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Naphthalene, phenanthrene and surfactant biodegradation

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Abstract

The impact of surfactants on naphthalene and phenanthrene biodegradation and vice versa after surfactant flushing were evaluated using two anionic surfactants: sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS); and two nonionic surfactants: POE (20) sorbitan monooleate (T-maz-80) and octylphenol poly(ethyleneoxy) ethanol (CA-620). Naphthalene and phenanthrene biodegradation varied differently in the presence of different surfactants. Naphthalene biodegradation was not impacted by the presence of SDS. In the presence of T-maz-80 and CA-620, naphthalene biodegradation occurred at a lower rate (0.14 d^{-1} for T-maz-80 and 0.19 d^{-1} for CA-620) as compared to un-amended control (0.29 d^{-1}). Naphthalene biodegradation was inhibited by the presence of SDBS. In the presence of SDS, phenanthrene biodegradation occurred at a lower rate (0.10 d^{-1} as compared to un-amended control of 0.17 d^{-1}) and the presence of SDBS, CA-620 and T-maz-80 inhibited phenanthrene biodegradation. The surfactants also responded differently to the presence of naphthalene and phenanthrene. In the presence of naphthalene, SDS biodegradation was inhibited; SDBS and T-maz-80 depleted at a lower rate (0.41d^{-1} and 0.12 d^{-1} as compared to 0.48 d^{-1} and 0.22 d^{-1}). In the absence of naphthalene, CA-620 was not degradable, while in the presence of naphthalene, CA-620 began to degrade at a comparatively low rate (0.12 d^{-1}). In the presence of phenanthrene, SDS biodegradation occurred at a lower rate (1.2 d^{-1} as compared to 1.68 d^{-1}) and a similar trend was observed for T-maz-80. The depletion of SDBS and CA-620 did not change significantly. The choice of SDS for naphthalene-contaminated sites would not adversely affect the natural attenuation of naphthalene, in addition, naphthalene was preferentially utilized to SDS by naphthalene-acclimated microorganisms. Therefore, SDS was the best choice. T-maz-80 was also found to be usable in naphthalene-contaminated sites. For phenanthrene contaminated sites, SDS was the only choice.

Abbreviations: SDS – sodium dodecyl sulfate, SDBS – sodium dodecyl benzene sulfonate, T-maz-80 – POE (20) sorbitan monooleate, CA-620 – octylphenol poly(ethyleneoxy) ethanol, PAHs – polycyclic aromatic hydrocarbons, AS – activated sludge, NMO – naphthalene-acclimated microorganisms, PMO – phenanthrene-acclimated microorganisms, CMCs – critical micelle concentrations.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds in the environment as a result of contamination by coalgasification plants and wood preserving industries. The *in-situ* biodegradation of the PAH contaminants in the subsurface is restricted by the sorption processes or mechanisms. Recently, this problem has been addressed with the development of surfactant-enhanced remediation (Aronstein & Alexander 1991). Surfactants can affect the contaminant mobilization by lowering the interfacial tension and increasing the contaminant aqueous solubility by forming micelles. Increase of the desorption of contaminants sorbed to the soil with the addition of surfactants has been observed by Fu & Alexander

(1995) and Thibault et al. (1996). Abriola et al. (1995) performed a surfactant flushing to mobilize DNAPL and Gabr et al. (1995) used SDS to flush the PAHs from soil. Although this technology shows great potential, further research is required to characterize the fate of the PAHs and the surfactants. Easily degradable surfactants are not efficient due to the increase in cost; while non- or low-degradable surfactants result in high residual concentrations. Therefore, an ideal surfactant should be slowly degradable. In addition, as not all the sorbed PAHs are washed off, the surfactant should not inhibit PAH biodegradation.

The objective of this research was to address the impact of the presence of surfactants on PAH biodegradation and vice versa. It built upon previous research that investigated the affect of the surfactant on the attenuation of BTpX (Goudar et al. 1999). While retaining some generality, the research was designed for relevance to the higher molecular weight contaminants, naphthalene and phenanthrene, which are commonly present in the petroleum contaminated sites. Based on previous use in remedial applications (Rouse et al. 1996, 1993 and Shiau et al. 1995), four synthetic surfactants were used in this research, of which sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS) are anionic surfactants; POE (20) sorbitan monooleate (T-maz-80) and octylphenol poly(ethyleneoxy) ethanol (CA-620) are nonionic surfactants. The results of this research will further our understanding concerning the residual surfactant biodegradation in PAH contaminated sites after flushing. It is anticipated that this research will provide an important guideline for the development of more effective remediation strategies in the future field applications of the surfactant flushing remediation technology.

Materials and methods

Microorganisms and media

Activated sludge (AS) was used as a base consortium to investigate the surfactant biodegradation, which was obtained from Norman Municipal Wastewater Treatment Plant, Norman, OK and was used without further acclimation or enrichment. The naphthalene-acclimated microorganisms (NMO) and phenanthrene-acclimated microorganisms (PMO) were isolated from a petroleum- contaminated site in Norman, OK. For culturing, 10 g (dry weight) soil from this site was added to a 250 ml Teflon-sealed vial containing 100 ml carbon-free mineral salts media that has a composition of KH_2PO_4, 160 mg l^{-1}; K_2HPO_4, 420 mg l^{-1}; Na_2HPO_4, 50 mg l^{-1}; NH_4Cl, 40 mg l^{-1}; $MgSO_4 \cdot 7H_2O$, 50 mg l^{-1}; $CaCl_2$, 50 mg l^{-1}; $FeCl_3(6H_2O$, 0.5 mg l^{-1}; $MnSO_4 \cdot 4H_2O$, 0.05 mg l^{-1}; H_3BO_3, 0.1 mg l^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.05 mg l^{-1}; $(NH_4)_6Mo_7O_{24}$, 0.03 mg l^{-1}. The vial was repetitively exposed to naphthalene or phenanthrene at incremental concentrations until no appreciable lag time was observed. The vial was tumbled at 120 RPM for 7 days before being transferred to a subsequent higher concentration. After four enrichment cycles were completed, the soil was transferred to an aliquot of dilution buffer (1.9 g Na_2HPO_4 and 1.8 g NaH_2PO_4 in 1 l deionized water). The culture was then agitated and plated on the mineral salt agar with naphthalene or phenanthrene serving as the sole carbon source.

Surfactants

The surfactants used in this research, sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate (SDBS), POE (20) sorbitan monooleate (T-maz-80), and octylphenol poly(ethyleneoxy) ethanol (CA-620), have been extensively evaluated for their usage in the surfactant-enhanced subsurface remediation (Rouse et al. 1993 and Shiau et al. 1995). Of these four surfactants, SDS, the linear alkyl sulfate, has been reported to be easily degraded by *Pseudomonas* via an initial cleavage of the inorganic sulfate by alkyl sulfatase, after which the liberated alcohol is oxidized to lauric acid by appropriate alcohol dehydrogenase through the *alk* pathway. The lauric acid is further degraded in beta-oxidation. SDBS, the linear alkylbenzene sulfonate, is moderately recalcitrant (Schmitt 1992). T-maz-80 is an edible nonionic surfactant comprised of a sorbitan ring and 20 ethylene oxide units. It has little or no activity as an anti-bacterial agent (Dawson 1985). On the contrary, it has been shown to have an adverse effect on the anti-bacterial effect of methyl paraben and related compounds (Block 1991). CA-620 is a polyethoxylate non-ionic surfactant and has been reported to be nonedible.

SDS and SDBS were obtained from Aldrich, Milwaukee, WI in powder form. T-maz-80 was obtained from Sigma, St. Louis, Mo. and CA-620 was from Rhone-Poulene, Cranbury, NJ. They were both in liquid form. To simulate the actual field concentration after surfactant flushing application, the surfactants were studied at a concentration of 1/4 of their critical

micelle concentrations (CMCs). Specifically, initial concentration of SDS was 600 mg l^{-1}; SDBS, 100 mg l^{-1}; T-maz-80, 7.5 mg l^{-1}; and CA-620, 12 mg l^{-1}.

Naphthalene and phenanthrene

Among the PAHs, naphthalene (a double cyclic aromatic hydrocarbon) and phenanthrene (a tricyclic aromatic hydrocarbon) are the two simplest forms. Naphthalene is slightly soluble in water (31 mg l^{-1}, 1 atm, 25 °C). Once in groundwater, biodegradation may occur if conditions are aerobic. Phenanthrene is weak soluble in water (1.2 mg l^{-1}, 1 atm, 25 °C), therefore, the biodegradation is strictly limited by its bioavailability. Phenanthrene biodegradation has been reported to be greatly enhanced in the presence of surfactants (Cuny et al. 1999; Macur et al. 1998).

Both naphthalene and phenanthrene used in this research were obtained from Sigma, St. Louis, MO in powder form. In this study, naphthalene and phenanthrene were studied at a concentration of 30 mg l^{-1} and 1.0 mg l^{-1} to simulate the contaminant aqueous concentrations after surfactant flashing, which were equal to their solubility.

Analytical methods

Naphthalene and phenanthrene were analyzed using a HPLC (Water Associates, Milford, MA) equipped with an ODS hypersil column from Hewlett Packard, Palo Alto, CA. Quantification of naphthalene and phenanthrene was accomplished using a UV detector (Water Associates, Milford, MA). The mobile phase was 80% methanol (high-pressure liquid chromatography grade) mixed with 20% nanopure DI water (v/v) (Barnstead Thermodyne, Debuque, IO). The flow rate was 1 ml min^{-1} and the injection was 25 μl. Naphthalene and phenanthrene concentrations were quantified against external standards.

Surfactant concentrations were analyzed using either liquid chromatography for nonionic surfactants or ion chromatography for anionic surfactants. Liquid chromatography/ion chromatography analysis was conducted with a HP 1100 series liquid chromatograph (Palo Alto, CA) equipped with a 250 mm × 4 mm Spherisorb C18 column (Supelco, Bellefonte, PA). Anionic surfactants were processed by passing the separated analytes through an Alltech 335 suppressor (Alltech, Deerfield, IL). Quantification was accomplished with an Alltech 320 Conductivity Detector connected in series with the suppressor with an isocratic mobile phase of methanol/water 80/20 (v/v) at a flow rate of 1 ml min^{-1}. For noninoic surfactants, a variable wavelength UV detector was used. All the surfactant concentrations were quantified against external standards.

Surfactant and PAH biodegradation experiments

Biodegradation experiments for the four surfactants were performed in triplicate with the average values being reported using activated sludge (AS), naphthalene-acclimated microorganisms (NMO) and phenanthrene-acclimated microorganisms (PMO), respectively (Table 1). The surfactant biodegradation experiments were conducted in a closed 500 ml amberglass reactor with a Teflon-lining septum sampling port. Oxygen was supplied from a pressure cylinder at a flow rate of 0.1 mg min^{-1}. Excess oxygen passed through the oxygen pressure regulator to provide an atmospheric seal and to maintain a constant pressure inside the reactor. Carbon dioxide produced was removed from the atmosphere by potassium hydroxide held in a glass tube inside the reactor. Each reactor contained 250 ml mineral salt medium with one of the four surfactants, SDS (600 mg l^{-1}), SDBS (100 mg l^{-1}), T-maz-80 (7.5 mg l^{-1}), or CA-620 (12 mg l^{-1}) serving as the sole carbon source and was incubated at 30 °C. The inoculum was 5 mg l^{-1} as COD of AS, NMO or PMO. The contents of the flask were continuously stirred at 150 RPM throughout the course of the experiments. Samples were withdrawn periodically from the reactor and analyzed for surfactant concentrations and total protein for biomass determination.

Naphthalene and phenanthrene biodegradation experiments were conducted similarly to the surfactant biodegradation experiments, except that 30 mg l^{-1} naphthalene or 1.0 mg l^{-1} of phenanthrene served as the carbon source, and the medium was inoculated with 5 mg l^{-1} as COD of NMO or PMO (Table 1). Samples were withdrawn periodically from the reactor and analyzed for either naphthalene or phenanthrene concentrations and total protein for biomass determination.

Surfactant and naphthalene or phenanthrene mixture biodegradation experiments

Surfactant and naphthalene or phenanthrene mixture biodegradation experiments were performed in triplicate using the same apparatus and oxygen supply as previously described. One of the four surfactants,

Table 1. Summary of biodegradation experiments

Substrate	Inoculum
SDS	AS, NMO, PMO
SDBS	AS, NMO, PMO
T-maz-80	AS, NMO, PMO
CA-620	AS, NMO, PMO
Naphthalene	NMO
Phenanthrene	PMO
Naphthalene + SDS	NMO
Naphthalene + SDBS	NMO
Naphthalene + T-maz-80	NMO
Naphthalene + CA-620	NMO
Phenanthrene + SDS	PMO
Phenanthrene + SDBS	PMO
Phenanthrene + T-maz-80	PMO
Phenanthrene + CA-620	PMO

SDS (600 mg l^{-1}), SDBS (100 mg l^{-1}), T-maz-80 (7.5 mg l^{-1}) or CA-620 (12 mg l^{-1}) in the presence of naphthalene (30 mg l^{-1}) or phenanthrene (1.0 mg l^{-1}) served as the carbon source. The medium was inoculated with 5 mg l^{-1} as COD of NMO (in the presence of naphthalene) or PMO (in the presence of phenanthrene) (Table 1). The contents of the flask were continuously stirred at 150 RPM and sampled periodically for the analysis of surfactant and naphthalene or phenanthrene concentrations as well as total protein analysis for biomass determinations. Abiotic losses of the surfactant and naphthalene or phenanthrene were monitored using sterilized uninoculated controls.

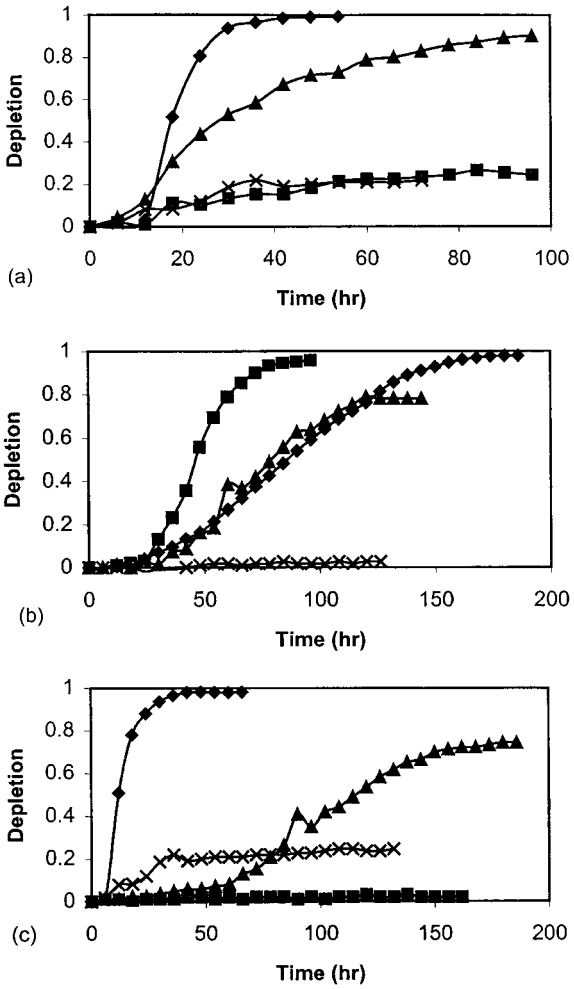

Figure 1. Surfactant depletion curves by activated sludge (a), naphthalene-acclimated culture (b), and phenanthrene-acclimated culture (c). [(♦), SDS; (■), SDBS; (▲), T-maz-80; (×), CA-620].

Results

Surfactant biodegradation

The inconsistency of the triplicate results was within 5% (95% CI, t-test) and a representative result is illustrated in Figure 1. Activated sludge (AS) can degrade SDS, T-maz-80, and about 20% of SDBS and CA-620 (Figure 1a). After initial lag period, SDS was depleted at a very high rate (normalized rate coefficient of 1.44 $\pm$ 0.13 d^{-1}), after which the depletion decreased significantly (95% CI, t-test). The degradation was nearly completed within 30 hrs. T-maz-80 depletion (after initial lag period) occurred at a comparatively lower rate than that of SDS (0.38 $\pm$ 0.02 d^{-1}), followed by a gradually decreasing rate and about 90% was depleted within 100 hrs. SDBS and CA-620 were depleted at a very low and similar rate after initial lag period (0.07 $\pm$ 0.01 d^{-1} and 0.09 $\pm$ 0.02 d^{-1} respectively). Using naphthalene-acclimated microorganisms (NMO), only CA-620 was not biodegradable (Figure 1b). SDBS had the highest depletion rate (0.41 $\pm$ 0.02 d^{-1}) (after initial lag period), after which the depletion decreased significantly (95% CI, t-test) and the depletion was completed within 100 hrs. SDS and T-maz-80 had a similar depletion rate (0.17 $\pm$ 0.02 d^{-1} and 0.12 $\pm$ 0.02 d^{-1} respectively) (after initial lag period) until 120 hrs, after which SDS was depleted at a gradually decreasing rate and the depletion was completed within 200 hrs, and T-maz-80 depletion halted. Phenanthrene-acclimated microorganisms (PMO) degraded SDS, T-maz-80, and about 20% of CA-620,

but it could not degrade SDBS at all (Figure 1c). SDS had a very high depletion rate after initial lag period ($1.68 \pm 0.14\ d^{-1}$), after which the depletion decreased significantly and the depletion was complete within 50 hrs. T-maz-80 had an initial lag period of 50 hrs, after which it was depleted at a comparatively lower rate than that of SDS ($0.12 \pm 0.02\ d^{-1}$) followed by a gradually decreasing rate. About 80% of T-maz-80 was depleted within 200 hrs. CA-620 was depleted at a rate of $0.14 \pm 0.05\ d^{-1}$ after initial lag period until 40 hrs, after which the depletion halted.

Naphthalene and phenanthrene biodegradation

93% of naphthalene and 78% of phenanthrene were degraded within 120 and 140 hrs, respectively (Figure 2). The mineralization of naphthalene was characterized by a relatively constant rate ($0.29 \pm 0.05\ d^{-1}$), followed by a gradually decreasing rate. The degradation of phenanthrene occurred at a lower rate than that of naphthalene ($0.17 \pm 0.02\ d^{-1}$), and also followed by a gradually decreasing rate.

Surfactant biodegradation in the presence of naphthalene or phenanthrene

Surfactant depletion profiles in the presence of naphthalene differed from those in the absence of it, and the responses to the presence of naphthalene varied among surfactants. In the presence of naphthalene, an initial lag of 100 hrs (as compared to 24 hrs in the absence of naphthalene) was observed for SDS, and nearly 90% of naphthalene was degraded within this lag period (Figure 3a). Therefore, naphthalene was preferentially utilized to SDS. There was no significant change in initial lag period for the SDBS and T-maz-80 as compared to un-amended controls, but the depletion rates decreased slightly. SDBS and T-maz-80 depleted at $0.41 \pm 0.02\ d^{-1}$ and $0.12 \pm 0.02\ d^{-1}$ (as compared to $0.48 \pm 0.05\ d^{-1}$ and $0.22 \pm 0.02\ d^{-1}$). In the absence of naphthalene, CA-620 was not significantly degraded; while in the presence of naphthalene and an initial lag period of 30 hrs, NMO were able to degrade CA-620 at a rate of $0.12 \pm 0.01\ d^{-1}$, however, biodegradation stopped when ~30% was depleted.

Surfactant depletion profiles by PMO in the presence of phenanthrene did not follow the NMO trend (in the presence of naphthalene). There was no significant change in initial lag time for SDS and T-maz-80 in the presence of phenanthrene as compared to the un-amended controls. SDS depletion rate decreased from $1.68 \pm 0.14\ d^{-1}$ for un-amended controls to $1.20 \pm 0.09\ d^{-1}$ for phenanthrene amended systems. A similar trend was observed for T-maz-80 biodegradation, with a rate of $0.12 \pm 0.02\ d^{-1}$ for phenanthrene amended systems as compared to $0.19 \pm 0.01\ d^{-1}$ for un-amended controls. There was no significant difference of SDBS and CA-620 depletion between phenanthrene amended systems and un-amended controls (95% CI, t-test). Like un-amended controls, SDBS was not biodegradable by PMO. The depletion rate of CA-620 in phenanthrene amended systems was $0.12 \pm 0.02\ d^{-1}$ as compared to $0.14 \pm 0.02\ d^{-1}$ in un-amended controls.

Naphthalene and phenanthrene biodegradation in the presence of the surfactant

The naphthalene mineralization profiles by NMO in the absence and presence of SDS were superimposable, which indicated that the presence of SDS had no impact on naphthalene biodegradation (Figure 4a). In the presence of T-maz-80 and CA-620, the initial lag period of naphthalene biodegradation was not affected as compared to un-amended controls, however, naphthalene biodegradation rates were lower with a rate of $0.14 \pm 0.02\ d^{-1}$ for T-maz-80 amended systems and $0.19 \pm 0.02\ d^{-1}$ for CA-620 amended systems as compared to $0.29 \pm 0.05\ d^{-1}$ for un-amended controls. In the presence of SDBS, naphthalene biodegradation by NMO had a lag period of ~40 hrs, after which naphthalene displayed a similar depletion rate of $0.26 \pm 0.07\ d^{-1}$ as compared to SDBS un-amended control ($0.29 \pm 0.05\ d^{-1}$).

The initial lag period did not change significantly for phenanthrene biodegradation by PMO in SDS amended systems as compared to the un-amended control. However, the phenanthrene depletion rate in the presences of SDS ($0.10 \pm 0.01\ d^{-1}$) was lower than the un-amended control ($0.17 \pm 0.02\ d^{-1}$). In the presence of SDBS, T-maz-80 and CA-620, the initial lag period for phenanthrene biodegradation displayed a similar trend: the presence of each surfactant increased the initial lag period. The average initial lag periods observed were 42 hrs for SDBS, 54 hrs for CA-620 and 120 hrs for T-maz-80. Phenanthrene depletion rates were also affected as compared to un-amended controls. Un-amended controls had a phenanthrene depletion rate of $0.17 \pm 0.02\ d^{-1}$, while CA-620 amended systems lowered the phenanthrene depletion rate to $0.10 \pm 0.01\ d^{-1}$ and T-maz-80 amended systems displayed a similar trend with a phenanthrene depletion rate of $0.12 \pm 0.02\ hr^{1}$. Con-

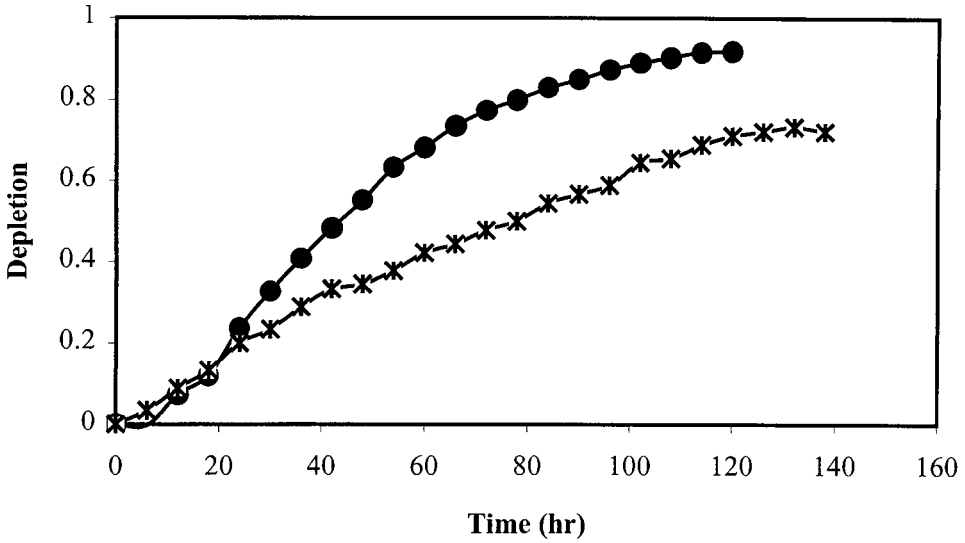

Figure 2. Naphthalene (a) and phenanthrene (b) depletion curves by naphthalene- and phenanthrene-acclimated cultures respectively [(●), naphthalene; (*), phenanthrene].

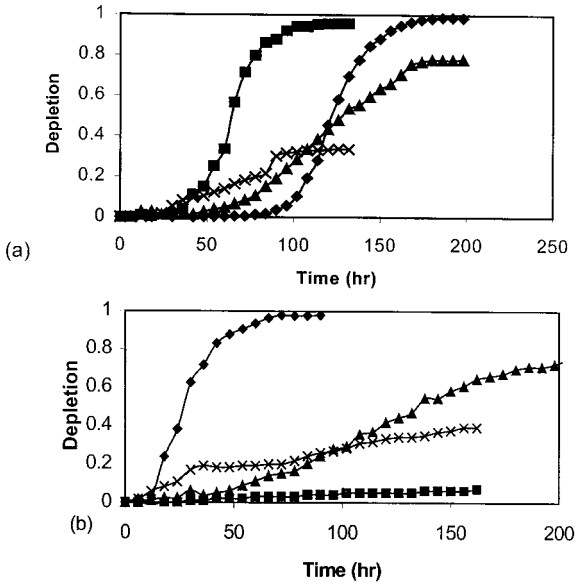

Figure 3. Surfactant depletion curves in the presence of naphthalene (a) and phenanthrene (b) [(♦), SDS; (■), SDBS; (▲), T-maz-80; (×), CA-620].

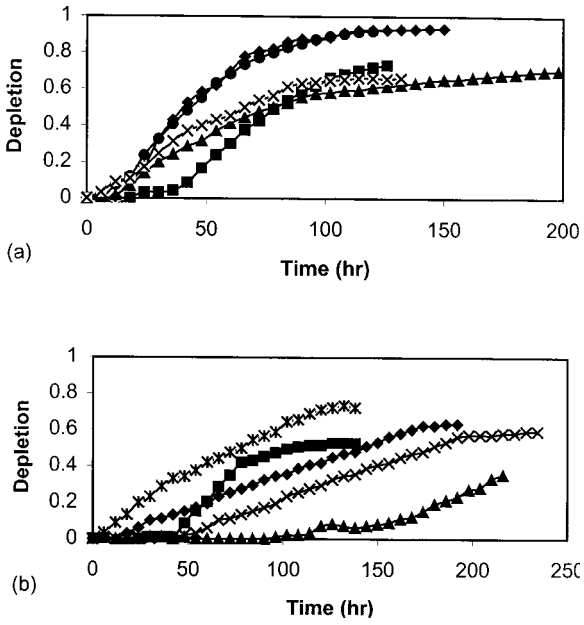

Figure 4. Naphthalene (a) and phenanthrene depletion curves in the presence of the surfactants [(●), naphthalene in the absence of the surfactants; (*), phenanthrene in the absence of the surfactants; (♦), naphthalene (a) or phenanthrene (b) in the presence of SDS; (▲), naphthalene (a) or phenanthrene (b) in the presence of SDBS; (▲), naphthalene (a) or phenanthrene (b) in the presence of T-maz-80; (×), naphthalene (a) or phenanthrene (b) in the presence of CA-620].

versely, even though there was an increased initial lag period for SDBS amended systems, the depletion rate increased slightly with an observed rate of 0.24 ± 0.05 hr^{-1} for SDBS amended systems as compared to un-amended controls (0.17 ± 0.02 d^{-1}).

Discussion

Surfactant biodegradation

As demonstrated by the surfactant biodegradation experiments using activated sludge (AS), both SDS and T-maz-80 were easily degradable. This result is consistent with other T-maz-80 and SDS biodegradation studies (Marchesi et al. 1991 and Goudar et al. 1999). Using *Pseudomonas* C12B, Marchesi et al. (1991) observed a complete SDS biodegradation at an initial concentration of 60 μM in about 12.5 hrs. Goudar et al. (1999) observed a biodegradation of 0.69 mM SDS in about 12 hrs. Rapid SDS biodegradation by AS is attributed to the SDS chemical structure which does not contain an aromatic ring but rather a linear alkyl hydrophobe group. Similarly, T-maz-80, a sorbitan ring with 20 ethylene oxide units, has been reported to be rapidly biodegraded (0.15 mM degraded in about 14 hrs, Goudar et al. 1999). Therefore, the hydrocarbon-adapted AS should have no difficulty acclimating to SDS and T-maz-80. On the contrary, as both SDBS and CA-620 contain aromatic rings, AS cannot degrade them easily. However, presumably due to the diversity of the microbes in activated sludge, AS was able to degrade about 20% of SDBS and CA-620.

In addition to the readily degradable surfactants (SDS and T-maz- 80), naphthalene-acclimated microorganisms (NMO) could also degrade SDBS, while phenanthrene-acclimated microorganisms (PMO) were unable to produce any significant depletion as compared to sterile controls. To further investigate the reason for this phenomenon, naphthalene dioxygenase assay was conducted for NMO and PMO respectively, using the method described by Riis et al. (1996). The results showed that NMO had a naphthalene dioxygenase activity of 3.7 ± 0.3 nmol min^{-1} mg·protein^{-1} when grown on SDBS alone, 6.6 ± 0.2 nmol min^{-1} mg·protein^{-1} when grown on naphthalene and SDBS, and 6.8 ± 0.2 nmol min^{-1} mg·protein^{-1} for un-amended control (NMO grown on naphthalene as sole carbon source). While PMO had a low naphthalene dioxygenase activity of 0.8 ± 0.5 nmol min^{-1} mg·protein^{-1} when grown on SDBS alone and 1.1 ± 0.4 nmol min^{-1} mg·protein^{-1} for un-amended control (PMO grown on phenanthrene alone). It has been demonstrated that naphthalene dioxygenase plays an important role in the degradation of aromatic compounds ranging in size from benzene to beno[a]pyrene (Lee et al. 1997). Naphthalene dioxygenase, a membrane-bonded enzyme, appears critical, however not conclusive, in SDBS degradation. For high molecular weight compound degradation, membrane-bonded enzymes usually play a more important role that those of cytosolic enzymes because it is generally considered difficult for the high molecular weight compounds to enter into the cells. Riis et al. (1996) found that some strains in the natural environment had a certain naphthalene dioxygenase activity. For example, *Sphingomonas* sp., which is commonly found in freshwater sediment (Karlson et al. 1995) and nitrogen fixation (Anderson 1955) showed a naphthalene dioxygenase activity of 2.1 nmol min^{-1} mg·protein^{-1}. Common naphthalene-degrading microorganisms in the literature are *Pseudomonas* and *Burkholderia* strains with *Pseudomonas* dominating (Hedlund et al. 1999) while phenanthrene-degrading microorganisms are usually identified as *Archrobacter, Acidovorax* and *Brevibacterium*, etc. (Samanta et al. 1999).

Impact of the presence of naphthalene or phenanthrene on surfactant degradation and vice versa

The four surfactants responded to the presence of naphthalene or phenanthrene in different ways. The first response was that a lag occurred, such as SDS in the presence of naphthalene. A four-fold increase in initial lag period was observed for SDS and naphthalene mixture systems as compared to SDS alone. During this increased lag period, naphthalene was preferentially degraded by NMO. The second response was that there was no change in lag period for surfactant degradation, but a change in the degradation rate, which were the most cases with NMO. The third response was that the non- degradable surfactant was degraded after a lag period, such as SDBS and CA-620 in the presence of naphthalene.

Naphthalene and phenanthrene also responded differently to the presence of the surfactant. The first response was no change in degradation, such as naphthalene in the presence of SDS. The second response was also that the depletion had no change in lag period but occurred at lower rates, such as naphthalene in the presence of T-maz-80 and CA-620, phenanthrene in the presence of SDS. The third response was that a increased lag occurred, such as naphthalene in the presence of SDBS and phenanthrene in the presence of SDBS, T-maz-80 and CA-620.

Naphthalene was preferentially utilized by NMO to SDS, which was shown by the response of SDS to

the presence of naphthalene (Figure 3a) and the response of naphthalene to the presence of SDS (Figure 4a). SDS degradation did not start until naphthalene degradation was nearly completed. As more biomass was formed (based on total protein analysis, data not shown) when SDS began to degrade, SDS depleted at a much higher rate. For naphthalene in the presence of T-maz-80 and CA-620, phenanthrene in the presence of SDS, there was no lag change but naphthalene, phenanthrene and the surfactants had a lower depletion rate than when they alone served as the carbon source (Figures 3a, 3b, 4a, and 4b). Therefore, the degree of the preference was hard to define. The presence of SDBS inhibited naphthalene and phenanthrene degradation and the presence of T-maz-80 and CA-620 inhibited phenanthrene degradation. The degradation of CA-620 in the presence of naphthalene was induced by the degradation of naphthalene. During the degradation of naphthalene or phenanthrene, some intermediates such as catechol, salicylate and phthalate that had a similar structure to CA-620 were formed (data not shown). The enzymes that can break down these intermediates, could also react with CA-620. This could explain why CA-620 did not start to degrade until naphthalene biodegradation began.

Using the same culture, kinetic parameters, such as maximum specific growth rate μ_m and half-saturation coefficient K_s, should be the same for the same substrate. For naphthalene in the presence of T-maz-80 and CA-620 and phenanthrene in the presence of SDS, an assumption that the initial inoculum was subdivided into two consortia with one group continuing naphthalene or phenanthrene degradation and another acclimating to the surfactant, was made. Assuming that there was no co-metabolism or inhibition between naphthalene or phenanthrene and the surfactant, the competition between naphthalene or phenanthrene and the surfactant for the substrate can be expressed by the distribution ratio of the initial biomass between naphthalene or phenanthrene and the surfactant.

If microbial growth is coupled with substrate depletion, and Monod-type kinetics are assumed to describe microbial growth, the substrate and biomass concentrations over time can be described by the following equation (Grady et al. 1999):

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{\mu_m S X}{K_s + S} - \frac{bX}{K_s + S} \tag{1}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\mu_m S X}{K_s + S} \tag{2}$$

where X is the biomass concentration (mg l^{-1}); t time elapsed (hr); μ_m maximum specific growth rate (hr^{-1}); S substrate concentration (mg l^{-1}); K_s half-saturation coefficient (mg l^{-1}); b microbial decay coefficient (hr^{-1}) and Y growth yield coefficient (mg biomass per mg substrate). Ignoring microbial decay, Y can be used to estimate the biomass production based on substrate depletion such that:

$$Y = -\frac{\Delta X}{\Delta S} \tag{3}$$

$$X = X_0 + Y(S_0 - S). \tag{4}$$

By substituting Equation (4) into Equation (2), the substrate depletion can be expressed as:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\frac{1}{Y}\frac{\mu_m S[X_0 + Y(S_0 - S)]}{K_s + S} \tag{5}$$

where X_0 and S_0 refer to the initial biomass and substrate concentration (mg l^{-1}). Define $\alpha_0 X_0$ the actual initial biomass for naphthalene or phenanthrene degradation and $\alpha_1 X_0$ the actual initial biomass for surfactant degradation. The competition between naphthalene or phenanthrene and the surfactant for substrate can be expressed by α_0/α_1. In addition, α_0 and α_1 should be in a relationship of:

$$\alpha_0 + \alpha_1 = 1. \tag{6}$$

Naphthalene, phenanthrene and surfactant depletion profiles, using NMO or PMO, when they served as the sole carbon source were simulated by means of non-linear regression based on simplex optimization of least squares to estimate their relevant Monod kinetic parameters. Based on these values, naphthalene and phenanthrene depletion profiles in the presence of the surfactant and the surfactant depletion profiles in the presence of naphthalene or phenanthrene were evaluated to estimate the actual initial biomass concentration $\alpha_0 X_0$ and $\alpha_1 X_0$. The results are summarized in Table 2. Based on the α_0/α_1 values, it can be predicted that naphthalene had a higher utilization preference to T-maz-80 than to CA-620.

Implication of surfactant flushing

For PAH contaminated sites where naphthalene dominates, selection of SDS is more appropriate based on its biodegradability and results of this study. SDS was not preferentially utilized by naphthalene-acclimated microorganisms; also, SDS itself is an easily degradable compound and can be degraded by either NMO

Table 2. Summary of kinetic and stoichiometric parameters and α_0/α_1 values

Substrate	Inoculum	μ_m (hr^{-1})	K_s (mg l^{-1})	Y (mg biomass/ mg substrate)
Naphthalene	NMO	0.49	0.08	0.61
Phenanthrene	PMO	0.18	0.07	0.72
CA-620	NMO	N/A	N/A	N/A
T-maz-80	NMO	0.71	0.12	0.75
SDS	PMO	0.37	0.10	0.34
	α_0		α_1	α_0/α_1 or $\alpha_0/(1-\alpha_1)$
Naphthalene in the presence of CA-620	0.65	CA-620 in the presence of naphthalene	N/A	1.85
Naphthalene in the presence of T-maz-80	0.46	T-maz-80 in the presence of naphthalene	0.55	0.84
Phenanthrene in the presence of SDS	0.42	SDS in the presence of phenanthrene	0.60	0.82

or the indigenous microbes. The second choice would be T-maz-80. Although CA-620 can remain longer in the contaminated sites in the presence of residual naphthalene than T-maz-80 due to its higher α_0/α_1 value, CA-620 is not a good choice due to its low biodegradability. In addition, the degradation of CA-620 is induced by the degradation of naphthalene. It should be kept in mind that only 80% of T-maz-80 can be degraded, which means that the other 20% will remain in the subsurface for an extended time period. As SDBS slightly inhibits naphthalene degradation, it should not be considered for surfactant flushing at sites where naphthalene dominates.

For the PAH contaminated sites where phenanthrene dominates, SDS is the best choice among the surfactants studied. Also, it can be easily and completely degraded. As all the three other surfactants inhibit phenanthrene degradation, they should not be considered for such usage. Based on above discussion, for the PAH contaminated sites with both naphthalene and phenanthrene existing, SDS is the best and the only choice.

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