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Mathematical evaluation of intermediates accumulation during microbial phenanthrene degradation

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Abstract–Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the soil environment, and considered to be hazardous due to their toxic and carcinogenic properties. Intermediates accumulation during PAHs degradation significantly alters the overall biodegradation rate and toxicity of the soil environment. The biodegradation pathway of phenanthrene, a 3-ring PAH, consisting of 14 enzymatic steps was analyzed to determine the release pattern of the intermediates by mathematical calculation of permeability using a membrane transport model. The intermediates with high permeability such as 1-hydroxy-2-naphthoic acid were consistent with the compounds frequently observed in laboratory or field in the literature.

Key words: Biodegradation, Intermediate, Membrane Transport, Modeling, Phenanthrene

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are among the most common organic pollutants found in air, water, soil, and sediment. PAHs are released to the environment in high concentrations mainly by industrial activities, handling petrochemical products, such as oil spills and leaks, gasification, wood preservation, and waste incineration [Woo et al., 2004]. Two or three ring PAHs are acutely toxic, while high molecular weight PAHs are considered to be genotoxic [Cerniglia, 1992]. Due to their stability of condensed ring structure, the intrinsic biodegradation rate is very low. Their hydrophobicity and tendency to sorb to organic matters greatly decrease the portion of PAHs available to microorganisms [Cho et al., 2005; Woo et al., 2001]. Numerous intermediates are formed during complete biodegradation of PAHs, which complicate their biodegradation mechanism by altering bioavailability, carbon balance, and toxicity.

The biodegradation pathway of low-molecular-weight PAHs (two- or three-ring) has been demonstrated by a number of researchers [Cerniglia, 1992]. For example, phenanthrene, a 3-ring PAH, is degraded by some bacteria through one of two different routes. Both routes have the same "upper pathway" for the degradation of phenanthrene to 1-hydroxy-2-naphthoic acid. However, the "lower pathway" is divided into two routes [Kiyohara and Nagao, 1978]. In one route, 1-hydroxy-2-naphthoic acid is oxidized to 1,2-dihydroxynaphthalene, which is further degraded via the naphthalene pathway to salicylic acid, which can be further metabolized. In the other pathway, the ring of 1-hydroxy-2-naphthoic acid is cleaved and further metabolized via the phthalate pathway.

Complete degradation of phenanthrene requires a number of enzymatic steps (at least 14 steps in the naphthalene pathway, Fig. 1) and consequently produces numerous intermediates. However, only a few common intermediates such as 1-hydroxy-2-naphthoic acid have been found in laboratory or field studies [Adachi et al., 1999; Balashova et al., 2001; Barnsley, 1983; Cerniglia and Yang, 1984; Doddamani and Ninnekar, 2000; Evans et al., 1965; Moody et al., 2001; Prabhu and Phale, 2003; Samanta et al., 1999]. The limited intermediate accumulation may be explained by genetic regulation and flux control of enzymatic steps [Woo and Rittmann, 2000]. As another reason, this may be related to the chemical properties of intermediates affecting their release by transport through cell membrane. The objective of this study was to estimate the permeability of the intermediates mathematically by using a membrane transport model in order to explain why only a few common intermediates are found in extracellular media.

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MATHEMATICAL MODELING

1. Membrane Transport Model

The general structure of biological membrane is a phospholipid bilayer. Fig. 2 shows a schematic illustration of the biodegradation and transport of intermediates in the cell membrane. Phenanthrene on the outer surface of the cell membrane is transported into the cell membrane mainly by a diffusion process. After that, phenanthrene begins to be degraded by membrane-bound enzymatic reactions and consequently produces various intermediates. The intermediates produced at a specific enzymatic step have two different fates: rapid transfer to the next enzymatic reaction or release out of the outer membrane by diffusion. Therefore, some intermediates can be found in liquid media by the release mechanism. The amount of the intermediates released into liquid media depends on its permeability, its concentration gradient between cell membranes, and thickness of cell membranes. Other factors except for permeability can be assumed to be identical for all the intermediates. The permeability of the intermediate of interest is related to its partition coefficient between biological membrane and water. The permeabil-

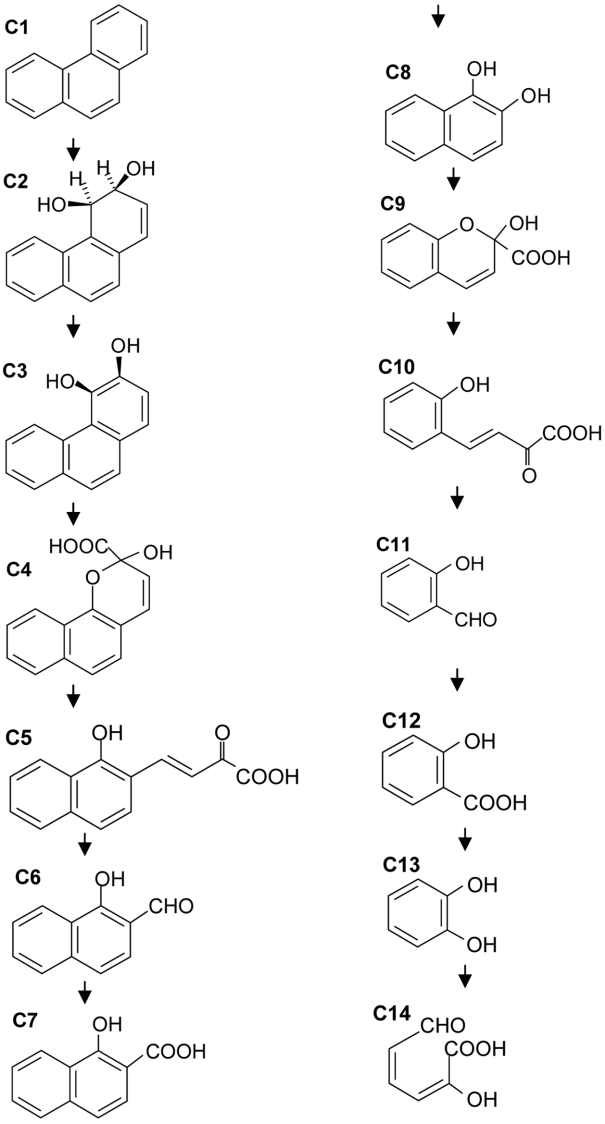

Fig. 1. Biodegradation pathway of phenanthrene via the naphthalene pathway [Woo and Rittmann, 2000].
C1: Phenanthrene, C2: *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene, C3: 3,4-dihydroxyphenanthrene, C4: 2-hydroxy-2H-benzo[h]chromene-2-carboxylic acid, C5: *cis*-4-(1'-hydroxynaphth-2'-yl)-2-oxo-but-3-enoic acid, C6: 1-hydroxy-2-naphthaldehyde, C7: 1-hydroxy-2-naphthoic acid, C8: 1,2-dihydroxynaphthalene, C9: 2-hydroxychromene-2-carboxylic acid, C10: *trans*-o-hydroxybenzylidene-pyruvic acid, C11: salicylaldehyde, C12: salicylic acid, C13: catechol, C14: 2-hydroxymuconic semialdehyde

ity (P(cm/s)) can be expressed by Eq. (1) using a selective coefficient f to account for the difference between membrane/water partition coefficient and octanol/water partition coefficient [Wang et al., 2001].

$$\log P = \log\left(\frac{D_e}{\Delta L}\right) + f\log K_{ow} + \beta M_r \quad (1)$$

where, D_e (cm^2/s) is the diffusivity of the intermediate that is calculated from the effect of the molar volume, ΔL is the thickness of cell membrane (assumed to be 10 nm [Michael et al., 2003]). β is a

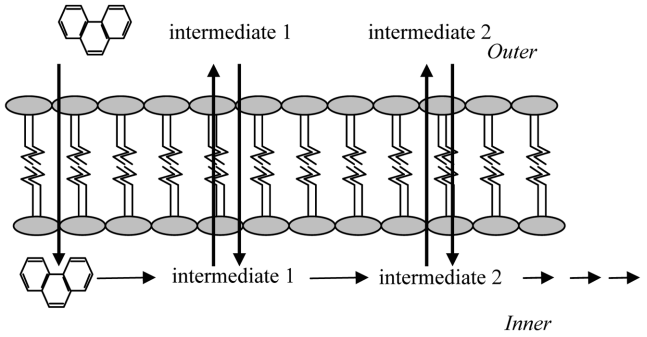

Fig. 2. A schematic illustration of two parts within a lipid bilayer.

constant, and M_r (g/mol) is the molecular weight of the intermediates. The coefficient f is proposed 2.4 by Xing and Anderson [1994]. Camennish et al. [1998] showed that compounds with a molecular weight below 350±150 g/mol could readily diffuse through the membrane without any restriction. Thus the value of βM_r is negligible because the molecular weights of phenanthrene and its intermediates are ranging from 110 to 242 g/mol. On the basis of these assumptions, Eq. (1) becomes

$$\log P = \log\left(\frac{D_e}{\Delta L}\right) + 2.4\log K_{ow} \quad \text{or} \quad P = \frac{D_e K_{ow}^{2.4}}{\Delta L} \quad (2)$$

2. Parameter Estimation by Group Contributions Theory

A semi-empirical equation can be utilized to obtain solution phase diffusivities of organic molecules as:

$$D_e = \frac{13.26\times 10^{-5}}{\mu^{1.14}\times(\overline{V})^{0.589}} \quad (3)$$

where, D_e is diffusivity of the chemical in water (cm^2/s), μ is the solution viscosity in centipoise (10^{-2} g/cm·s, μ=0.8904 centipoise at 25 °C), and $\overline{V}$ is the molar volume of the chemical (cm^3/mol) [Hayduk and Laudie, 1974].

The molar volume ($\overline{V}$) of the chemical can be obtained experimentally by measuring molecular mass and liquid density. However, these experimental data of many intermediates formed from phenanthrene degrading pathway are difficult to obtain in the literature. The molar volume can be estimated by using the structural group contributions theory, which was deduced by regression of available diffusion data [Fuller et al., 1966]. It is simply obtained from the sum of the diffusion size of the atoms making up the structure of a molecule. For example, phenanthrene consists of 14 carbon atoms (C), 10 hydrogen atoms (H) and 3 benzene rings (Rings). From this component the molar volume of phenanthrene is estimated as:

$$\begin{aligned}\overline{V} &= 14(C)+10(H)+3(Rings)\\ &= 14\times 16.5+10\times 2.0+3\times(-20.2)=190.4\end{aligned} \quad (4)$$

Octanol/water partition coefficient (K_{ow}) of chemicals of interest can be estimated from the chemical structure by using the following equation.

$$\log K_{ow} = \sum_i f_i + \sum_j F_j \quad (5)$$

where f_i values are fragment constants that quantify the contribu-

tions arising from each building block i in the particular chemical, and F_j values are intramolecular interaction factors that account for special intramolecular interaction, j between these fundamental pieces which cause them to act a little special in the particular chemical. For example, since phenanthrene consists of 10 carbon atoms within aromatic rings ($f_{C\,aromatic}$), 4 carbon atoms between aromatic rings ($f_{C\,between\,ring}$), and 10 hydrogen atoms bonded to aromatic carbons (f_H^{ϕ}), its K_{ow} value is calculated as:

$$\log K_{ow}=10f_{C\,aromatic}+4f_{C\,between\,ring}+10f_H^{\phi}$$
$$=10\times0.13+4\times0.23+10\times0.23=4.52 \quad (6)$$

where, superscript ϕ indicates constant for substituents bonded to aromatic carbons. The observed result of phenanthrene octanol/water partition coefficients is $\log K_{ow}=4.46$.

RESULTS AND DISSCUSSION

The parameters estimated from structural group contributions theory were summarized in Table 1. Generally, molar volume of the intermediate during degradation of phenanthrene decreases as degradation proceeds. However, in some cases, molar volume ($\overline{V}$) increases because larger aliphatic parts can be added during cleavage of aromatic structure. A lower value of molar volume has a positive effect on membrane transport of the chemical. Diffusivity (D_e) is inversely proportional to molar volume of the chemical. Therefore, the intermediates in the lower pathway have high diffusivity. Thus, from the viewpoint of chemical size, the intermediates in the lower pathway are expected to be easier to release out the cell membrane.

However, the octanol/water partition coefficient ($\log K_{ow}$) is larger in the intermediates in the upper pathway. This is because the number of hydrophobic parts such as carbons in the aromatic ring compared to hydrophilic functional groups (-OH, -COOH, -COH, and -CO) is higher in the upper pathway. This trend is opposite to molar volume or diffusivity in terms of easiness to release out of the membrane. The intermediates in the upper pathway are positive by partition coefficient but negative by diffusivity and molar volume. The combined effects of the parameters are reflected in the permeability value (logP). Eq. (1) suggests that the partition coefficient is more effective on the permeability value due to high exponent of partition coefficient. As a result, the trend of permeability was almost identical to the value of partition coefficient. Among the intermediates, *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene (C2), 3,4-dihydroxyphenanthrene (C3), 1-hydroxy-2-naphthaldehyde (C6), 1-hydroxy-2-naphthoic acid (C7), 1,2-dihydroxynaphthalene (C8), and salicylic acid (C12) showed higher value of permeability (Table 1).

Table 1. Estimation of parameters of the intermediates in the degradation pathway of phenanthrene

Compound	$\overline{V}$ (cm^3/mol)[a]	D_e ($\times10^{-6}$ cm^2/s)[b]	$\log K_{ow}$ (-)	logP (cm/s)
C1	190.4	6.88	4.52	11.69
C2	205.4	6.58	3.20	8.50
C3	201.4	6.65	3.18	8.45
C4	212.4	6.45	2.21	6.11
C5	232.6	6.11	0.64	2.32
C6	168.1	7.40	2.81	7.61
C7	173.6	7.26	3.51	9.28
C8	151.6	7.86	3.16	8.48
C9	162.6	7.55	1.03	3.33
C10	182.8	7.04	−0.59	−0.57
C11	118.3	9.10	1.58	4.75
C12	123.8	8.86	2.28	6.42
C13	101.8	9.94	1.93	5.63
C14	133.0	8.49	−0.96	−1.38

[a]Calculated from Fuller et al. [1996].

[b]Diffusivities of organic molecules in water, calculated from Hayduk and Laudie [1974].

Volume contribution (cm^3/mol) is 16.5 for C, 2.0 for H, 5.5 for O, and −20.2 for a ring.

Table 2. Intermediate compounds in the degradation pathway of phenanthrene observed in the literature

Reference	C2	C7	C8	C12
Samanta et al. [1999]		•		•
Evans et al. [1965]		•	•	
Doddamani and Ninnekar [2000]		•		
Adachi et al. [1999]		•		
Prabhu and Phale [2003]		•	•	•
Moody et al. [2001]	•	•		
Balashova et al. [2001]		•	•	•
Cerniglia and Yang [1984]	•			
Barnsley [1983]		•		

Table 2 summarizes experimentally observed intermediates during phenanthrene degradation in the laboratory or field in the literature. The experimental results show good agreement to the results of the permeability value estimated from the chemical structure. In general, the intermediates (C2, C7, C8, or C12) with high permeability have been found in laboratory experiments or natural environments. The intermediate 1-hydroxy-2-naphthoic acid, in particular, is the most frequently accumulated one in the medium during phenanthrene degradation as observed in a number of previous studies [Adachi et al., 1999; Balashova et al., 2001; Barnsley, 1983; Cerniglia and Yang, 1984; Doddamani and Ninnekar, 2000; Evans et al., 1965; Moody et al., 2001; Prabhu and Phale, 2003; Samanta et al., 1999]. The highest value of permeability (logP=9.28) of 1-hydroxy-2-naphthoic acid would be one of the reasons why this compound is most frequently accumulated among the intermediates. The release and accumulation of intermediates are also affected by other factors such as enzyme location. In some cases, enzymes are bound to the surface of a membrane in adsorptive arrays or are more strongly incorporated into the lipid bilayer of the membrane as integral arrays. Through structural organization, the proximity of active sites may then render it possible for the product of the first enzyme to get rapid access to the second enzyme. This physical juxtaposition of enzymes in a sequence can markedly accelerate the overall reaction rate without the spilling of intermediates [Gaertner, 1978]. Thus, intermediates with high permeability such as C3 and C6 which have not been frequently released out of the cell membrane would be due to the structural organization of enzyme clusters.

CONCLUSIONS

A membrane transport model for intermediates during the microbial phenanthrene degradation was developed to explain the release pattern by mathematical calculation of why only a few intermediates are found in extracellular media. The permeability results have good agreement with experimental results. Thus, the results from model estimation provide a good explanation why only a few intermediates are found during phenanthrene degradation. While the permeability results of some intermediates (C3, C6) were mismatched experimental data due to more complicated mechanisms, this simple approach will be applicable to predict the releasing pattern of intermediates during degradation of various compounds.

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NOMENCLATURE

β : the constant related with molecular weight [-]
D_e : diffusivity of the intermediates [cm^2/s]
f : selective coefficient constant related with the difference between membrane/water partition coefficient and octanol/water partition coefficient [-]
f_i : fragment constant of each building block i [-]
F_j : intramolecular interaction factors of interactions for fundamental pieces of j [-]
K_{ow} : octanol/water partition coefficient [-]
ΔL : the thickness of cell membrane [nm]
M_r : molecular weight of intermediates [g/mol]
P : permeability of intermediates [cm/s]
μ : the solution viscosity [10^{-2} g/cm·s]
$\overline{V}$: molar volume of the intermediates [cm^3/mol]

Superscript
ϕ : constant for substituents bonded to aromatic carbons

REFERENCES

Adachi, K., Iwabuchi, T., Sano, H. and Harayama, S., "Structure of the ring cleavage product of 1-hydroxy-2-naphthoate, an intermediate of the phenanthrene-degradative pathway of *Nocardioides* sp. strain KP7," *J. Bacteriol.*, **181**, 757 (1999).

Balashova, N. V., Stolz, A., Knackmuss, H. J., Kosheleva, I. A., Naumov, A. V. and Boronin, A. M., "Purification and characterization of a salicylate hydroxylase involved in 1-hydroxy-2-naphthoic acid hydroxylation from naphthalene and phenanthrene-degrading bacterial strain *Pseudomonas putida* BS202-P1," *Biodegradation*, **12**, 179 (2001).

Barnsley, E. A., "Phthalate pathway of phenanthrene metabolism: Formation of 2'-carboxybenzalpyruvate," *J. Bacteriol.*, **154**, 113 (1983).

Camenisch, G, Alsenz, J., Van de Waterbeemd, H. and Folkers, G, "Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight," *Eur. J. Pharm. Sci.*, **6**, 313 (1998).

Cerniglia, C. E., "Biodegradation of polycyclic aromatic hydrocarbons," *Biodegradation*, **3**, 351 (1992).

Cerniglia, C. E. and Yang, S. K., "Stereoselective metabolism of anthracene and phenanthrene by the fungus *Cunninghamella elegans*," *Appl. Environ. Microb.*, **47**, 119 (1984).

Cho, D., Youn, S. H. and Rhee, I. H., "Interaction of b-cyclodextrin with lipophilic ring compounds deposited in a sandy soil matrix during flushing," *Korean J. Chem. Eng.*, **21**, 389 (2004).

Doddamani, H. P. and Ninnekar, H. Z., "Biodegradation of phenanthrene by a *Bacillus* species," *Curr. Microbiol.*, **41**, 11 (2000).

Evans, W. C., Fernley, H. N. and Griffiths, E., "Oxidative metabolism of phenanthrene and anthracene by soil *pseudomonads*: The ring fission mechanism," *Biochem. J.*, **95**, 819 (1965).

Fuller, E. N., Schettler, P. D. and Giddings, J. C., "A new method for prediction of binary gas-phase diffusion coefficients," *Ind. Eng. Chem. Res.*, **58**, 19 (1966).

Gaertner, F. H., "Unique catalytic properties of enzyme clusters," *Trends. Biochem. Sci.*, **3**, 63 (1978).

Hayduk, W. and Laudie, H., "Prediction of diffusion coefficients for nonelectrolytes in dilute aqueous solutions," *AIChE. J.*, **20**, 611 (1974).

Kiyohara, H. and Nagao, K., "The catabolism of phenanthrene and naphthalene by bacteria," *J. Gen. Microbiol.*, **105**, 69 (1978).

Michael, T. M., John, M. M. and Jack, P., *Biology of microorganisms*, pp. 66-67, 10th ed. Pearson Education, Inc. Upper Saddle River, New Jersey (2003).

Moody, J. D., Freeman, J. P., Doerge, D. R. and Cerniglia, C. E., "Degradation of phenanthrene and anthracene by cell suspension of *Mycobacterium* sp. strain PYR-1," *Appl. Environ. Microbiol.*, **67**, 1476 (2001).

Prabhu, Y. and Phale, P. S., "Biodegradation of phenanthrene by *Pseudomonas* sp. Strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation," *Appl. Microbiol. Biotechnol.*, **61**, 342 (2003).

Samanta, S. K., Chakraborti, A. K. and Jain, R. K., "Degradation of phenanthrene by different bacteria: evidence for novel transformation sequences involving the formation of 1-naphthol," *Appl. Microbiol. Biotechnol.*, **53**, 98 (1999).

Wang, H. Y., Komolpis, K., Kaufman, P. B., Malakul, P. and Shotipruk, A., "Permeabilization of metabolites from biologically viable soybeans (*Glycine max*)," *Biotechnol. Prog.*, **17**, 424 (2001).

Woo, S. H., Jeon, C. O. and Park, J. M., "Phenanthrene biodegradation in soil slurry systems: Influence of salicylate and Triton X-100," *Korean J. Chem. Eng.*, **21**, 412 (2004).

Woo, S. H., Park, J. M. and Rittmann, B. E., "Evaluation of the interaction between biodegradation and sorption of phenanthrene in soil-slurry systems," *Biotechnol. Bioeng.*, **73**, 12 (2001).

Woo, S. H. and Rittmann, B. E., "Microbial energetics and stoichiometry for biodegradation of aromatic compounds involving oxygenation reactions," *Biodegradation*, **11**, 213 (2000).

Xiang, T.-X. and Anderson, B. D., "The relationship between permeant size and permeability in lipid bilayer membranes," *J. Membrane Biol.*, **140**, 111 (1994).