APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1994, p. 258–263
0099-2240/94/$04.00+0

Vol. 60, No. 1

Degradation of Polycyclic Aromatic Hydrocarbons in the Presence of Synthetic Surfactants

ANDREAS TIEHM*

Lehrstuhl für Mikrobiologie, Universität Karlsruhe, D-76128 Karlsruhe, Federal Republic of Germany

Received 6 July 1993/Accepted 24 October 1993

The biodegradation of polycyclic aromatic hydrocarbons (PAH) often is limited by low water solubility and dissolution rate. Nonionic surfactants and sodium dodecyl sulfate increased the concentration of PAH in the water phase because of solubilization. The degradation of PAH was inhibited by sodium dodecyl sulfate because this surfactant was preferred as a growth substrate. Growth of mixed cultures with phenanthrene and fluoranthene solubilized by a nonionic surfactant prior to inoculation was exponential, indicating a high bioavailability of the solubilized hydrocarbons. Nonionic surfactants of the alkylethoxylate type and the alkylphenolethoxylate type with an average ethoxylate chain length of 9 to 12 monomers were toxic to a PAH-degrading *Mycobacterium* sp. and to several PAH-degrading mixed cultures. Toxicity of the surfactants decreased with increasing hydrophilicity, i.e., with increasing ethoxylate chain length. Nontoxic surfactants enhanced the degradation of fluorene, phenanthrene, anthracene, fluoranthene, and pyrene.

Polycyclic aromatic hydrocarbons (PAH) have been released into the environment by incomplete combustion of fossil fuels and oil spills throughout past decades. Remediation of contaminated sites is of public interest because of the toxic, carcinogenic, and mutagenic potential of PAH (7). Biodegradation of PAH containing up to four rings by pure and mixed cultures has been demonstrated (7, 33). Even a decrease in PAH with more than four rings was reported after biological treatment of contaminated sites (24).

The biodegradation of PAH often is limited by the low water solubility and dissolution rates of these hydrophobic substrates (5, 27, 29, 34). Degradation rates depend on the mass transfer rates of the PAH from the solid phase to the water phase (31). Enhanced mass transfer can be achieved by the use of small substrate particles, thereby increasing the surface area and resulting in a higher dissolution rate (29, 31).

Surfactants mediate between immiscible phases because of their hydrophobic and hydrophilic moieties. The addition of surfactants increases the concentration of hydrophobic compounds in the water phase by solubilization or emulsification. Solubilization occurs above a specific threshold, the critical micellar concentration (CMC), where surfactant molecules aggregate to micelles. The slope of the curve relating the concentration of solubilized PAH to the surfactant concentration above CMC can be expressed as the solubilization ratio (SR). The solubilization of PAH and other hydrophobic pollutants by nonionic surfactants has been reported earlier (13, 17). An important parameter describing physical properties of surfactants is the hydrophil-lipophil-balance (HLB), which is determined by the relationship of the hydrophilic and the hydrophobic parts of the surfactant molecule (22). The HLB value of a surfactant increases with its hydrophilicity.

The potential of surfactants to increase the concentration of hydrophobic compounds in the water phase suggested that the degradation of hydrocarbons might be accelerated by the addition of surfactant. Attempts to enhance the metabolization rates of nonpolar substrates by the use of surfactants gave contradictory results (3, 6, 8, 14, 15). Although surfactants have been studied in complex water-soil systems (2, 19, 23), the effects are not well understood. This study was done to investigate the fundamental interactions of PAH, surfactants, and bacteria. Experiments focused on the toxicity of surfactants and the bioavailability of solubilized PAH.

MATERIALS AND METHODS

Media and culture conditions. Chemicals were obtained from Aldrich, Fluka, Merck, or Sigma at the highest available purity. The mineral medium used for all incubations contained (per liter) 3.58 g of Na_2HPO_4, 2.32 g of NaCl, 0.54 g of NH_4Cl, 0.15 g of KCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.04 g of $CaCl_2 \cdot 2H_2O$, and 10 ml of trace element solution (25). The pH was adjusted to 7.0. For enrichment and cultivation, PAH were provided in the medium as the sole source of carbon and energy.

All incubations were done in 300-ml Erlenmeyer flasks containing 200 ml of medium on a rotary shaker at 180 rpm and 30°C. Media containing only PAH, which were solubilized before inoculation (presolubilized), were prepared as described below. In experiments with crystalline PAH that were solubilized to a certain degree during the incubation, addition of PAH to the surfactant-containing mineral medium and inoculation were done simultaneously.

Small particles of phenanthrene were produced by recrystallization from acetone solutions. The kinetics of degradation of solid PAH were measured by using replicate inoculations, which were extracted at the times indicated.

Enrichment of PAH-degrading bacteria. Soil samples contaminated with PAH were obtained from a former coal gasification site at Karlsruhe, Germany. Enrichment of PAH-degrading bacteria was done in mineral medium containing 1 mM fluorene, phenanthrene, anthracene, fluoranthene, or pyrene as the sole source of carbon and energy (4). The mixed cultures were transferred to fresh medium every 3 weeks in the case of fluorene and phenanthrene and every

* Mailing address: Lehrstuhl für Mikrobiologie, Universität Karlsruhe, Kaiserstr. 12, D-76128 Karlsruhe, Germany.

TABLE 1. Chemical and physical properties of surfactants employed in this study

Surfactant	Avg mol formula	Avg mol wt	HLB[a]	CMC (mM)	MSR[b]	wtSR[c] (mg/g)
SDS	$C_{12}H_{25}OSO_3^-Na^+$	288	40.0	1.25	0.034	21.0
Marlipal 013/90	$C_{13}H_{27}O(CH_2CH_2O)_9H$	596	13.3	0.25	0.116	34.7
Triton X-102	$C_8H_{17}C_6H_4O(CH_2CH_2O)_{12}H$	756	14.6	0.40	0.112	26.4
Genapol X-150	$C_{13}H_{27}O(CH_2CH_2O)_{15}H$	860	15.0	0.50	0.068	14.1
Brij 35	$C_{12}H_{25}O(CH_2CH_2O)_{23}H$	1,200	17.0	0.10	0.152	22.5
Arkopal N-300	$C_9H_{19}C_6H_4O(CH_2CH_2O)_{30}H$	1,550	17.0	0.50	0.093	10.7
Sapogenat T-300	$(C_4H_9)_3C_6H_2O(CH_2CH_2O)_{30}H$	1,600	17.0	0.35	0.150	16.7
Pluronic PE6400	$HO(CH_2CH_2O)_x(C_2H_4CH_2O)_{30}(CH_2CH_2O)_{28-x}H$	3,000	N.A.[e]	1.40	0.067	4.0
Tegopren 5851	$(CH_3)_3SiO(SiCH_3XO)_5(SiCH_3CH_3O)_{20}Si(CH_3)_3$[d]	5,900	N.A.	0.05	0.401	12.1

[a] Data from manufacturers.
[b] Determined for phenanthrene.
[c] Determined for phenanthrene.
[d] $X = (CH_2)_3O(CH_2CH_2O)_{13}(C_2H_4CH_2O)_3H$.
[e] N.A., not applicable.

6 weeks in the case of anthracene, fluoranthene, and pyrene. A *Mycobacterium* sp. able to utilize several PAH was isolated from the same contaminated site. Characterization of the *Mycobacterium* sp. was published previously (5).

Surfactants. Sodium dodecyl sulfate (SDS), Brij 35, and Triton X-102 were obtained from Serva. Marlipal 013/90 was a gift from Hüls AG, Marl, Germany. Genapol X-150, Arkopal N-300, and Sapogenat T-300 were made available from Hoechst AG, Frankfurt, Germany. Pluronic PE6400 was a gift from BASF AG, Ludwigshafen, Germany. Tegopren 5851 was a gift from Goldschmidt AG, Essen, Germany. The detergents were used as obtained without further purification.

Specifications of the surfactants are given in Table 1.

Solubilization of PAH. Solid PAH at a concentration of 1 mM were shaken in surfactant-containing mineral medium for 18 h at 180 rpm and 30°C. The solubilized PAH were separated from remaining PAH particles by filtration through 0.2-μm-pore-size polycarbonate membrane filters. Determination of CMCs and the SRs for phenanthrene were done after this procedure. For phenanthrene, an equilibrium between crystalline and solubilized phenanthrene after 18 h was demonstrated.

For experiments done with the *Mycobacterium* sp., supernatants were additionally aseptically filtered into sterile flasks.

Analytical procedures. Bacterial growth was determined by measuring the protein concentration (32) and by visual examination for turbidity.

The concentration of SDS was measured by the methylene blue method (28). Determination of dissolved organic carbon was conducted after photochemical oxidation to CO_2 in a Dohrmann analyzer.

The concentration of particulate PAH was measured after extraction with 0.3 ml of *n*-octane ml^{-1} culture for 60 min on a magnetic stirrer. Emulsions that developed in the presence of surfactants were broken by repeated freezing or by centrifugation. *n*-Octane extracts were analyzed by gas chromatography as described previously (5). Recovery of PAH was at least 95% in sterile media with and without surfactants. *n*-Dodecanol, a degradation product of SDS, was identified by comparison of the retention time with that of the commercially available reference substance by gas chromatography.

Solubilized and dissolved aromatic compounds were measured by reversed-phase high-performance liquid chromatography (HPLC). Twenty microliters of culture supernatant was injected into an HPLC (Beckman) fitted with a Nucleosil column (5 μm, C-18; Knauer). Isocratic elution was carried out with 77.5:22.5 methanol to water acidified with 0.76 ml of H_3PO_4 $liter^{-1}$. Peaks were measured at 254 nm or scanned from 220 to 400 nm in flow-through mode in a spectrophotometer (Beckman model 165). Compounds were identified by their retention times and absorbance spectra in comparison with reference substances.

RESULTS

Determination of CMC and SR. Determination of the CMCs and the solubilizing properties was a prerequisite for knowing the amounts of the individual surfactants necessary to enhance the concentration of PAH in the water phase. The solubilizing capacities of the surfactants were determined with phenanthrene as a model compound. The relationship of solubilized phenanthrene to surfactant concentration can be expressed as moles of hydrophobic compound solubilized per mole of surfactant (molar SR [MSR]) or as mass solubilizate per mass of surfactant (weight SR [wtSR]).

The CMCs and both expressions for the SRs determined for phenanthrene are given in Table 1.

Effect of SDS on phenanthrene degradation. SDS has the potential to solubilize phenanthrene (Table 1). In spite of this, the degradation of solid phenanthrene by the mixed culture adapted to phenanthrene degradation was inhibited by the addition of SDS (Fig. 1). Primary degradation and

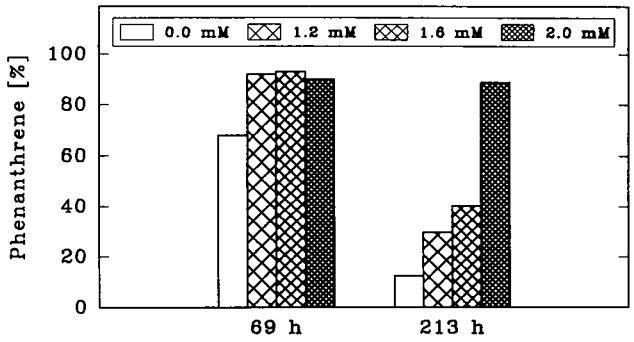

FIG. 1. Inhibition of phenanthrene degradation in a mixed culture by SDS. Incubation was done with 200 mg of phenanthrene per liter after addition of SDS at various concentrations.

TABLE 2. Degradation of solubilized PAH by mixed cultures enriched on crystals of the respective PAH[a]

Surfactant	Concn (mM)	Solubilized PAH with surfactant[b]			
		Fluorene	Phenanthrene	Fluoranthene	Pyrene
Marlipal 013/90	5	−	+	+	−
Triton X-102	4	−	+	+	−
Genapol X-150	8	−	+	+	+
Brij 35	3	+	+	+	+
Arkopal N-300	6	+	+	+	+
Sapogenat T-300	4	+	+	+	+
Pluronic PE 6400	10	+	+	+	+
Tegopren 5851	1	+	+	+	+

[a] Degradation was indicated by disappearance of the PAH and increase of turbidity of the medium within 7 days.
[b] Degradation (+) or no degradation (−) of the PAH is indicated.

subsequent mineralization of SDS occurred within 44 h. The hydrolytic cleavage of SDS led to the temporary formation of *n*-dodecanol that was detected after 20 h. Release of the other product of hydrolysis, HSO_4^-, resulted in the acidification of the medium. After 44 h of incubation, the pH values were 7.0, 6.5, 6.2, and 6.0 in the growth media with 0, 1.2, 1.6, and 2.0 mM SDS, respectively. The differences in phenanthrene degradation increased with prolonged incubation time (Fig. 1). Phenanthrene degradation was fastest without the addition of SDS.

Toxicity of nonionic surfactants on PAH-degrading bacteria. PAH were solubilized by the nonionic surfactants prior to inoculation with mixed cultures or the *Mycobacterium* sp. The surfactants were used in different concentrations in order to achieve the solubilization of similar amounts of PAH. Solubilized PAH were in the range of 20 to 100 mg/liter, with the highest concentration for phenanthrene and the lowest for pyrene.

Utilization of phenanthrene and of fluoranthene by mixed cultures enriched on these PAH occurred within 7 days, regardless of the solubilizing surfactant. Whether solubilized fluorene or pyrene was degraded by the respective mixed cultures depended on the solubilizing agents (Table 2).

Growth of the *Mycobacterium* sp. which is able to utilize acetate, phenanthrene, fluoranthene, and pyrene was tested with the solubilized PAH and with 3 mM acetate in the presence of the surfactants. Surfactants were used in the same concentrations as in the experiment with the mixed cultures (Table 2). No growth of the *Mycobacterium* sp. occurred with Marlipal 013/90 or Triton X-102 in the medium, either with solubilized PAH or with acetate. Degradation of solubilized PAH and growth on acetate occurred within 7 days in the presence of the other surfactants.

Kinetics of growth on solubilized phenanthrene and fluoranthene. Growth of the phenanthrene-degrading mixed culture was exponential, with a doubling time of 4.7 h when incubated with solubilized phenanthrene. Degradation of the hydrocarbon corresponded with biomass increase (Fig. 2A and B). In contrast, linear growth and degradation occurred on solid phenanthrene. Reduction of average crystal sizes and thereby an increase in the surface area resulted in faster growth (Fig. 2A and B). Degradation of 120 mg of phenanthrene per liter presolubilized by 10 mM Marlipal 013/90 required 31 h. Metabolism of the same weight of small crystals of phenanthrene occurred within 68 h, and 90% of the untreated large crystals were degraded after 115 h.

Degradation of phenanthrene was accompanied by the

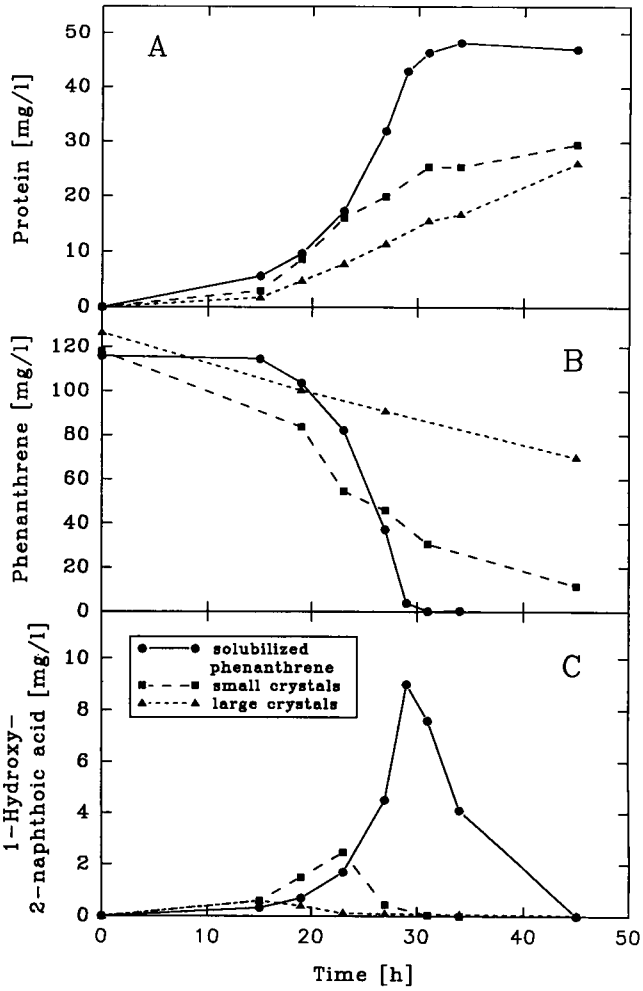

FIG. 2. Increase in protein (A), degradation of phenanthrene (B), and temporary accumulation of 1-hydroxy-2-naphthoic acid (C) during the degradation of phenanthrene by a mixed culture. Incubation was done with phenanthrene solubilized by 10 mM Marlipal 013/90, with small particles, and with larger particles of phenanthrene. 1-Hydroxy-2-naphthoic acid was identified by HPLC retention time and absorbance spectra (maxima at 250 and 334 nm, minima at 234 and 269 nm).

release of 1-hydroxy-2-naphthoic acid, an early intermediate of phenanthrene metabolism. The amount of 1-hydroxy-2-naphthoic acid increased with enhanced substrate availability. Degradation of 120 mg of phenanthrene per liter led to the temporary accumulation of 0.6, 2.5, and 9.0 mg of the metabolite per liter in the media with large crystals, small crystals, and phenanthrene presolubilized by Marlipal 013/90, respectively. Release of 1-hydroxy-2-naphthoic acid followed exponential kinetics where growth was on presolubilized phenanthrene (Fig. 2C). The temporary release of three other, unidentified metabolites also was increased in the presence of the surfactant (data not shown).

Incubation of 120 mg of solid phenanthrene per liter in the presence of 10 mM Marlipal 013/90 led to simultaneous solubilization of the crystals and degradation of phenanthrene. Since 100 mg of solubilized phenanthrene per liter was detected in the water phase after 16 h, the rate of solubilization exceeded the rate of degradation. Because of

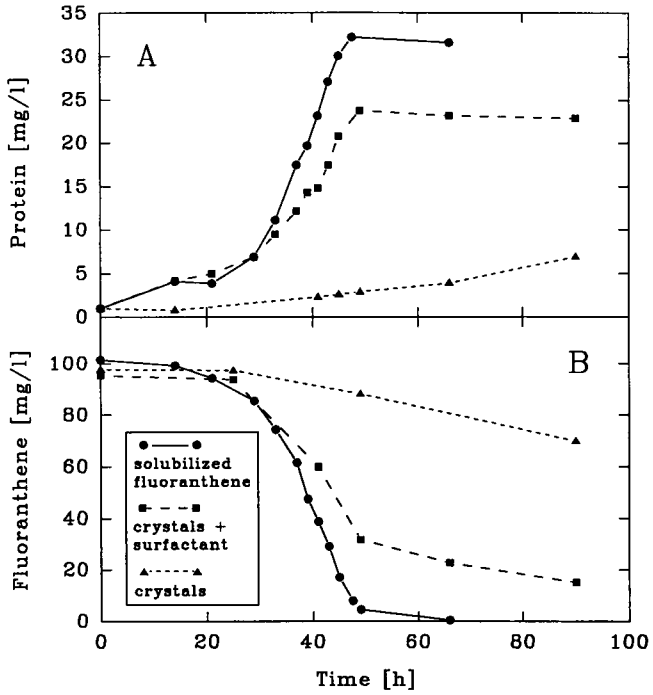

FIG. 3. Growth of bacteria (A) and degradation of fluoranthene (B). Incubation was done with fluoranthene presolubilized by 10 mM Marlipal 013/90 (●), with solid fluoranthene and the addition of 10 mM Marlipal 013/90 (■), and with solid fluoranthene without the addition of surfactant (▲).

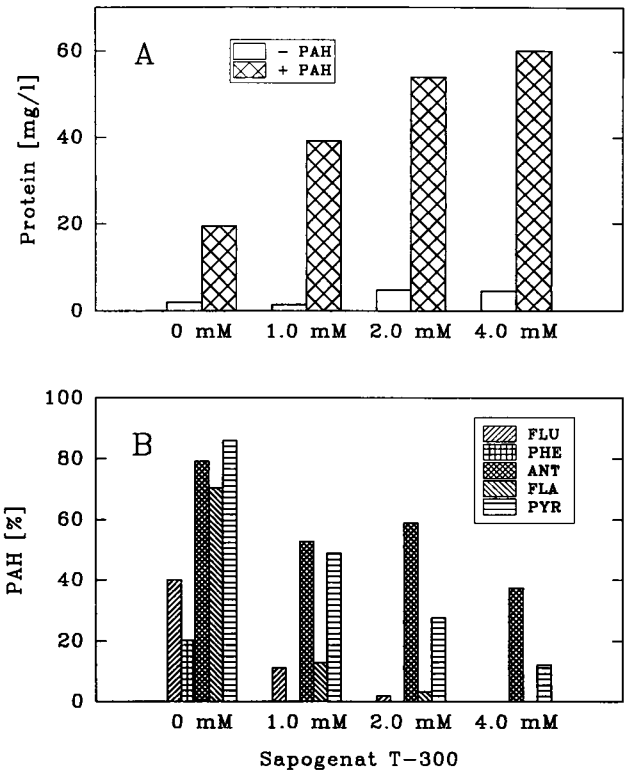

FIG. 4. Growth of bacteria (A) and degradation of PAH (B) within 7 days in the presence of Sapogenat T-300 at various concentrations. (A) Comparison of the biomass productions with surfactant and PAH and with surfactant alone. (B) Degradation of fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), and pyrene (PYR). PAH were provided as particles in a concentration of 50 mg of each per liter.

this, a decrease of the phenanthrene that was solubilized after inoculation during the incubation occurred, with kinetics similar to those for the degradation of the presolubilized phenanthrene. 1-Hydroxy-2-naphthoic acid reached a temporary concentration of 6.3 mg/liter under these conditions.

Growth of the fluoranthene-degrading mixed culture on solubilized fluoranthene was exponential, with a doubling time of 7.6 h. The degradation rate of solid fluoranthene in the presence of 10 mM Marlipal 013/90, where processes of solubilization and degradation occurred simultaneously, was exponential in the early phase of the experiment and then changed to linear. Growth with solid fluoranthene without addition of surfactant was linear (Fig. 3). Degradation of 90% of the crystalline fluoranthene required 105 h in the presence of 10 mM Marlipal 013/90 and 330 h without surfactant.

Effect of surfactants on degradation of PAH mixtures. This experiment could not be done with Marlipal 013/90 because of its toxicity against the fluorene- and pyrene-degrading cultures. A useful surfactant without toxicity was Sapogenat T-300, which was chosen for experiments with mixtures of 50 mg each of fluorene, phenanthrene, anthracene, fluoranthene, and pyrene per liter. Degradation of PAH and biomass production were enhanced by the addition of surfactant. The biomass production was not due to the utilization of Sapogenat T-300 (Fig. 4A). Fast degradation of the PAH occurred in the cases of phenanthrene and fluorene even without the addition of surfactant. The degradation rates of the PAH were increased with increased concentrations of surfactant (Fig. 4B). The same kind of experiment was done with the silicon block copolymer Tegopren 5851. Because this surfactant contained an easily degradable part, differences in biomass production related to PAH degradation could not be measured. Determination of PAH content confirmed the enhancement of hydrocarbon degradation by the nontoxic surfactant (Fig. 5). Without surfactant addition, 61.8% of the PAH remained, compared with 14.6% where incubation occurred in the presence of 2.0 mM Tegopren 5851.

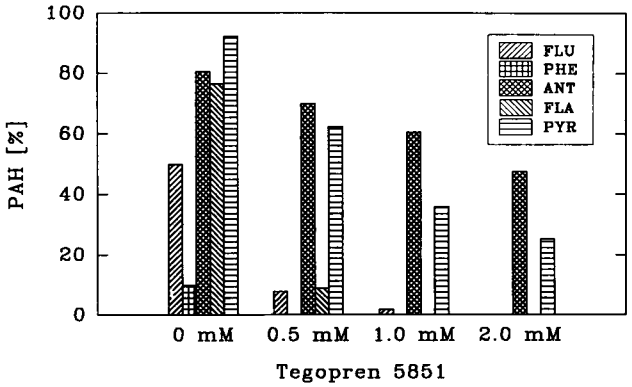

FIG. 5. Degradation of fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), and pyrene (PYR) within 7 days in the presence of Tegopren 5851 at various concentrations. PAH were provided as particles in a concentration of 50 mg of each per liter.

DISCUSSION

The surfactants varied considerably in their solubilizing properties. Because of differences in aggregation behavior, different amounts of surfactants were needed for an increase in phenanthrene concentration in the water phase. The SRs determined for the alkylethoxylates and alkylphenolethoxylates were in the same range as reported for comparable nonionic surfactants (13). The high MSR of Tegopren 5851 results from the high molecular weight of this siloxan block copolymer, and the low MSR of SDS is caused by its low molecular weight. On the basis of weight ratios, the differences between Tegopren 5851 and SDS become much smaller (Table 1). Data for SRs were determined for phenanthrene. Higher values for smaller PAH such as naphthalene and lower values for larger PAH such as pyrene have to be expected, because the SR decreases with increasing molecular size of the solubilizate (13, 18). The block polymer Pluronic PE6400 had a relatively small effect in solubilization because of a high CMC and a low SR.

SDS, although a surfactant with good solubilizing properties, did not support degradation of phenanthrene (Fig. 1). The mixed culture, which was enriched and subcultured several times on phenanthrene so that microorganisms were adapted to phenanthrene degradation, rapidly mineralized the surfactant. Such an inhibitory effect of a readily degradable substrate on the degradation of phenanthrene was observed previously with glucose (16). The inhibition of phenanthrene degradation even after complete degradation of SDS might have been due to the lowering of the pH of the medium (12, 26).

To determine the bioavailability of solubilized PAH, surfactants had to be chosen that were not easily degraded. Biodegradation of nonionic surfactants is rendered more difficult when the hydrophobic part of the molecule is branched and when the length of the ethoxylate chain is increased. An aromatic ring within the hydrophobic part of the molecule further hinders rapid biodegradation. Block polymers and block copolymers are degraded slowly (28).

Mycobacterium sp. did not grow on acetate in the presence of the surfactants Marlipal 013/90 and Triton X-102. This experiment clearly demonstrates that growth inhibition is due to toxic effects of the surfactants. This conclusion can be drawn because a pure culture was used. In mixed cultures, the sensitivity of the PAH-degrading strains to the surfactants may be completely different from the sensitivities of other strains of the mixed culture which metabolize easily degradable substrates such as acetate or glucose. In this study, the surfactants with the highest lipophilicity, Marlipal 013/90 and Triton X-102, inhibited growth of the *Mycobacterium* sp. The same surfactants inhibited the degradation of solubilized fluorene and pyrene by the mixed cultures (Table 2). The more hydrophilic surfactants with an HLB of 17.0 enabled the degradation of solubilized PAH in all cases.

Therefore, this study supports the suggestion that the toxicity of surfactants to PAH-degrading microorganisms is related to the surfactants' lipophilicity. It has been demonstrated for several soil bacteria that the toxicity of surfactants is related to the HLB values. Nonionic surfactants with hydrophobic parts similar to those of the surfactants in this study were most toxic with 6 to 13 ethylene groups per molecule (9, 10). In another investigation, toxicity decreased with shorter or longer ethoxylate chains and was related to membrane-damaging effects. Surfactants with ethylene oxide chains consisting of fewer than six monomers were buried in the lipid layer of liposomes, and the long ethylene oxide chains, e.g., those with 30 monomers, had no effect on the membrane permeability (11). The inhibition of physiological activity by Triton X-100 (HLB, 13.5), another surfactant of the Triton series containing two ethoxylate monomers less than Triton X-102, is documented. Triton X-100 decreased the phenanthrene degradation by a mixed culture (19) and completely inhibited the sterol transformation by *Mycobacterium fortuitum* (3). Thus, the more hydrophobic surfactants that seem to exhibit better solubilizing properties (3, 17) are tolerated by only a few bacteria.

The degradation of solubilized PAH by the mixed cultures gave protein yields of 0.40 mg/mg of phenanthrene and 0.32 mg/mg of fluoranthene (Fig. 2 and 3). Yields of 0.24 mg of protein per mg of phenanthrene and 0.23 mg of protein per mg of fluoranthene have been reported for pure cultures (33). Since the values of the present study are high compared with the reported data, the utilization of the impurities of the surfactant has to be expected.

The exponential degradation of solubilized phenanthrene and fluoranthene indicated that cultures were not limited by substrate availability (Fig. 2 and 3). Solubilized *n*-decane and *n*-tetradecane also supported exponential growth of pure cultures, as was reported recently (6). Thus, solubilized PAH and alkanes are bioavailable, although the hydrocarbons are originally enclosed by surfactant molecules. In this context, it should be noted that a constant dynamic exchange of aromatic hydrocarbons between surfactant micelles and the water phase has been demonstrated (1), although hydrophobic organic compounds are preferably localized within the micelles (30). The high diffusion rate from the micelle's core into the aqueous medium should enable a continuous uptake of the PAH from the water phase. Therefore, at least two mechanisms might be responsible for the transfer of PAH from micelles to the microorganisms: (i) uptake of PAH from the water phase after diffusion out of the micelles or (ii) a close contact of micelles with the cell envelope, perhaps accompanied by membrane fusion as was postulated for phosphatidylcholine liposomes (21).

The high bioavailability of solubilized PAH only results in an exponential degradation of crystalline PAH when the mass transfer from the crystalline form to the solubilized state is faster than the degradation by the bacteria. During a batch cultivation, the mass transfer rate will decline because of a decrease in the surface area of the crystals and the degradation rate will rise because of an increase in biomass. Mass transfer of crystalline phenanthrene in the presence of Marlipal 013/90 was fast enough to promote exponential growth. Mass transfer from fluoranthene crystals to the surfactant-containing water phase supported exponential growth only in the initial phase (Fig. 3). This may be due to the bigger crystals and lower solubility of fluoranthene and subsequent slower solubilization. The linear growth and degradation occurring on the solid PAH without addition of surfactant confirm observations that the dissolution rates limit the degradation of hydrophobic compounds (27, 29, 31, 34).

Enhancement of PAH degradation occurred for all PAH present in a mixture to which nontoxic surfactants that were not preferred as growth substrates were added. Even in the presence of a surfactant, the degradation rates of the PAH were correlated with their water solubility (Fig. 4B and 5). Anthracene, the PAH with the lowest water solubility of 57 μg/liter (20), had the slowest degradation rate, followed by pyrene with a water solubility of 162 μg/liter. Of course, the

size and form of crystals may alter the dissolution and solubilization rates.

The enhancement of PAH degradation by the addition of surfactants is a helpful technique for laboratory investigations. Experiments can be done with minor or no limitation by substrate availability. Furthermore, the addition of surfactants without toxicity may be a useful tool to accelerate bioremediation of contaminated areas.

ACKNOWLEDGMENT

This work was supported by a fellowship to A. Tiehm by the DECHEMA, Frankfurt, Germany, and by a grant to W. Zumft from the Bundesminister für Forschung und Technologie.

REFERENCES

1. **Almgren, M., F. Grieser, and J. K. Thomas.** 1979. Dynamic and static aspects of solubilization of neutral arenes in ionic micellar solutions. J. Am. Chem. Soc. **101**:279–291.
2. **Aronstein, B. N., Y. M. Calvillo, and M. Alexander.** 1991. Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. Environ. Sci. Technol. **25**:1728–1731.
3. **Atrat, P. G., B. Koch, B. Szekalla, and C. Hörhold-Schubert.** 1992. Application of newly synthesized detergents in the side chain degradation of plant sterols by *Mycobacterium fortuitum*. J. Basic Microbiol. **32**:147–157.
4. **Boldrin, B.** 1992. Ph.D. thesis. Universität Karlsruhe, Karlsruhe, Germany.
5. **Boldrin, B., A. Tiehm, and C. Fritzsche.** 1993. Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a *Mycobacterium* sp. Appl. Environ. Microbiol. **59**:1927–1930.
6. **Bury, S. J., and C. A. Miller.** 1993. Effect of micellar solubilization on biodegradation rates of hydrocarbons. Environ. Sci. Technol. **27**:104–110.
7. **Cerniglia, C. E., and M. A. Heitkamp.** 1989. Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment, p. 41–68. *In* U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Inc., Boca Raton, Fla.
8. **Cox, D. P., and A. L. Williams.** 1980. Biological process for converting naphthalene to *cis*-1,2-dihydroxy-1,2-dihydronaphthalene. Appl. Environ. Microbiol. **39**:320–326.
9. **Cserhati, T., Z. Illes, and I. Nemes.** 1991. Effect of non-ionic tensides on the growth of some soil bacteria. Appl. Microbiol. Biotechnol. **35**:115–118.
10. **Cserhati, T., M. Szoegyi, and B. Bordas.** 1982. QSAR study on the biological activity of nonyl-phenyl-ethylene-oxide polymers. Gen. Physiol. Biophys. **1**:225–231.
11. **Cserhati, T., M. Szoegyi, B. Bordas, and A. Dobrovolszky.** 1984. Structural requirements for the membrane damaging effect of non homologous series of nonionic tensides. Quant. Struct.-Act. Relat. **3**:56–59.
12. **Dibble, J. T., and R. Bartha.** 1979. Effect of environmental parameters on the biodegradation of oil sludge. Appl. Environ. Microbiol. **37**:729–739.
13. **Edwards, D. A., R. G. Luthy, and Z. Liu.** 1991. Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. Environ. Sci. Technol. **25**:127–133.
14. **Foght, J. M., D. L. Gutnick, and D. W. S. Westlake.** 1989. Effect of Emulsan on biodegradation of crude oil by pure and mixed bacterial cultures. Appl. Environ. Microbiol. **55**:36–42.
15. **Guerin, W. F., and G. E. Jones.** 1988. Mineralization of phenanthrene by a *Mycobacterium* sp. Appl. Environ. Microbiol. **54**:937–944.
16. **Keuth, S., and H.-J. Rehm.** 1991. Biodegradation of phenanthrene by *Arthrobacter polychromogenes* isolated from a contaminated soil. Appl. Microbiol. Biotechnol. **34**:804–808.
17. **Kile, E. D., and C. T. Chiou.** 1989. Water solubility enhancements of DDT and trichlorobenzene by some surfactants below and above the critical micelle concentration. Environ. Sci. Technol. **23**:832–838.
18. **Klevens, H. B.** 1950. Solubilization of polycyclic hydrocarbons. J. Phys. Colloid Chem. **54**:283–298.
19. **Laha, S., and R. G. Luthy.** 1991. Inhibition of phenanthrene mineralization by nonionic surfactants in soil-water systems. Environ. Sci. Technol. **25**:1920–1930.
20. **McElroy, A. E., J. W. Farrington, and J. M. Teal.** 1989. Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment, p. 1–39. *In* U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Inc., Boca Raton, Fla.
21. **Miller, R. M., and R. Bartha.** 1989. Evidence from liposome encapsulation for transport-limited microbial metabolism of solid alkanes. Appl. Environ. Microbiol. **55**:269–274.
22. **Myers, D.** 1991. Surfaces, interfaces, and colloids; principles and applications. VCH Publishers, Inc., New York.
23. **Oberbremer, A., R. Müller-Hurtig, and F. Wagner.** 1990. Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. Appl. Microbiol. Biotechnol. **32**:485–489.
24. **Park, K. S., R. C. Sims, R. R. Dupont, W. J. Doucette, and J. E. Matthews.** 1990. Fate of PAH compounds in two soil types: influence of volatilization, abiotic loss and biological activity. Environ. Toxicol. Chem. **9**:187–195.
25. **Pfennig, N., and K. D. Lippert.** 1966. Über das Vitamin B_{12} Bedürfnis phototropher Schwefelbakterien. Arch. Mikrobiol. **55**:245–256.
26. **Strandberg, G. W., T. J. Abraham, Jr., and G. C. Frazier.** 1986. Phenanthrene degradation by *Beijerinckia* sp. B8/36. Biotechnol. Bioeng. **28**:142–145.
27. **Stucki, G., and M. Alexander.** 1987. Role of dissolution rate and solubility in biodegradation of aromatic compounds. Appl. Environ. Microbiol. **53**:292–297.
28. **Swisher, R. D.** 1987. Surfactant biodegradation. Surf. Sci. Ser. **18**:415–517.
29. **Thomas, J. M., J. R. Yordy, J. A. Amador, and M. Alexander.** 1986. Rates of dissolution and biodegradation of water-insoluble organic compounds. Appl. Environ. Microbiol. **52**:290–296.
30. **Valsaraj, K. T., and L. J. Thibodeaux.** 1989. Relationships between micelle-water and octanol-water partition constants for hydrophobic organics of environmental interest. Water Res. **23**:183–189.
31. **Volkering, F., A. M. Breure, A. Sterkenburg, and J. G. van Andel.** 1992. Microbial degradation of polycyclic aromatic hydrocarbons: effect of substrate availability on bacterial growth kinetics. Appl. Microbiol. Biotechnol. **36**:548–552.
32. **Wang, C.-S., and R. L. Smith.** 1975. Lowry determination of protein in the presence of Triton X-100. Anal. Biochem. **63**:414–417.
33. **Weissenfels, W. D., M. Beyer, and J. Klein.** 1990. Degradation of phenanthrene, fluorene, and fluoranthene by pure bacterial cultures. Appl. Microbiol. Biotechnol. **32**:479–484.
34. **Wodzinski, R. S., and J. E. Coyle.** 1974. Physical state of phenanthrene for utilization by bacteria. Appl. Microbiol. **27**: 1081–1084.