

Scale-up performance of a partitioning bioreactor for the degradation of polyaromatic hydrocarbons by *Sphingomonas aromaticivorans*

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Abstract

A partitioning bioreactor, consisting of an aqueous phase containing *Sphingomonas aromaticivorans* and an immiscible organic phase (dodecane), loaded with naphthalene and phenanthrene, was operated at two scales, 5 l and 150 l. Complete degradation of 15 g and 300 g, respectively, of these polyaromatic hydrocarbon (PAH) mixtures was achieved in 21 h in both cases resulting in a volumetric PAH degradation rate of 238 mg l⁻¹ h⁻¹ based on reactor aqueous volumes.

Introduction

Polyaromatic hydrocarbons (PAHs) are 2 to 5 ring aromatic compounds derived from both natural and anthropogenic sources and arise from the incomplete combustion of fuels or biomass. Many PAHs are toxic or carcinogenic (Keith & Telliard 1979), and are exceedingly recalcitrant to degradation due to their inhibitory nature and their very low aqueous solubility, properties that generally become more pronounced as the number of rings increases.

Although numerous approaches have been used to degrade PAHs [e.g. packed bed systems (Guieysse *et al.* 2000)] the use of two-phase partitioning bioreactors (TPPBs) holds significant promise when dealing with toxic or poorly soluble substrates (Daugulis 2001). TPPBs are characterized by an aqueous phase containing the cells, and an immiscible, and biocompatible, organic phase that partitions toxic/insoluble substrates to the cells based on their metabolic demand. Although very large amounts of organic substrates can be added to a bioreactor, the cells 'see' only very low concentrations, and the rate of delivery of the xenobiotics from the organic phase to the microbes is demand based and driven by the system trying to maintain thermodynamic equilibrium (Daugulis & Collins 2001). TPPB technology has been demon-

strated for the destruction of large quantities and concentrations of phenol (Collins & Daugulis 1997), benzene (Yeom & Daugulis 2001, Yeom *et al.* 2000), benzene/toluene/xylene (BTX) (Collins & Daugulis 1999), and most recently PAHs (Guieysse *et al.* 2001, Janikowski 2001).

In the present work we have examined the performance of a TPPB system to degrade two relatively simple PAHs, naphthalene and phenanthrene, at 5 l and 150 l, as a preliminary evaluation of the potential to scale up TPPB operation.

Materials and methods

Microorganism

Sphingomonas aromaticivorans B0695, previously isolated from deep subsurface sediment of the Atlantic Coastal Plains, was generously provided by Dr David Balkwill of the Department of Biological Science, Florida State University, USA.

Medium and culture conditions

Maintenance medium was based on a modified Luria Bartani broth developed by Lantz *et al.* (1995). Stock

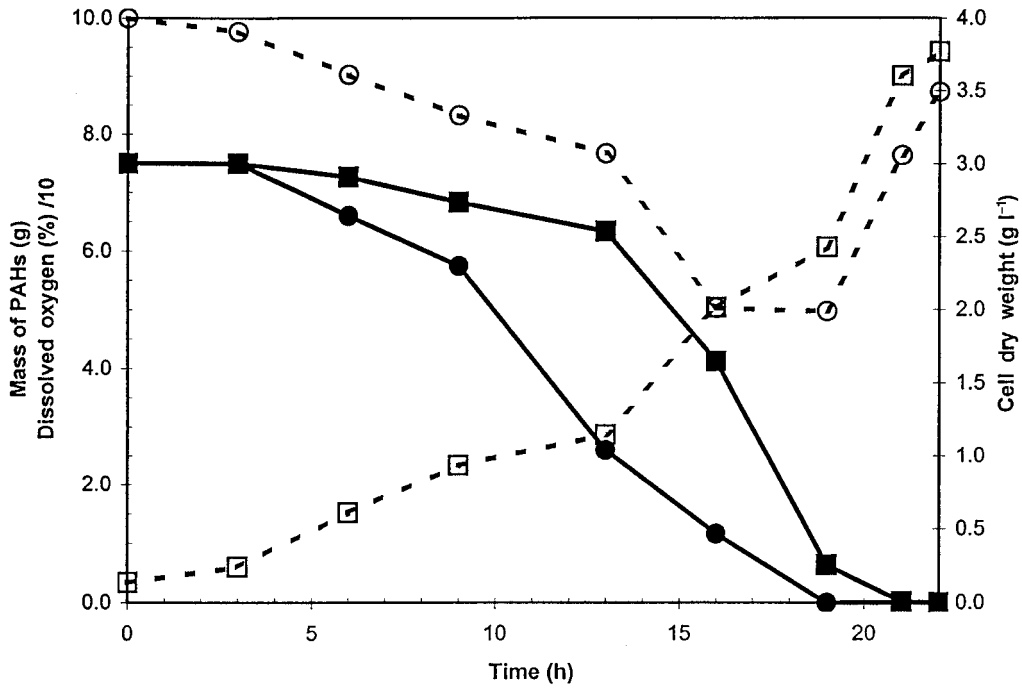


Fig. 1. Degradation of 7.5 g naphthalene and 7.5 g phenanthrene in a 5 l partitioning bioreactor. ●, Naphthalene; ■, phenanthrene; ○, dissolved oxygen; □, cells. Operating conditions: pH 6.2, 300 rpm, 1 vvm.

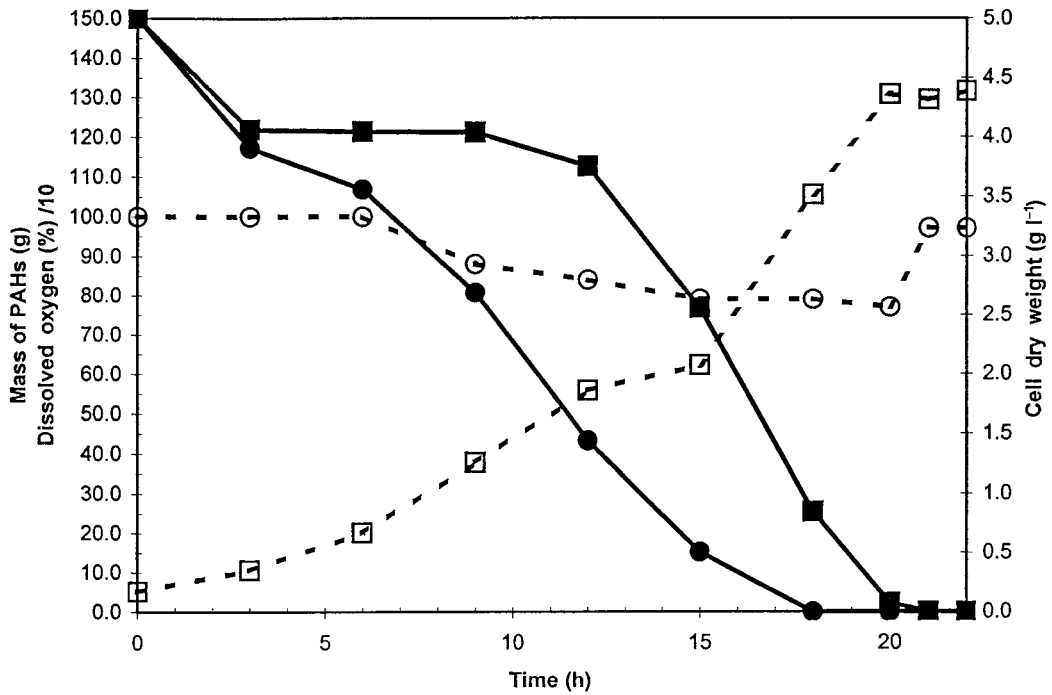


Fig. 2. Degradation of 150 g naphthalene and 150 g phenanthrene in a 150 l partitioning bioreactor. ●, Naphthalene; ■, phenanthrene; ○, dissolved oxygen; □, cells. Operating conditions: pH 6.2, 200 rpm, 3 vvm.

Table 1. Properties of dodecane related to its use as a solvent in a TPPB for the destruction of PAHs.

Melting point (°C)	Boiling point (°C)	Density (g ml ⁻¹)	Log <i>P</i>	Solubility of naphthalene ^a (g l ⁻¹)	Solubility of Phenanthrene ^a (g l ⁻¹)
-9.6	216.3	0.749	6.1	95.1	38.0

^aFrom Janikowski (2001).

cultures were maintained on sterilized maintenance medium with the addition of 15 g agar per l and sub-cultured monthly. Inoculum for the two scales of operation consisted of 24 h cultures grown in shake flasks on maintenance medium with 1 g glucose per l as carbon source. Inoculum volumes were 110 ml for the 5 l bioreactor (aqueous volume of 3 l) and 2.2 l for the 150 l bioreactor (aqueous volume 60 l). During PAH degradation experiments the medium formulation was altered to ensure that PAHs were the sole carbon source [with the exception of small amounts of tryptone (1.5 g l⁻¹) and yeast extract (0.75 g l⁻¹); thus, glucose was omitted entirely and, instead, naphthalene and phenanthrene were added via the solvent (dodecane) phase. The solvent volumes and masses of PAHs were: 0.5 l, 7.5 g naphthalene, 7.5 g phenanthrene for the small scale; and 10 l, 150 g naphthalene and 150 g phenanthrene for the pilot scale. During operation at each scale, a small bolus of additional yeast extract and peptone (at the concentrations noted above) was added approximately mid-way through the experiments. The bioreactors consisted of a 5 l Bioflo III (New Brunswick Scientific) and a 150 l MBR (Sulzer Biotech) fermenter operating with pH control (pH of 6.2), 30 °C, 300 rpm and an aeration rate of 1 vvm for the 5 l unit, and 200 rpm and 3 vvm for the pilot scale system. Dissolved O₂ was monitored by means of a polarographic electrode. Antifoam 289 (Aldrich) was added, as required, to each vessel.

Analytical procedures

Cell concentration in the aqueous phase was monitored by centrifuging samples, aspirating off the solvent phase (the system operated as a dispersion with complete mixing between the two phases), vortexing, and measuring the turbidity at 650 nm. The actual cell concentration was calculated from a previously prepared calibration curve. PAH concentration changes in the organic phase were monitored by diluting the samples 10- or 20-fold in dichloromethane in order to improve peak elucidation and injecting 1 μl of the diluted sample directly into a Perkin Elmer Gas Chro-

matograph equipped with a flame ionization detector and an Agilent J&W DB-5.625 nonpolar column. N₂ was used at 10 ml min⁻¹. The PAHs were detected using the following temperature program: the column temperature was held at 40 °C for 2 min, then increased by 5 °C min⁻¹ to 160 °C, then increased by 20 °C min⁻¹ to 270 °C and held at constant temperature for 15 min. The injector and detector were maintained at 250 °C and 300 °C, respectively.

Results and discussion

Table 1 shows some of the key properties of dodecane, which had been previously identified as an appropriate solvent for PAH degradation with *Sphingomonas aromaticivorans* B0695 in a TPPB (Janikowski 2001). The log *P* of a solvent is defined as the logarithm of its partition coefficient when placed into a two-phase system consisting of octanol and water. The critical log *P* for an organism is defined as the log *P* of a solvent at which organism growth in the aqueous phase is not adversely affected by the presence of the solvent. From Table 1, the log *P* of dodecane (6.1) was above the critical log *P* of this organism (3.8) as previously determined (Janikowski 2001), the boiling point is relatively high to minimize losses due to volatilization, good phase stability is obtained due to dodecane's low density, and the PAHs used in this study are highly soluble in this solvent.

The degradation of naphthalene and phenanthrene in the TPPB at the 5 l scale is depicted in Figure 1. After a short lag, both PAHs were degraded rapidly, and simultaneously, although naphthalene was consumed somewhat more quickly than was phenanthrene. During PAH degradation, the dissolved O₂ (DO) decreased and cell concentration increased until the PAHs were consumed after 21 h of operation. Shortly after this time the cell concentration leveled off and the DO returned to near its saturation level, reflecting the fact that the substrates had become depleted. The cell yield in this experiment was estimated to be 0.73 g cells g⁻¹ PAH consumed, which

is also consistent with an earlier study (Janikowski 2001), and the volumetric rate of PAH degradation was $238 \text{ mg PAH l}^{-1} \text{ h}^{-1}$ based on the aqueous volume of the system (3 l).

A similar performance was obtained for the pilot scale system (Figure 2), with an initial short lag (the early drop in PAH concentration was likely due to volatilization arising from a brief condenser malfunction) followed by rapid and simultaneous PAH degradation. The DO and cell concentration profiles were similar to those found in the 5 l case, although the DO did not decline as much at the larger scale, likely due to enhanced oxygen transfer arising from the higher aeration rate. The estimated cell yields and volumetric degradation rates were also similar and were found to be $0.84 \text{ g cells g}^{-1} \text{ PAH consumed}$, and $238 \text{ mg PAH l}^{-1} \text{ h}^{-1}$, respectively, based on the aqueous volume of 60 l.

Qualitatively, operation at both scales resulted in similar behaviour, with extensive foaming being experienced during the rapid degradation periods (controlled by the addition of antifoam), and significant colour transformation occurring as the cultures changed from off-white to pale yellow to yellow-orange over the course of 24 h. As noted above, the systems were operated as dispersions with the phases being completely mixed during the fermentation, but the phases in the samples easily separated after allowing for a period to stand.

Conclusion

The degradation rates of the PAHs in this study were similar to those found in other work from our laboratory (Janikowski 2001), and substantially higher than that seen for another recent TPPB study (Guieysse *et al.* 2001) of approximately $1.4 \text{ mg l}^{-1} \text{ h}^{-1}$ vs. $238 \text{ mg l}^{-1} \text{ h}^{-1}$ in the present work, based on aqueous volume. Dodecane is an excellent solvent for use in a TPPB, both from the standpoint of having a high capacity for PAHs and for physical (non-volatile, no tendency to form emulsions) and biological reasons (biocompatible, non-bioavailable). Performance of the

TPPB system for PAH degradation would also appear to be highly reproducible at both the laboratory and pilot scales.

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