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Quantifying the Biodegradation of Phenanthrene by *Pseudomonas stutzeri* P16 in the Presence of a Nonionic Surfactant

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The low water solubility of polycyclic aromatic hydrocarbons is believed to limit their availability to microorganisms, which is a potential problem for bioremediation of polycyclic aromatic hydrocarbon-contaminated sites. Surfactants have been suggested to enhance the bioavailability of hydrophobic compounds, but both negative and positive effects of surfactants on biodegradation have been reported in the literature. Earlier, we presented mechanistic models of the effects of surfactants on phenanthrene dissolution and on the biodegradation kinetics of phenanthrene solubilized in surfactant micelles. In this study, we combined the biodegradation and dissolution models to quantify the influence of the surfactant Tergitol NP-10 on biodegradation of solid-phase phenanthrene by *Pseudomonas stutzeri* P16. Although micellized phenanthrene does not appear to be available directly to the bacterium, the ability of the surfactant to increase the phenanthrene dissolution rate resulted in an overall increase in bacterial growth rate in the presence of the surfactant. Experimental observations could be predicted well by the derived model with measured biokinetic and dissolution parameters. The proposed model therefore can serve as a base case for understanding the physical-chemical effects of surfactants on nonaqueous hydrocarbon bioavailability.

Many contaminants in soil and subsurface environments are poorly soluble in water and therefore persist in nonaqueous-phase liquids (NAPLs) or are partitioned into soil organic matter. Polycyclic aromatic hydrocarbons (PAH) are an important example of hydrophobic pollutants found in contaminated soils and groundwater at many sites across the United States, particularly at creosote wood treatment facilities (20, 25) and former manufactured gas plants (6, 16). Many PAH are subject to bacterial degradation (2), but slow rates of mass transfer from solid phases, NAPLs, or soil have been found to limit the availability of PAH to microorganisms (5, 6, 11, 18, 22, 31, 33, 35, 37).

The use of surfactants has been proposed to enhance remedial efforts for hydrocarbon-contaminated soils (1, 4, 27, 36). These strategies primarily enhance NAPL removal rates by increasing NAPL solubility in the liquid phase and/or by mobilizing NAPL. The use of surfactants for solubilization in combination with in situ or ex situ biodegradation processes has been suggested recently (15, 26).

Conflicting results have been reported in the literature describing the effect of surfactants on biodegradation. The effects have ranged from inhibition of biodegradation to no effect to stimulation of biodegradation, and a variety of factors have been proposed to explain these findings (14, 15, 21, 26, 32, 34). Conflicting results have even been observed within an individual study. For example, the addition of a rhamnolipid biosurfactant to a silt loam soil slurry inhibited phenanthrene mineralization by *Pseudomonas* sp. strain UG14r, but the same biosurfactant at the same concentration enhanced phenanthrene mineralization when added to a soil which originated from a creosote-contaminated site (23). A variety of experimental systems and initial conditions have been used to study the effects of surfactants on biodegradation, making direct comparisons between the findings of these studies difficult. To date, most experimental approaches described in the literature have been empirical. No mechanistic model of the influences of surfactants on biodegradation of hydrophobic substrates has been developed, and a recent attempt to do so in a well-defined system did not provide a satisfactory quantitative model (34).

In situations in which there is no excess nonaqueous phase present and bacteria have access only to aqueous-phase hydrocarbons, a decrease in biodegradation rates in the presence of surfactants can be explained by the partitioning of hydrocarbon into the micelles (8). In order to understand the impact of surfactants on hydrocarbon biodegradation when the hydrocarbon is present in a nonaqueous phase or is in the adsorbed state, the effects of the surfactants on the mass transfer processes (dissolution or desorption) have to be quantified. Previously, we presented a model which described the effect of nonionic surfactants on the rate of dissolution of phenanthrene (10).

The focus of the work described in this paper was to study and quantify the biodegradation of solid phenanthrene, by using a well-defined experimental system with known biodegradation parameters and known effects of surfactants on phenanthrene dissolution. The organism chosen for this investigation, *Pseudomonas stutzeri* P16, was shown earlier not to interact with crystalline phenanthrene and therefore appeared to degrade only soluble phenanthrene (29). The objective of this study was to test the hypothesis that bacterial growth rates and liquid-phase phenanthrene concentrations in the experimental system could be quantified by assuming that the organ-

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ism had access only to phenanthrene present in the aqueous phase and did not have direct access either to solid phenanthrene or to phenanthrene solubilized in surfactant micelles. The hypothesis was translated into a mathematical model by combining biodegradation, partitioning, and dissolution models.

THEORY

Model description. When bacterial growth occurs in a batch system in the presence of solid phenanthrene, the phenanthrene concentration in the liquid phase, C_T, depends on both the biodegradation rate and the rate of phenanthrene dissolution (equation 1).

$$\frac{dC_T}{dt} = -r_{\text{bio}} + r_{\text{diss}} \tag{1}$$

The biodegradation rate, r_{bio}, can be described as follows:

$$r_{\text{bio}} = qX \tag{2}$$

where q is the specific rate of phenanthrene degradation and X is the biomass concentration. For *P. stutzeri* P16, q can be described by Monod kinetics (29, 30):

$$q = \frac{q_{\max} C_a}{K_s + C_a} \tag{3}$$

where $q_{\max}$ is the maximum specific phenanthrene degradation rate, C_a is the aqueous-phase phenanthrene concentration, and K_s is the Monod half-saturation coefficient. For phenanthrene solubilized in surfactant micelles, it can be assumed that the aqueous phase is in equilibrium with the micelles (10). If it is also assumed that the microorganism does not have direct access to micellized phenanthrene, a modified Monod equation can be written in terms of the total liquid-phase phenanthrene concentration, C_T (9):

$$\frac{q}{q_{\max}} = \frac{C_T/C_{T,\text{sat}}}{K_s/C_{a,\text{sat}} + C_T/C_{T,\text{sat}}} = \frac{C_T}{K_s\left(\frac{C_{T,\text{sat}}}{C_{a,\text{sat}}}\right) + C_T} \tag{4}$$

where $C_{a,\text{sat}}$ and $C_{T,\text{sat}}$ represent the saturation concentration of phenanthrene in the absence and presence of surfactant, respectively. The saturation concentration in the presence of surfactant at concentrations above the critical micelle concentration (cmc) can be described by

$$C_{T,\text{sat}} = \text{SC}(C_{\text{surf}} - \text{cmc}) + C_{a,\text{sat}} \tag{5}$$

where SC is the solubilization capacity of the surfactant for phenanthrene (10) and C_{surf} is the surfactant concentration.

The phenanthrene dissolution rate, r_{diss}, can be described by a first-order rate model (10):

$$r_{\text{diss}} = k_{l,\text{obs}} \cdot a \cdot [C_{T,\text{sat}} - C_T] \tag{6}$$

where $k_{l,\text{obs}}$ is an observed mass transfer coefficient and a is the specific surface area of the solid phenanthrene (surface area per unit volume). In the presence of a surfactant, the observed mass transfer coefficient is the weighted average of the mass transfer coefficients for two individual processes (Fig. 1): (i) the dissolution of molecular phenanthrene and (ii) the transport of phenanthrene-saturated micelles away from the solid phenanthrene interface (10). The relationship between $k_{l,\text{obs}}$ and surfactant concentration is shown in equation 7:

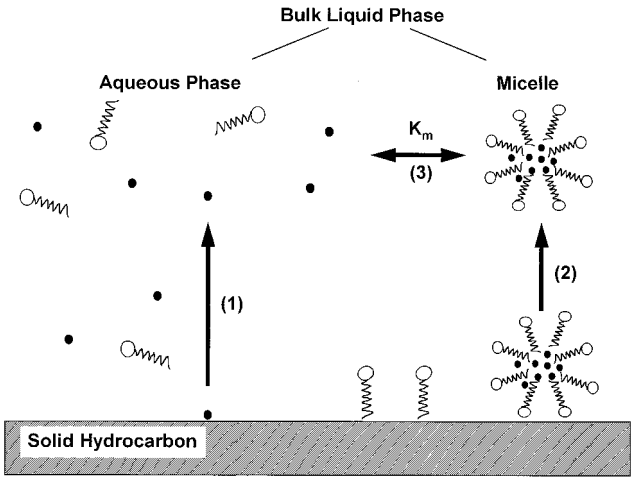

FIG. 1. Conceptual model of surfactant-mediated hydrocarbon dissolution into an overlying liquid phase. (1) Dissolution of molecular phenanthrene. (2) Dissolution of phenanthrene present in surfactant micelles. (3) Equilibrium partitioning of phenanthrene between micelles and aqueous phase.

$$k_{l,\text{obs}} = \frac{k_{l,a} + \left(\frac{\text{SC}}{C_{a,\text{sat}}}\right) \cdot (C_{\text{surf}} - \text{cmc}) \cdot k_{l,m}}{\left(\frac{\text{SC}}{C_{a,\text{sat}}}\right) \cdot (C_{\text{surf}} - \text{cmc}) + 1} \tag{7}$$

where $k_{l,a}$ is the mass transfer coefficient for dissolution of phenanthrene directly into the aqueous phase and $k_{l,m}$ is the mass transfer coefficient associated with the micellar transport mechanism.

Thus, the change of liquid-phase phenanthrene concentration over time in a biologically active batch system is described by equation 8:

$$\frac{dC_T}{dt} = k_{l,\text{obs}} \cdot a \cdot [C_{T,\text{sat}} - C_T] - \frac{q_{\max} C_T X}{\left[\left(\frac{\text{SC}}{C_{a,\text{sat}}}\right)(C_{\text{surf}} - \text{cmc}) + 1\right] K_s + C_T} \tag{8}$$

The net rate of growth in the system is defined by equation 9:

$$\frac{dX}{dt} = qYX \tag{9}$$

where Y is the yield coefficient.

The two first-order differential equations (equations 8 and 9) were solved simultaneously by applying a fourth-order Runge-Kutta algorithm. The following initial conditions were used:

$$C_T(t = 0) = C_{T,0}$$

$$X(t = 0) = X_0$$

All input parameters with the exception of $k_{l,m}$ could be measured independently. The value of $k_{l,m}$ for Tergitol NP-10 for the experimental apparatus was obtained by the method described in the work of Grimberg et al. (10).

MATERIALS AND METHODS

Mathematical terms used. The mathematical terms used in this paper are as follows: C_a, aqueous-phase phenanthrene concentration (mass length^{-3}); $C_{a,\text{sat}}$, aqueous-phase saturation concentration (mass length^{-3}); C_T, total bulk liquid

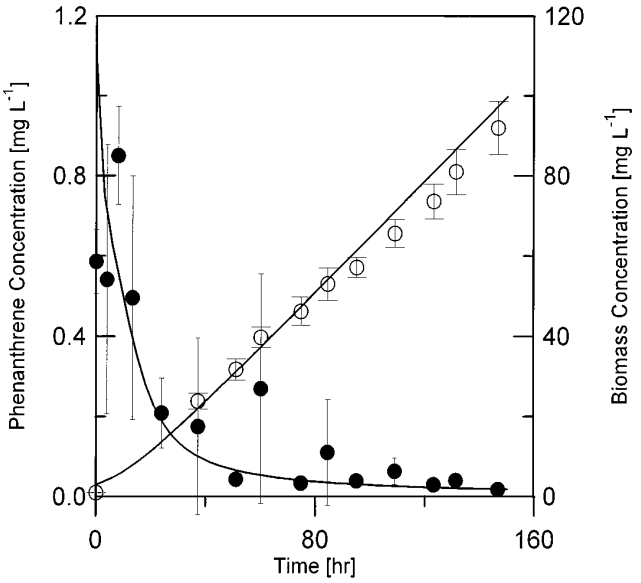

FIG. 2. Bacterial biomass (○) and liquid phenanthrene concentration (●) during growth of *P. stutzeri* P16 in the presence of solid phenanthrene. Lines depict model simulations. Symbols and error bars represent means and standard deviations, respectively, of triplicate flasks.

(aqueous plus micellar) phenanthrene concentration (mass length^{-3}); $C_{T,0}$, initial bulk liquid phenanthrene concentration (mass length^{-3}); $C_{T,\text{sat}}$, bulk liquid saturation concentration (mass length^{-3}); C_{surf}, surfactant concentration (mass length^{-3}); cmc, critical micelle concentration (mass length^{-3}); $k_{l,a}$, mass transfer coefficient for phenanthrene dissolution into aqueous phase (length time^{-1}); $k_{l,m}$, mass transfer coefficient for micelle transport away from solid-liquid interface (length time^{-1}); $k_{l,\text{obs}}$, observed mass transfer coefficient for phenanthrene dissolution (length time^{-1}); K_S, Monod half-saturation coefficient (mass length^{-3}); $q_{\max}$, maximum specific phenanthrene removal rate (mass mass^{-1} time^{-1}); q, specific phenanthrene removal rate (mass mass^{-1} time^{-1}); r_{diss}, rate of phenanthrene dissolution into water (mass length^{-3} time^{-1}); SC, solubilization capacity (mass mass^{-1}) (phenanthrene/surfactant ratio); X, biomass concentration (mass length^{-3}); Y, yield coefficient (mass mass^{-1}).

Chemicals. Reagent-grade phenanthrene (purity, >96%) was obtained from Sigma Chemical Co. (St. Louis, Mo.) and used without further purification. Phenanthrene is a three-ring PAH with a melting point of 98°C (17) and a water solubility of 1.12 mg/liter (9). Tergitol NP-10, a nonylphenol ethoxylate, was obtained from Sigma Chemical Co. The surfactant has a molecular weight of 682, a cmc of 82 mg/liter, and an SC for phenanthrene of 0.029 g/g (10).

Microorganism and culture media. *P. stutzeri* P16 was isolated from soil contaminated with creosote and has been well characterized with respect to phenanthrene biodegradation (28–30). *P. stutzeri* P16 was maintained on slants with R2A agar (Difco) supplemented with phenanthrene (29). For quality control and to confirm the purity of the culture, *P. stutzeri* P16 was periodically streaked on sheep's blood agar and nutrient agar plates.

Biomass estimation. Growth of *P. stutzeri* P16 was monitored by measuring optical density at 420 nm (A_{420}) with a double-beam UV/vis scanning spectrophotometer (Hitachi Instruments, Inc.). In the absence of surfactant, one absorbance unit corresponded to 500 mg (dry weight) of biomass per liter (29). Some coagulation of the biomass was observed at higher surfactant concentrations, so that the correlation of absorbance to biomass dry weight was determined by preparing standard curves for each surfactant concentration. Standard curves were prepared by measuring A_{420} in the absence of surfactants and then adding surfactant and measuring A_{420} again. Triplicate measurements were performed for each of three biomass concentrations at four different surfactant concentrations.

Analytical techniques. Phenanthrene and Tergitol NP-10 concentrations were measured by high-performance liquid chromatography (HPLC) (8). The HPLC system consisted of a Waters (Millipore, Marlborough, Mass.) model 600E HPLC quaternary pump, model 470 fluorescence detector, and model 717 autosampler and a C_{18} reversed-phase column (25 cm by 4.5 mm; Supelco, Inc., Bellefonte, Pa.). The mobile phase (1 ml/min) was 70:30 (vol/vol) acetonitrile in water for 0 to 6 min, was increased linearly to 100% acetonitrile over 6 to 8 min, and was maintained at this composition through 18 min. Phenanthrene was analyzed at an excitation wavelength of 259 nm and emission wavelength of 370 nm (24), while Tergitol NP-10 was analyzed at 225 and 295 nm, respectively (19).

Growth experiments. Flasks containing solid phenanthrene with a constant interfacial area were prepared as described earlier (9, 10). A total of 200 ml of liquid solution (tap water buffer [TWB] alone or TWB plus surfactant) was added to each flask. Several loops (at least three) of *P. stutzeri* P16 grown on R2A-phenanthrene agar for 24 h were suspended in 7 ml of TWB and vortexed for 30 s. One milliliter of this *P. stutzeri* P16 suspension was used to inoculate each of six growth flasks. After inoculation, the flasks were placed on a constant temperature incubator (25°C) and agitated at 150 rpm. Growth of *P. stutzeri* P16 and concentrations of phenanthrene and surfactant (where added) were monitored over time. Biomass concentrations were determined by withdrawing 1-ml aliquots and measuring optical density at 420 nm. Separate 1-ml samples were taken to determine liquid-phase phenanthrene and Tergitol NP-10 concentrations by HPLC. Formaldehyde (10% [vol/vol] final concentration) was added to the sample to prevent phenanthrene biodegradation in the sample vial before analysis.

In later experiments, the phenanthrene removal kinetics of *P. stutzeri* P16 were measured for each set of flasks (controls and flasks containing surfactant) by combining the liquid phases from replicate flasks. The culture from a given set of flasks was centrifuged and washed, and then phenanthrene removal kinetics in the absence of surfactant were determined by a spectrophotometric rate assay described earlier (30). Measured rates were then used in model simulations.

RESULTS

Partitioning of phenanthrene between micelles and the aqueous phase has been observed to inhibit phenanthrene biodegradation by *P. stutzeri* P16 when all of the phenanthrene was solubilized in surfactant micelles (8). However, surfactants also were observed to increase the dissolution rate of solid phenanthrene (10), so that it was of interest to determine whether increased rates of dissolution would correspond to increased rates of bacterial growth and phenanthrene degradation in the presence of excess phenanthrene.

In the absence of surfactants, the growth of *P. stutzeri* P16 was observed to be limited by phenanthrene dissolution in the batch system containing solid phenanthrene with a known and constant surface area (Fig. 2). The culture was inoculated in this system after phenanthrene had reached equilibrium with the liquid phase (1.12 mg/liter). The phenanthrene concentration decreased rapidly over time, and linear growth of *P. stutzeri* P16 was observed as the liquid-phase phenanthrene concentration was depleted (Fig. 2). The lines in Fig. 2 represent predicted estimates of the dissolution-limited growth model (equations 8 and 9) with published input parameters, which are summarized in Table 1 (9, 29).

Results (Fig. 3) from experiments in which Tergitol NP-10 was added indicate that significantly faster growth was observed for the systems containing surfactant relative to the control, which had no surfactant present (Fig. 3a). All six flasks were inoculated as soon as the liquid solution was added ($C_{T,0} \approx 0$). Growth rates varied from near exponential as long as liquid phenanthrene concentrations could be detected (Fig. 3b) to linear when the liquid-phase phenanthrene was depleted to low concentrations. The phenanthrene concentration in the

TABLE 1. Input parameters for P16 growth simulation in Fig. 2

Parameter or condition	Value
Biokinetic parameters[a]	
$q_{\max}$ (1/min)	0.004
K_S (mg/liter)	0.24
Y (g/g)	1.3
System parameters[b]	
$C_{a,\text{sat}}$ (mg/liter)	1.12
a (1/cm)	0.09
$k_{l,a}$ (cm/min)	0.11
Initial conditions	
$C_{T,0}$ (mg/liter)	1.12
X_0 (mg/liter)	3

[a] From reference 29.
[b] From reference 9.

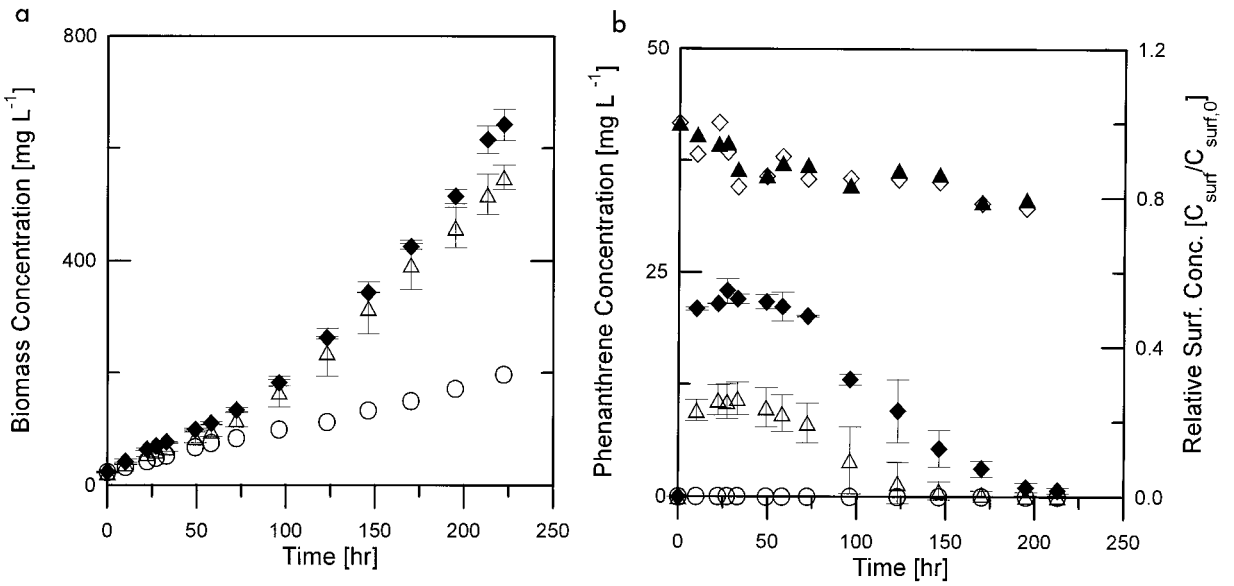

FIG. 3. (a) Batch growth of *P. stutzeri* P16 in the absence of surfactant (○) or in the presence of Tergitol NP-10 at a starting concentration of 500 mg/liter (△) or 1,000 mg/liter (◆). (b) Phenanthrene concentration in the control flask (○) and phenanthrene (△ and ◆) and surfactant (▲ and ◇) concentrations for the systems containing surfactant at 500 mg/liter (triangles) or 1,000 mg/liter (diamonds). Symbols and error bars represent means and standard deviations, respectively, of triplicate flasks for 500-mg/liter surfactant concentration and duplicates for 1,000-mg/liter Tergitol NP-10 concentration. The control with no surfactant was a single flask.

500-mg/liter Tergitol system was depleted faster than that for the higher surfactant concentration used (Fig. 3b), so that linear growth was observed earlier at the lower surfactant concentration. The slopes of the linear growth phase ($C_T \approx 0$) predicted by the model were 4.5 mg liter^{-1} h^{-1} for 1,000 mg of Tergitol NP-10 liter^{-1} and 2.3 mg liter^{-1} h^{-1} for 500 mg liter^{-1}. They correlated relatively well with the observed slopes, which were 4.9 mg liter^{-1} h^{-1} ($r^2 = 0.991$, $n = 3$) for 1,000 mg liter^{-1} and 3.0 mg liter^{-1} h^{-1} ($r^2 = 0.999$, $n = 5$) for 500 mg liter^{-1}, respectively (fits not shown).

Relatively minor losses of surfactant concentration seemed to occur during the first 25 h of the experiment, but surfactant concentrations remained essentially constant for the remainder of the experiment (Fig. 3b).

Except for the linear growth phase, the data shown in Fig. 3 could not be simulated well with published kinetic coefficients for *P. stutzeri* P16 grown in the absence of surfactants (29). Therefore, an additional experiment was conducted in which the specific phenanthrene removal rates were measured for the bacterium after growth in the presence of the surfactant (Table 2). Results of this experiment (Fig. 4) compared well with model predictions.

DISCUSSION

Various explanations have been proposed for the inhibitory effects of surfactants on biodegradation (15, 26), but few have been verified experimentally. Among the proposed effects is the partitioning of hydrophobic substances into surfactant micelles (14, 34), with the assumptions that microorganisms do not have direct access to compounds inside micelles and that the mass transport of hydrophobic substances exiting micelles limits biodegradation. The dynamics of micellar exchange of hydrophobic compounds with the aqueous phase have been studied extensively in the recent past by time-resolved fluorescence quenching techniques (3, 7). Kinetic coefficients for the exit of the hydrocarbon from the micelles were observed to be generally lower than those for the entrance (3, 7). However, both entrance and exit rates are likely to be far higher than biodegradation rates in the presence of microbial activity. Inhibitory effects of surfactants on biodegradation of solubilized phenanthrene can be explained well even if it is assumed that aqueous and micellar concentrations of phenanthrene are in equilibrium (8).

The concept of partitioning between the aqueous phase and micelles formed a basis for understanding the effects of surfactants on biological activity in systems containing excess phenanthrene. An experimental system containing solid phenanthrene with a constant interfacial area was designed to study the effect of surfactants on mass transfer processes and on

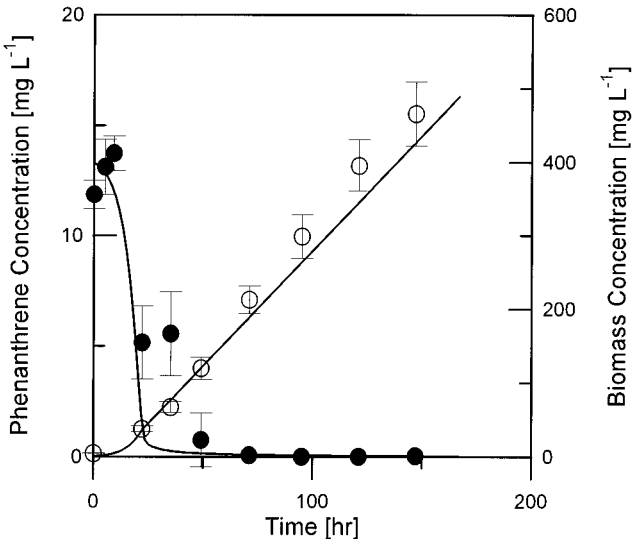

FIG. 4. Bacterial biomass (○) and liquid phenanthrene concentration (●) during batch growth of P16 in the presence of solid phenanthrene and 500 mg of Tergitol NP-10 liter^{-1}. Lines depict model simulations. Symbols and error bars represent means and standard deviations, respectively, of triplicate flasks.

TABLE 2. Input parameters for P16 growth simulation in Fig. 4

Parameter or condition	Value
Biokinetic parameters	
q_{max} (1/min)	0.003
K_S (mg/liter)	0.08
Y (g/g)	1.3[a]
System parameters	
$C_{a,sat}$ (mg/liter)	1.12[b]
a (1/cm)	0.09[b]
$k_{l,a}$ (cm/min)	0.11[b]
$k_{l,m}$ (cm/min)	0.02[c]
SC (g/g)	0.03[c]
C_{surf} (mg/liter)	500
cmc (mg/liter)	82[c]
Initial conditions	
$C_{T,0}$ (mg/liter)	13.3
X_0 (mg/liter)	2

[a] From reference 29.
[b] From reference 9.
[c] From reference 10.

biodegradation. In an experiment on growth of *P. stutzeri* P16 in the absence of surfactants, the phenanthrene mass transfer rate was not high enough to replenish the degraded phenanthrene from the liquid phase, which is indicated by the rapid decrease in liquid phenanthrene concentration over time (Fig. 2). Linear growth of *P. stutzeri* P16 was observed as the liquid phenanthrene concentration approached zero, indicating that, during that phase, growth was limited by the dissolution of phenanthrene. Using published coefficients (Table 1), the model described well the observed decline in phenanthrene concentration and the growth of *P. stutzeri* P16 (Fig. 2). Similar results have been reported for bacterial growth on solid naphthalene (33).

Since phenanthrene dissolution rate increases in the presence of surfactants (10), the growth of *P. stutzeri* P16 under dissolution-limited conditions ($C_T \approx 0$) would be expected to increase with increased surfactant concentration. This was observed as soon as the liquid concentration was depleted in flasks containing surfactant at two different concentrations above the cmc (Fig. 3). The model predicted phenanthrene concentrations and *P. stutzeri* P16 growth well for the control flask in which no surfactant was present (not shown). However, a more rapid decline in liquid phenanthrene concentrations for both surfactant concentrations would have been predicted by the model with published input parameters (not shown). The observed lag was not likely due to the use of the surfactant as an alternate carbon source by *P. stutzeri* P16, since the surfactant concentration was essentially constant after about 25 h of incubation (Fig. 3b). Minor initial losses in surfactant may have been due to adsorptive processes.

Previous experiments indicated that Tergitol NP-10 did not significantly influence the growth of *P. stutzeri* P16 on peptone (8). However, it is possible that the surfactant could affect the specific activity of the organism during growth on phenanthrene. Also, it is reasonable to expect some variation in specific activity between batch cultivations of any organisms. Therefore, an experiment similar to that reported for Fig. 3 in which biokinetic parameters were measured at the end of the experiment was conducted. The measured q_{max} (Table 2) was lower than reported earlier (29), suggesting that the culture was less active than in previous experiments with *P. stutzeri* P16. Model simulations using the measured parameters predicted well both the liquid phenanthrene concentration and *P. stutzeri* P16 growth (Fig. 4). The assumptions of the model—that phenanthrene present in the solid phase or in micelles is not directly available for biodegradation and that partitioning between micelles and the aqueous phase occurs instantaneously—therefore seem to be justified by the results of this study. Volkering et al. (34) proposed a mathematical model which incorporated a first-order rate expression to describe mass transfer of phenanthrene and naphthalene between the micellar and aqueous phases. The model, however, was unable to describe their observations of PAH concentration in the liquid phase and microbial growth.

For the bacterial culture used in this study, it appears that micelles can be treated as a distinctly different phase with respect to the influence of surfactants on the biodegradation of phenanthrene. While the conceptual and quantitative model derived agrees well with the experimental observations, this might not necessarily be the case for different combinations of surfactants, hydrocarbons, and bacteria. For example, microorganisms that have a tendency to attach to hydrophobic surfaces might alter potential mass transfer processes, which might in turn modify the effect of surfactant addition. It also has been suggested that bacteria which produce biosurfactants might gain direct access to hydrocarbons within micelles or microemulsions via complex transport mechanisms (13). Interactions between micelles and microorganisms, or between microorganisms and hydrocarbon surfaces, were ignored in the development of the model. Such interactions would result in higher rates of biodegradation than would be predicted if it were assumed that only the amount of hydrocarbon present in the aqueous phase were bioavailable. Two recent studies have suggested that biodegradation of surfactant-solubilized PAH was greater than could be accounted for by ignoring direct access to micellized PAH (12, 15).

The proposed biodegradation model describes the biodegradation of solid hydrophobic compounds in the presence of surfactants. It accounts for the physical effects of surfactants on partitioning of the compound between the aqueous and micellar phases and on the effects of solid hydrocarbon dissolution. More complex mechanisms, such as the effect of surfactants on liquid nonaqueous-phase surfaces, on emulsification mechanisms, or on the direct interaction of microorganisms with micelles or with nonaqueous phases, would require extension of the proposed model. An important implication of this work is that surfactants should stimulate biodegradation processes when biodegradation is limited by dissolution processes and for as long as the substrate is present in excess. An overdosing of surfactant to the point that all of the hydrophobic substrate would be solubilized into micelles, however, could have a negative effect on the rate of biodegradation because of a resulting decline in aqueous-phase hydrocarbon concentration. The amount of surfactant at which such inhibition would occur depends on the specific system but can be evaluated experimentally on a case-by-case basis.

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