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Phenanthrene Biodegradation in Soil Slurry Systems: Influence of Salicylate and Triton X-100

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Abstract–The effects of a nonionic surfactant (Triton X-100) and a metabolic inducer (salicylate) were investigated in order to enhance the biodegradation rate of phenanthrene in soil slurry systems. The addition of salicylate reduced the time for the complete degradation of phenanthrene up to about 3 times (12.9 mg/L-d) even at highly concentrated soils of 650 mg/kg. The inducer was beneficial not only by increasing metabolic activity of existing cells, but also by increasing cell mass since it was utilized as an additional carbon source. The fraction of fast growing bacteria in total with salicylate addition was much higher compared to that without salicylate. The addition of Triton X-100 ranging from 0 to 10 g/L increased the apparent solubility of phenanthrene in soil slurry, but significantly inhibited the phenanthrene degradation in both slurry and pure liquid systems without any inhibition to cell growth. The phenanthrene degradation was inhibited much more with increasing the surfactant concentration. The inhibition by surfactant addition might be due to the prevention of bacterial adhesion to phenanthrene sorbed to soil and/or decrease of micellar-phase bioavailability.

Key words: Biodegradation, Soil Remediation, Phenanthrene, Salicylate, Surfactant

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) used to be released to the environment as a result of incomplete combustion of fossil fuels or by accidental discharge during the transport, use, and disposal of petroleum products [Cerniglia, 1992]. Remediation of contaminated sites with these compounds is of great environmental concern due to their toxic and carcinogenic properties [Cerniglia, 1992; Keith and Telliard, 1979]. 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 [Mihelcic and Luthy, 1993]. Therefore, the natural degradation process of PAHs is very slow, and enhancing their biodegradation rate is highly required for successful soil bioremediation.

Recently, various strategies adding supplements have been tried to enhance the biodegradation rate of PAHs by either increasing their bioavailability or stimulating growth and metabolism of PAH-degrading microorganisms. Surfactants may be an attractive alternative to increase bioavailable concentrations of PAHs, since they increase apparent solubility of PAHs in the water phase by reducing interfacial energy [Su and Liu, 2003]. A number of studies to test the effects of surfactants on PAH biodegradation have been carried out, but the results have been inconclusive. While some reports suggest that surfactants may increase PAH biodegradation rates [Tiehm, 1994; Liu et al., 1995], others have shown that the effect of their application may be negligible or even detrimental [Chen et al., 2000; Stelmack et al., 1999; Guha and Jaffe, 1996].

One of the strategies proposed to enhance the degradation of specific PAH is to offer bacteria metabolic or pathway inducers to stimulate both selective growth of PAH degraders and induction of PAH metabolism. The potential pathway inducers, which are produced as intermediates during PAH degradation, include salicylate, salicylaldehyde, 1-hydroxy-2-naphthoate, catechol, phthalate, gentisate, and cinnamate. The previous studies have shown that some of the pathway inducers could stimulate biodegradation of PAHs [Chen and Aitken, 1999; Ogunseitan and Olson, 1993]. For example, salicylate was one of the most effective inducers for the biodegradation of various molecular weight PAHs; naphthalene [Ogunseitan and Olson, 1993; Stringfellow et al., 1995], benz[a]anthracene [Mahaffey et al., 1988], phenanthrene, fluoranthene, pyrene, benz[a]pyrene, benz[a]anthracene, and chrysene [Chen and Aitken, 1999]. However, in some cases, it has been reported there is no effect or even negative effect by salicylate addition on degradation of naphthalene and phenanthrene [Cidaria et al., 1994; Carmichael and Pfaender, 1997; Marcoux et al., 2000]. The effectiveness of the addition of metabolic inducers is significantly dependent on the conditions of the target system including contaminants, soil, and bacteria.

Thus, the metabolic inducer and surfactant could be considerable options for enhancing the rate of bioremediation for soil contaminated with PAHs. Previous studies have not considered the effect of metabolic inducer and surfactant at the same time in a soil slurry system. The objective of this study is to investigate effects of two representative supplements (Triton X-100 as a surfactant and salicylate as a metabolic inducer) in a soil slurry system on phenan-

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threne biodegradation and microbial population.

MATERIALS AND METHODS

1. Materials and Microbial Cultures

A mixed culture of phenanthrene-degrading microorganisms was previously isolated from PAH-contaminated soil [Woo and Park, 1999]. The mixed culture was subcultured in a soil slurry containing phenanthrene every two weeks for three months. The soil-slurry culture was harvested by centrifugation, freeze-dried, and stored at −70 °C. The frozen stock was used to prepare seed cultures for all experiments. For inoculation, the frozen stock was cultivated in a nutrient broth (Difco, USA) for three days, and bacterial cells were harvested by centrifugation and washed twice with mineral salt medium (MSM). The cells were resuspended in MSM and used as an inoculum for biodegradation experiments.

The mineral salt medium for biodegradation experiments contained 50 mM NH_4Cl, 5 mM NaH_2PO_4, 11.3 mM Na_2HPO_4, 10 mM KCl, 2 mM Na_2SO_4, 1.25 mM $MgSO_4 \cdot 7H_2O$, 0.02 mM $CaCl_2$, 0.1 mM $Na_2MoO_4 \cdot 2H_2O$, 1 mM EDTA, and 5 mL/L of a trace solution containing 0.12 mM HCl, 5 mM $ZnSO_4 \cdot 7H_2O$, 20 mM $FeSO_4 \cdot 7H_2O$, 10 mM $MnSO_4 \cdot H_2O$, 1 mM $CuSO_4 \cdot 5H_2O$, 2 mM $CoCl_2 \cdot 6H_2O$, and 0.8 mM H_3BO_3. The final pH was 7.0.

Soil was previously prepared and described in detail by Woo and Park [1999]. A fraction of silt/clay in the soil was used in all the experiments in order to have a homogeneous slurry. The content of organic matter of this soil fraction was 0.58%.

2. Solubilization Experiments

The solubilization experiments were performed in 15-mL borosilicate glass vials sealed with Teflon-lined septa on a rotary shaker (150 rpm) at 30 °C. An individual sample consisted of a 5 mL solution containing sterile deionized water, excess phenanthrene particles, and Triton X-100 ranging from 0 to 10 g/L. After shaking the vials for 24 h, we withdrew about 1 mL sample solution by syringe and filtered through a preconditioned filter (0.2 μm PTFE filter, Whatman, USA) to remove solid-phase crystalline phenanthrene. The concentration of phenanthrene in a dissolved state was analyzed by using the HPLC system.

In order to examine the change of solubilizing power of surfactant due to its sorption to soil, phenanthrene solubilized in liquid phase in a soil slurry was measured with various surfactant doses. Each batch test sample in a 250-mL Erlenmeyer flask contained 100 mL sterile deionized water, 2 g soil spiked with 1.3 mg phenanthrene, and 0-10 g/L Triton X-100. After the flasks were shaken for 24 h, the filtered liquid sample was analyzed to measure phenanthrene concentration.

3. Biodegradation Experiments

Biodegradation experiments for completely soluble phenanthrene in MSM in the absence of soil were performed at various concentrations of Triton X-100 ranging from 0 to 10 g/L. Phenanthrene solubilized in methylene chloride was added in a 250 mL Erlenmeyer flask and evaporated at 45 °C. The flask containing 100 mL MSM at variable total surfactant concentrations was autoclaved at 121 °C for 20 min. In case of low concentration of phenanthrene (0.7 mg/L), the medium saturated with excess phenanthrene crystals was filtered and adjusted to the target concentration with sterile medium. The flasks were received microbial inoculum and shaken at 150 rpm in a rotary shaker at 25 °C. .Control tests were also performed in order to examine abiotic phenanthrene loss from the medium with or without surfactant.

In soil slurry tests, phenanthrene solubilized in methylene chloride was impregnated into soil at loadings ranging from 25 to 650 mg/kg soil, and the methylene chloride was evaporated at 45 °C. The phenanthrene concentration in methylene chloride was adjusted so that all the soil samples could be completely soaked with methylene chloride to induce homogeneous sorption in soil particles. Sterile MSM and microbial inoculum were added, and the flasks were incubated at 25 °C in a rotary shaker at 150 rpm. Soil content was 20 g/L in all tests.

To examine the effect of a metabolic inducer on biodegradation of phenanthrene, salicylate at a concentration of 200 mg/L was added in the liquid medium or soil slurry containing different phenanthrene strength. The effect of different initial concentration of phenanthrene (25, 50, and 75 mg/kg) was also examined in soil slurry tests. The effect of surfactant (Triton X-100) in soil slurry was tested at various concentrations ranging from 0-10 g/L. Duplicate runs were performed for all the experiments. Liquid samples were taken at appropriate times from the flasks and used for the measurement of CFU, phenanthrene, and salicylate.

4. Analytical Methods

Phenanthrene was analyzed by HPLC (High Performance Liquid Chromatography, Dionex, USA) using a UV detector at 254 nm. The analytical column was a reversed phase column, Supelcosil™ LC-PAH column (150 mm×4.6 mm). The mobile phase (74.5% acetonitrile, 24.5% deionized water, and 1% acetate) was eluted at a flow rate of 1.5 mL/min. The minimum detectable concentration was approximately 0.01 mg/L for phenanthrene and 1 mg/L for salicylate.

For total phenanthrene analysis, slurry samples were extracted with the sonication method described previously by Woo and Park [1999]. The dissolved-phase phenanthrene was very carefully measured due to its strong tendency of sorption on the sampling apparatus. Approximately 1 mL liquid or slurry sample was withdrawn with a disposable glass Pasteur pipette and moved into a 1.5-mL borosilicate glass vial. This was immediately centrifuged at 6,000 g for 10 min. The supernatant was injected directly into the HPLC system. All liquid samples were immediately measured within 30 min after sampling in order to minimize adsorption onto the wall of the sample vial.

The colony forming units (CFUs) were counted after incubation on nutrient agar (Difco, USA) at 25 °C. They represented the total number of culturable heterotrophic microorganisms in the slurry or water sample. Fast growing bacteria (FGB) and slow growing bacteria (SGB) were enumerated after 2-day and 7-day incubation, respectively.

RESULTS AND DISCUSSION

1. Phenanthrene Biodegradation in Soil Slurry

Fig. 1 shows the time courses of phenanthrene degradation in soil slurries at various initial concentrations of 25, 50, 75 mg/kg soil. The liquid concentration at equilibrium was 0.23, 0.47, and 0.7 mg/L for the initial phenanthrene concentration 25, 50, and 75 mg/kg soil, respectively. These liquid concentrations for all cases were be-

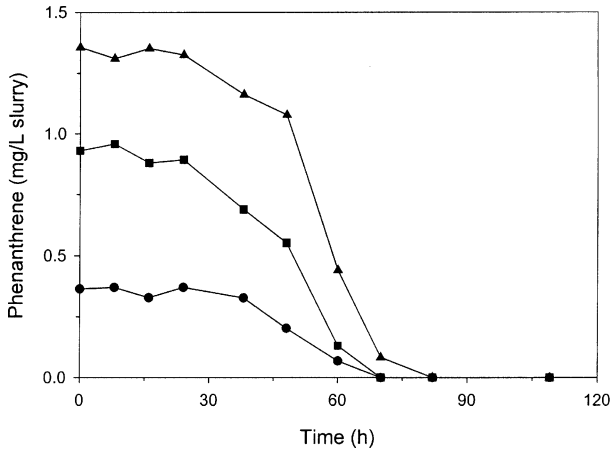

Fig. 1. Effect of initial phenanthrene concentration in soil slurry treatment. Symbols: 25 mg/kg, ●; 50 mg/kg, ■; 75 mg/kg, ▲.

low the liquid solubility of phenanthrene (1.1 mg/L), indicating the separate phase of phenanthrene was not present in the systems. The degradation pattern was similar for all three cases, even though the strength of phenanthrene was different. The lag time before active degradation of phenanthrene was similar, around 30 h, for all cases and the noticeable degradation of phenanthrene began after the lag time. The time for complete degradation (around 70-80 h) was not significantly extended with increasing initial phenanthrene concentration. The maximum degradation rate during active degradation period increased with increasing phenanthrene concentration; 0.27, 0.84, and 1.28 mg/L-d for each initial phenanthrene concentration of 25, 50, and 75 mg/kg soil, respectively.

2. Effect of Inducer Addition

The effect of salicylate as a metabolic inducer on phenanthrene degradation was examined in soil slurry with different initial phenanthrene concentrations. As seen in Fig. 2(a), the phenanthrene degradation was greatly enhanced by salicylate addition. The maximum phenanthrene degradation rate was 2.3 mg/L-d at the initial concentration of 0.9 mg/L, which is 2.7 times faster than that of the control (0.84 mg/L-d). The time for complete degradation was reduced to 57% of the control from 70 to 30 h. Enhanced biodegradation with salicylate addition was observed even at thirteen-times increased initial concentrations (Fig. 2(b)). The complete phenanthrene degradation was delayed from 70 to 120 h at high initial phenanthrene concentration without salicylate addition, while it was not much delayed from 30 h with salicylate addition. This suggests that the capacity of bacteria induced by salicylate exceeded the level of substrate available at low initial phenanthrene concentration. The lag time was also dramatically reduced from 30 to 10 h at both ranges of initial concentration. The higher cell number, 1.32×10^8 CFU/mL, was obtained with the salicylate addition compared to the control (2.6×10^7 CFU/mL). In case of the initial concentration at 650 mg/kg, the liquid concentration reached the liquid solubility and the excess phenanthrene is present in separate phase as crystalline, which was about 8% of total mass of phenanthrene.

The stimulatory effect of salicylate addition on the degradation of various PAHs has been elucidated in several previous studies. Stringfellow et al. [1995] observed that the transformation of naph-

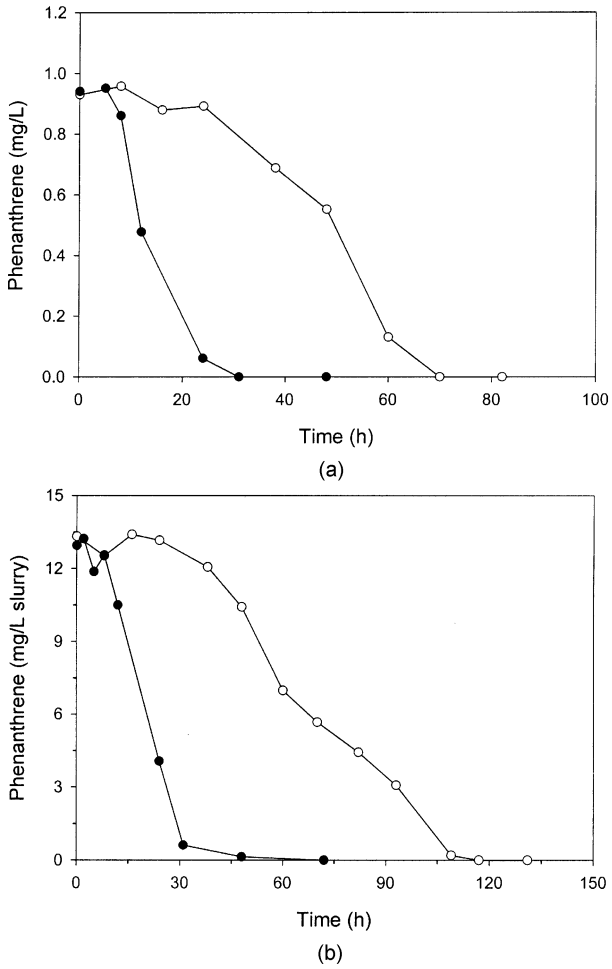

Fig. 2. Effect of salicylate addition on phenanthrene sorbed to soil in slurry treatment at different phenanthrene concentration. Symbols: 0 mg salicylate/L, ○; 200 mg salicylate/L, ●. (a) 50 mg phenanthrene/kg soil. (b) 650 mg phenanthrene/kg soil.

thalene, phenanthrene, fluoranthene, and pyrene by *Pseudomonas saccharophila* P15 was stimulated by salicylate. Chen and Aitken [1999] reported that salicylate greatly enhanced initial rates of removal of high molecular weight PAHs; benz[a]anthracene, chrysene, and benzo[a]pyrene, which are not used for growth of the strain P15. However, Carmichael and Pfaender [1997] reported that salicylate or other pathway inducers (phthalate, gentisate, and cinnamate) decreased the mineralization of phenanthrene or pyrene in their study using various natural soil samples that have an abundance of other competitive carbon sources. Thus, induction by the pathway inducers could not always be effective and would be dependent on environmental conditions. In this study, low content of soil (2 wt%) and soil organic matter (0.58 wt%) might protect phenanthrene utilization by salicylate induction from competition of other carbon sources. For this reason, induction by salicylate could be highly effective and produce rapid degradation of phenanthrene even in highly concentrated soil. In this study, the salicylate dose was selected at a concentration of 200 mg/L, which was the best condition to effectively observe phenanthrene degradation (data not shown).

Fig. 3 shows the change of cell number during phenanthrene de-

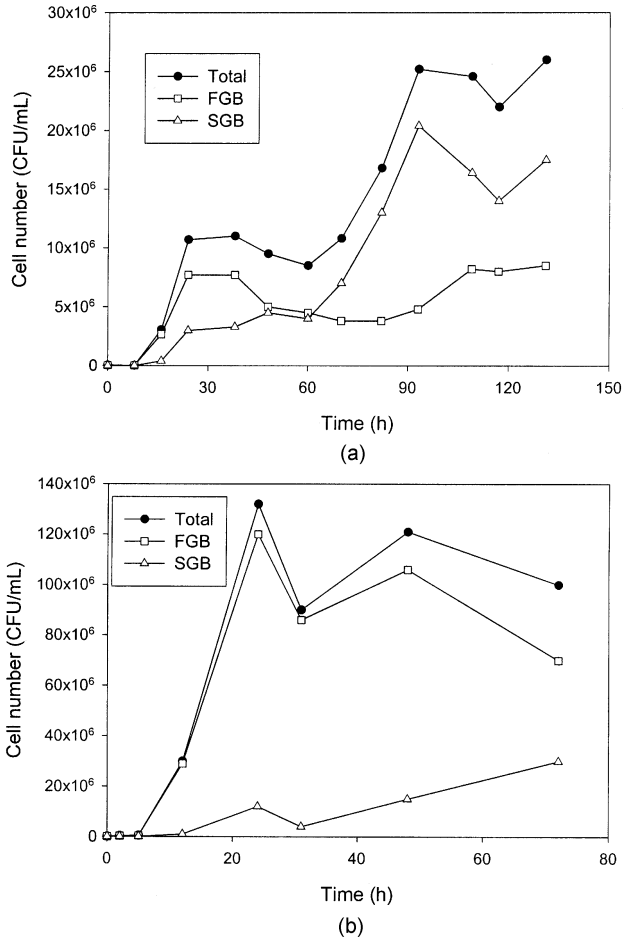

Fig. 3. Change of bacterial community during phenanthrene degradation in slurry treatment at an initial concentration of 50 mg phenanthrene/kg soil. FGB and SGB represent fast and slow growing bacteria, respectively. (a) 0 mg salicylate/L. (b) 200 mg salicylate/L.

gradation with or without salicylate addition. Total cell number was much higher with salicylate since salicylate was an additional carbon source. The pattern of the change of total cell number reflected the community change of different bacteria. The total cell number shows two flourishing peaks: at 24 h and 90 h. The number of FGB (fast growing bacteria) increased rapidly to 0.77×10^7 CFU/mL but decreased thereafter. The major population was switched from FGB to SGB (slow growing bacteria) at about 60 h. The number of SGB reached to 2.0×10^7 CFU/mL, which is 2.9 times higher than the maximum of the cell number of FGB. The results seem to reflect the dynamic effect of different bacterial groups possessing different growth rates or degradability. Considering the time course of phenanthrene degradation, the initial step of phenanthrene degradation (dioxygenase reaction) seems to occur mainly by FGB. Although SGB can grow on phenanthrene as a single carbon source, the flourish of SGB in a later period might be due to the consumption of more easily-degradable intermediates compared to phenanthrene itself. When salicylate was added, the fraction of FGB in total was much higher compared to that without salicylate. This is attributed to competitiveness of FGB to SGB on additional salicylate as a primary substrate. Thus, the addition of inducers would alter the bacterial community in the system in view of supplying easily degradable substrates.

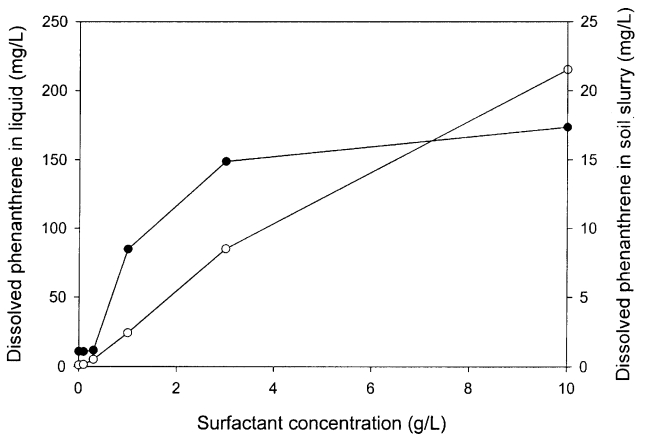

Fig. 4. Dissolved phenanthrene concentration in pure liquid solution (○) and soil slurry (●) supplemented with Triton X-100 at different concentrations (0-10 g/L).

3. Solubilization of Phenanthrene by Surfactant Addition

Fig. 4 shows the enhanced solubility of phenanthrene in the presence of a nonionic surfactant, Triton X-100. The amount of maximum solubilized phenanthrene in liquid is not noticeably increased until the critical micelle concentration (CMC) of the surfactant is reached and, above this concentration, the solubilized phenanthrene concentration increases approximately linearly with the surfactant concentration. The CMC value (0.18 g/L) was obtained from the concentration denoted by the intersection of the two linear portions (increase and non-increase of solubility) of a curve showing solubility variation in Fig. 4. The molar solubilization ratio (MSR) was 0.1 mol phenanthrene/mol surfactant. The values of CMC and MSR fall in the similar range reported by others: 0.15 g/L for CMC [Laha and Luthy, 1992] and 0.111 for MSR [Edwards et al., 1991]. The enhanced solubility of phenanthrene by surfactant addition is significantly reduced when soil is present. For example, at a concentration of 0.3 g Triton X-100/L, the dissolved phenanthrene was 15 mg/L that is 5.5 times lower than that of solubility (85 mg/L) without soil. This is due to the sorption of surfactant to soil, which resulted in the increase of CMC value. In a separate test, the amount of sorbed surfactant was about 0.02 g/g soil, which is consistent to 0.4 g/L at a soil content of 20 g soil/L liquid. The dissolved concentration of phenanthrene was not linearly increased with increasing surfactant addition due to phenanthrene partitioning between soil and water phase as described in the other report [Edwards et al., 1994]. Thus, the presence of soil changes the surfactant micellization pattern due to surfactant sorption onto the soil, resulting in the aqueous-phase surfactant being considerably less than the total added.

4. Phenanthrene Degradation in Water/Surfactant System

The results of primary biodegradation tests during 7-day culture with various conditions are summarized in Table 1. No appreciable removal of phenanthrene (below 10%) occurred for the abiotic control with (Test B1) or without (Test A1) surfactant addition. Using phenanthrene as a single carbon source (Test A2), 91.4% of the initial phenanthrene (0.7 mg/L) was degraded with degradation rate

Table 1. Effect of Triton X-100 on phenanthrene degradation in liquid tests with completely soluble phenanthrene

	Tx [g/L]	P (0d) [mg/L]	P (7d) [mg/L]	CFU (0d) [/mL]	CFU (7d) [/mL]	P degraded (%)	r [mg/L-d]	q [/d]
A1	0	0.7	0.63±0.03	0	0	9.34±3.7	0.009±0.004	-
A2	0	0.7	0.06±0.09	2.10×10^{4}	$1.25\pm0.33\times10^{6}$	91.4±12.2	0.091±0.012	0.17±0.02
B1	1	0.7	0.64±0.04	0	0	8.62±5.9	0.009±0.006	-
B2	1	0.7	0.24±0.12	2.10×10^{4}	$2.03\pm0.40\times10^{6}$	66.1±17.0	0.066±0.017	0.079±0.035
C1	1	20	15.6±1.0	2.10×10^{4}	$1.77\pm0.18\times10^{7}$	22.1±5.0	0.63±0.14	0.082±0.010
C2	3	20	16.7±2.4	2.10×10^{4}	$2.57\pm0.42\times10^{7}$	16.6±12.1	0.47±0.35	0.041±0.025
C3	10	20	17.7±2.1	2.10×10^{4}	$2.89\pm0.23\times10^{7}$	11.2±10.4	0.32±0.30	0.025±0.022

Tx : Triton X-100, P : phenanthrene, r : phenanthrene degradation rate, and q : specific phenanthrene degradation rate. Data presented are the means of duplicate samples±standard deviations.

of 0.091 mg/L-d and specific degradation rate of 0.17 d^{-1}. The microorganisms were grown from 2.1×10^{4} CFU/mL to 1.25×10^{6} CFU/mL for 7 days culture. The biodegradation of phenanthrene was much lower in pure liquid tests compared with the slurry tests as seen in Fig. 1. Our previous study demonstrated that the more rapid degradation in soil slurry was due to the rapid utilization of just-desorbed chemicals by microorganisms very near the soil surfaces [Woo et al., 2001].

In Test B2, 66.1% of the initial phenanthrene with 1 g/L of Triton X-100 was degraded, which is about 30% decrease compared to the control Test A2. The cell number was increased in greater amount with surfactant addition in spite of lower degradation of phenanthrene, indicating biodegradability per unit cell (probably enzymatic activity) was decreased. The microorganisms could not use the surfactant Triton X-100 as a carbon source, but instead increased organic impurities in the surfactant solution probably increased the cell number. It was indirectly confirmed from the fact that the cell number was increased with increasing the surfactant concentration (Test C). Comparing Test B2 with Test C1, the percentage phenanthrene degraded was lower at high concentration of phenanthrene. In contrast, phenanthrene degradation was inhibited much more with increasing the surfactant concentration. Possible explanation for surfactant-related inhibition of PAH degradation includes decreased bioavailability of micelle-solubilized PAH [Guha and Jaffe, 1996; Zhang et al., 1997], or surfactant interference with cellular processes without inhibitory effects on cell growth, e.g., inhibition of enzymatic dependent processes in biodegradation pathways [Allen et al., 1999].

5. Phenanthrene Degradation in Water/Soil/Surfactant System

The effect of a nonionic surfactant (Triton X-100) on phenanthrene degradation and cell growth in soil slurry is shown in Fig. 5. The inhibition of phenanthrene biodegradation by surfactant addition is clearly observed. The maximum biodegradation rate was 12.9, 7.18, 2.0 mg/L-d when the surfactant concentration was at 0, 3, and 10 g/L, respectively. The biodegradation rate was decreased and the lag time was delayed proportionally with increasing surfactant concentration. However, the cell growth was not distinguished at different surfactant addition (Fig. 5b). The increase of cell number was caused mainly by the growth on salicylate and soil organic matter, which are more abundant compared to phenanthrene.

In many previous studies on surfactant effect, the results were inconsistent [Volkering et al., 1998]. The surfactant addition inhibited degradation in some cases and enhanced it in others, depending on the specifics of the target compound, bacterial species and choice of surfactant. The inhibition of surfactant can be explained by several reasons: (a) toxicity of surfactants due to changing membrane permeability, sticking to membrane enzyme, and membrane lysis; (b) toxicity of surfactant-enhanced aqueous PAH concentrations; (c) prevention of bacterial adhesion to the hydrophobic substrate [Chen et al., 2000]; (d) decreased availability of the surfac-

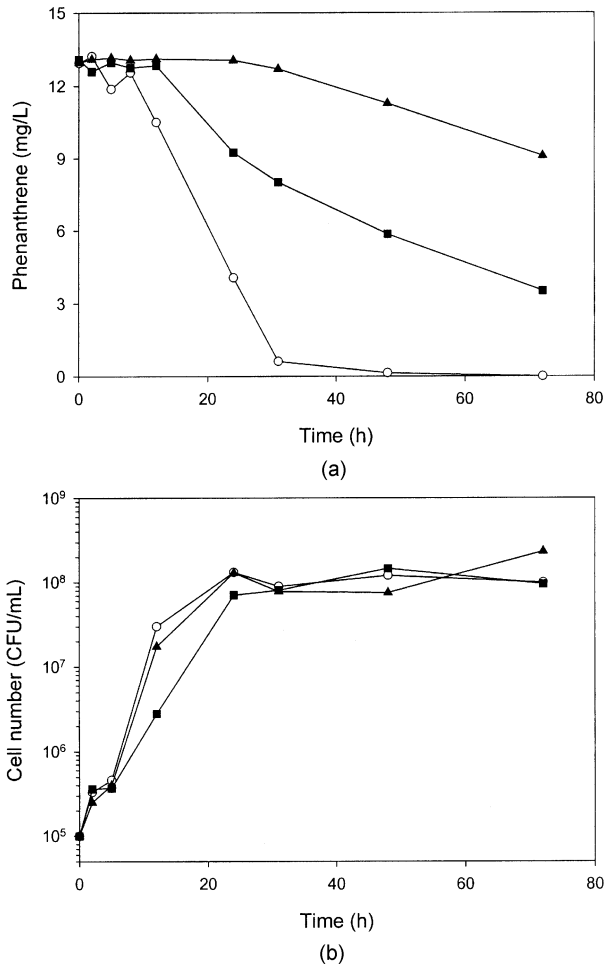

Fig. 5. Effect of Triton X-100 at different concentrations on the degradation of phenanthrene in soil slurry. Symbols: 0 g/L, ○; 3 g/L, ■; 10 g/L, ▲. (a) phenanthrene. (b) cell number.

tant-micelle-solubilized PAH [Guha and Jaffe, 1996]; and (e) competitive substrate utilization [Liu et al., 1995]. In our study, the inhibition of surfactant addition was not caused by the toxicity of surfactant-added solution as (a) and (b) since the evidence of decrease in cell growth was not observed in liquid (Table 1) and slurry tests (Fig. 5b). The surfactant, Triton X-100, was not used for growth substrate as (e).

The prevention of bacterial adhesion to phenanthrene sorbed to soil seems one of the possible reasons for the surfactant inhibition. Attached cells can readily capture the compounds sorbed to soil surfaces prior to their diffusion into bulk liquid, which can lead to enhanced biodegradation in soil slurry systems compared to pure liquid [Guerin and Boyd, 1992; Woo et al., 2001]. However, the addition of surfactant might cover the upper surface of phenanthrene sorbed to soil particles and consequently interfere with the direct utilization of phenanthrene by attaching bacteria. Stelmack et al. [1999] showed similar results that the presence of surfactant inhibited adhesion of bacteria to the surfaces of NAPL (non-aqueous phase liquids)-coated test tubes. It has been reported that the growth of bacteria on solid anthracene was inhibited by the addition of Triton X-100 even at low concentration (0.09 CMC) [Chen et al., 2000].

Bioavailability of phenanthrene in micellar phase would be another possible reason for the decreased biodegradation rate. Since the surfactant micelle functions as another organic sorbent, phenanthrene concentration in aqueous pseudophase decreases with increasing surfactant dose [Guha and Jaffe, 1996; Edwards et al., 1994]. Phenanthrene concentration in the aqueous pseudophase would be lower than 1.1 mg/L, solubility in pure water, even though the apparent concentration in water was increased up to 18 mg/L at the surfactant concentration of 10 g/L (Fig. 4). If bacteria cannot directly utilize phenanthrene in the micellar phase, the overall bioavailability of phenanthrene in the system decreases with increasing surfactant dose. This micellar-phase bioavailability was known to depend on the type of surfactant, substrate, and microorganism [Guha and Jaffe, 1996]. The micellar-phase bioavailability seems to be significantly limited in the system used in this study, although it is not clear whether the bioavailability is completely zero.

CONCLUSIONS

The effects of supplements were investigated as a technology to enhance the rate of bioremediation of soil contaminated with hazardous organic chemicals. Triton X-100 as a surfactant and salicylate as a metabolic inducer were selected for the biodegradation experiments. The biodegradation rates of phenanthrene were dramatically increased by the addition of salicylate. The inducer was beneficial not only by increasing metabolic activity of existing cells but also by increasing cell mass since it was utilized as an additional carbon source. However, Triton X-100 inhibits the phenanthrene degradation in both slurry and pure liquid systems. The extent of inhibition was increased with increasing the amount of the surfactant added. The inhibition by surfactant addition might be due to the interference between bacteria and phenanthrene sorbed to soil and/or decrease of micellar-phase bioavailability. The metabolic inducers would be a good supplement, while surfactant should be cautiously considered as a supplement for effective soil bioremediation.

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