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Short Communication

Biodegradation of pyrene by *Mycobacterium frederiksbergense* in a two-phase partitioning bioreactor system

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Abstract

Biodegradation of pyrene by *Mycobacterium frederiksbergense* was studied in a two-phase partitioning bioreactor (TPPB) using silicone oil as non-aqueous phase liquid (NAPL). The TPPB achieved complete biodegradation of pyrene; and during the active degradation phase, utilization rates of 270, 230, 139, 82 mg l^{-1} d^{-1} for initial pyrene loading concentrations (in NAPL) of 1000, 600, 400 and 200 mg l^{-1}, respectively, were obtained. The degradation rates achieved using *M. frederiksbergense* in TPPB were much higher than the literature reported values for an *ex situ* PAH biodegradation system operated using single and pure microbial species. The degradation data was fitted to simple Monod, logistic, logarithmic, three-half-order kinetic models. Among these models, only exponential growth form of the three-half-order kinetic model provided the best fit to the entire degradation profiles with coefficient of determination (R^2) value >0.99. From the experimental findings, uptake of pyrene by the microorganism in TPPB was proposed to be a non-interfacial based mechanism.

Keywords: *Mycobacterium frederiksbergense*; Pyrene degradation; Two-phase partitioning bioreactor; Kinetics; Three-half-order kinetic model

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) compose of two or more fused aromatic rings, and are ubiquitous chemicals produced via natural and anthropogenic sources. Many of these compounds are toxic, carcinogenic and mutagenic in nature (Yuan et al., 2000). Because of strict environmental legislations concerning their discharge, several treatment methods have been proposed in the past and recent years (Venkata-Mohan et al., 2006). One such microbe-based method of recent interest is in the use of two-phase partitioning bioreactors (TPPBs). TPPBs are characterized by an immiscible organic phase loaded with a target substrate that partitions into the aqueous phase containing microorganisms, and the partitioning is based on equilibrium considerations and real-time demand of the organisms (Daugulis, 2001). Hydrophobic nature of PAHs makes them ideal candidates of degradation in TPPB systems since high concentration and large surface area could be achieved by dissolving PAHs in the dispersed organic phase (Daugulis and Janikowski, 2002).

Déziel et al. (1999) proposed mainly three mechanisms by which microorganisms utilize substrates in TPPB systems: uptake of dissolved substrate from the aqueous phase, aqueous-organic interfacial uptake and uptake of substrate by direct contact with the organic phase. However, a predominant uptake mechanism has been shown to be the interfacial uptake (MacLeod and Daugulis, 2005), where the substrate is taken up by microorganism present in the interfacial area and therefore the degradation rate is limited mainly by agitation rate and phase volume ratio of the two phases in TPPB system.

Mycobacterium frederiksbergense – a fast growing PAH degrading strain has shown good potential to degrade pyrene as sole carbon source (Willumsen et al., 2001). But

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there are no reports available to establish its pyrene degradation potential in TPPB systems. This is the first time we are reporting the successful use of *M. frederiksbergense* in TPPB system for biodegradation of pyrene, which seem to follow a non-interfacial based uptake mechanism.

2. Methods

2.1. Chemicals and reagents

Analytical grade pyrene and silicone oil (dimethylpolysiloxane) used in the study were purchased from Sigma–Aldrich Chemicals, India and Loba Chemie, India, respectively; media components were purchased from Himedia laboratory, India. All the other chemicals, reagents and solvents used were of analytical grade and purchased from Merck, India.

2.2. Microorganism and culture conditions

M. frederiksbergense NRRL B-24126 was obtained from Microbial Genomics and Bioprocessing Research unit, USDA, Illinois, USA. Nutrient Broth was used for routine growth (at 28 °C and 140 rpm shaking) and maintenance of *M. frederiksbergense*. Bushnell Hass (BH) media having the composition ($g\,l^{-1}$): $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; K_2HPO_4, 1.0; NH_4NO_3, 1.0; $FeCl_3$, 0.05 and supplemented with trace elemental solution (2 ml l^{-1} media) containing ($g\,l^{-1}$) KI, 0.30; $SnCl_2 \cdot 2H_2O$, 0.43; LiCl, 0.20; $CuSO_4 \cdot 5H_2O$, 0.80; $AlK(SO_4)_2 \cdot H_2O$, 2.10; $NiCl_2$, 0.55; $CoCl_2 \cdot 6H_2O$, 0.85; boric acid 0.60; $MnSO_4 \cdot H_2O$ 0.37 and $FeSO_4 \cdot 7H_2O$, 0.30 was used to study pyrene biodegradation.

2.3. Pyrene biodegradation in bioreactor

All biodegradation experiments in this study were carried out in two identical (3 l) autoclavable glass fermenters (Applikon® with ADI 1010 BioController and ADI 1025 BioConsole) equipped with pH, temperature, dissolved oxygen sensors for monitoring and/or control. The bioreactors were operated at 28 °C, pH 7.0, aeration rate of 1.5 vvm and agitation rate, 600 rpm.

For pyrene biodegradation study in slurry phase system, calculated amount of pyrene stock solution (25 $g\,l^{-1}$ in acetone) was added to the empty reactor vessels so as to give a final pyrene concentration of 50 $mg\,l^{-1}$. Following evaporation of acetone, 1.5 l BH media supplemented with trace elemental solution was added to the vessel, and then autoclaved. Fifty milliliters of overnight grown *M. frederiksbergense* culture was aseptically added into the bioreactor as inoculum. Duplicate samples, of 1.5 ml each, were collected every 24 h for 12 d to measure residual pyrene concentration.

Pyrene biodegradation in TPPB system was studied using the same set of fermenters under similar operating conditions as previously mentioned. One liter BH medium supplemented with trace elemental solution constituted the aqueous phase of the system. Organic phase was prepared by dissolving required amount of pyrene in 250 ml of silicone oil and incubating the mixture in an ultrasonic water bath for 1 h, so as to give pyrene concentrations in the range of 200–1000 $mg\,l^{-1}$ in silicone oil. Both the aqueous and organic phases were loaded in the reactor vessels, autoclaved and then inoculated with the organism, as described above. During its operation, duplicate samples were withdrawn from the reactor every 24 h for 15 d for measuring residual pyrene concentration in the organic phase.

2.4. Analytical methods

For analysis of pyrene in silicone oil samples, the samples were pretreated with anhydrous sodium sulphate to remove any trace amount of water. Treated sample (0.1 ml) was then extracted with 1 ml of methanol by vortexing for 1 min and then centrifuging at 10 000*g* for 10 min to allow phase separation.

As aqueous solubility of pyrene is very low (0.13 $mg\,l^{-1}$), added pyrene remained as finely suspended particle and samples from slurry phase bioreactor were treated separately. One ml of the sample was extracted twice with equal volumes of ethyl acetate by vortexing for 1 min and then centrifuging at 10 000*g* for 10 min to allow phase separation. Ethyl acetate extracts were then pooled and total volume adjusted to 1 ml.

The sampling and extraction in both the cases were highly reproducible with relative standard deviation of ±4.29% and ±5.12% for slurry phase and TPPB systems, respectively.

Pyrene concentrations in all samples were quantified using synchronous fluorescence spectroscopy (Patra and Mishra, 2001) following proper dilution. Synchronous spectra were collected using a FluoroMax-3® (HORIBA Jobin Yvon, USA) fluorescence spectrometer. Excitation and emission slits were set to 2.5 nm and 5 nm, respectively, for all the measurements. The synthetic quartz cuvettes used were of Hellma® type 104.045Q with a path length of 10 mm. Synchronous scans were performed to generate unique peak for pyrene. The detection condition for the synchronous scan of pyrene was: $\Delta\lambda = 36.0$ nm, emission peak maximum = 369.7 nm and integration area = 366–375 nm.

3. Results and discussion

3.1. Pyrene biodegradation

3.1.1. Biodegradation in slurry phase system

Pyrene in slurry phase reactor was completely degraded within 200 h for initial concentration of 50 $mg\,l^{-1}$. No initial lag phase in degradation was observed with the slurry reactor but overall degradation rate was only 6 $mg\,l^{-1}\,d^{-1}$.

Pyrene degradation in slurry phase system has been widely studied. For instance, Habe et al. (2004) have