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Biodegradation of Naphthalene in Aqueous Nonionic Surfactant Systems

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The principal objective of this study was to quantify the bioavailability of micelle-solubilized naphthalene to naphthalene-degrading microorganisms comprising a mixed population isolated from contaminated waste and soils. Two nonionic surfactants were used, an alkylethoxylate, Brij 30 ($C_{12}E_4$), and an alkylphenol ethoxylate, Triton X-100 ($C_8PE_{9.5}$). Batch experiments were used to evaluate the effects of aqueous, micellized nonionic surfactants on the microbial mineralization of naphthalene and salicylic acid, an intermediate compound formed in the pathway of microbial degradation of naphthalene. The extent of solubilization and biodegradation under aerobic conditions was monitored by radiotracer and spectrophotometric techniques. Experimental results showed that surfactant concentrations above the critical micelle concentration were not toxic to the naphthalene-degrading bacteria and that the presence of surfactant micelles did not inhibit mineralization of naphthalene. Naphthalene solubilized by micelles of Brij 30 or Triton X-100 in liquid media was bioavailable and degradable by the mixed culture of bacteria.

Hydrophobic organic compounds (HOCs) have low aqueous solubilities, and the biodegradation of such compounds may be restricted because of the low solubility coupled with strong binding/sorption onto solids. There is interest in evaluating whether the biotreatment of HOCs may be facilitated through surfactant addition, since surfactants can increase the amount of sparingly soluble HOCs present in the liquid phase by incorporation in surfactant micelles or aggregates. Micellized HOCs may have increased bioavailability to microorganisms and may thereby improve the biodegradation rate.

Experimental observations on the effects of surfactant additions on microbial degradation of HOCs are not consistent, nor has a general explanation been advanced for the influence of the surfactants. Results of studies on the effects of surfactants in biodegradation experiments are very diverse. Table 1 is a summary of recent research work; the presence of surfactant has been reported to be beneficial, detrimental, or ineffective to microbial substrate utilization rates and growth yields. Clearly, the effect of surfactant on microbial degradation depends on various factors of the systems studied, including the physical and chemical aspects and the concentration of surfactant and the substrate.

Surfactants can solubilize both solid-phase and sorbed-phase HOCs, such as polycyclic aromatic hydrocarbons (PAHs). Volkering et al. (32) found that for compounds with low aqueous solubility or high adsorptive capacity (e.g., PAHs), mass transfer from the solid to the liquid phase may be the rate-limiting step in the microbial degradation process. It has been shown that the presence of nonionic surfactant micelles in aqueous solution results in effective removal of sorbed PAHs from soil through the process of solubilization (10, 11, 16, 24). Once the PAH substrate is present in a micellar pseudophase, an important question becomes whether PAH solubilized in micelles is bioavailable, so that mass transfer to the aqueous pseudophase is not a rate-limiting step. In this study, we set out to provide information to answer this question. An important difference between our study and many other investigations is that we have used concentrations of PAHs that are much in excess of the PAH pure-compound aqueous solubility limit. This is not the case for most other studies described in Table 1. We chose such concentrations since most of the PAH is then solubilized in the micelles. This may be representative of effective use of surfactant in remediation applications, which optimally should have micelles containing significant amounts of PAHs. Thus, in this study the micellar pseudophase served as a source for as much of 75% of the PAH present in each system.

To understand more fully the possible effects of surfactant on microbial mineralization of PAHs, it is necessary to evaluate the physicochemical processes of equilibrium partitioning of PAHs and the mass exchange of PAHs between the micellar and aqueous pseudophases. As microorganisms deplete the PAH in the aqueous pseudophase, the micelle-solubilized PAH diffuses to the aqueous pseudophase. The micellar pseudophase thus serves as a source for the aqueous pseudophase.

This investigation included solubilization and biomineralization experiments with naphthalene. The research addressed the specific issue of whether micelle-solubilized naphthalene was bioavailable to PAH-degrading microorganisms. We present here the results of our study with PAH-degrading bacteria, pure organic compounds, and synthetic nonionic surfactants.

MATERIALS AND METHODS

Compounds. The surfactants used in this work were two nonionic polyethoxylate ethers: an alkylethoxylate ether, Brij 30 ($C_{12}E_4$), and an alkylphenol ethoxylate ether, Triton X-100 ($C_8E_{9.5}$). These surfactants were a subset of those selected initially on the basis of a literature survey of surfactant-aided soil washing and screening tests to assess which surfactants demonstrated the best PAH-solubilizing capacities in soil-aqueous systems (24). The critical micelle concentrations (CMC) of Brij 30 and Triton X-100 were 2.3×10^{-5} M (8.8×10^{-4}%, vol/vol) and 1.7×10^{-4} M (1.0×10^{-2}%, vol/vol), respectively (23). Laboratory-grade Brij 30 and Triton X-100 were purchased from Aldrich Chemical Co., Milwaukee, Wis., and used without further purification.

The PAH solubilizate used in this work was naphthalene. The other two organic compounds were glucose and salicylic acid. Salicylic acid was used in this study because it is a naphthalene metabolite (8, 9). The aqueous solubilities of salicylic acid ($C_7H_6O_3$) and naphthalene ($C_{10}H_8$) are approximately 2,200 and 32 mg/liter, respectively. Reagent-grade glucose, salicylic acid, and naphthalene

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TABLE 1. Summary of reported effects of surfactants on microbial degradation

Overall effect[a]	Observation	Explanation	Reference
+	Biodegradation of naphthalene solubilized in Triton X-100 micelles; mineralization of soil-bound naphthalene and phenanthrene in the presence of Triton X-100	Dissolved HOCs are bioavailable in both aqueous and micellar phases	16
+	Enhanced bacterial growth rate and increased rate of n-alkane consumption	Surfactant solubilization increases aqueous solubility of hydrocarbon	7
+	Addition of Alfonic 810-60 or Novel 11 1412-56 enhanced biodegradation of phenanthrene and biphenyl in the presence of aquifer material	Enhancement may be due to removal from the solid and subsequent partitioning in the aqueous pseudophase	3
+	Extent of biodegradation of phenanthrene in soil increased by low doses of surfactant in absence of surfactant-induced desorption	No explanation	4
+	Triton X-100 increased both the rate and extent of mineralization of naphthalene	Although bacterial adherence prevented, there was sufficient aqueous naphthalene concentration	13
+	Increased hydrocarbon degradation rate and extent with biosurfactant addition	Reduction in interfacial tension	27
+	Microorganisms were able to rapidly incorporate significant quantities of octadecane	Liposomes facilitated substrate transport through the microbial cell wall	25
+	Increased degradation rate of oil in soil-water slurries	Reducing interfacial tension promotes formation of more interfacial area	29
+	Different Tween-type nonionic surfactants enhanced phenanthrene biodegradation	Surfactant solubilization of HOCs increases bioavailability	17
+	Nonionic detergents stimulated growth on hexadecane	Emulsifying action permits effective contact between cells and substrate	6
+	Enhanced rate of biodegradation of PCBs[b] in ligninsulfonate emulsion	Emulsifying action overcomes interfacial area limitation	22
+	Increased rate of hydrocarbon degradation	Emulsifying action increases interfacial area	30
0	No effect on phenanthrene mineralization in soil-water systems at low surfactant dose	Surfactant sorbed onto soil	19
0	Aromatic biodegradation by pure cultures either unaffected or slightly stimulated by emulsification of oil	Microbial uptake only of solubilized substrate, implying prior adherence not required	14
0	Bioemulsifier addition has no significant effect on PAH degradation	No explanation	15
−	Mineralization of phenanthrene inhibited at higher surfactant doses	Possible bacterium-surfactant interactions and/or competitive substrate utilization	19, 20
−	Reduced effectiveness or inhibition observed at higher surfactant concentration	Toxicity of surfactants to microorganisms	4
−	Triton X-100 completely prevents mineralization of hexadecane dissolved in heptamethylnonane	Surfactant prevented bacterial adherence to solvent-water interface and/or affects cell membranes	13
−	Decreased biodegradation of HC[b] in emulsan-treated oil	Surfactant prevents adherence to hydrophobic surfaces	14
−	Crude oil biodegradation retarded by chemical dispersants	Nontoxic surfactants used as preferential substrate	26
−	Decrease in growth rate of yeast cells on alkanes in the presence of Tween 20	Surfactant prevented large fraction of cells from adhering to hydrocarbon	1

[a] +, beneficial effect; −, detrimental effect; 0, no effect.
[b] PCB, polychlorinated biphenyl; HC, hydrocarbon.

with a purity greater than 98% were purchased from Fisher Scientific Co., Pittsburgh, Pa. D-[U-^{14}C]glucose with a specific activity value of 286 mCi/mmol was purchased from Amersham Corp., Arlington Heights, Ill. [*carboxy*-^{14}C]salicylic acid (specific activity, 11.4 mCi/mmol) and [1-^{14}C]naphthalene (specific activity, 4.5 mCi/mmol) were purchased from Sigma Chemical Co., St. Louis, Mo. The inorganic reagents were analytical grade (Fisher Scientific). All glassware was washed in 1:1 water-concentrated nitric acid before use.

Medium. Biodegradation experiments were conducted at room temperature on an orbital shaker (Lab-Line Instruments, Inc., Melrose Park, Ill.) with autoclaved mineral salts medium (MSM), consisting of 170 mg of KH_2PO_4, 435 mg of K_2HPO_4, 668 mg of $Na_2HPO_4 \cdot 7H_2O$, 85 mg of NH_4Cl, 22.5 mg of $MgSO_4 \cdot 7H_2O$, 27.5 mg of $CaCl_2$, and 0.25 mg of $FeCl_3 \cdot 6H_2O$ per liter of deionized water. Nonionic surfactant stock solutions for solubilization experiments or mineralization experiments were made by dissolving the required volume of surfactant into deionized water or autoclaved MSM. The solutions were sonicated for 24 h or more to ensure that the surfactant was completely dissolved.

Organisms. The PAH-degrading bacteria (RET-PA-101) were a mixed population that had been isolated from contaminated wastes and soils. For the purpose of inoculation, the initial enrichments were incubated for 3 to 4 days at room temperature on a reciprocating shaker at 60 strokes/min with a timer set to operate the shaker for 15 min every 0.5 h. The culture of PAH-degrading bacteria was grown in autoclaved MSM containing naphthalene in excess of saturation as the sole carbon source. Standard sterile techniques were used for plate counts and inoculation. The PAH-degrading bacteria were enumerated by plating aliquots from appropriate dilutions of the culture on the spread plates. The plates were made by spreading autoclaved MSM containing 1.5% agar. The inoculated plates were incubated with naphthalene vapors at room temperature for 5 days or longer. The colonies of the PAH-degrading bacteria were identified as clear circular zones against a cloudy field.

Analytical methods. Radiotracer techniques were used to measure the extent of solubilization and mineralization. Naphthalene-spiking solutions, consisting of mixtures of labeled and unlabeled compound, were prepared in methanol. The spiking solution of glucose or salicylic acid was prepared in deionized water or in 0.05 M sodium bicarbonate. Analysis of ^{14}C-compound was taken as representative of the behavior of the compound as a whole. Samples were counted for ^{14}C on a Beckman LS 5000 TD liquid scintillation counter by the H number quench monitoring technique with automatic quench compensation and computer data logging. Residual methanol in solubilization tests did not exceed 1%.

Spectrophotometric techniques were used to measure the absorbance of micellar surfactant solutions in order to determine whether there was substantial

biodegradation of Brij 30 and Triton X-100. An azo dye, *p*-dimethylaminoazobenzene (DMAB), was used to determine the concentration of Brij 30 by excess dye solubilization, filtration through a 0.45-μm-pore-size Supor-450 membrane filter (Gelman Sciences, Ann Arbor, Mich.) to remove excess DMAB, and absorbance measurement. The absorbance measurement was made on an HP 8541A diode array spectrophotometer (Hewlett Packard Co.) and measured A_{414} or A_{278} for the bulk aqueous solutions of Brij 30 plus DMAB and of Triton X-100, respectively. In tests with surfactant and naphthalene, the PAH was volatilized from the liquid phase prior to DMAB solubilization for Brij 30 or absorbance measurement for Triton X-100.

Solubilization tests. Batch tests for solubilization of naphthalene by surfactants in water were performed at 25°C. The amount of [1-^{14}C]naphthalene used in each batch test was approximately 0.5 μCi, corresponding to approximately 10^6 dpm. The mass of naphthalene used was 20 times the mass of naphthalene required to attain the aqueous solubility limit in pure water in order to ensure that the progress of solubilization upon addition of surfactant could be observed over a range of surfactant concentrations above and below the CMC. Each batch test was performed in duplicate. An individual batch test sample consisted of a 5-ml solution containing deionized water, naphthalene stock, and surfactant stock, all of which were in a glass vial having a capacity of 8-ml. The vial was sealed with an open-port screw cap, which was fitted with a Teflon-lined septum to prevent loss of naphthalene from solution. The prepared vials were placed in a water bath at 25°C and reciprocated at 80 strokes per min for an equilibration period of approximately 24 h. Aliquots of the sample solution were withdrawn by syringe and expressed through a preconditioned 0.22-μm Teflon membrane filter to remove solid-phase crystalline naphthalene while passing dissolved naphthalene in the aqueous pseudophase and solubilized naphthalene in the micellar pseudophase. Filter preconditioning involved purging the filter with a few milliliters of sample solution to allow sorption saturation of internal surfaces of the syringe, filter, and needle. Duplicate aliquots were injected into two 20-ml polyethylene counting vials with 10 ml of liquid scintillation cocktail (Scintiverse II; Fisher Scientific) and counted for [1-^{14}C]naphthalene.

Biodegradation tests. Batch tests designed to assess the mineralization of glucose, salicylic acid, and naphthalene by PAH-degrading bacteria in aqueous systems were conducted by adding approximately 10^6 dpm of a specific labeled compound plus unlabeled compound with or without surfactant to 250-ml biometer flasks. The mineralization of naphthalene included these three configurations of aqueous solutions: (i) naphthalene in excess of saturation in aqueous solution without surfactant, (ii) naphthalene in excess of saturation with 0.00022% (vol/vol) Brij 30 or 0.0025% (vol/vol) Triton X-100, and (iii) micelle-solubilized naphthalene with 0.15% (vol/vol) Brij 30 or 0.20% (vol/vol) Triton X-100. Naphthalene-spiking solution in methanol was added to each biometer flask. After methanol evaporation, the mass of naphthalene remaining in the biometer flasks was measured by determining [1-^{14}C]naphthalene through addition of a high concentration of surfactant solution. For the three sets of experiments, the total amount of naphthalene was 3.0, 3.2, and 5.7 mg, respectively. Brij 30 or Triton X-100 stock solutions in autoclaved MSM at various predetermined amounts of surfactant were then added to the biometer flasks along with sufficient autoclaved MSM to bring the total solution volume of each biometer to 50 ml. The concentration of glucose or salicylic acid for the mineralization tests in aqueous solutions was 100 mg/liter. Biodegradation tests for selected compounds involved an aerobic, closed system consisting of a biometer flask fitted with a side arm containing sodium hydroxide solution (5, 28). The pH of the systems at the start of experiments was buffered to 7, which is neither too low to inhibit mineralization nor too high to restrict the evolution of $^{14}CO_2$.

Batch tests designed to assess the biodegradation of surfactant by PAH-degrading bacteria in aqueous systems were conducted by adding Brij 30 or Triton X-100 at 0.04% (vol/vol) with naphthalene to Erlenmeyer flasks. Surfactant biodegradation tests involved an aerobic, closed system. The pH of the systems at the start of experiments was buffered to 7.

Biometer flasks and Erlenmeyer flasks were autoclaved prior to use in the experiments. The biometer flasks containing selected compounds with or without surfactant and the Erlenmeyer flasks containing Brij 30 or Triton X-100 with naphthalene were equilibrated for 24 h or more prior to inoculation. Each flask received a 2-ml culture of PAH-degrading bacteria containing approximately 5×10^7 CFU/ml. The flasks were immediately sealed with foil-covered neoprene stoppers. The sealed flasks were continuously shaken at approximately 60 rpm to ensure that the aqueous solution was well mixed. The flasks were purged briefly with oxygen at the time of sampling to preclude the possibility of oxygen limitation to the systems.

Microbial mineralization of selected compounds was monitored by measuring the evolution of $^{14}CO_2$. Each mineralization test was performed in duplicate or triplicate. Abiotic controls were set up to evaluate any abiotic losses. The $^{14}CO_2$ evolved was trapped in 5.0 ml of 2 M NaOH solution contained in the sidearm of the biometer flask. The NaOH solution was replaced with fresh alkali at the time of sampling. Total $^{14}CO_2$ evolved during these periods was measured by placing a 0.5-ml subsample of the NaOH solution in triplicate for each sampling in 20-ml scintillation vials containing 10 ml of scintillation cocktail (OptiFluor; Packard Instrument Co., Downers Grove, Ill.) and counted for ^{14}C activity after being stored overnight in the dark to minimize chemiluminescence. Cumulative mineralization was determined from average values of disintegrations per minute for each triplicate set of samples, accounting for the disintegrations per minute removed at each sampling period.

Biodegradation of surfactant was monitored qualitatively by measuring the absorbance of the bulk aqueous solution. A 20-ml sample of the bulk aqueous solution was withdrawn from Erlenmeyer flasks and heated at 60 to 70°C to remove naphthalene by evaporation. When the solution was evaporated, a fresh volume of MSM was added to reconstitute the surfactant solution. Naphthalene remaining in the reconstituted samples was assessed by measuring [1-^{14}C]naphthalene; residual naphthalene was less than 3% of the initial [1-^{14}C]naphthalene. This amount of naphthalene had little effect on the solubilization of DMAB by Brij 30 or on the absorbance measurement of Brij 30 plus DMAB or of Triton X-100. The reconstituted surfactant solutions of either Brij 30 plus DMAB or Triton X-100 were prepared by sonication to ensure that surfactant was dissolved. The samples of Brij 30 plus DMAB or Triton X-100 were then shaken for an equilibration period of 64 or 40 h, respectively. Aliquots of Brij 30 plus DMAB and of Triton X-100 were withdrawn with a syringe and expressed through a preconditioned 0.45-μm Supor-450 membrane filter. Filter preconditioning involved purging with several milliliters of sample. The filtrate was used for absorbance measurement to determine surfactant concentrations for Brij 30 and Triton X-100.

RESULTS

Bacterial growth and cell structure in the presence of surfactant. The growth of *Escherichia coli* on glucose was studied in the absence and presence of Triton X-100. The density of bacterial cells was monitored by measuring the A_{560}. The specific growth rates were observed in the absence and presence of 0.10% (vol/vol) surfactant; the results were similar for both systems. This indicates that Triton X-100 nonionic surfactant at concentrations above the CMC did not inhibit the growth of the gram-negative *E. coli* strain on glucose. This confirms that the surfactant concentrations tested did not produce a readily observable, inherent toxic effect.

Little et al. (21) have examined the cellular structures of bacteria and biofilms by advanced environmental-sample scanning electron microscopy techniques. Environmental-sample scanning electron microscopy analyses were performed to compare the cell structures of PAH-degrading bacteria grown on naphthalene plus either Brij 30 or Triton X-100 at 10× CMC. This comparison showed that there were no significant structural differences between the bacteria grown on naphthalene in the absence and presence of surfactant micelles. This confirms that the surfactant concentrations tested had no observable effect on rupture of the cell membrane or lysis of the PAH-degrading bacteria.

Mineralization of glucose and salicylic acid in the presence of surfactant. Mineralization experiments were performed with glucose in the absence and presence of the nonionic surfactants Brij 30 and Triton X-100 in aqueous systems. The presence of surfactant had no adverse effect on glucose mineralization with 0.20% (vol/vol) Brij 30 (~225× CMC) or Triton X-100 (~20× CMC). The data showed that glucose mineralization proceeded at a high initial rate that declined after 2 days, with approximately 28 to 41% of glucose being mineralized; 33 to 45% of the initial glucose was mineralized in 5 days. The results of mineralization of glucose in the presence of these surfactants are qualitatively similar to those of Laha and Luthy (19).

Biological mineralization of salicylic acid by PAH-degrading bacteria in the absence and presence of Brij 30 or Triton X-100 was performed. The mineralization of salicylic acid with Brij 30 proceeded at almost the same rate as without surfactant. About 60% of the salicylic acid was mineralized in 7 days. The initial rates of mineralization of salicylic acid in the absence and presence of Triton X-100 were similar, but the extents of mineralization of salicylic acid were increased for samples containing Triton X-100. About 60, 73, and 74% of the initial salicylic acid was mineralized in aqueous systems containing 0, 0.0025, and 0.20% (vol/vol) Triton X-100, respectively. These

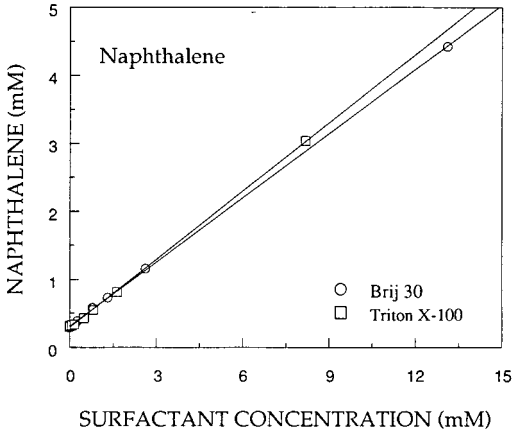

FIG. 1. Solubilization of naphthalene by Brij 30 and Triton X-100. The slope of the solubilization curve is the molar solubilization ratio.

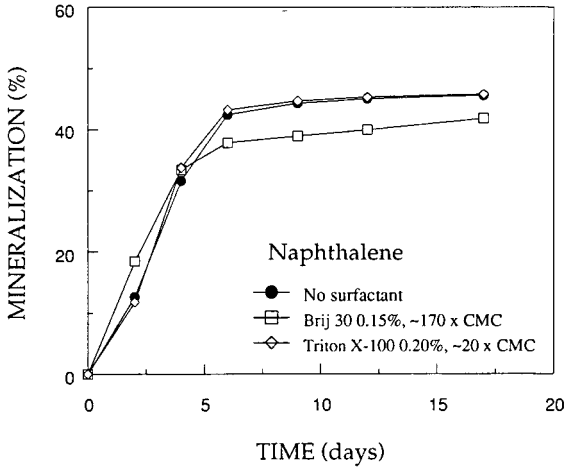

FIG. 2. Mineralization of naphthalene in 50 ml of aqueous solution without surfactant and with 0.15% (vol/vol) Brij 30 (~170× CMC) or 0.20% (vol/vol) Triton X-100 (~20× CMC) with 5.7 mg of naphthalene.

results show that the mineralization of salicylic acid was not inhibited by surfactant micelles. Both glucose and salicylic acid are very much more hydrophilic than are nonpolar PAHs. As a consequense, glucose and salicylic acid are not micellized substantially at surfactant concentrations above the CMC.

Solubilization of naphthalene by nonionic surfactant. Data in Fig. 1 show that significant naphthalene solubilization is observed only at surfactant concentrations greater than the CMC. The aqueous solubility of pure naphthalene is about 0.25 mmol/liter, which is approximately equal to the ordinate intercept value of about 0.3 mmol/liter for the surfactant solubilization plot (Fig. 1).

The slope of the linear portion of the apparent solubility curve for Brij 30 and Triton X-100 at concentrations greater than the CMC is the numerical value for the molar solubilization ratio (MSR). Values of a dimensionless mole fraction partition coefficient, K_m, micelle-phase (X_m)/aqueous-phase (X_{aq}) (see Discussion for definitions of X_m and X_{aq}), may be computed from the MSR (12):

$$K_m = \frac{55.4}{S_{CMC}}\left(\frac{MSR}{1 + MSR}\right)$$

where S_{CMC} is the naphthalene solubility at the CMC. Experimentally measured values of aqueous S_{CMC}, MSR, and log K_m (12) are summarized in Table 2. The solubilization experiments show that substantial amounts of naphthalene are incorporated into the micellar pseudophase.

Mineralization of naphthalene in the presence of surfactant. Mineralization experiments were conducted with naphthalene as the target compound. The results are shown in Fig. 2 to 4. The cumulative disintegrations per minute flushed from sterile controls was less than 0.1% of the total disintegrations per minute therefore, naphthalene losses through abiotic degradation were not used to correct microbial mineralization of naphthalene. The mineralization of naphthalene with Brij 30 or Triton X-100 proceeded at almost the same initial rate as without surfactant. The extent of mineralization of naphthalene in the presence of Brij 30 was equal to or somewhat lower than in the absence of surfactant, whereas the extent of mineralization of naphthalene in the presence of Triton X-100 was equal to or somewhat greater than in the absence of surfactant. In summary, the results in Fig. 2 to 4 show that the microbial degradation of naphthalene was not significantly affected by the addition of Brij 30 or Triton X-100.

Biodegradation of surfactant with naphthalene. The biodegradation of Brij 30 or Triton X-100 with naphthalene was determined qualitatively. The data showed that the concentration of Brij 30 in inoculated samples decreased as the naphthalene degradation process proceeded, indicating that Brij 30 was degraded along with naphthalene. By comparison, there was little change in the noninoculated surfactant samples. The results showed that the concentrations of Triton X-100 with

TABLE 2. Naphthalene solubility and mole fraction micelle-phase/aqueous-phase partition coefficients

Surfactant	Naphthalene solubility[a] (mol/liter)		MSR	log K_m
	No surfactant	Surfactant dose = CMC		
Brij 30	3.0×10^{-4}	3.4×10^{-4}	0.32	4.59
Triton X-100	3.0×10^{-4}	3.2×10^{-4}	0.34	4.64

[a] With ≤1% (by volume) methanol.

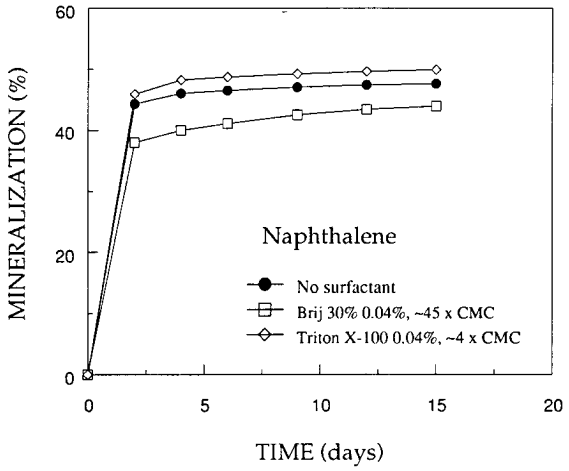

FIG. 3. Mineralization of naphthalene in 50 ml of aqueous solution without surfactant and with 0.04% (vol/vol) Brij 30 (~45× CMC) or 0.04% (vol/vol) Triton X-100 (~4× CMC) with 3.2 mg of naphthalene.

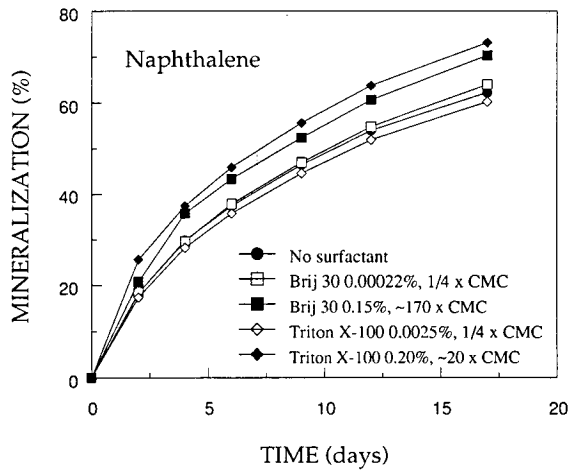

FIG. 4. Mineralization of naphthalene in 50 ml of aqueous solution without surfactant and with 0.00022% (vol/vol) Brij 30 (1/4× CMC), and 0.15% (vol/vol) Brij 30 (~170× CMC) or with 0.0025% (vol/vol) Triton X-100 (1/4× CMC) and 0.20% (vol/vol) Triton X-100 (~20× CMC) with 3.0 mg of naphthalene.

and without inoculation were almost the same as the initial value. From this information and comparison with results in Fig. 3, these data indicate that Triton X-100 was not degraded while naphthalene was degraded and that Brij 30 was degraded simultaneously with naphthalene. The degradation of Triton X-100 by the PAH-degrading microorganisms was not evident by UV spectral analysis of surfactant samples used in these experiments. Despite evidence for the degradation of Brij 30, there were apparently no substantive competitive effects between the two substrates, Brij 30 and naphthalene, to the exclusion of mineralization of the PAH.

DISCUSSION

The mineralization of glucose, salicylic acid, and naphthalene is characterized by a high initial rate followed by an approach to a limited rate. The extent of mineralization of glucose, salicylic acid, and naphthalene in the absence or presence of surfactant doses from 0 to 0.20% (vol/vol) ranged from 33 to 45%, 59 to 75%, and 42 to 75%, respectively. The carbon substrate not mineralized would consist of degradation products remaining in the aqueous solution and of carbon assimilated into cell material. Some naphthalene was lost to the atmosphere during sampling and reoxygenation, which over the course of the experiments described here could amount at most to a theoretical maximum of 25 to 35% in some samples, assuming complete loss of gas-phase naphthalene in equilibrium with the liquid phase during each of six sampling intervals.

One objective of this study was to evaluate any specific effects of surfactant micelles on PAH-degrading bacteria. Environmental-sample scanning electron microscopy data showed that there were no significant structural differences between the PAH-degrading bacteria in the absence and presence of either Triton X-100 or Brij 30 micelles. Additionally, the growth of *E. coli* on glucose was not adversely affected by the presence of Triton X-100 micelles. Biodegradation tests were performed with glucose and salicylic acid because glucose is a readily degradable substrate while salicylic acid is a metabolic intermediate of naphthalene oxidation (8, 9). Both of these compounds are polar organic compounds, and thus they are not appreciably solubilized in micelles and would be bioavailable to the microorganisms as aqueous-phase solutes. Mineralization tests show that surfactant concentrations above and below the CMC had little or no effect on the mineralization of glucose and salicylic acid. These results suggest that microbial mineralization of glucose and salicylic acid is not impaired by the presence of the surfactant micelles. The surfactant concentrations tested did not have a toxic effect on the PAH-degrading bacteria.

The key interest in this research was focused on determining the distribution of micelle-solubilized naphthalene versus aqueous-phase naphthalene and finding whether biodegradation of micelle-solubilized naphthalene was occurring. The process of naphthalene solubilization by nonionic surfactant micelles in aqueous systems results in the simultaneous transfer of naphthalene from the solid phase to the aqueous phase, and from the aqueous phase to the hydrophobic interior of surfactant micelles. Solubilization by micelles is modeled as a partition process. The partitioning of solubilizate between the aqueous pseudophase and the micellar pseudophase can be quantified by using either one of two parameters, the MSR in the presence of excess solid-phase naphthalene or, more generally, the equilibrium mole fraction micelle-phase/aqueous-phase partition coefficient (K_m). Both MSR and K_m have constant values in systems in which naphthalene is present as a separate solid phase. The value of K_m is constant in a system in which naphthalene is not present as a separate solid phase, while the value of MSR in such a system varies with the surfactant dose as a result of changes in the aqueous-phase concentration of solubilizate (10). In contrast to the MSR, the parameter K_m represents, in a thermodynamically rigorous manner, the equilibrium partitioning of solubilizate between surfactant as micelles and monomers of surfactant:

$$K_m = X_m/X_{aq}$$

where X_m is the micellar pseudophase mole fraction of solubilizate and X_{aq} is the aqueous pseudophase mole fraction of solubilizate. The value X_m, can be computed by using the equation

$$X_m = \frac{S_{mic}}{C_{mic} + S_{mic}}$$

where C_{mic} is the molar concentration of surfactant in micellar form; in a dilute solution, X_{aq} is approximately

$$X_{aq} = S_{aq} V_w$$

where V_w is the molar volume of water, i.e., 0.01805 liters/mol at 25°C, so that K_m is given as

$$K_m = \frac{S_{mic}}{(C_{mic} + S_{mic}) S_{aq} V_w} \quad (1)$$

where S_{mic} and S_{aq} are the molar concentration of solubilizate in the micellar pseudophase and the aqueous pseudophase, respectively, both based on bulk aqueous solution volume. By using equation 1 and the mass balance for the total amount of naphthalene in the system, the initial naphthalene distribution between the aqueous and micellar pseudophases was calculated for the various systems and is summarized in Table 3. The range of surfactant concentrations listed in Table 3 corresponds to the formation of micelles in the aqueous solution, causing micellar solubilization of naphthalene.

Surfactant solubilization of the PAH may facilitate the biodegradation rate if the exchange rate between the aqueous and micelle-bound PAH is higher than the dissolution rate of the

TABLE 3. Initial values of naphthalene distribution between the micellar and aqueous pseudophases

Surfactant and concn (%)[a]	S_{mic} (M)	Amt of naphthalene (mg)		
		Total	Micellar pseudophase	Aqueous pseudophase
Brij 30				
0.15%	3.90×10^{-3}	5.7	4.3	1.4
0.15%	3.90×10^{-3}	3.0	2.2	0.8
0.04%	1.02×10^{-3}	3.2	1.5	1.7
Triton X-100				
0.20%	3.29×10^{-3}	5.7	4.3	1.4
0.20%	3.29×10^{-3}	3.0	2.2	0.8
0.04%	5.22×10^{-4}	3.2	1.1	2.1

[a] Total volume, 50 ml.

TABLE 4. Estimate of the initial mass distribution of naphthalene between the micellar and aqueous pseudophases and comparison with the amount of naphthalene mineralized

Surfactant and concn (%)	Total amt of naphthalene (mg)	% of total naphthalene in:		% of total naphthalene mineralized
		Micelle phase	Aqueous phase	
Brij 30				
0.15%	5.7	75	25	44
0.15%	3.0	73	27	70
Triton X-100				
0.20%	5.7	75	25	46
0.20%	3.0	73	27	73

PAH from a solid phase or hydrocarbon phase. The literature suggests that there is a rapid exchange of PAHs between the aqueous pseudophase and the surfactant micelles. The residence time for naphthalene in micelles of sodium dodecyl sulfate and cetyltrimethylammonium bromide has been reported to range from 4 to 13 μs, while the residence time for pyrene in micelles of sodium dodecyl sulfate and cetyltrimethylammonium bromide is 243 to 588 μs (18). The exit rates reported for naphthalene, biphenyl, and 1-methylnaphthalene from ionic micelles are higher than 5×10^4 s^{-1}, the exit rates for anthracene and pyrene are reported by be higher than 10^3 s^{-1}, and that for perylene is approximately 3×10^2 s^{-1} (2). In other studies, the exit rates for benzyl radicals from sodium dodecyl sulfate micelles were reported to be higher than 10^6 s^{-1} (31). These short residence times and high exit rates suggest that micelle-solubilized PAHs should be bioavailable to PAH-degrading microorganisms.

Results in Fig. 2 to 4 show that the surfactant systems considered in this study released naphthalene from the micelles in response to microbial degradation of the aqueous pseudophase naphthalene. These results include data that describe mineralization of naphthalene in a micellar surfactant system with no excess solid-phase naphthalene. In such a system there is a decrease in the aqueous pseudophase naphthalene concentration owing to the equilibrium between the two pseudophases. For example, in Fig. 4 with supra-CMC surfactant the aqueous pseudophase naphthalene concentration at the start of the experiment is calculated to be about 16 mg/liter, which is lower than the pure-naphthalene solubility that would have been observed in the absence of surfactant. The modest reduction in the free aqueous pseudophase concentration of naphthalene that occurs in the presence of surfactant micelles with no excess solid-phase PAH present does not affect the naphthalene mineralization significantly.

An estimate of the initial mass distribution of naphthalene between the micellar pseudophase and the aqueous pseudophase and a comparison with the amount of naphthalene mineralized are given in Table 4 for the results shown in Fig. 2 and 4 with 0.15% Brij 30 (~170× CMC) and 0.20% Triton X-100 (~20× CMC). These estimates are based on percent mineralization of the initial total amount of naphthalene. Thus, the estimate of the amount mineralized is conservative, i.e., assuming no losses. These data show clearly that mineralization of micelle-solubilized naphthalene occurred. For example, in the test with 3.0 mg of total naphthalene (Fig. 4), the naphthalene mineralized from the micellar pseudophase accounted for the majority of the total amount of naphthalene mineralized. These results suggest that neither the surfactant micelle nor the micelle-solubilized naphthalene affected naphthalene mineralization.

An additional goal of this work was to assess in a preliminary manner the role of surfactants as competitive substrates. Our experiments show that although the nonionic surfactant Brij 30 was degradable, this did not impair to a significant degree the biodegradation of naphthalene, glucose, or salicylic acid. Some of the differences observed in the mineralization of glucose, salicylic acid, and naphthalene with Brij 30 and Triton X-100 could reflect differences between the degradability of Brij 30 and Triton X-100. Further investigation of the competitive-substrate issue requires a quantitative determination of the effects of nonionic surfactant on the biodegradation of other candidate PAHs, especially for PAHs with much lower aqueous solubilities. In these cases, competition or preferential utilization of the surfactant over the PAH may be more significant.

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