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Effect of NAPL(non-aqueous phase liquid) on enhanced biodegradation of phenanthrene

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ABSTRACT

NAPL이 다핵방향족화합물의 하나인 phenanthrene 의 생분해에 미치는 영향을 알아보았다. *Pseudomonas putida* CRE7 을 이용한 실험에서 NAPL 의 첨가로 인한 가장 큰 차이는 미생물의 소수성의 변화였다. 소수성이 증대됨으로써 phenanthrene 의 가용성이 증대되었으며, 이로 인해 더 많은 양의 오염물 분해가 이루어졌다. 생물학적 분해의 관찰은 발생되어지는 $^{14}CO_2$의 radioactivity 측정을 통해 이루어졌으며, 미생물의 소수성 측정은 bacterial adhesion to hydrocarbon (BATH) assay 를 이용하였다.

key word : NAPL, phenanthrene, radioactivity, biodegradation

Ⅰ. Introduction

Polycyclic aromatic hydrocarbons (PAH) are toxic, carcinogenic and mutagenic compounds (Amgren et al., 1979) which are found in the ground of industrial plants like gas works, coking plants, and petroleum refineries. As constituents of NAPL such as coal tar, creosote, and petroleum distillates, PAH serve as long-term source of groundwater contamination while remaining trapped in the pores of soil and aquifer matrix (Mercer et al., 1990).

In the biodegradation of PAH, it is apparent that the presence of multiple chemicals in various amounts and phases modifies biological activity and biodegradation rate (Armstrong et al., 1991). Most of previous works on the biodegradation of PAH from NAPL has been focused on the elucidation of physicochemical effect of NAPL such as the partitioning of PAH between NAPL and aqueous phase, overlooking the possible change of cells in the presence of NAPL (Köhler et al., 1994; Efroymson and Alexander, 1994; Labare and Alexander, 1995; Gamerdinger et al., 1995; Carroquino and Alexander,

1998).

In this work, emphasis has been put on the change of cells brought by the introduction of NAPL and the enhancement effect of NAPL is discussed with the quantitative comparison of the mineralization of phenanthrene with and without NAPL.

Ⅱ. Materials and methods

1. Microorganism

Pseudomonas putida CRE7 was obtained from Mike Montgomery, Geo-Center, Inc., Naval Research Lab, Washington, DC. This culture was maintained at room temperature on mineral salts medium (MSM) agar plates using phenanthrene as sole carbon source and transferred monthly. This strain did not pruduce or utilize biosurfactants during growth on MSM containing phenanthrene (Zhang and Miller, 1992).

2. Chemicals

Phenanthrene (98% pure) was purchased from Aldrich Chemical Company (Milwaukee, WI). [9-^{14}C]phenanthrene (13.1 mCi/mmol), unlabeled hexadecane and [1-^{14}C]hexadecane (2.2 mCi/mmol) were purchased from Sigma Chemical Co. (St. Louis, MO). Radioactivity was determinied using a Packard liquid scintillation analyzer Model TR (Packard Instruments, Meriden, CT). Unless otherwise noted, the scintillation cocktail used in this study was Scintiverse BD (Fisher Scientific, Pittsburgh, PA). Other chemicals used were reagent grade.

3. Mineralization of phenanthrene

Biodegradation of phenanthrene was quantified by measurement of $^{14}CO_2$ evolved during growth on [^{14}C]phenanthrene. For mineralization experiments, a mixture of phenanthrene and [^{14}C]phenanthrene was prepared by adding 50 uL of [^{14}C]phenanthrene into stock solutions (10 mL of chloroform containing 100 mg of dissolved phenanthrene) to make the mixture solution to have <105 dpm. 0.5 mL of the mixture solution was carefully added to the bottom of 125-mL micro-Fernbach flask (Wheaton, Millville, NJ). After evaporation of chloroform, 10 mL of MSM was added to the flask. The concentration of phenanthrene in the MSM comes to be 500 mg/L (500 ppm). Finally, each flask was inoculated (2%) with a culture pregrown on MSM containing phenanthrene (500 ppm) as the sole carbon and energy source at room temperature for 1 day. The flasks were sealed with specially designed caps, incubated and purged periodically to collect $^{14}CO_2$. Hexadecane was chosen to represent NAPL, and was added after the inoculation.

4. Bacterial adhesion to hydrocarbons (BATH) assay

General protocol was refered to Zhang and Miller (1994). In brief, cells grown for 2 days were centrifuged and pellets were washed with MSM two times to remove any interfering solutes, particulary hexadecane which was added in the experiments. Cells were then resuspended in MSM and adjusted to an optical density of 1.0 at 400 nm. Hexadecane (1.0 mL) was added to 4 mL of cell suspension in a 16 x 100 mm capped test tube, and vortexed for 60 s. The mixture was then allowed to separate for 30 min and turbidity of the aqueous phase at 400 nm was measured.

Ⅲ. Results and discussion

Effect of NAPL on the biodegradation of phenanthrene was investigated. Mineralization of phenanthrene was monitored by measuring the radioactivity from evolved $^{14}CO_2$ during growth. As shown in Fig. 1, enhanced mineralization of phenanthrene over control was observed for the experiments of hexadecane addition. Cells grown on the hexadecane as sole carbon source did not show any growth, and for the expeirment of phenanthrene and labelled hexadecane, growth was observed from the change of medium color but no significant amount of $^{14}CO_2$ evolution was detected. Thus, CRE7 does not seem to utilize hexadecane as carbon source.

Table 1 shows the result of BATH assay for the measurement of the relative hydrophobicity of cells. Cells grown in the presence of NAPL, were adhered to hexadecane phase two times more than the cells grown without NAPL. It suggests that in the presence of NAPL, cells are not only in aqueous phase but also in interface of NAPL and aqueous medium, which gives additional chance to contact with phenanthrene in NAPL which had been distributed from aqueous phase. On the other hand, cells are in aqueous phase only, in the absence of NAPL.

This change in cell hydrophobicity and the resulting difference in bioavailability seem to be related with enhancement in the biodegradation of phenanthrene. In this context, it is apparent that the enhancement over control was achieved due to the increased availability of carbon substrate.

In summary, the results of this study present that the effect of NAPL is the enhancement of PAH mineralization by increasing the hydrophobicity of cells and by supplying additional carbon dissolved in NAPL.

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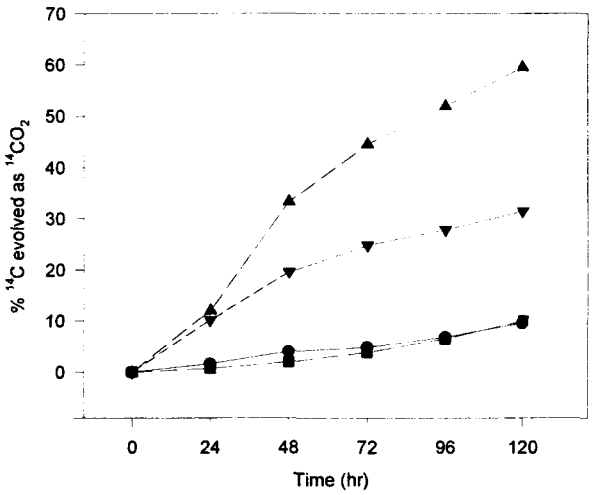

Figure 1. Effect of NAPL (hexadecane) on the mineralization of phenanthrene. (▼) 5 mg phenanthrene*, (▲) 5 mg phenanthrene* and 100 mg NAPL, (●) 5 mg phenanthrene and 100 mg hexadecane*, (■) 100 mg hexadecane* only. *: denotes radioactive-labelled., Averages of duplicates were shown in the plot.

Table 1. Hydrophobicity of *P. putida* CRE7 (% adhered) grown on phenanthrene in the presence or absence of hexadecane. Cells were grown in mineral salts medium with or without NAPL at room temperature for 2 days, washed and assayed.

Phenanthrene (mg)	NAPL (mg)	% Adhered to hexadecane (mean±SD)
0.5	0	35±3
0.5	10	73±6
5	100	75±5