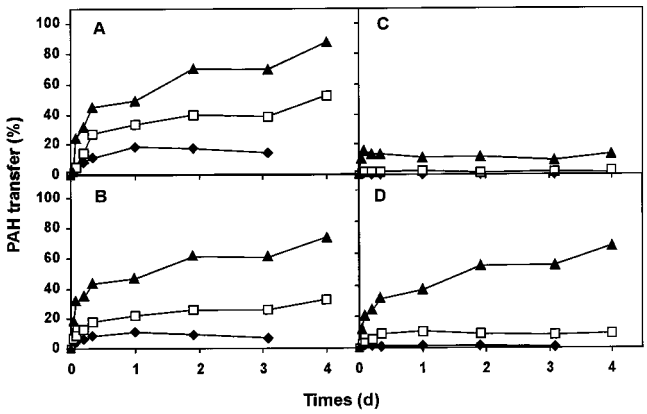

Figure 1. PAH transfer from soil slurries into silicone oil. Phenanthrene (A), pyrene (B), chrysene (C), and benzo[*a*]pyrene (D) were added to sterilized soil (120 g), then mixed with water (30% w/v soil) and silicone oil. PAH concentrations were determined in soil and silicone oil. PAH transfer was presented as the percentage of the PAH amount found in silicone oil from the initial PAH amount in soil. Data are means of duplicates. Variations were no more than 50% between duplicate values. Silicone oil (v/v): ◆, 2.5%; □, 7.5%; ▲, 15%.

transfers in TLP slurry systems (20% [v/v] of silicone oil) containing pyrene, chrysene, benzo[*a*]pyrene, and perylene added at 50 mg/L each. The performance of the consortium was evaluated after the fifth transfer. Pyrene was completely degraded in less than 15 days. Most of the chrysene was degraded in 15 days, whereas 65% of benzo[*a*]pyrene was degraded in 32 days. PAH mineralization assays confirmed that the consortium was capable of metabolizing HMW PAH molecules. Figure 2 shows that approximately 80% of ^{14}C-pyrene was mineralized in 11 days. ^{14}C-chrysene mineralization started after a 7 day lag and reached more than 70% after 54 days.

Efficiency of HMW PAH Biodegradation in TLP Slurry Bioreactors. Biodegradation of a mixture of pyrene, chrysene, and benzo[*a*]pyrene was compared between classical slurry and TLP slurry biosystems. Three TLP slurry bioreactors were prepared with the same sterilized soil artificially contaminated with pyrene, chrysene, and benzo[*a*]pyrene, and then inoculated with the HMW PAH-degrading microbial consortium. Because the results illustrated in Figure 1 showed that PAH transfer was still occurring in TLP slurry system containing 15% silicone oil, the TLP slurry bioreactors were done respectively with 10, 20, or 30% (v/v) silicone oil. The concentration of each PAH was followed in soil and

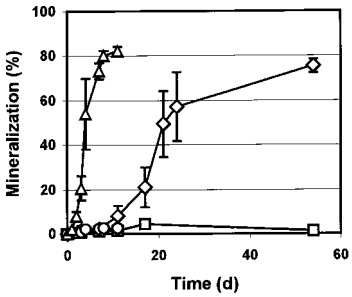

Figure 2. Mineralization of pyrene and chrysene by the HMW PAH-degrading microbial consortium. ^{14}C-pyrene (△, ○) and ^{14}C-chrysene (◇, □) were added separately to TLP slurry bioreactors containing 10% (w/v) soil, 20% (v/v) silicone oil, pyrene, chrysene, benzo[*a*]pyrene, and perylene at 50 mg/L each, and the consortium. Mineralization is expressed as a percentage of radioactivity released as $^{14}CO_2$. TLP slurry bioreactors (△, ◇) and abiotic controls (○, □).

in silicone oil. PAH concentration in water was not determined because of the very low aqueous solubility of these molecules (below detection limits using the analytical methods in this study). Figure 3 shows the results obtained with the TLP slurry bioreactors containing 10 and 30% silicone oil along with the classical slurry bioreactor and the abiotic control. Similar results were observed between the TLP slurry bioreactors containing 20% or 30% silicone oil. The concentration of each PAH in the abiotic control, that contained sterilized soil but no silicone oil and no inoculum, dropped by approximately 40% in 5−10 days then stabilized at this level afterward.

The rate of pyrene degradation was similar in TLP slurry bioreactors containing 10 and 30% silicone oil (Figure 3A). After a rapid initial drop during the first 2 days, this PAH was no longer detectable after 4 days, whereas more than 50% pyrene was still present in the slurry bioreactor. During this period, the pyrene concentration increased in silicone oil after 1 day and decreased afterward. Considering a 30% abiotic loss in the first 4 days (based on the abiotic control), the rate of pyrene degradation was estimated at 19 mg L^{-1} day^{-1} (59.5 mg of pyrene degraded; 0.8 L total volume) in the three TLP slurry bioreactors during that period, and 4.3 mg L^{-1} day^{-1} (13.7 mg degraded) in the classical slurry bioreactor.

Chrysene degradation was more extensive in the TLP slurry bioreactor containing 30% silicone oil than the one containing 10% (Figure 3B). In the 30% TLP bioreactor,

Table 2. Percentage of PAH Transferred from Soil to Silicone Oil in the TLP Slurry Systems[a]

	t (days)	phenanthrene	pyrene	chrysene	benzo[*a*]pyrene
		TLP slurry system with 2.5% silicone oil			
PAH in soil (mg)	0	26.8	24.8	33.4	32.4
	4	22.7	16.9	26.7	29.6
PAH in silicone oil (mg)	3	3.8	1.7	0.03	0.3
% transfer		14	6.8	0.1	0.8
		TLP slurry system with 7.5% silicone oil			
PAH in soil (mg)	0	30.1	30.7	34.1	28.9
	4	1.3	12.8	24.2	20.0
PAH in silicone oil (mg)	4	15.7	10.0	0.4	2.7
% transfer		52	33	1.3	9.4
		TLP slurry system with 15% silicone oil			
PAH in soil (mg)	0	36.5	27.3	16.5	18.1
	4	1.5	4.7	10.1	5.9
PAH in silicone oil (mg)	4	31.9	20.0	2.2	11.7
% transfer		88	74	13	69
% silicone oil saturation[b]	4	10	16	50	45

[a] Values were the mean of two measurements and variation was between 0 and 7%. [b] Based on Table 1 with 60 mL of silicone oil.

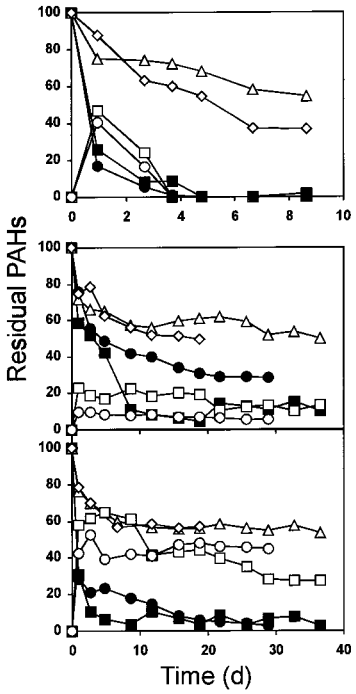

Figure 3. PAH concentrations in slurry and in TLP slurry bioreactors. Pyrene (A), chrysene (B), and benzo[*a*]pyrene (C) were added together to a sterilized soil. The TLP slurry bioreactors contained 30% (w/v) soil, silicone oil (10 or 30% v/v), and the PAH-degrading consortium. The slurry bioreactor contained the same components and culture as the TLP slurry bioreactors except for the silicone oil. Abiotic controls contained the same components except for the silicone oil and the microbial culture. PAH concentrations were determined in silicone oil and in soil. The residual PAHs was calculated as the percentage of the PAH amount found in the silicone oil or soil to the initial PAH amount added in soil. Data are means of duplicates. Variations were no more than 50% between duplicate values. Residual PAHs in soil (■, ●) or in silicone oil (□, ○) in bioreactors containing 0% (◇; slurry bioreactor), 10% (●, ○) or 30% (■, □) silicone oil and in the abiotic controls (△).

10% of the initial chrysene concentration was detected in soil after 9 days. Afterward, the concentration in soil stayed approximately at the same level. In the 10% TLP slurry bioreactor, 30% of the initial concentration was still detected in soil after 19 days, and then no further decrease was observed. Chrysene concentration in silicone oil reached its maximum level after 1 day in the 10% and 30% TLP slurry bioreactors. Similarly to what was observed in the PAH transfer assays (Figure 1), the chrysene concentration remained at approximately the same level afterward. Overall, 23% of chrysene remained in the 30% TLP slurry bioreactor after 29 days compared to 34% for the 10% TLP slurry bioreactor. Considering a 40% abiotic loss in the first 9 days, the rate of chrysene degradation was estimated at 2.6 and 3.5 mg L^{-1} day^{-1} in the 10% and 30% TLP slurry bioreactors, respectively, during that period (18.6 and 25.5 mg chrysene degraded, respectively; 0.8 L total volume). No significant degradation occurred in the classical slurry bioreactor compared to that in the abiotic control.

A strong decrease in benzo[*a*]pyrene concentration in soil after the first day was observed in the 10% and 30% TLP slurry bioreactors that paralleled an increase in the silicone oil phase (Figure 3C). In both bioreactors, approximately 5% benzo[*a*]pyrene was still detected in the soil after 19 days. After a sharp increase in the first day, the level of benzo[*a*]pyrene in silicone oil remained the same in the 10% TLP slurry bioreactor in which approximately 45% of the initial benzo[*a*]pyrene amount was found. In the 30% TLP slurry bioreactor, benzo[*a*]-pyrene concentration in silicone reached a plateau between days 1 and 9 in which approximately 60% of the initial benzo[*a*]pyrene amount was found. Afterward, the concentration decreased from days 12 to 37, resulting from biodegradation. Overall, 35% of benzo[*a*]pyrene remained in the 30% TLP slurry bioreactor after 29 days compared to 48% for the 10% TLP slurry bioreactor. By taking into account a 44% abiotic loss in the first 29 days, the rate of benzo[*a*]pyrene degradation was estimated at 0.73 and 0.94 mg L^{-1} day^{-1} in the 10% and 30% TLP slurry bioreactors, respectively, during that period (17.0 and 21.9 mg benzo[*a*]pyrene degraded, respectively; 0.8 L total volume). No significant biodegradation was observed in the classical slurry bioreactor since benzo-[*a*]pyrene concentrations were similar between the slurry bioreactor and the abiotic control.

Discussion

Improving microbial growth conditions is an important aspect to optimize soil bioremediation. Since HMW PAHs are not readily available for microbial degradation, improving microbial degrading capacities will not lead to higher biotransformation rates because the rate of mass transfer is the limiting factor. The bioavailability of PAHs is controlled by various physicochemical properties such as aqueous solubility and sequestration by soil and sediment (*11, 36*). Since the partition of hydrophobic compounds into a NAPL is in many ways comparable to sorption to soil organic matter, the presence of a water-immiscible solvent can promote the extraction of hydrophobic pollutants from the soil. The ensuing facilitated mass-transfer and increased surface area for microbial contact should increase the rate of biodegradation.

In this study, silicone oil was used as the water-immiscible solvent in a TLP slurry bioreactor because of its hydrophobicity, biocompatibility, chemical stability, and resistance to hydrolytic and oxidative breakdown. Our results showed that the solubility of several PAHs was greater in silicone oil than in water by 3−5 orders of magnitude. Ascon-Cabrera and Lebeault (*31*) used this compound to enhance the biodegradation of 1,2-dichlorobenzene, 1,2,3- and 1,2,4-trichlorobenzene, ethyl butyrate, and 2-ethylbutyraldehyde by a mixed culture, and ethyl butyrate and 2,4,6-trichlorophenol by defined cultures (*32, 33*). Better degradation of styrene (*37, 38*) and several PAHs (*34*) was also achieved by mixed cultures in TLP bioreactors in which silicone oil was used as the water-immiscible liquid. This is the first report however in which a TLP system was combined with a slurry system to improve the biodegradation of target compounds into soil.

A sterile slurry artificially contaminated with phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene was used to study the capacity of silicone oil to extract these PAHs from soil. In the TLP slurry bioreactor containing 15% silicone oil, a high percentage of phenanthrene, pyrene, and benzo[*a*]pyrene was transferred into the silicone oil. Rapid PAH transfer occurred during the first 8 h, probably from the extraction of nonsolubilized and poorly sorbed PAHs. In the remaining 88 h, a slower but constant PAH transfer occurred, suggesting extraction of more tightly bound PAHs. The desorption of many chemicals from soils is known to show a rapid phase followed by a period of slow desorption (*39*). For chrysene, its concentration in silicone oil reached a maximum within 2 h and then remained approximately the same for the remaining 95 h of the experiment. A similar trend was observed in the TLP slurry bioreactors in the

presence of the HMW PAH-degrading consortium (Figure 3B). Interestingly and in both cases, the silicone oil was not saturated since the observed chrysene concentration was approximately half of its maximal value. This could be explained by an equilibrium state between the soil matrix and the silicone oil phase. Another possibility is that substances from the soil fraction were dissolved in the oil phase and interfered with the dissolution of chrysene into silicone oil.

The enrichment of a consortium with microorganisms able to degrade poorly water-soluble compounds is an important factor for the optimization of biodegradation processes. The addition of a water-immiscible, hydrophobic liquid has the potential to shorten the time required to enrich a consortium with the desired degrading microorganisms because more substrate becomes available for growth. Ascon-Cabrera and Lebeault (*31*) used a TLP bioreactor with silicone oil for the rapid selection of consortia able to degrade two mixtures of chlorinated and nonchlorinated hydrophobic compounds, while very little or no growth was observed in the control monoliquid aqueous enrichment systems. This was attributed to the high concentrations of the substrates that could be added to the enrichment culture via the water-immiscible phase.

We have obtained a HMW PAH-degrading consortium with a TLP slurry system, by enrichment of a microbial population isolated from a creosote-contaminated soil. Experiments with radio-labeled PAHs in TLP slurry bioreactors demonstrated that the enriched microbial population mineralized PAHs. We used this consortium along with an artificially contaminated soil to develop TLP slurry bioreactors. Within 5 days, all pyrene were degraded in the three TLP slurry bioreactors that contained 10, 20, or 30% silicone oil with a degradation rate of 19 mg L^{-1} day^{-1}, compared to 4.3 mg L^{-1} day^{-1} in the control slurry bioreactor. For chrysene and benzo[*a*]pyrene, the TLP slurry bioreactor containing 30% silicone oil was the most efficient. In this bioreactor, the degradation rates were 3.5 mg L^{-1} day^{-1} for chrysene and 0.94 mg L^{-1} day^{-1} for benzo[*a*]pyrene through a period of 9 and 29 days, respectively. The TLP slurry bioreactors were much more efficient than the classical slurry bioreactor in which no significant degradation of chrysene and benzo[*a*]pyrene occurred. Thiem et al. (*17*) reported that a mixed culture enriched from a contaminated soil degraded 4.5 mg/L of benzo[*a*]pyrene in 23 days (0.20 mg L^{-1} day^{-1}) with 2 mM of the surfactant Sapogenat T-300; however, no concurring degradation of chrysene was observed. *Mycobacterium* strain PYR-1 was capable of slowly degrade benzo[*a*]pyrene but not chrysene (*40*), whereas *Burkholderia cepacia* VUN 10 001 degraded 20.5 mg/L of benzo[*a*]pyrene in 56 days (0.36 mg L^{-1} day^{-1}) (*41*). Finally, with 5 g/L of the surfactant Tergitol NP-10 added in the culture, 32 mg/L (0.64 mg L^{-1} day^{-1}) of chrysene and 41 mg/L (0.82 mg L^{-1} day^{-1}) of benzo[*a*]pyrene were degraded in 50 days by a *Stenotrophomonas maltophilia* strain (*42*). The TLP slurry bioreactor containing 30% silicone oil appears, therefore, to provide degradation performances at least as good as that in most previously reported systems.

Improved performance of the TLP-enriched consortium in the TLP slurry bioreactors could be explained by three factors (*30*): (1) a better enrichment of HMW PAH-degrading microorganisms because of the improved availability of the substrate, (2) a better adaptation of the consortium to TLP growth conditions, e.g., enrichment of adherent microorganisms to the hydrophobic liquid phase, or simply (3) an increased bioavailability offered by the TLP system.

Abiotic controls was carried out only for the slurry system in which 30−40% of the PAHs was no longer detectable after 10 days. This may have been caused by an increasingly strong sorption/sequestration of PAHs to soil particles or organic matter, probably promoted by the addition of water and the intense mixing of the slurry. A diminished availability for solvent extraction with increasing period of time of contact between hydrophobic compounds and soil is well-known (*43, 44*). Unfortunately, no TLP slurry abiotic controls were carried out in our assays. However, we recently reported (*45*) the optimization of the TLP slurry bioreactors in which such controls were included. Overall, the abiotic losses were between 10 and 30%. Consequently, the degradation rates of PAHs in the TLP slurry bioreactors determined in this report in which only the abiotic losses from slurry system were taken into account are probably minimal estimates of what was really achieved by TLP slurry bioreactors.

TLP slurry bioreactors can provide an efficient way to bioremediate contaminated soils. For example, in a large scale process, a TLP slurry bioreactor could be used to extract pollutants from a contaminated soil by transfer to the water-immiscible, nondegradable liquid phase. The solvent would then be recovered and the treated soil returned to the site. This could be repeated a number of times with the same solvent. Once the water-immiscible liquid has reached a certain concentration of contaminant, the extraction vessel could then be used as a TLP bioreactor to degrade the extracted contaminants.

Acknowledgment

We thank Sylvain Milot for assistance with PAH analyses.

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Accepted for publication August 30, 2000.

BP000118J