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ORIGINAL PAPER

Enhanced degradation of a mixture of polycyclic aromatic hydrocarbons by a defined microbial consortium in a two-phase partitioning bioreactor

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Abstract Biological treatment methods are effective at destroying polycyclic aromatic hydrocarbons (PAHs), and some of the highest rates of PAH degradation have been achieved using two-phase-partitioning bioreactors (TPPBs). TPPBs consist of a cell-containing aqueous phase and a biocompatible and immiscible organic phase that partitions toxic and/or recalcitrant substrates to the cells based on their metabolic demand and on maintaining the thermodynamic equilibrium of the system. In this study, the degradation of a 5-component mixture of high and low molecular weight PAHs by a defined microbial consortium of *Sphingomonas aromaticivorans* B0695 and *Sphingomonas paucimobilis* EPA505 in a TPPB was examined. The extremely low aqueous solubilities of the high molecular weight (HMW) PAHs significantly reduce their bioavailability to cells, not only in the environment, but in TPPBs as well. That is, in the two-phase system, the originally selected solvent, dodecane, was found to sequester the HMW PAHs from the cells in the aqueous phase due to the inherent high solubility of the hydrophobic compounds in this solvent. To circumvent this limitation, the initial PAH concentrations in dodecane were increased to sufficient levels in the aqueous phase to support degradation: LMW PAHs (naphthalene, phenanthrene) and fluoranthene were degraded completely in 8 h, while the HMW PAHs, pyrene and benzo[*a*]pyrene, were degraded by 64% and 11%, at rates of 42.9 mg l^{-1} d^{-1} and 7.5 mg l^{-1} d^{-1}, respectively. Silicone oil has superior PAH partitioning abilities compared to dodecane for the HMW PAHs, and was used to improve the extent of degradation for the PAH mixture. Although silicone oil increased the bioavailability of the HMW PAHs and greater extents of biodegradation were observed, the rates of degradation were lower than that obtained in the TPPB employing dodecane.

Keywords Biodegradation · Consortium · Polycyclic Aromatic Hydrocarbons (PAHs) · *Sphingomonas* · Two-Phase Partitioning Bioreactor (TPPB)

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are composed of two or more fused, aromatic rings and are ubiquitous organic pollutants occurring in the environment as a result of fossil fuel combustion, as by-products of industrial processes and via natural causes. Massive soil contamination

with PAHs originated from extensive industrial coal gasification during most of the 20th century and these contaminants continue to be generated and released into the environment by the incomplete combustion of organic matter, for example in forest fires, home heating, traffic, and waste incineration (Johnsen et al. 2005). The presence of PAHs in the environment is a considerable public health hazard because some of these compounds have been shown to have mutagenic and carcinogenic properties (Cerniglia 1992). Consequently, the US Environmental Protection Agency (EPA) has identified 16 PAHs as priority pollutants (Shuttleworth and Cerniglia 1995; Kanaly and Harayama 2000) and significant effort is being made to develop strategies for removing these pollutants from the environment.

PAHs are lipophilic in nature and have a high potential to accumulate in the solid phases of the terrestrial environment as they tend to sorb to the organic fraction of soil. Nevertheless, many physical, chemical, and biological methods for decontamination exist and among these, microbial degradation is an environmentally clean and versatile mode of PAH treatment (Ashok and Saxena 1995). Biodegradation however, is limited by the low aqueous solubility inherent to PAHs and the bioavailability of these compounds decreases almost logarithmically with increasing molecular weight (Cerniglia 1992; Juhasz and Naidu 2000).

Many different methods for enhancing biodegradation of PAHs by bacteria have been examined (Woo and Park 2004) and because the bioavailability of PAHs is critical for effective bioremediation, a significant amount of research has been devoted to increasing the availability of these hydrophobic compounds in the aqueous phase. The addition of surfactants is a very common approach for enhancing the bioavailability of PAHs (Efroymson and Alexander 1991; Tiehm 1994; Volkering et al. 1995), although a general consensus on the actual effectiveness of surfactants has not yet been reached. Specially designed bioreactors are another method for enhancing bioavailability and thereby biodegradation rates (Shuttleworth and Cerniglia 1995). Two-phase partitioning bioreactors (TPPBs) have been used to increase the bioavailability of poorly soluble compounds in the aqueous phase and very effective degradation of PAHs has been observed in these systems (Bouchez et al. 1997; Marcoux et al. 2000; Janikowski et al. 2002; MacLeod and Daugulis 2003). A TPPB consists of a cell containing aqueous phase and an immiscible organic phase that can be loaded with high concentrations of the target substrate. The substrate will partition from the delivery phase to the biomass in the aqueous phase based on equilibrium considerations and in response to the metabolic demand of the microorganisms. The mass transfer rate is favored by the increased surface area for partitioning, which results in enhanced biodegradation rates (MacLeod and Daugulis 2005).

In the present work, the degradation of a mixture of low molecular weight (LMW) PAHs (naphthalene (NAP), phenanthrene (PHE)), and high molecular weight (HMW) PAHs (pyrene (PYR), fluoranthene (FLU), and benzo[*a*]pyrene (BAP)) in a TPPB using a defined consortium of *Sphingomonas aromaticivorans* B0695 and *Sphingomonas paucimobilis* EPA505 was examined. Two solvents, dodecane and silicone oil, with differing partitioning characteristics were examined for their ability to enhance the bioavailability of PAHs.

Materials and methods

Chemicals

The PAHs (naphthalene, phenanthrene, pyrene, fluoranthene, and benzo[*a*]pyrene) used in this study were obtained from Sigma-Aldrich Canada, Oakville, Ontario at the highest purity grade available. Silicone oil, Poly(dimethylsiloxane) 200 fluid at viscosity of 5 cSt, was also purchased from Sigma-Aldrich. All salts and *n*-dodecane were purchased from Fisher Scientific, Ottawa, Ontario, Canada.

Microorganisms

S. aromaticivorans B0695, isolated from the deep subsurface of the Savannah River Site and identified by Fredrickson et al. (1999), was generously provided by Dr. David Balkwill, Florida State University. *S. paucimobilis* EPA505, isolated

from soil highly contaminated with coal tar creosote in Gulf Breeze, Florida and identified by Mueller et al. (1990), was purchased from Microbiological Properties Research at the National Centre of Agricultural Utilization Research, Peoria, Illinois, USA.

Medium and culture conditions

Maintenance medium was based on a modified Luria Bertani broth developed by Lantz et al. (1995) and contained 10 g l^{-1} tryptone; 5 g l^{-1} yeast extract; 1.0 g l^{-1} glucose; 1.0 g l^{-1} KH_2PO_4; 1.0 g l^{-1} K_2HPO_4; 1.0 g l^{-1} NH_4NO_3; 0.2 g l^{-1} $MgSO_4{\cdot}7H_2O$; 0.02 g l^{-1} $CaCl_2$ and 1 ml of a trace element solution. The trace element solution (containing 16.20 mg l^{-1} $FeCl_3{\cdot}6H_2O$; 40.0 mg l^{-1} citric acid; 9.44 mg l^{-1} $CaHPO_4$ and 0.15 mg l^{-1} $CuSO_4{\cdot}5H_2O$) was prepared as a stock solution at a 1000-fold concentration and then diluted to give the amounts indicated. Stock cultures were maintained in liquid maintenance medium with the addition of dimethyl sulfoxide (10 v/v%) and frozen down to –80°C. Inoculum was prepared using the maintenance medium for all experiments. The growth medium for *S. paucimobilis* EPA505 was amended with FLU at a concentration of 0.1 g l^{-1} (which is above its saturation concentration) as this strain apparently loses its ability to degrade PAHs without such selection pressure. During PAH degradation experiments, the medium formulation was altered to ensure that PAHs were the main source of carbon (minimal medium). The concentrations of tryptone and yeast extract were varied according to the amount of PAHs present for each experiment and glucose was omitted entirely and replaced by a solvent phase containing a mixture of PAHs during the bioreactor experiments. For culture maintenance, all medium components were dissolved in distilled water and the pH was adjusted to 6.2 using 2 M H_2SO_4. All medium was sterilized prior to use.

Analytical procedures

The optical density of the aqueous phase was measured using a Biochrom Ultraspec 3000 at a wavelength of 650 nm to track cell densities, which were converted to cell dry weight (CDW) by means of a calibration curve. PAHs in the organic layer were quantified via fluorescence spectroscopy. Fluorescence spectra were collected using a QuantaMaster QM-2000-6 fluorescence spectrometer (Photon Technology International, London, Ontario, Canada) equipped with a 72 W xenon arc lamp and Czerney-Turner excitation and emission monochromators. Excitation and emission slits were set to 2 nm bandpass for all measurements. Samples were held in quartz cuvettes, type 3H, with a path length of 10 mm, obtained from NSG Precision Cells, Farmingdale, New York, USA). All samples were diluted by a factor of 10,000 in anhydrous HPLC-grade ethanol to be in the linear range of detection for PAHs (from 0 to 0.1 mg/l); because of the large sample dilution factor the detection limit for this method was extremely good. Synchronous scans were performed to generate unique peaks for each PAH and the Felix software package, version 32, was used to collect the data from this device. The detection conditions for the synchronous scan of each PAH [change in wavelength ($\Delta\lambda$), peak maximum, integration area] were, in nanometers: NAP (104.0, 322.0, 315–330), PHE (53.0, 346.0, 343–351), PYR (37.0, 371.5, 369–375), FLU (175.5, 460.0, 459–468), and BAP (108.0, 404.0, 398–415). PAH concentration measurements could be reproduced with +5% accuracy using the fluorescence spectrometer and the spectra obtained showed no indication of interference due to PAH degradation products.

Degradation of PAHs in aqueous phase by individual species and consortium

S. aromaticivorans B0695 and *S. paucimobilis* EPA505 were grown individually in maintenance medium for 24 h and 40 h respectively. After incubation, the cell cultures were centrifuged at 3400 rpm for 20 min. The cell pellets were washed twice with sterile buffer solution and then resuspended in minimal medium (containing 0.5 g l^{-1} and 0.25 g l^{-1} of tryptone and yeast extract respectively) to an optical density of $OD_{650} = 1$. Aliquots of 2 ml of the cell suspensions (1 ml of *S. aromaticivorans* B0695 and 1 ml

of *S. paucimobilis* EPA505 for the consortium) were added to sterile 20 ml scintillation vials and the PAHs (NAP, PHE, PYR, FLU, and BAP) were added in hexane to final concentrations of 10 mg l^{-1}. An abiotic control was prepared using 2 ml of sterile minimal medium instead of the cell suspensions. The vials were incubated on a rotary shaker at 160 rpm and 30°C for 4 days. After incubation, hexane was used to extract the residual PAHs from the vials and these solutions were analyzed via fluorescence spectroscopy, to determine the capabilities of these organisms to degrade the selected PAHs.

Abiotic operation

Three liters of sterilized distilled water were placed in 5-l New Brunswick Scientific BioFlo III fermentor and a solvent layer (500 ml of dodecane or silicone oil) loaded with 0.1 g each of NAP, PHE, PYR, FLU, and BAP was then added. The fermentor was operated at an aeration rate of 1.5 l/min and an agitation speed of 350 rpm for 7 days, with the excess sparged air exiting the system via a condenser. An initial organic phase sample was taken and analyzed using fluorescence spectroscopy to determine the concentration of PAHs and additional samples were taken every 24 h to monitor any losses of PAHs due to volatilization.

Batch fermentation 1: proof of concept

An initial experiment to determine the ability of *S. aromaticivorans* B0695 and *S. paucimobilis* EPA505 as a consortium to degrade a mixture of PAHs compared to the individual species was conducted using two LMW PAHs (NAP and PHE) and three HMW PAHs (PYR, FLU and BAP) in a 5-l New Brunswick Scientific BioFlo III fermentor. Three liters of medium containing 0.5 g l^{-1} and 0.25 g l^{-1} of tryptone and yeast extract respectively was prepared in the fermentation vessel and autoclaved at 121°C and 15 psig for 20 min. Inoculum preparation was consistent during all experiments. The inoculum was grown in maintenance medium for 24 h and 40 h for *S. aromaticivorans* B0695 and *S. paucimobilis* EPA505 respectively, and a 150 ml volume of each cell species was added to the reactor. A solution of 500 ml of dodecane loaded with 0.5 g of each NAP, PHE, PYR, FLU and BAP was filter sterilized and added to the bioreactor. Samples of the aqueous and organic phases were taken every 2 h for the first 12 h of operation, every 4–8 h for the next 36 h and once a day thereafter and analyzed for optical density of the aqueous phase and PAH concentration in the solvent phase. For each fermentation, the agitation speed, aeration rate, pH, and temperature were maintained at 350 rpm, 1.5 l min^{-1}, 6.3°C and 30°C respectively. The pH of the system was controlled by the automatic delivery of acid (2 M H_2SO_4) and base (2 M KOH) solutions.

Batch fermentation 2: increased PAH concentrations in dodecane

In an attempt to increase the bioavailability of the HMW PAHs in the aqueous phase a TPPB was operated with increased initial PYR and BAP concentrations in the dodecane delivery phase. The bioreactor was prepared and operated as described in Batch Fermentation 1, however, the solvent (500 ml of dodecane) was loaded with 0.5 g of each NAP, PHE and FLU and 1.0 g of each PYR and BAP, and the tryptone and yeast extract concentrations in the aqueous phase medium (3 l) were tripled in response to the higher PAH levels and to prevent nutrient limitation. These PAH levels are readily soluble in dodecane.

Batch fermentation 3: use of silicone oil as organic delivery phase

To assess the ability of silicone oil as the second phase to increase the bioavailability of the HMW PAHs in the aqueous phase compared to dodecane a TPPB was prepared and operated as described in Batch Fermentation 2. The aqueous phase consisted of 3 l of medium containing the same levels of tryptone and yeast extract as in Batch Fermentation 1, and the solvent phase (500 ml of silicone oil) was loaded with 0.5 g of NAP, PHE, FLU and PYR and 0.25 g of BAP. The levels of PYR and BAP used in this experiment are approaching the solubility limits of these

PAHs in silicone oil, and higher concentrations were found to crystallize out in the solvent.

Mass partitioning coefficient determination

A 3-ml aliquot of sterile maintenance medium was added to a quartz cuvette, and then 100 μl of either dodecane or silicone oil containing 2000 mg/l of PYR (used as a proxy for all PAHs used in this study) was slowly pipetted on top of the aqueous phase. The two-phase system was allowed to reach equilibrium for 24 h and the concentration of PYR in the aqueous phase was then measured directly in the cuvette via fluorescence spectroscopy. The mass partitioning coefficient, K_{SW}, was determined as the ratio of the concentration of the solute in the organic phase to the concentration of the solute in the aqueous phase.

Results

Degradation of PAHs in aqueous phase by individual species and consortium

After 4 days of incubation, complete degradation of NAP, PHE, and FLU by the individual and combined cell cultures was achieved (Fig. 1). These results are consistent with previous studies that have confirmed degradation of these PAHs by *S. aromaticivorans* B0695 (Fredrickson et al. 1995, Shi et al. 2001, Janikowski et al. 2002) and EPA505 (Mueller et al. 1990, Story et al. 2004). Following incubation with 10 mg/l of each PAH, B0695 degraded 65.8% and 1.9% of PYR and BAP respectively. *S. aromaticivorans* B0695 is a well known LMW PAH degrader, however, to date there have been no reports of HMW PAH degradation by B0695, with the exception of fluoranthene and 2,3-benzofluorene in the presence of Tween 80 (Shi et al. 2001). *S. paucimobilis* EPA505 was able to degrade 73.6% and 41.5% of PYR and BAP respectively, after 4 days of incubation (Fig. 1). The degradation of PYR and BAP by EPA505 is consistent with the results reported by Ye et al. (1996). Mueller et al. (1990) found that *S. paucimobilis* EPA505 could not grow on these HMW PAHs as sole carbon sources, therefore, it is possible that they are degraded co-metabolically and the LMW PAHs and/or FLU are capable of inducing the enzyme(s) responsible for the microbial degradation of these HMW PAHs. Finally, the consortium degraded PYR and BAP by 94.8% and 62.1% respectively (Fig. 1). Greater extents of HMW PAH degradation were observed for the consortium compared to the individual species, suggesting a synergistic relationship between the two organisms that may enhance the degradation of these PAHs.

Abiotic operation of TPPB

After 168 h of operation at a high rate of aeration and agitation, no losses of the six PAHs present were detected from the organic phase, with the exception of NAP when silicone oil acted as the second phase. The NAP concentration decreased gradually over 7 days which can be attributed to volatilization from the fermentation vessel. However, this loss was not significant during the

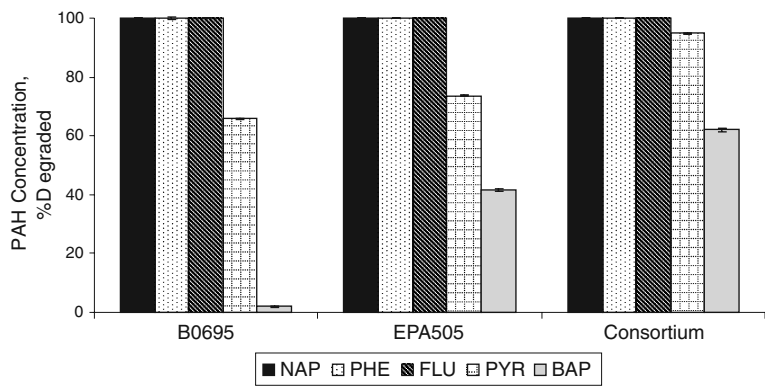

Fig. 1 Aqueous phase degradation of five PAHs by *S. aromaticivorans* B0695, *S. paucimobilis* EPA505 and the B0695/EPA505 consortium

biotic fermentations because in all cases NAP was observed to be metabolized within the first 12 h of operation. It can be concluded that, largely due to the condenser and the low volatility of the PAHs, any loss of these compounds from the bioreactor during fermentations was a result of microbial degradation and not volatilization.

Batch fermentation 1: proof of concept

NAP, PHE, and FLU disappeared completely from the system after 10, 16, and 20 h, respectively (Fig. 2). These rates of degradation correspond to an overall volumetric rate of 400 mg l^{-1} d^{-1} of NAP, 250 mg l^{-1} d^{-1} of PHE, 200 mg l^{-1} d^{-1} of FLU, and an overall PAH consumption rate of 600 mg l^{-1} d^{-1} based on aqueous volume. The HMW PAHs did not experience significant degradation and PYR and BAP were degraded by 26% and 3% after 120 h, respectively. Although a small amount of HMW PAH degradation was observed, the extents of PYR and BAP consumption were lower than those obtained by Daugulis and McCracken (2003). Improvements on the system were therefore sought in an attempt to achieve greater extents of HMW PAH degradation.

Batch fermentation 2: Increased PAH concentrations in dodecane

NAP was degraded at a rate of 667 mg l^{-1} d^{-1}, while PHE and FLU were degraded at a rate of 500 mg l^{-1} d^{-1}, corresponding to an overall PAH consumption rate of 1500 mg l^{-1} d^{-1} (Fig. 3). Janikowski et al. (2002) and McLeod and Daugulis (2003) are the only reports in the literature to date to achieve greater rates of degradation of these PAHs in TPPBs. The higher rates achieved with dodecane suggest that this solvent does not sequester the LMW PAHs and FLU as it seems to do to the HMW PAHs. PYR was degraded by 64% 72 h into the fermentation at a rate of 71 mg l^{-1} d^{-1}, after which time no further PYR decrease was observed. The increased concentration of this PAH shows that higher initial PYR concentrations will result in higher aqueous phase concentrations sufficient for microbial transformation. The BAP concentrations remained relatively constant throughout the fermentation and after 120 h, only 11% had been degraded, corresponding to a rate of 7.5 mg l^{-1} d^{-1}. The aqueous solubility of BAP is approximately 100-fold smaller than that of PYR and it is possible that the increase in the initial BAP made in this two-phase system was still not high enough to generate sufficient concentrations in the aqueous phase. A substantially larger biomass concentration was obtained during this bioreactor experiment and can be attributed to the higher tryptone and yeast extract concentrations which may be acting as additional carbon sources. Although PYR was degraded at a rapid rate during this fermentation due to its higher loading, a second strategy for enhancing PAH availability (the use of a delivery phase with improved partitioning capacity) was also investigated.

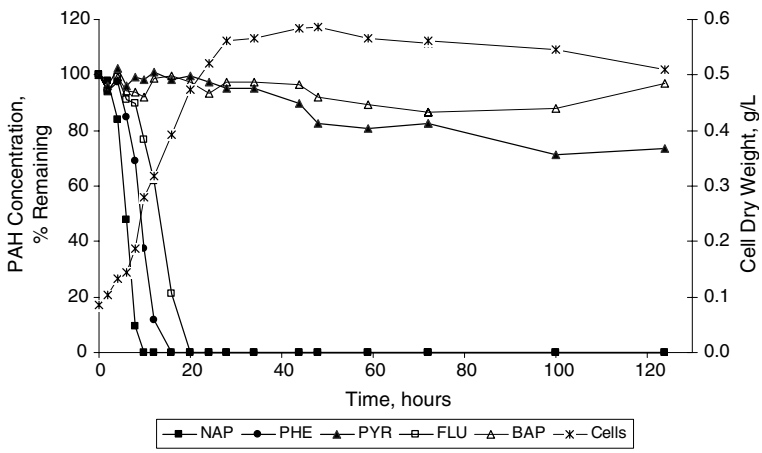

Fig. 2 Degradation of five PAHs and cell dry weight values for a defined consortium of *S. aromaticivorans* B0695 and *S. paucimobilis* EPA505 using a TPPB with a dodecane solvent phase

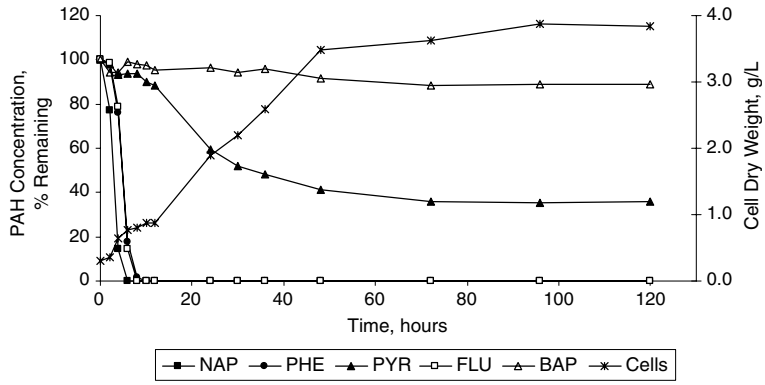

Fig. 3 Degradation of five PAHs with higher initial concentrations of the HMW PAHs and cell dry weight values for a defined consortium of *S. aromaticivorans* B0695 and *S. paucimobilis* EPA505 using a TPPB with a dodecane solvent phase

Comparison of K_{SW} for dodecane and silicone oil

The K_{SW} and log K_{SW} values determined experimentally for dodecane and silicone oil are presented in Fig. 4. Silicone oil was found to have a lower log K_{SW} value (3.9) compared to dodecane (4.3). The lower partitioning coefficient for silicone oil indicates that this solvent has a lower affinity for PYR and will release more PAHs into the aqueous phase where microbial degradation can take place. Silicone oil has a lower log K_{SW} value because HMW PAHs are much less soluble in silicone oil (Villemur et al. 2000) compared to dodecane. Therefore, the concentration of pyrene in the solvent will be much nearer to the saturation concentration of the solvent and, as a result, when the two phases are at equilibrium, the concentration of pyrene in the aqueous phase will also be closer to the saturation concentration. From the general principle, 'like dissolves like', or more specifically, polar solvents will dissolve polar solutes and non polar solvents will dissolve non polar solutes, the hydrophobicity inherent to PAHs makes them extremely soluble in non polar solvents, like dodecane. As the solubility of PAHs increases in specific solvents, the solvent will tend to sequester the PAH molecules and less will be transferred to the aqueous phase. PAHs are more soluble in dodecane than silicone oil; for example, phenanthrene is soluble in dodecane at 38.0 g l^{-1} (Janikowski et al. 2002) and is only soluble in silicone oil at 5.2 g l^{-1} (Villemur et al. 2000). It was concluded from these results that silicone oil would be a superior HMW PAH organic delivery phase for use in the TPPB in order to increase the PAH concentration in the aqueous phase. Silicone oils have been used in two-phase systems with mixed cultures because of their hydrophobicity, chemical stability, and resistance to hydrolytic and oxidative breakdown (Déziel et al. 1996).

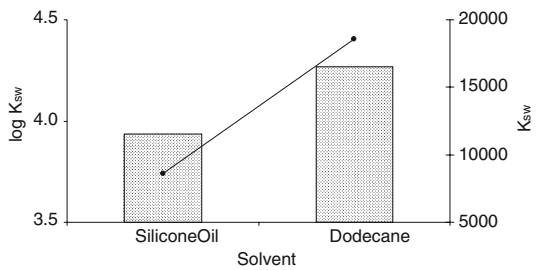

Fig. 4 The partitioning coefficients, K_{SW}, represented by the line graph and the log of these values, represented by the bar graph, for PYR in dodecane and silicone oil

Batch fermentation 3: use of silicone oil as organic delivery phase

The LMW PAHs and FLU were rapidly degraded (Fig. 5), with rates of 667 mg l^{-1} d^{-1} for NAP and PHE, 400 mg l^{-1} d^{-1} for FLU and an overall PAH consumption of 1200 mg l^{-1} d^{-1}. These rates are very similar to those obtained in the dodecane fermentation with higher initial concentrations of PAHs (Batch Fermentation 2) which suggests that the bioavailability of these PAHs is not hindered by the delivery phase used. PYR degradation commenced upon the near consumption of FLU, which is consistent with the sequential PAH degradation observed in the previous fermentations. Multiple PAHs are transformed by a common enzyme system and competitive

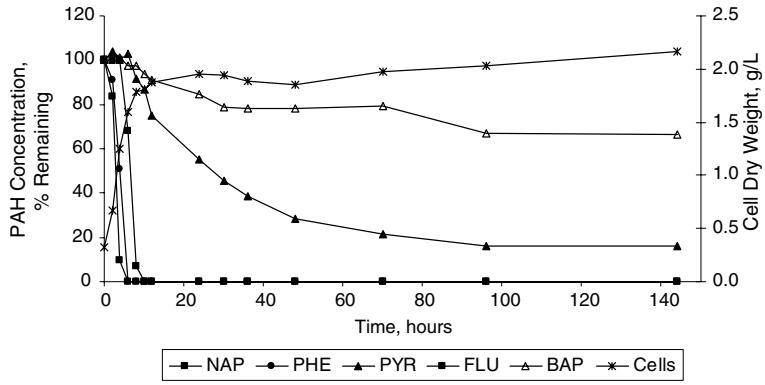

Fig. 5 Degradation of five PAHs and cell dry weight values for a defined consortium of *S. aromaticivorans* B0695 and *S. paucimobilis* EPA505 using a TPPB with a silicone oil solvent phase

inhibition has been suggested to cause sequential utilization of PAHs (Stringfellow and Aitken 1995; Trzesicka-Mlynarz and Ward 1995). After 96 h, 84% of the PYR had been degraded and this corresponds to a volumetric rate of 35 mg l^{-1} d^{-1}. This rate of PYR degradation exceeded that obtained in Batch Fermentation 1 and can be attributed to the ability of silicone oil to release PYR into the aqueous phase. BAP degradation was initiated upon the degradation of PYR and also leveled off at the same time. After 96 h, the BAP concentration had decreased by 34%, at a rate of 7 mg l^{-1} d^{-1}, based on aqueous volume, which was similar to the rate obtained in Batch Fermentation 2. Once again, the higher biomass accumulation is a result of the increased tryptone and yeast extract concentrations in the fermentation broth. The higher rates of PAH degradation using silicone oil has demonstrated this solvent's effectiveness at partitioning these molecules into the aqueous phase, making them more bioavailable for bacterial consumption.

Discussion

When using microbial consortia for the degradation of contaminants the overall degradative capacity is not merely the sum of the capacities of the individual strains forming the association (Ghazali et al. 2004). Rather, the enhanced degradative performance of consortia is often attributed to the effects of synergistic interactions among members of the microbial community, although the mechanisms by which these degraders benefit from the synergistic interactions may be very complex. Enhanced PAH degradation by microbial consortia compared to the individual species has been shown in the past (Trzesicka-Mlynarz and Ward 1995, Richard and Vogel 1999) and was also achieved during this work (Fig. 1). The mixed B0695/EPA505 culture showed a greater capacity for degrading the HMW PAHs compared to the pure cultures something that we have also observed in TPPBs as well (data not shown). The individual cultures and the consortium exhibited efficient PAH degradation performance with more water-soluble PAHs (NAP, PHE, and FLU), but a decreased PAH degradation capacity with the more hydrophopbic PAHs (PYR and BAP), which was a similar observation made by Trzesicka-Mlynarz and Ward (1995) for the degradation of a PAH mixture including BAP, anthracene, PHE, acenaphthalene, and fluorene by a mixed culture and the individual isolates (*Pseudomonas putida*, *Flavobacterium* sp., and *Pseudomonas aeruginosa*). The increased PAH degradation obtained with the consortium in this study may also be attributed to a synergism between B0695 and EPA505, although the mechanisms by which these species benefit from each other is still unknown. Synergistic interactions may include one species removing toxic metabolites, that may otherwise hinder microbial activities, of the species preceding it, or one species being able to degrade compounds that another species can only partially degrade (Ghazali et al. 2004).

During this study, modifications were made to the two-phase system in an attempt to improve the HMW PAH degradation achieved by the B0695/EPA505 consortium in Batch Fermentation 1.

Increasing the aqueous concentrations of the HMW PAHs enhanced their uptake by the microorganisms and subsequent transformation. These higher aqueous PAH concentrations were achieved by increasing the initial concentrations of the HMW PAHs in dodecane to obtain greater partitioning into the aqueous phase (Batch Fermentation 2). The rate of PYR biodegradation achieved in this two-phase system is almost two-fold greater than the degradation rates reported widely in the literature to date (Bouchez et al. 1997, Marcoux et al. 2000, Villemur et al. 2000, Guieysse et al. 2001, Mutnuri et al. 2005) and is second only to reports by MacLeod and Daugulis (2003) and Daugulis and McCracken (2003). Also, the rate of BAP degradation is at least 4 times greater than the degradation rates reported for this PAH in two-phase systems (Vanneck et al. 1995, Marcoux et al. 2000, Villemur et al. 2000, Kanaly and Watanabe 2004). Increasing the HMW PAH concentrations in the organic phase reduced the ability of this solvent to sequester the more hydrophobic PAHs leading to enhanced degradation by the B0695/EPA505 consortium.

The limited HMW PAH degradation obtained in Batch Fermentation 1 may be attributed to the low aqueous solubilities inherent to these PAHs. The lack of HMW PAH degradation is a common occurrence in nature as these contaminants are often associated with non-aqueous phase liquids or the organic content in soil resulting in insufficient aqueous phase concentrations for microbial degradation to take place. The results of the mass partitioning coefficient experiment showed that using silicone oil as the organic delivery phase should permit greater partitioning of the HMW PAHs into the aqueous phase resulting in elevated HMW PAH concentrations to support microbial growth, compared to when dodecane is used as the second phase. In another study, the greatest extent of HMW PAH (PYR, chrysene, BAP, and perylene) degradation by a microbial consortium in a two-phase system was observed using silicone oil, compared to 2,2,4,4,6,8,8-heptamethylnonane, paraffin oil, hexadecane, and corn oil when these materials were used as the second-phase (Marcoux et al. 2000). It was believed that the other solvents may have sequestered the PAHs, thus decreasing their solubilization in the aqueous phase and their availability to the microorganisms. A similar occurrence may have been observed in Batch Fermentation 1 that employed dodecane as the delivery phase, which has a high affinity for PAHs and was likely sequestering them from the aqueous phase. The higher extents of PAH degradation in Batch Fermentation 3 using silicone oil compared to that in Batch Fermentation 1 using dodecane has proven this solvent's effectiveness at partitioning these molecules into the aqueous phase, making them more bioavailable for bacterial consumption.

Solvent selection is critical when operating TPPBs and from the results obtained in this study it is evident that the chosen delivery phase can have a significant impact on the extents and rates of degradation achieved. Solvents to be employed in TPPBs are primarily chosen based on their water immiscibility, biocompatibility, and non-bioavailability to the microorganism(s) present in the aqueous phase (Collins and Daugulis 1999). Solvents that are cheap, non-toxic to humans, and possessing a high boiling point and a low volatility to prevent solvent and contaminant volatilization from the fermentor during air sparging, are all beneficial characteristics. Also, solvents with a high affinity for the target substrate are generally an advantage as they allow for high initial substrate loadings and the use of smaller solvent volumes, thus reducing costs. However, this high affinity can also be a disadvantage as the solvent may potentially sequester the insoluble substrate from the aqueous phase rendering it non-bioavailable.

The two solvents, dodecane and silicone oil, utilized in this study have both advantages and disadvantages associated with their potential and effectiveness as delivery phases in TPPBs. Although both solvents were non-toxic and non-biodegradable to both *S. aromaticivorans* B0695 and *S. paucimobilis* EPA505, as well as the consortium, these solvents vary in terms of cost, ability to be manipulated, and capability of partitioning PAHs into the aqueous phase. The price of silicone oil is twice that of dodecane and as the viscosity of silicone oil decreases, the price increases. Less viscous solvents are beneficial as they are more manageable in two-phase systems.

The chemical structure of silicone oil cannot be easily manipulated compared to organic solvents such as alkanes, where their properties can be changed by manipulating the chemical structures (i.e. selecting different chain lengths and/or extents of branching) to benefit the desired process. However, one advantage of silicone oil over dodecane is its increased capacity to partition the extremely hydrophobic HMW PAHs into the aqueous phase. Silicone oil provided the greatest extents of PYR and BAP degradation and depending on the clean-up requirements this may be an advantageous outcome compared to using dodecane. A similar effect can be achieved by increasing the concentrations of these PAHs in dodecane to attain the threshold concentration required for degradation, however, depending on the application these initial concentrations may not be realistic. The high affinity of dodecane for these PAHs inherently sequesters them from the microorganisms in the aqueous phase and reduces microbial degradation. Although this solvent may sequester the contaminant, the high solubilization capacity of dodecane for PAHs results in a decreased volume of solvent required. Silicone oil and dodecane are not the only potential solvents for delivering this PAH mixture to the B0695/EPA505 consortium and further screening and testing of various solvents may result in a second-phase that essentially alleviates the limitations associated with both of the solvents employed in this study.

Conclusions

The bioavailability of extremely hydrophobic contaminants, such as PAHs, in TPPBs may limit the rates of degradation achieved. The use of different solvents with differing partitioning capacities, as well as increased initial concentrations of the contaminant can alleviate these limitations and improve the extents and rates of biodegradation. Using such strategies this work has shown that a defined consortium of bacteria from the genus *Sphingomonas* is capable of PAH degradation rates of 1200–1500 mg l^{-1} d^{-1}, among the highest reported in the literature to date. PAH degradation in TPPBs has also been successfully conducted at pilot scale (Daugulis and Janikowski 2002) as a continuing effort to implement TPPB technology.

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References

Ashok BT, Saxena S (1995) Biodegradation of polycyclic aromatic hydrocarbons—A review. J Sci Indust Res 54:443–451

Bouchez M, Blanchet D, Vandecasteele JP (1997) An interfacial uptake mechanism for the degradation of pyrene by a *Rhodococcus* strain. Microbiology 143:1087–1093

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351–368

Collins LD, Daugulis AJ (1999) Benzene/toluene/*p*-xylene degradation. Part I. Solvent selection and toluene degradation in a two-phase partitioning bioreactor. Appl Microbiol Biotechnol 52:354–359

Daugulis AJ, Janikowski TB (2002) Scale-up performance of a partitioning bioreactor for the degradation of polyaromatic hydrocarbons by *Sphingomonas aromaticivora*ns. Biotechnol Lett 24:591–594

Daugulis AJ, McCracken CM (2003) Microbial degradation of high and low molecular weight polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp. Biotechnol Lett 25:1441–1444

Déziel E, Paquette G, Villemur R, Lepine F, Bisaillon JG (1996) Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. Appl Environ Biotechnol 62:1908–1912

Efroymson RA, Alexander M (1991) Biodegradation by an *Arthrobacter* species of hydrocarbons partitioned into and organic solvent. Appl Environ Biotechnol 57:1441–1447

Fredrickson JK, Balkwill DL, Drake GR, Romine MF, Ringelberg DB, White DC (1995) Aromatic-degrading *Sphingomonas* isolates from the deep subsurface. Appl Environ Biotechnol 61:1917–1922

Fredrickson JK, Balkwill DL, Romine MF, Shi T (1999) Ecology, physiology, and phylogeny of deep subsurface *Sphingomonas* sp. J Indust Microbiol Biotechnol 23:273–283

Ghazali FM, Rahman RNZA, Salleh AB, Basri M (2004) Biodegradation of hydrocarbons in soil by microbial consortium. Int Biodeterioration Biodegradation 54:61–67

Guieysse B, Cirne MdDTG, Mattiasson B (2001) Microbial degradation of phenanthrene and pyrene in a two-liquid phase-partitioning bioreactor. Appl Microbiol Biotechnol 56:796–802

Janikowski TB, Velicogna D, Punt M, Daugulis AJ (2002) Use of a two-phase partitioning bioreactor for degrading polycyclic aromatic hydrocarbons by a *Sphingomonas* sp. Appl Microbiol Biotechnol 59:368–376

Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133:71–84

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

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

Kanaly RA, Watanabe K (2004) Multiple mechanisms contribute to the biodegradation of benzo[*a*]pyrene by petroleum-derived multicomponent nonaqueous-phase liquids. Environ Toxicol Chem 23:850–856

Lantz S, Lin JE, Mueller JG, Pritchard PH (1995) Effects of surfactants on fluoranthene mineralization by *Sphingomonas paucimobilis* EPA 505 In: Hinchee RE, Brockman FJ, Vogel CM (Eds) Microbial processes for bioremediation, vol 3. Batelle, Columbus, Ohio, pp 7–14

MacLeod CT, Daugulis AJ (2003) Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent. Appl Microbiol Biotechnol 62:291–296

MacLeod CT, Daugulis AJ (2005) Interfacial effects in a two-phase partitioning bioreactor: degradation of polycyclic aromatic hydrocarbons (PAHs) by hydrophobic *Mycobacterium*. Process Biochem 40:1799–1805

Marcoux J, Déziel E, Villemur R, Lépine F, Bisaillon JG, Beaudet R (2000) Optimization of high-molecular-weight polycyclic aromatic hydrocarbons' degradation in a two-liquid-phase bioreactor. J Appl Microbiol 88:655–662

Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH (1990) Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl Environ Microbiol 56:1079–1086

Mutnuri S, Vasudevan N, Kaestner M (2005) Degradation of anthracene and pyrene supplied by microcrystals and non-aqueous-phase liquids. Appl Microbiol Biotechnol 67:569–576

Richard JY, Vogel TM (1999) Characterization of a soil bacterial consortium capable of degrading diesel fuel. Int Biodeterioration Biodegradation 44:93–100

Shi T, Fredrickson JK, Balkwill DL (2001) Biodegradation of polycyclic aromatic hydrocarbons by *Sphingomonas* strains isolated from the terrestrial subsurface. J Indust Microbiol Biotechnol 26:283–289

Shuttleworth KL, Cerniglia CE (1995) Environmental aspects of PAH biodegradation. Appl Biochem Biotechnol 54:291–302

Story SP, Kline EL, Hughes TA, Riley MB, Hayasaka SS (2004) Degradation of aromatic hydrocarbons by *Sphingomonas paucimobilis* Strain EPA505. Environ Contamin Toxicol 47:168–176

Stringfellow WT, Aitken MD (1995) Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading pseudomonads. Appl Environ Microbiol 61:357–362

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

Trzesicka-Mlynarz D, Ward OP (1995) Degradation of polycyclic aromatic hydrocarbons (PAHs) by a mixed culture and its component pure cultures, obtained from PAH-contaminated soil. Can J Microbiol 41:470–476

Vanneck P, Beeckman M, De Saeyer N, D'Haene S, Verstraete W (1995) Biodegradation of polycyclic aromatic hydrocarbons in a two-liquid-phase system. In: Hinchee RE, Anderson DB, Heoppel RE (Eds) Bioremediation of recalcitrant organics, vol 7. Battelle Press, Columbus, Ohio, pp 55–62

Villemur R, Déziel E, Benachenhou A, Marcoux J, Gauthier E, Lépine F, Beaudet R, Comeau Y (2000) Two-liquid-phase slurry bioreactors to enhance the degradation of high-molecular-weight polycyclic aromatic hydrocarbons in soil. Biotechnol Prog 16:966–972

Volkering F, Breure AM, van Andel JG, Rulkens WH (1995) Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons. Appl Environ Microbiol 61:1699–1705

Woo SH, Park JM (2004) Microbial degradation and enhanced bioremediation of polycyclic aromatic hydrocarbons. J Indust Eng Chem 10:16–23

Ye D, Siddiqi MA, MacCubbin AE, Kumar S, Sikka HC (1996) Degradation of polynuclear aromatic hydrocarbons by *Sphingomonas paucimobilis*. Environ Sci Technol 30:136–142