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Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp.

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Abstract

A mixture of six polyaromatic hydrocarbons (naphthalene, phenanthrene, fluoranthene, pyrene, chyrysene and benzo[a]pyrene), varying in size from 2 to 5 rings, was dissolved in dodecane, and used as the delivery phase of a partitioning bioreactor. Two species of *Sphingomonas* were then used individually, and as a consortium, to determine which of the PAHs were degraded. Only low molecular weight PAHs (naphthalene, phenanthrene and fluoranthene) were degraded by the individual strains, but the consortium degraded all substrates either to completion or near completion.

Introduction

Two Phase Partitioning Bioreactors (TPPBs) consist of an aqueous phase containing a microorganism and an immiscible and biocompatible organic phase into which large amounts of toxic or hydrophobic substrates can be dissolved. In the case of toxic substrates, equilibrium partitioning to the aqueous phase results in sub-inhibitory concentrations being delivered based on cellular demand (Daugulis 2001, Malinowski 2001) and, in the case of hydrophobic substrates, very large surface areas can be generated by dispersing the two phases, resulting in high access to the substrate, with corresponding enhanced reaction rates (Déziel *et al.* 2000, Daugulis & Janikowski 2002, Marcoux *et al*. 2000).

Polyaromatic hydrocarbons (PAHs) are poorly soluble materials that have been successfully degraded at unprecedented rates in TPPBs, although only the low molecular weight species, as biodegradability decreases dramatically with the number of rings (Table 1). We have previously shown rapid and effective degradation of low molecular weight PAHs using *Sphingomonas aromaticivorans* B0695 in a TPPB with dodecane as the delivery solvent (Daugulis & Janikowski 2002, Janikowski *et al.* 2002).

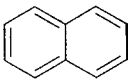
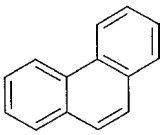
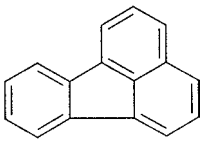
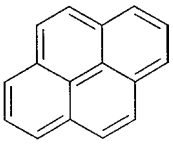
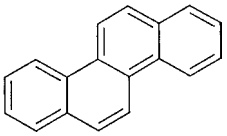
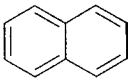
Here, we report on the use of B0695 in pure culture with a mixture of six PAHs in a TPPB. We have also examined another species of *Sphingomonas*, *Sph. paucimobilis* EPA 505 (able to degrade high molecular weight PAHs) to degrade the same mixture, and have also used a consortium of the 2 strains to seek possible synergies in degrading the PAH mixture.

Materials and methods

Substrates

The characteristics of the six PAHs used in this study are shown in Table 1. All compounds have a low solubility in water, but are highly soluble in dodecane, which makes them amenable for use in TPPB systems by virtue of the large masses that can be dissolved and the high surface area (and hence access) that can be provided via a dispersed second liquid phase.

Table 1. Properties of polyaromatic hydrocarbons used in this study.

PAH	Structure	Aqueous solubility at 25 °C (mg l^{-1})	Half-life in soil and sediment[a] (d)	EPA carcinogen class[b]
Naphthalene LMW		31.7	2.1–30.8	C
Phenanthrene LMW		1.29	16–126	D
Fluoranthene LMW		0.26	137–377	D
Pyrene HMW		0.135	19.4–630	D
Chrysene HMW		0.002	129–407	B
benzo[a]pyrene HMW		0.0038	211–>1400	B

[a]Shuttleworth & Cerniglia (1995).
[b]Under the US EPA's classification of carcinogenicity, class A compounds are 'known carcinogens', class B are 'probable carcinogens', class C are 'possible carcinogens', class D have insufficient evidence of carcinogenicity in their data to place them in any of the three higher classifications, and class E are non-carcinogenic.

Organisms and cultivation conditions

Sphingomonas aromaticivorans B0695 is relatively well characterized (Frederickson *et al.* 1995, 1999), and *Sphingomonas paucimobilis* EPA 505 has also received attention (Mueller *et al.* 1990, Ye *et al.* 1996) as a possible high molecular weight PAH degrading organism. In previous work (Janikowski *et al.* 2002), the critical logP of B0695 and solvents that B0695 cannot degrade were identified, leading to the selection of dodecane as the delivery solvent. Similar behaviour (data not shown) has recently been found for EPA505, and thus dodecane was used as the delivery solvent for both organisms. Cultivation conditions (Janikowski *et al.* 2002) were the same for both organisms, except that fluoranthene crystals were added in all sub-cultivations of EPA505, as this strain apparently loses its ability to degrade PAHs without such selection pressure.

Analytics

PAH concentrations in the organic phase were determined by fluorescence spectroscopy and cell concentration was determined by turbidity at 650 nm.

Bioreactor studies

Fermentations of the individual cultures, as well as the dual consortium, were run using an NBS BioFlo III fermentor. Three litres of minimal (carbon-free) medium (Janikowski *et al.* 2002) were added to the reactor and autoclaved; inocula of both B0695 and EPA505 were grown in liquid maintenance medium

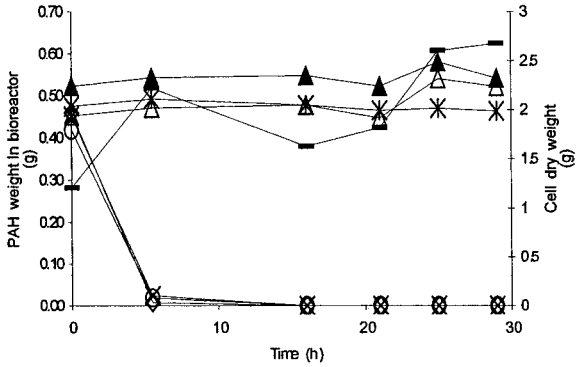


Fig. 1. Degradation of a mixture of low molecular weight and high molecular weight PAHs by *Sphingomonas aromaticivorans* B0695. Naphthalene, open diamond; phenanthrene, open circle; pyrene, closed triangle; fluoranthene, cross; chrysene, asterisk; benzo[a]pyrene, open triangle; cells, dash.

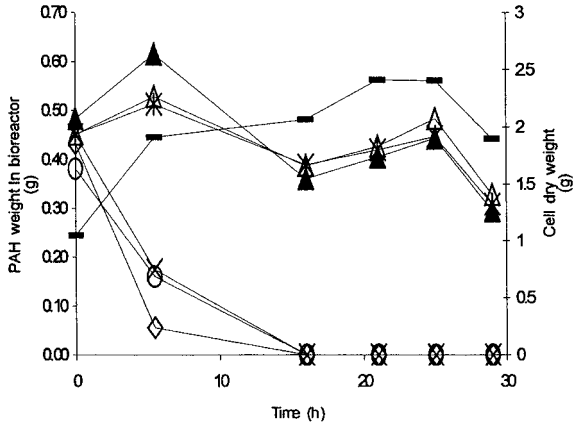


Fig. 2. Degradation of a mixture of low molecular weight and high molecular weight PAHs by *Sphingomonas paucimobilis* EPA505. Naphthalene, open diamond; phenanthrene, open circle; pyrene, closed triangle; fluoranthene, cross; chrysene, asterisk; benzo[a]pyrene, open triangle; cells, dash.

for 48 and 24 h respectively, and 300 ml of culture were added to the reactor (150 ml each of B0695 and EPA505 for the consortium fermentation). A solution of 0.5 g of each of naphthalene, fluoranthene, phenanthrene, pyrene, chrysene, and benzo[a]pyrene (or 0.25 g of each for the consortium fermentation) was prepared in 500 ml dodecane and added to the bioreactor. For each fermentation, the agitation speed, aeration rate, and temperature were maintained at 350 rpm, 2 l min^{-1}, and 30 °C, respectively.

Results and discussion

The results of the cultivation of B0695 and EPA505 on the mixtures of six PAHs are shown in Figures 1 and

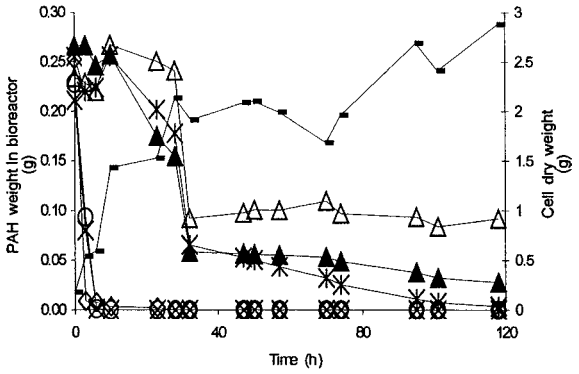


Fig. 3. Degradation of a mixture of low molecular weight and high molecular weight PAHs by B0695 and EPA505. Naphthalene, open diamond; phenanthrene, open circle; pyrene, closed triangle; fluoranthene, cross; chrysene, asterisk; benzo[a]pyrene, open triangle; cells, dash.

2, respectively. Note that there are some small fluctuations in the reported levels of some PAHs (particularly seen in Figure 2) due to the challenge associated with making the very large ($>10^6$) dilutions of the viscous solvent that are required by the fluorescence spectroscopy method. Each species degraded naphthalene, phenanthrene and fluoranthene very rapidly but the others remained untouched (even after 100 h, although only the first 30 h of fermentation are shown in these figures). For B0695 (Figure 1), naphthalene, phenanthrene, and fluoranthene are almost completely degraded in the first 6 h (98, 96, 95% degradation, respectively) and all three are 100% degraded in the first 16 h. This resulted in an overall volumetric degradation rate of 82 mg l^{-1} h^{-1} based on aqueous volume, and these rates of degradation of naphthalene and phenanthrene are consistent with the findings of Janikowski *et al.* (2002).

For EPA505 (Figure 2) the amounts of naphthalene, phenanthrene, and fluoranthene decreased by 87%, 58%, and 34%, respectively, in the first 5 h, and all were degraded to undetectable levels within 16 h. This results in an overall volumetric degradation rate of 32 mg l^{-1} h^{-1} based on aqueous volume. These results suggest that, in our hands, and/or in TPPB systems, each individual species of *Sphingomonas* is extremely efficient at degrading smaller PAHs, but their individual substrate spectra are limited to low molecular weight PAHs. That B0695 can degrade only low molecular weight PAHs is consistent with our own earlier work with this organism (Janikowski *et al.* 2002), but the inability of EPA505 to attack the high molecular weight PAHs is contrary to the observations

of Mueller *et al*. (1990) and Ye *et al.* (1996) who reported activity against high molecular weight PAHs by this organism.

In light of these results, the data in Figure 3 were unanticipated as all of the PAHs were degraded when a mixture of the two cell lines was used. The degradation of naphthalene, phenanthrene, and fluoranthene was again rapid, with 97, 59, and 62%, respectively, being degraded in the first 3 h, and each reaching 100% degradation within the first 28, 10, and 10 h, respectively. This degradation is comparable to that seen in the previous fermentations for both B0695 and EPA505 alone. Of particular interest, however, is the degradation of pyrene, chrysene, and benzo[a]pyrene. All three of these high molecular weight PAHs were initially degraded between 28 h and 32 h of the fermentation, after which they are further degraded more slowly to maximum degradations of 93, 99, and 64%, respectively. Although the degradation rates are modest relative to the low molecular weight PAHs, the extent and rate of degradation of these high molecular weight PAHs are significant.

As noted earlier, the ability to make insoluble substrates available to cells (by dissolving and dispersing large masses of PAHs) can be attributed to the TPPB process concept. Enhancing the solubility of PAHs through the use of surfactants has previously led to modest enhancements of degradation (Mueller *et al.* 1990), although these researchers also pointed out the potential toxicity of such solubilizing agents, and their possible preferential use as carbon sources. The somewhat contradictory, yet positive, result of being able to degrade all six PAHs by means of the consortium, even though we were not able to show individual utilization of all substrates, is unexplained and under investigation by us. Our observed result is, however, consistent with the statement by Mueller *et al.* (1990), '...the ability to degrade these chemicals may be provided through the action of other organisms acting in concert with EPA 505'.

In summary, our preliminary results have shown that, individually, *Sphingomonas aromaticivorans* B0695 and *Sphingomonas paucimobilis* EPA 505 can degrade only low molecular weight PAHs in TPPBs, although they can do so at very substantial rates. Neither can, on its own, degrade high molecular weight PAHs in TPPBs. As a consortium, they are much more effective in this role, and can degrade 4 and 5 ring PAHs relatively rapidly, with no apparent effect on low molecular weight degradation. The individual effects will be important to ascertain, and efforts are underway to establish a robust means of differentiating between these two strains, in co-culture, in TPPBs. Preliminary results have indicated that these two organisms have significantly different sensitivity to a range of antibiotics, and we plan to exploit this feature as a means of distinguishing and enumerating individual populations.

Acknowledgement

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