EXTRACTION AND BIODEGRADATION OF PAHS FROM CONTAMINATED SOIL WITH THE AID OF AQUEOUS DNA SOLUTION

Navarro R, Iimura Y, Ichikawa H, Tatsumi K
National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan

Abstract
The extraction of PAHs from a contaminated soil using aqueous DNA and the biodegradability of PAHs in the resulting effluent were investigated. Using a garden soil that was spiked with 72 mg kg^{-1} anthracene, 102 mg kg^{-1} phenanthrene and pyrene 99 mg kg^{-1}, extractions of close to 88%, 78% and 94%, respectively, were attained with 5% DNA at a 1:50 soil:extractant ratio. Better performance of DNA over cyclodextrin was observed with regards to its ability to bind even larger PAHs such as pyrene. Though DNA displayed almost similar extraction property with Tween 80, extensive emulsion formation that complicates handling as well as recycling operation did not occur in the former. Complete disappearance of PAHs in a model DNA-phenanthrene effluent was also found feasible using a pure culture of *Sphingomonas sp*. Enhanced degradation of PAHs with DNA was also observed.

Introduction
Polycyclic aromatic hydrocarbons (PAHs) are highly toxic pollutants and their contamination of many industrial sites throughout the world has become an utmost concern.[1] Their hydrophobicity and strong affinity for organic matter leads to their persistency and removal difficulty from the soil environment.[2] Though PAHs removal through soil flushing using a combination of water miscible organic solvents is highly effective, the exhaustive nature of this process has repercussions on important physical as well as chemical characteristics of soil. In this work, aqueous DNA was used as a non-destructive soil washing agent for common PAHs such as anthracene, phenanthrene and pyrene. The PAHs affinity of DNA has been generally attributed to the intercalation of planar PAHs molecules in the hydrophobic spaces between adjacent base pairs of the DNA molecule.[3,4] The biodegradability of PAHs in the resulting effluent after extraction was also evaluated.

Materials and Methods
Materials. Anthracene, phenanthrene and pyrene (purity > 98%) were purchased from Nacalai Tesque (Kyoto, Japan). Salmon DNA powder (>90% purity) of molecular weight ranging from 50,000-200,000 was purchased from Nippon Chemical Feeds (Hakodate, Japan).

Spiking of soil with PAHs. The PAHs-free gardening soil was obtained from a local home center. It has an organic carbon content (f_{OC}) of 9.67% ± 0.22. The soil was combined with PAHs solution in acetone. The mixture was then subjected to rotary evaporation to dryness at 35°C under vacuum. The spiked soil was transferred in a wide rectangular pan and then left under the fume hood for 3 days to evaporate any traces of acetone. The final samples were stored in glass bottles and then kept in the refrigerator. Analysis indicated a composition of 487 mg kg^{-1} for pyrene-spiked soil and 72 mg kg^{-1}, 102 mg kg^{-1} and 99 mg kg^{-1} for anthracene, phenanthrene and pyrene, respectively, for the soil spiked with a mixture of PAHs.

Bacterial strain and culture conditions. A PAHs degrader, *Sphingomonas sp.* AJ1, was purchased from the National Institute for Technology and Evaluation (NTTE) Biological Resource Center, Japan. The received microorganism was rejuvenated as indicated in the accompanying protocol. During inoculum build-up, cells from an agar dish were used to inoculate 10 ml of 3.74% Difco 2216 Marine broth. The cell culture was incubated at 30°C for 48 hours under continuous shaking to an average cell concentration of 2 x 10^9 CFU/ml.

Comparative extraction of PAHs from spiked soil by DNA and other extractants. PAHs-spiked soil (0.50 grams) was combined with 5 ml of 1% aqueous solutions of DNA, methyl-beta (M-β) and gamma (γ) cyclodextrins and Tween 80. The mixtures were shaken for 1 day. The samples were centrifuged and the PAHs and DNA content of the supernatants were analyzed.

Kinetics and equilibrium extractions of PAHs with DNA. To evaluate the required time for maximum PAHs

extraction, 10 grams of pyrene-spiked soil were combined with 100 ml of 1% DNA solution and then shaken. Three ml of sample were taken at regular time intervals and then centrifuged. The DNA and pyrene concentrations of the supernatants were analyzed.

To evaluate the maximum PAHs extraction**,** 0.20 grams of PAHs-spiked soil were combined with 10 ml of aqueous DNA of different initial concentrations. The use of lower soil to extractant ratio of 1:50 was considered in this case due to the high viscosity of DNA solution beyond 5%. After 4 hrs of shaking, the samples were centrifuged and the supernatants were analyzed.

Degradation of PAH with and without DNA in model system. A measured volume (0.03 ml) of 1000 mg L^{-1} phenanthrene solution in acetone was transferred in L-tubes. The acetone was allowed to completely evaporate under the fume hood. Approximately 2.5 ml of 1% DNA aqueous solution was combined with the PAH-containing tubes and then shaken for 4 hours to allow for initial PAHs solubilization. The samples were inoculated with 0.5 ml of *Sphingomonas sp.* culture and then subjected to continuous shaking. The PAHs and DNA concentrations were determined at specified incubation times. Phenanthrene solutions in Bushnell Haas minimum salt medium (BH-MSM) was used as control to compare the degradation rate with DNA.

Analytical Methods. PAHs analyses were conducted by combining the PAHs-containing supernatants with hexane. The resulting mixture was subjected to vortex mixing for 2-3 minutes. The two layers were allowed to separate (by centrifugation if necessary) and the PAHs in the organic layer were analyzed using a Shimadzu GC-mass spectrometer (GCMS-QP 2010, Kyoto, Japan) equipped with a mass spectrometric detector and auto-injector (AOC-20i). Analysis of DNA concentration was conducted by measuring the absorbance at 260 nm wavelength using a spectrophotometer (Bio-rad SmartSpec TM Plus, Tokyo, Japan).

Results and Discussion

Comparisons of PAHs solubiization by DNA and other agents. Figure 1 shows the extraction of different PAHs using aqueous solutions of DNA and other commonly employed surfactants such as Tween 80 and cyclodextrin. Cyclodextrin was effective only for binding low molecular weight PAHs such as anthracene and phenanthrene. This may be attributed to size limitation of the intercalation cavity. Note that even with the use γ-CD with a wider cavity size, extraction of large pyrene molecule did not improve. DNA and Tween 80 both displayed better performances, exhibiting high affinities for all the three PAHs. A selectivity trend of pyrene > phenanthrene > anthracene was apparent with DNA. This indicates that DNA's intercalation site may accommodate a wide range of PAHs molecular size. In addition, PAHs extraction with DNA was achieved without the formation of viscous emulsion that can complicate handling and recycling operations. This is one practical advantage of DNA over Tween 80 for soil washing purposes.

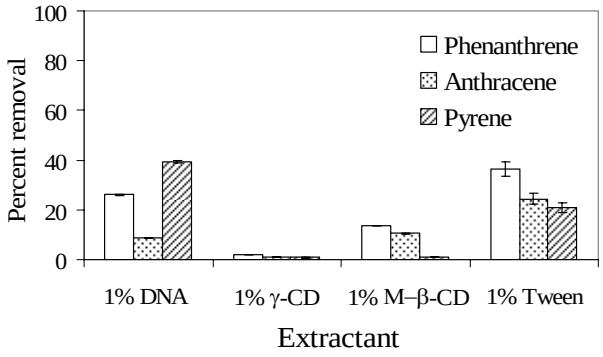

Figure 1. Extraction of different PAHs from spiked soil by different types of solubilizing agents. Soil to solution ratio was 1:10. Treatment time was 1 day.

Kinetics of PAHs extraction. Using pyrene as a model PAHs, the time course indicated an equilibrium time of around 4 to 6 hours. Except for the initial decline during the first hour, DNA concentration has also remained stable throughout the extraction period. The equilibrium pHs were maintained at around pH 6.2. However, it must be emphasized that this rapid kinetics for PAHs removal by DNA may not be generalized for all systems

particularly for aged soils. It has been reported that PAHs become increasingly difficult to extract from soils with time exposure.[5]

Equilibrium extraction of PAHs at different DNA concentrations The feasibility of complete PAHs removal by DNA in a single extraction stage using a treatment time of 4 hours was also evaluated. Results confirmed the dependence of PAHs extraction on DNA content. These data further supports the high selectivity for pyrene that was mentioned above. At 5% DNA concentration, phenanthrene, anthracene and pyrene extractions were satisfactorily high at 78% and 88% and 94%, respectively (Figure 2).

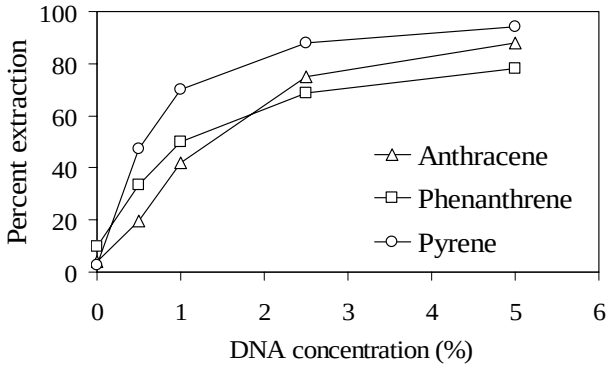

Figure 2. Extraction of different PAHs from spiked soil at different DNA concentrations. Soil to solution ratio was 1:50.

Biodegradation of DNA-partitioned phenanthrene by *Sphingomonas* sp. Data in Figure 3 show the biodegradation of DNA-intercalated PAHs, which represents the general speciation of PAHs' in soil washing effluents. Using phenanthrene in 1% DNA as model system, almost complete removal was feasible after 1 hour treatment with *Sphingomonas* sp. Detection of a hydroxylated phenanthrene intermediate confirmed the role of microbial enzyme activity on phenanthrene disappearance. More importantly, DNA was also found to enhance the degradation of phenanthrene relative to the powder form. Note the faster disappearance of phenanthrene in the1% DNA solution than in the Bushnell-Haas MSM alone. This indicates that the partitioning of PAHs in DNA does not deter but in fact it accelerates the overall rate of PAHs degradation.

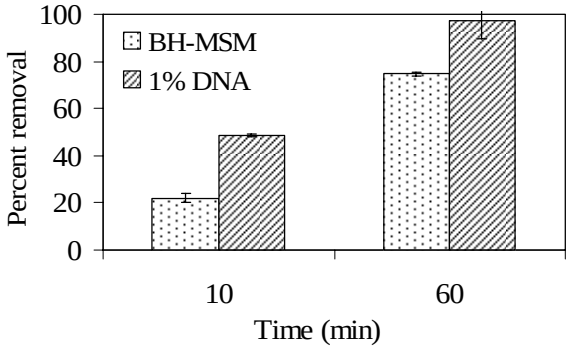

Figure 3. Phenanthrene removal by *Sphingomonas sp. w*ith and without DNA. The initial phenanthrene concentration was 9.0 mg L^{-1}.

References

1. Latimer, J, Zheng J. *In PAHs: An ecological perspective. Ecological and environmental toxicology series,* Douben, E. T. (ed.), John Wiley and Sons, Ltd, West Sussex, England, 2003.
2. Yamada M, Kato K, Nomizu M, Ohkawa K, Yamamoto H, Nishi N. *Environ Sci. Technol*. 2002: 36, 949-954.
3. Wang X, Brusseau M. *Environ. Sci. Technol.* 1993; 27: 2821-2625.
4. Wolfe A, Shimer G, Meehan T. *Biochemistry.* 1987; 26: 6392-6396.
5. Pinto L, Moore M. *Environ. Toxicol. Chem*. 2000; 19: 1741-1748.