ASSESSMENT OF THE USE OF SURFACTANTS FOR BIODEGRADATION OF HYDROPHOBIC POLLUTANTS: DEVELOPMENT OF PRINCIPLES

By

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ABSTRACT

A well-defined experimental system was developed to quantify the effects of a surfactant on biodegradation of phenanthrene, a polycyclic aromatic hydrocarbon and model hydrophobic chemical, under two situations relevant to experimental work reported by others and to potential field applications. When phenanthrene was completely solubilized in surfactant micelles, the rate of degradation decreased with increasing surfactant concentration. However, when phenanthrene was present in excess as a solid phase, the surfactant increased the rate of microbial growth relative to a control in which surfactant was not added. The effects of the surfactant were explained well with a mechanistic model based on partitioning of phenanthrene between the aqueous phase and surfactant micelles, and on the known ability of the surfactant to increase the rate of phenanthrene dissolution from the solid phase. Such effects are expected to be observable for hydrophobic pollutants in the field as well. Whenever biodegradation is limited by mass transfer processes between nonaqueous phases and the aqueous phase, surfactants can be expected to stimulate biodegradation. The degree of stimulation for a given surfactant concentration would depend on the extent to which the mass transfer process is enhanced. It is possible, however, that a surfactant could be overdosed to the point that all of the hydrophobic compound would be solubilized, in which case a negative effect on biodegradation might occur. Thus, tests to study the potential effect of a surfactant on biodegradation in the field must consider the effects on mass transfer rates and on solubilization of the compounds of interest. Together with information on specific rates of biodegradation in the absence of surfactant, the model proposed in this study can serve as a basis for estimating biodegradation rates in the field.
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SUMMARY AND CONCLUSIONS

Many of the pollutants found in contaminated soils and sediments are hydrophobic, and are found either as discrete nonaqueous phases or partitioned into natural organic matter. Aqueous phase concentrations of these pollutants tend to be quite low, so that biodegradation in the subsurface can be limited by rates at which they dissolve or desorb from nonaqueous phases. Surfactants are known to solubilize hydrophobic chemicals and therefore have been proposed as a means of increasing the bioavailability of these compounds, either in situ in the subsurface or above-ground in a pump-and-treat remediation scheme. However, the literature on the effects of surfactants on the biodegradation of hydrophobic compounds has been contradictory, with experimental observations ranging from inhibition to no effect to stimulation. Many of these experiments have been conducted in complex systems and with a wide range of initial conditions, so that it is difficult to establish cause-and-effect relationships or to compare different studies directly.

The purpose of this study was to evaluate the effects of a surfactant on biodegradation of a model hydrophobic compound, phenanthrene, by an approach based on first principles. A well-defined experimental system was constructed to provide quantifiable rates of (a) phenanthrene dissolution; (b) growth of a phenanthrene degrading bacterium, \textit{Pseudomonas stutzeri} P16; and (c) accumulation of phenanthrene in the liquid phase (comprising the true aqueous phase and surfactant micelles) in the presence of a nonionic surfactant, Tergitol NP-10. This system was then used to test a hypothesis that the bacterium could degrade phenanthrene only when present in the aqueous phase, and that it did not have direct access to phenanthrene either in the solid phase or in surfactant micelles. Results support this hypothesis both conceptually and quantitatively. In the absence of excess phenanthrene (no nonaqueous phase present), biodegradation rates were found to decrease with increasing surfactant concentration. Such inhibition of biodegradation is due to partitioning of phenanthrene in surfactant micelles, effectively lowering the concentration available to the bacteria at any instant in time. In the presence of nonaqueous phenanthrene, however, increasing surfactant concentrations resulted in increased bacterial growth rates. The increase in growth rate was due to the ability of the surfactant to increase the rate of phenanthrene dissolution. A biodegradation model using independently measured parameter values was able to simulate experimental results accurately.

The results of this work provide a benchmark case to describe biodegradation of hydrophobic compounds in the presence of surfactants. It accounts only for the physical effects of surfactants on partitioning of the compound between the aqueous and micellar phases, and on the dynamics of exchange between the nonaqueous, aqueous and micellar phases. More complex mechanisms involving direct interactions of microorganisms with micelles or with nonaqueous phases would require extension of the model provided in this report. An implication of this work is that surfactants will stimulate biodegradation processes whenever biodegradation is limited by mass transfer processes and for as long as the hydrophobic substrate is present in a nonaqueous phase. Unless a surfactant has deleterious effects on metabolic processes within an organism, the extent of stimulation by a surfactant at a given concentration should be proportional to its effect on the mass transfer process(es). Overdosing
the surfactant to the point of solubilizing all of the hydrophobic substrate could, however, decrease the rate of biodegradation, particularly if the amount of the compound solubilized is well below its saturation concentration. The amount of surfactant at which such decreases would occur depends on the specific system but can be evaluated experimentally on a case-by-case basis.
The concepts illustrated in this experimental study can be extended to more complex situations and potentially to field applications. However, careful experimental testing is still required on a case-by-case basis before the effects of surfactants on biodegradation can be evaluated in a more complex system. First, some knowledge of the equilibrium behavior of surfactants, particularly in the presence of soil (e.g., sorption to soil), is necessary. The recent publications of Luthy and co-workers (Edwards et al., 1991; Laha and Luthy, 1991; Laha and Luthy, 1992; Edwards et al., 1994a; Edwards et al., 1994b) are especially useful in this regard. The concepts and methods described in these publications can be used to determine the critical micelle concentrations (CMC) of surfactants in the presence of soil, from which an assessment can then be made of the solubilization capacity for one or more hydrophobic compounds. The solubilization capacity in the presence of soil is not likely to be the same as in clean aqueous systems, since some soil organic matter and other hydrophobic chemicals might be solubilized in addition to the contaminants of concern. It is recommended that the solubilization capacity be determined using actual soil samples while permitting a long enough equilibration time to be satisfied that the system is truly at or near equilibrium.

Once the solubilization capacity of a surfactant for a particular compound is known, the concentration of surfactant required to completely solubilize all of the compound(s) in a given system can be estimated. This concentration should be considered an upper limit for the initial range of surfactant concentrations of interest, since it is possible that the surfactant will actually inhibit biodegradation at concentrations higher than this. The lower limit on surfactant concentration should be the in-soil CMC. Although some stimulating effects of surfactants on biodegradation have been suggested at sub-CMC concentrations, mechanisms to explain these effects have not been proposed and long-term tests have not verified the findings of short-term laboratory studies. Thus, there is a window of surfactant concentrations for which a significant effect on biodegradation might occur.

After a range of surfactant concentrations has been selected for testing, the effect of the surfactant on rates of dissolution (relevant to systems with nonaqueous phase liquids present) or desorption (relevant to systems with low concentrations of hydrophobic pollutants) should be tested. If different surfactant concentrations are tested, empirical or semi-empirical mass transfer models could be used to estimate dissolution or desorption rates as a function of surfactant concentration. The driving force for mass transfer typically involves a difference between the actual and saturation concentrations of a substance in the liquid phase, so that maximum rates of mass transfer can be estimated by assuming the actual concentration in the liquid phase is equal to zero. When biodegradation of a compound is occurring under mass transfer-limited conditions, the concentration in the liquid phase will be at or near zero, so that it is important to quantify maximum rates of mass transfer.

In most soil systems it would be difficult to follow the growth of microorganisms as was done in the present study. Instead, it probably would be easier to conduct biodegradation tests by following the removal of specific target compounds. Biodegradation tests can be done on compounds already present in the soil or on compounds added exogenously. Tests on
Exogenously added compounds would be useful for determining maximum specific rates of biodegradation (and, if relevant, Monod half-saturation coefficients). To preclude confounding effects of sorption of added compounds, such tests should be conducted on very dilute soil suspensions or, if necessary, on enrichment cultures derived from the target soil.

Exogenously added chemicals are not representative of the same chemicals already present in the soil if mass transfer processes govern the bioavailability of those chemicals in the soil system. Biodegradation tests therefore must be conducted on the compound(s) already present in the soil to determine whether biodegradation is likely to be mass-transfer limited. If not, there is no need to consider the use of surfactants further. Dissolution-limited biodegradation should be observable as a linear decrease in concentration of the target compound(s) over extended periods of time. This linear rate of removal should be equal to the maximum rate of dissolution in the absence of surfactants. Desorption-limited biodegradation might be more difficult to diagnose, since desorption rates would not necessarily be linear even with biodegradation occurring. However, in an active biological system, continuously increasing rates of removal should be observed if desorption is not limiting, since microbial populations should continuously increase with time. Conversely, rates of target compound removal will continuously decrease if biodegradation is not the rate-limiting step for sorbed compounds, since desorption rates should, in general, decrease as the sorbed concentration decreases. Empirical desorption models might be useful in estimating desorption rates at zero or near-zero concentrations of the compound in the aqueous phase, which would be relevant to biologically active systems.

If mass transfer limitations are verified to limit biodegradation, then models to describe dissolution or desorption in the presence of surfactant can be coupled to a biodegradation model. Tests such as those described above should be conducted to quantify all important model parameters. The complete model then can be used to estimate the impact of surfactant on the rate and overall extent of biodegradation. If a significantly positive impact of surfactant is indicated, then treatability tests using one or more stimulatory surfactant concentrations should be conducted.

Feasibility tests with surfactants can be followed by economic evaluations. Also, any surfactant considered for possible field use should first be evaluated for any potential adverse health or ecological effects. McCutcheon's handbook of surfactant data (McCutcheon's, 1993) identifies those surfactants which have been approved as food additives by the United States Food and Drug Administration. These food-grade additives are expected to be of limited human health concern, but their potential impacts on the environment should be considered on a case-by-case basis.
1. Introduction

The pollution of soil and sediment by industrial and commercial activities has been recognized as a national problem for several decades. Many of the pollutants found in contaminated soils and sediments are hydrophobic and are found either as discrete nonaqueous phases or partitioned into natural organic matter. Polycyclic aromatic hydrocarbons (PAH) represent a major class of hydrophobic pollutant found in the environment at many sites across the United States, and are of concern because several PAH have been identified as known carcinogens (Cerniglia and Heitkamp, 1989). PAH are typically present as major contaminants at sites associated with creosote treatment of wood (Mueller et al., 1989; Rosenfeld and Plumb, 1991) and with the former production of manufactured gas (Luthy et al., 1994). In North Carolina, for example, more than 30 manufactured gas plant (MGP) sites have been discovered in the last five years and are considered to represent a threat to both surface and ground water resources (B. Nicholson, Superfund section, N.C. Dept. of Environment, Health and Natural Resources, personal communication, January 1994).

Many of the PAH, particularly the lower molecular weight compounds (those with two or three aromatic rings), are known to be biodegradable (Cerniglia and Heitkamp, 1989). As a result, bioremediation has been attempted or proposed for many PAH-contaminated sites (U.S. Environmental Protection Agency, 1993). However, bioremediation is still not considered to be a proven technology for PAH contamination, and has not been effective for the extensive removal of high molecular weight compounds in particular (Wilson and Jones, 1993).

Aqueous phase solubilities of most PAH with three or more rings are in the sub-mg/L range (Mackay et al., 1992), so that biodegradation in the subsurface can be limited by rates at which these compounds dissolve or desorb from nonaqueous phases. Slow rates of dissolution or desorption have been suggested to be responsible for the limited biodegradation in soil of PAH that are otherwise known to be readily biodegradable (Manilal and Alexander, 1991; Weissenfels et al., 1992; Erickson et al., 1993). Developing methods to overcome limitations in the mass transfer of hydrophobic compounds has been suggested to be one of the most critical needs in bioremediation research (Alexander, 1991; Luthy, 1991).

Surfactants are known to solubilize hydrophobic chemicals and therefore have been proposed as a means of increasing the bioavailability of these compounds, either in situ in the subsurface or above-ground in a pump-and-treat remediation scheme. However, the literature on the effects of surfactants on the biodegradation of hydrophobic compounds has been contradictory, with experimental observations ranging from inhibition to no effect to stimulation (Rouse et al., 1994; Liu et al., 1995). Many of these experiments have been conducted in complex systems and with a wide range of initial conditions, so that it is difficult to establish cause-and-effect relationships or to compare different studies directly. To date, comprehensive models (conceptual or mathematical) to describe the effects of surfactants on biodegradation in various situations have not been developed. A recent attempt at developing a mathematical model to simulate experimental results in a well-defined laboratory system (Volkering et al., 1995) resulted in a poor fit to the data.
Scope and Objectives of Research

The purpose of this research project was to follow up on preliminary work on the effects of surfactants on biodegradation of phenanthrene as a model PAH (Aitken et al., 1996). A well-defined laboratory system was developed with known effects of surfactants on phenanthrene dissolution (Grimberg et al., 1995). This system was used to study the effects of a surfactant on the growth of the bacterium *Pseudomonas stutzeri* P16, which has been well characterized with respect to its ability to degrade phenanthrene and other PAH (Stringfellow and Aitken, 1994a; Stringfellow and Aitken, 1994b; Stringfellow and Aitken, 1995; Aitken et al., 1996). A mathematical model was then developed to account for the effects of the surfactant on growth of P16 by coupling mechanistic mathematical models for phenanthrene dissolution and biodegradation. The experimental system therefore permitted a direct analysis of the influences of surfactant on phenanthrene biodegradation using an approach that relied on first principles. Of primary interest was to determine if model simulations could accurately describe the experimental observations, which would justify the use of the model as a basis to analyze or predict the effects of surfactants on biodegradation in more complex situations.
Surfactants are molecules that consist of two regions with opposing affinities for water. Each molecule has a hydrophilic "head" group and one or more hydrophobic "tails." The head groups can be charged (anionic or cationic) or neutral species (nonionic). In water, surfactant molecules tend to accumulate at interfaces (such as the air-water interface) because of the low affinity of the hydrophobic tails to water. At low surfactant concentrations, surfactant monomers will exist primarily in solution or at surfaces. As the concentration of a surfactant in water is increased, the monomers of many types of surfactants will aggregate into structures referred to as micelles. Micelles are small (in the low nm size range), dynamic, three-dimensional colloids. The shape of a micelle depends on the surfactant and other factors, but for all micelles in water the hydrophobic tails of surfactant monomers are oriented inward while the hydrophilic group is exposed to the water.

**Solubilization of Hydrophobic Compounds by Surfactants**

Surfactant micelles are able to "solubilize" hydrophobic chemicals because of the strong affinity of the hydrophobic micelle core for other hydrophobic substances (Attwood and Florence, 1983). Since micelles are so small, any hydrophobic substance present inside them will appear to be dissolved in the liquid phase (i.e., they can not be removed by conventional filtration). Figure 2.1 illustrates the solubilization of a solid hydrocarbon (such as a PAH) by a surfactant. This figure is intended to show that the liquid phase in equilibrium with the solid hydrocarbon phase is actually a complex mixture of surfactant monomers, micelles, free hydrocarbon molecules in the aqueous phase and hydrocarbon solubilized in the micelles. In the presence of excess hydrocarbon, both the aqueous and micellar phases contain saturating concentrations of the hydrocarbon.

Several parameters can be used to quantify the behavior of surfactants and solubilized hydrocarbons in aqueous systems. For any given surfactant, the concentration at which micelles begin to form is the critical micelle concentration (CMC). At surfactant concentrations above the CMC, the concentration of surfactant monomers in the aqueous phase remains constant (Rosen, 1989). Once micelles begin to form, the solubilization of a hydrocarbon increases linearly with increasing surfactant concentration, as illustrated in Figure 2.2 for solubilization of phenanthrene. When the apparent solubility of the hydrocarbon is plotted as a function of surfactant concentration, the slope of the line obtained for surfactant concentrations above the CMC is referred to as the solubilization capacity (SC) (Rosen, 1989) or in molar units as the molar solubilization ratio (Edwards et al., 1991, 1994a, 1994b). The total concentration of hydrocarbon in the liquid phase (aqueous phase + micelles) at equilibrium is therefore:

\[ C_{T, sat} = C_{a, sat} + SC \cdot \left( C_{surf} - CMC \right) \]

where \( C_{T, sat} \) is the saturation concentration of the hydrocarbon in the liquid phase, \( C_{a, sat} \) is the saturation concentration in the aqueous phase alone and \( C_{surf} \) is the surfactant concentration.
Figure 2.1. Conceptual illustration of equilibrium conditions when a surfactant solution is in contact with a solid hydrocarbon (e.g., PAH).

Only the total liquid concentration, \( C_{T,sat} \), is easily measured by conventional measurement techniques.

The distribution of a hydrocarbon between the aqueous phase and micelles at equilibrium can be described by a partitioning coefficient, \( K_m \) (Figure 2.1), which has been defined as the mole fraction of hydrocarbon in micelles divided by the mole fraction of hydrocarbon in the aqueous phase (Valsaraj and Thibodeaux, 1989; Edwards et al., 1991; Jafvert et al., 1994).

Alternatively, the partitioning between the aqueous phase and micelles can be defined by the ratio of the aqueous saturation concentration, \( C_{a,sat} \), to the total liquid concentration, \( C_{T,sat} \), both of which are directly measurable quantities. As long as the aqueous phase is in equilibrium with micelles, this same ratio will hold at hydrocarbon concentrations below saturation (for example, in situations where more than enough surfactant has been added to solubilize all of the hydrocarbon). Therefore, the equilibrium aqueous phase concentration can be estimated from measurement of the total liquid concentration by:
Figure 2.2. Solubilization of phenanthrene by the surfactant T-Maz 20; SC = solubilization capacity, CMC = critical micelle concentration. Adapted from Grimberg et al., 1995.

\[ C_a = \left( \frac{C_{a,sat}}{C_{T,sat}} \right) C_T = \frac{C_{a,sat}}{C_{a,sat} + SC \cdot (C_{surf} - CMC)} \cdot C_T \]  

(2.2)

where \( C_T \) is the actual concentration of hydrocarbon in the liquid phase at equilibrium.

Dissolution Kinetics

Much work has been done recently on the effects of surfactants on solubilization and partitioning of hydrophobic pollutants in both well-defined systems and environmental matrixes such as soil (Valsaraj and Thibodeaux, 1989; Abdul and Gibson, 1991; Edwards et al., 1991; Anderson, 1992; Abriola et al., 1993; Park and Jaffé, 1993; Pennell et al., 1993; Edwards et al., 1994a; Edwards et al., 1994b; Jafvert et al., 1994; Shiau et al., 1994; Sun et al., 1995). However, relatively little research has been done on the kinetics of dissolution. In work on solubilization of dodecane in soil columns, Abriola and co-workers (Abriola et al., 1993; Pennell et al., 1993) found that hydrocarbon concentrations in the column effluent were far from equilibrium, illustrating the importance of dissolution kinetics in determining hydrocarbon transport in the presence of surfactants.
We recently developed a quantitative model of dissolution kinetics for solid phenanthrene in the presence of several nonionic surfactants, and quantified model parameters based on experimental measurements (Grimberg et al., 1994; Grimberg et al., 1995). Dissolution of solid phenanthrene is considered in this model to consist of two parallel mechanisms: direct dissolution into the aqueous phase, and dissolution into micelles near the hydrocarbon/water interface; furthermore, phenanthrene in the aqueous phase is assumed to be at equilibrium with that in micelles at all liquid phase concentrations. The rate of accumulation of phenanthrene in the bulk liquid phase was then assumed to be controlled by the rate of transport of free phenanthrene and phenanthrene-saturated micelles across a diffusive boundary layer at the hydrocarbon/water interface. These concepts are illustrated schematically in Figure 2.3. The direct dissolution of hydrocarbon into the aqueous phase is the same regardless of the surfactant concentration, but the transport of hydrocarbon via micelles increases with increasing surfactant concentration. Therefore, at high surfactant concentrations the overall transport is dominated by micellar diffusion through the boundary layer (Grimberg et al., 1995). Complex mechanisms have been suggested for the accumulation of surfactant monomers, formation of micelles and incorporation of hydrocarbon at the interface (Chan et al., 1976), but such mechanisms are not considered to be rate limiting in the proposed model.

Figure 2.3. Illustration of transport processes proposed to be involved in the dissolution of a solid hydrocarbon in the presence of surfactant in a well-mixed system. The hydrocarbon can dissolve directly into the aqueous phase or into micelles as they form near the hydrocarbon/water interface.
The experimental system used to study the effects of surfactant on dissolution kinetics was a completely mixed batch vessel containing a solid block of phenanthrene at the bottom of the vessel. The rate of phenanthrene dissolution in the system could be described by equations 2.3 and 2.4 (Grimberg et al., 1995):

\[
\begin{align*}
  r_{\text{diss}} &= k_{l,\text{obs}} \cdot \alpha \cdot (C_{T,\text{sat}} - C_T) \\
  &= k_{l,\text{obs}} \cdot \alpha \cdot \left[ SC \cdot \left( C_{\text{surf}} - C_{\text{MC}} \right) + C_{a,\text{sat}} - C_T \right] \\
  k_{l,\text{obs}} &= \frac{k_{l,a} + k_{l,m} \cdot \left( SC/C_{a,\text{sat}} \right) \cdot \left( C_{\text{surf}} - C_{\text{MC}} \right)}{(SC/C_{a,\text{sat}}) \cdot \left( C_{\text{surf}} - C_{\text{MC}} \right) + 1}
\end{align*}
\] (2.3) (2.4)

where \( k_{l,\text{obs}} \) is an observed mass transfer coefficient and \( \alpha \) is the specific surface area of the solid (surface area per unit volume). Note that both the observed mass transfer coefficient and the dissolution rate are functions of surfactant concentration. The maximum rate of phenanthrene dissolution occurs when the liquid phase concentration, \( C_T \), is zero or near-zero, which is relevant to situations in which phenanthrene would be consumed in the liquid phase as it dissolves. Figure 2.4 illustrates how the maximum dissolution rate varies as a function of surfactant concentration. Note that the absolute value of dissolution rate is system-specific, because the specific surface area available for dissolution is unique for a given system.

Figure 2.4. Effect of concentration of the surfactant T-Maz 20 on the maximum rate of phenanthrene dissolution in a completely mixed batch system. Adapted from Grimberg et al., 1995.
Biodegradation of Phenanthrene

We recently completed a large project (Aitken et al., 1996) in which we studied the biodegradation of phenanthrene and other PAH by bacteria isolated from PAH-contaminated soils. All of these bacteria were observed to degrade phenanthrene present in the aqueous phase by classic Monod kinetics:

$$q = \frac{q_{\text{max}} [S]}{K_S + [S]}$$

(2.5)

where $q$ represents the specific biodegradation rate of phenanthrene (rate per unit bacterial biomass), $q_{\text{max}}$ is the maximum specific rate of biodegradation, $[S]$ is the substrate (phenanthrene) concentration, and $K_S$ is the half-saturation coefficient (concentration of phenanthrene at which the specific biodegradation rate is half of the maximum rate). The dependence of the specific biodegradation rate on phenanthrene concentration is illustrated in Figure 2.5 for two of the strains isolated in the previous study.

![Figure 2.5](image)

**Figure 2.5.** Specific phenanthrene uptake rate (SPUR) as a function of phenanthrene concentration for two bacteria isolated from a creosote-contaminated soil, *Pseudomonas saccharophila* P15 and *Pseudomonas stutzeri* P16. The lines represent best fits of the Monod equation for each set of data. From Stringfellow and Aitken, 1994b.
The Monod equation to describe the overall biodegradation rate is obtained by multiplying $q$ by the biomass concentration:

$$r_{bio} = q \cdot X$$

(2.6)

where $r_{bio}$ is the overall phenanthrene biodegradation rate and $X$ is the biomass concentration.

The Monod equation is useful in describing the rate of biodegradation for substances present in the aqueous phase. In a system containing a separate hydrocarbon phase, however, continued growth of bacteria can deplete the amount available in the aqueous phase to a point where growth and biodegradation are limited by the rate of hydrocarbon dissolution (Volkering et al., 1992; Volkering et al., 1993). Based on experimental observations consistent with dissolution-limited growth, it has been assumed that bacteria can degrade hydrocarbons only in the aqueous phase (Wodzinski and Coyle, 1974; Stucki and Alexander, 1987; Volkering et al., 1992; Volkering et al., 1993; Efroymson and Alexander, 1995). There is current interest in understanding whether some microorganisms have direct access to hydrophobic compounds present in nonaqueous phases, but there is virtually no direct evidence for this. It has been suggested from indirect measurements that some organisms might degrade hydrophobic compounds faster than would be predicted by the maximum rate of dissolution (or desorption for sorbed compounds) into the aqueous phase (Stucki and Alexander, 1987; Efroymson and Alexander, 1991; Guerin and Boyd, 1992; Ortega-Calvo and Alexander, 1994; Harms and Zehnder, 1995). One of the explanations for this phenomenon is the attachment of bacteria directly to the hydrocarbon surface (Efroymson and Alexander, 1991; Ortega-Calvo and Alexander, 1994) or the sorbing surface (Harms and Zehnder, 1995), which presumably allows for more direct access to the substrate.

Despite the possibility that some bacteria might be able to influence the availability of hydrophobic substrates, the most general situation, and "worst-case" from a practical perspective, is that the rate of biodegradation is limited by the rate of dissolution or desorption. Thus, any means of increasing the rate of dissolution or desorption should increase the rate of biodegradation. In this project, therefore, we tested the hypothesis that the ability of a surfactant to increase the dissolution rate of phenanthrene would result in a corresponding increase in the rate of phenanthrene biodegradation.

**Biodegradation of Solubilized Phenanthrene**

As noted above, when a hydrocarbon such as phenanthrene is present in completely solubilized form (no excess hydrocarbon phase) in the presence of a surfactant, the equilibrium distribution of hydrocarbon between the surfactant micelles and the aqueous phase is readily quantified. When such a system contains bacteria capable of degrading the hydrocarbon, the distribution of hydrocarbon between micelles and the aqueous phase is continuously changing. It is reasonable to assume that the equilibrium distribution is reached virtually instantaneously because the exchange of hydrophobic solutes between micelles and water has been observed to be extremely rapid (Gehlen and De Schryver, 1993). This assumption was used to develop a model describing the biodegradation of phenanthrene completely solubilized by surfactant
micelles (Grimberg and Aitken, 1995). A modified Monod equation was used to quantify the effects of surfactant on biodegradation of solubilized phenanthrene:

\[
\frac{q}{q_{\text{max}}} = \frac{C_T / C_{T, \text{sat}}}{K_s / C_{a, \text{sat}} + C_T / C_{T, \text{sat}}} = \frac{C_T}{K_s \cdot \left( C_{T, \text{sat}} / C_{a, \text{sat}} \right) + C_T}
\]  

(eq. 2.7)

where \( C_{T, \text{sat}} \) is as defined in eq. 2.1.

Note that eq. 2.7 is exactly the same form as the Monod equation (eq. 2.5), except that the coefficient \( K_s \) is increased by an amount equal to the ratio of the total liquid solubility to the aqueous phase solubility. Simulation of experimental data with this equation demonstrated its utility (Figure 2.6) and justified the assumption that instantaneous equilibrium between the micellar and aqueous phases could be assumed.

**Figure 2.6.** Effect of the surfactant Tergitol NP-10 on the specific rate of phenanthrene biodegradation, \( q \) (normalized by \( q_{\text{max}} \)) by P. stutzeri P16 as determined in an initial rate assay. The total liquid phase concentration of phenanthrene, \( C_T \), was 0.8 mg/L for all surfactant concentrations, so the concentration in the aqueous phase decreases as surfactant concentration increases. The dashed line represents a simulation of \( q/q_{\text{max}} \) vs. surfactant concentration based on eq. 2.7 and independently measured Monod kinetic parameters. From Grimberg and Aitken, 1995.
In a system containing excess (nonaqueous) hydrocarbon, surfactant and bacteria, the simplest situation is one in which there is no significant interaction between the bacteria and the nonaqueous phase or between the bacteria and the surfactant. In such a situation it should be possible to quantify the rate of biodegradation by coupling expressions for hydrocarbon dissolution and for biodegradation of the hydrocarbon in the aqueous phase. This situation is illustrated schematically in Figure 2.7. The key features of the conceptual model are that hydrocarbon dissolution occurs as described in Figure 2.3, that there is instantaneous equilibrium between the hydrocarbon in micelles and that in the aqueous phase, and that the bacteria can consume only the hydrocarbon present in the aqueous phase.

In a batch system, therefore, the overall accumulation of phenanthrene in the liquid phase is equal to the combined rates of dissolution and biodegradation:
\[
\frac{dC_T}{dt} = r_{\text{diss}} + n_{\text{bio}} = k_{1,\text{obs}} \cdot a \cdot [C_{T,\text{sat}} - C_T] + qX
\]

\[
= a \cdot \frac{k_{1,a} + k_{1,m} \cdot \frac{SC/C_{a,\text{sat}}}{(C_{a,\text{sat}})(C_{surf} - CMC) + 1} \cdot [SC \cdot (C_{surf} - CMC) + C_{a,\text{sat}} - C_T]}{(SC/C_{a,\text{sat}})(C_{surf} - CMC) + 1}
\]

\[
+ \frac{q_{\text{max}} \cdot C_T \cdot X}{\left(\frac{SC}{C_{a,\text{sat}}}(C_{surf} - CMC) + 1\right)K_s + C_T}
\]

(2.8)

where \(r_{\text{diss}}\) and \(r_{\text{bio}}\) are as defined by equations 2.3 and 2.6, respectively. The rate of growth of organisms is:

\[
\frac{dX}{dt} = -q \cdot Y \cdot X = \frac{-q_{\text{max}} \cdot C_T \cdot Y \cdot X}{\left(\frac{SC}{C_{a,\text{sat}}}(C_{surf} - CMC) + 1\right)K_s + C_T}
\]

(2.9)

where \(Y\) is the growth yield (unit mass of bacteria grown per unit phenanthrene consumed). Equations 2.8 and 2.9 can be used to describe concentrations of both phenanthrene and bacteria as a function of time in a batch system.
3. EXPERIMENTAL MATERIALS AND METHODS

Microorganism and Culture Media

*Pseudomonas stutzeri* P16 was isolated from soil contaminated with creosote and has been well characterized with respect to phenanthrene biodegradation (Stringfellow and Aitken, 1994a; Stringfellow and Aitken, 1994b; Stringfellow and Aitken, 1995). In the absence of surfactants the organism is known to degrade phenanthrene by Monod kinetics and appears to degrade phenanthrene only in the aqueous phase (Stringfellow and Aitken, 1994a; Stringfellow and Aitken, 1994b). Strain P16 was grown in media containing peptone (5 % wt:wt) and phenanthrene (Stringfellow and Aitken, 1994b) or in tap water buffer (TWB) in the batch growth flasks described above. TWB (pH 6.5) was prepared by combining Na₂HPO₄·7H₂O (1.5 g), KH₂PO₄ (1.0 g), NH₄Cl (2.0 g), and Na₂S₂O₃·5H₂O (0.02 g) in one liter of tap water (Stringfellow and Aitken, 1994b).

*P. stutzeri* P16 was maintained on slants using R2A agar (Difco) supplemented with phenanthrene (R2A/PAT). R2A/PAT slants were prepared by dissolving 18.2 g R2A agar in 1 L of reagent water supplied with 0.05 g of phenanthrene. The solution was mixed, aliquoted into tubes and autoclaved for 20 min at 120 °C and 20 psi. For quality control and to confirm the purity of the culture, strain P16 was periodically streaked on sheep’s blood agar and nutrient agar plates (UNC Media Preparation Facility).

Inocula of P16 were prepared by suspending several loops (at least three) of cultures grown on R2A agar for 24 hrs in 7 mL TWB and vortexing for 30 sec. One mL of the suspension was used to inoculate each growth flask.

Degradation of Solubilized Phenanthrene

*P. stutzeri* P16 was used to study the effects of the nonionic surfactant Tergitol NP-10 on phenanthrene biodegradation in systems containing excess surfactant (all phenanthrene solubilized) and excess phenanthrene (solid phase phenanthrene present). For the experiment in which excess surfactant was used, the organism was added to replicate flasks containing Tergitol NP-10 at 500 or 1000 mg/L and phenanthrene at 6.8 mg/L. The phenanthrene concentration in these flasks was selected to be near its saturation concentration at the lower surfactant concentration. The flasks were inoculated with strain P16 at time zero, and phenanthrene concentration was followed over a four hr period. Phenanthrene concentration was also followed in uninoculated, replicate control flasks containing surfactant at 500 mg/L. Samples for determining phenanthrene concentration were obtained by withdrawing 1 mL aliquots and adding formalin (10% v:v) to quench the biological activity.

Monod rate coefficients for phenanthrene degradation by *P. stutzeri* P16 in the absence of surfactant were determined for the inoculum used in this experiment by the specific phenanthrene uptake rate assay described below. These coefficients were then used with eq. 2.7 and the integrated form of eq. 2.6 to simulate phenanthrene concentration as a function of time in the experimental system. Because of the short time interval used and the relatively low...
initial phenanthrene concentration, the value of $X$ in eq. 2.6 was assumed to be constant for simulation purposes. The values of $C_{\text{a,sat}}$, SC and CMC (required to calculate $C_{T,\text{sat}}$ in eq. 2.7) were as determined previously for Tergitol NP-10 (Grimberg et al., 1994; Grimberg et al., 1995).

**Degradation of Solid Phenanthrene**

The growth of P16 in the presence of excess (solid) phenanthrene was first studied in the absence of surfactant to verify that growth was limited by the rate of phenanthrene dissolution. A batch experimental system with a constant interfacial area of phenanthrene (Grimberg et al., 1994) was used. The batch growth apparatus consisted of an Erlenmeyer flask (500 mL) modified by welding a short cylindrical glass well (48 mm i.d. by 5 mm high) to the bottom. Approximately 2 g of crystalline phenanthrene was melted in a water bath (100 °C), then pipetted using sterile technique into the well at the bottom of an autoclaved flask, and allowed to cool slowly. After the phenanthrene had solidified, the flask was rinsed with filter sterilized (0.2 μm polycarbonate filter) methanol and autoclaved reagent water to remove any condensed phenanthrene from the glass walls and to smooth the phenanthrene surface. A total of 200 mL of growth medium was added, then P16 was inoculated to each flask. Triplicate flasks were prepared in this manner. After inoculation the flasks were placed on a constant temperature incubator (25 °C) and agitated at 150 rpm. Phenanthrene concentration and optical density at 420 nm (an indication of bacterial growth) were followed with time for each flask.

Simulated responses in this experiment were obtained by using eqs. 2.8 and 2.9, except the surfactant concentration was zero. Parameter values for the simulation are listed in Table 3.1. Equations 2.8 and 2.9 were numerically integrated using a Runge-Kutta technique.

<table>
<thead>
<tr>
<th>Table 3.1. Input parameters for simulation of <em>P. stutzeri</em> P16 growth in batch system.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biokinetic Parameters</strong></td>
</tr>
<tr>
<td>$q_{\text{max}}$ [min$^{-1}$]</td>
</tr>
<tr>
<td>$K_{a}$ [mg L$^{-1}$]</td>
</tr>
<tr>
<td>$Y$ [g g$^{-1}$]</td>
</tr>
<tr>
<td>$k_{d}$ [min$^{-1}$]</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Growth of strain P16 in the presence of Tergitol NP-10 was studied by preparing and inoculating six batch growth flasks as described above. For three of the flasks the growth medium was amended with 500 mg/L surfactant, for two others the medium was amended with 1,000 mg/L surfactant, and the remaining flask served as a control with no added surfactant. In addition to following growth (optical density), both the liquid phase phenanthrene concentration and the surfactant concentration were followed with time in each
flask. Samples were obtained by withdrawing 1 mL aliquots and quenching with formalin as described above.

In one experiment, the contents from each flask within a set of replicate flasks were pooled; the bacteria were removed by centrifugation and washed, then Monod kinetic coefficients were determined using the specific phenanthrene uptake rate assay described below. These coefficients were used with eqs. 2.8 and 2.9 to simulate phenanthrene and biomass concentrations as a function of time in the experimental system. The other parameters required in these equations were as determined previously (Stringfellow and Aitken, 1994b; Grimberg et al., 1994; Grimberg et al., 1995). All parameters used in the simulation are summarized in Table 3.2. Equations 2.8 and 2.9 were numerically integrated using a Runge-Kutta technique.

Table 3.2. Input parameters for simulation of batch growth of *P. putida* P16 in the presence of surfactants.

<table>
<thead>
<tr>
<th>Biokinetic Parameters</th>
<th>System Parameters</th>
<th>Initial Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{q}_{\text{max}} ) [min(^{-1})]</td>
<td>( \text{C}_{\text{i}, \text{sat}} ) [mg L(^{-1})]</td>
<td>1.2</td>
</tr>
<tr>
<td>( \text{K}_{\text{i}} ) [mg L(^{-1})]</td>
<td>( \text{a} ) [cm(^2) cm(^{-3})]</td>
<td>0.09</td>
</tr>
<tr>
<td>( \text{Y} ) [g g(^{-1})]</td>
<td>( \text{k}_{\text{i}, \text{s}} ) [cm min(^{-1})]</td>
<td>0.109</td>
</tr>
<tr>
<td>( \text{k}_{\text{d}} ) [min(^{-1})]</td>
<td>( \text{k}_{\text{i}, \text{m}} ) [cm min(^{-1})]</td>
<td>0.017</td>
</tr>
<tr>
<td>( \text{SC} ) [g g(^{-1})]</td>
<td>( \text{C}_{\text{surf}} ) [mg L(^{-1})]</td>
<td>0.029</td>
</tr>
<tr>
<td>( \text{CMC} ) [mg L(^{-1})]</td>
<td>( \text{X}_{\text{1}, \text{0}} ) [mg L(^{-1})]</td>
<td>2</td>
</tr>
<tr>
<td>( \text{C}_{\text{T}, \text{0}} ) [mg L(^{-1})]</td>
<td>( \text{X}_{\text{1}, \text{0}} ) [mg L(^{-1})]</td>
<td>13.3</td>
</tr>
</tbody>
</table>

**Biomass Measurement**

Biomass concentrations in growth experiments were determined by withdrawing 1 mL aliquots and measuring UV absorbance at 420 nm (\( A_{420} \)) using a double beam UV/vis scanning spectrophotometer (Hitachi Instruments, Inc.). In the absence of surfactant one absorbance unit corresponded to 500 mg/L of biomass dry weight (Stringfellow and Aitken, 1994b). Some coagulation of the biomass was observed in the presence of surfactant, 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 \( A_{420} \) prior to surfactant addition, then adding surfactant and measuring \( A_{420} \) again. Triplicate measurements were performed for each of three biomass concentrations at four different surfactant concentrations.
Specific Phenanthrene Uptake Rate (SPUR) Assay

Phenanthrene removal kinetics were determined using a spectrophotometric initial rate assay reported earlier (Stringfellow and Aitken, 1994b). Bacteria were concentrated by centrifuging at 3020 x G for 20 min, washed in TWB, centrifuged again for 20 min and resuspended in TWB. The washed cells were diluted with TWB to an absorbance of approximately 0.1 at 420 nm, and 2 mL of the cell suspension was placed in a 3 mL quartz cuvette in a spectrophotometer. The suspension was mixed using a Teflon-coated stir bar and a micromagnetic stirrer (Fisher Scientific). Ten μL of a concentrated phenanthrene solution in methanol was injected into the cuvette and the decrease in absorbance at 250 nm was measured over time for 90 seconds. Absorbance readings between 30 and 60 seconds (n = 6) were used to compute the linear decrease in absorbance by linear regression. The specific phenanthrene uptake rate at each phenanthrene concentration was calculated with the use of an extinction coefficient for phenanthrene of 6.46 x 10^4 L mole⁻¹ cm⁻¹ (Weast and Grasselli, 1989) and the biomass conversion factor of 0.5 g biomass per unit optical absorbance at 420 nm. Eq. 2.5 was fit to the SPUR data by non-linear regression (SYSTAT; Systat, Inc., Evanston, IL) to estimate values for q_max and K_s.

Analytical Techniques

Phenanthrene and Tergitol NP-10 concentrations were measured by high pressure liquid chromatography (HPLC) (Grimberg and Aitken, 1995). The HPLC system consisted of a Waters (Millipore, Marlborough, MA) 600E HPLC quaternary pump, 470 fluorescence detector and 717 autosampler, and a C18 reversed phase column (25 cm x 4.5 mm; Supelco, Inc. Bellefonte, PA). The mobile phase (1 mL/min) was 70:30 (v:v) acetonitrile in water for 0 to 6 min, increased linearly to 100 % acetonitrile over 6 to 8 min, and maintained at this composition through 18 min. Phenanthrene was analyzed at an excitation wavelength of 259 nm and emission wavelength of 370 nm (Risner and Connor, 1991), while Tergitol NP-10 was analyzed at 225 nm and 295 nm, respectively (Marcomini and Giger, 1987).

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 aromatic hydrocarbon with a melting point of 98 °C and a water solubility of 1.2 mg/L (Mackay et al., 1992). Tergitol NP-10, a nonylphenol ethoxylate, was obtained from Sigma Chemical Co. (St. Louis, MO) and used according to the manufacturer’s recommendations. The surfactant has a molecular weight of 682, a CMC of 82 mg/L and solubilization capacity (SC) for phenanthrene of 0.029 g/g (Grimberg et al., 1995). As a nonionic compound, this surfactant does not affect the pH of aqueous solutions.
4. RESULTS

Degradation of Solubilized Phenanthrene

We demonstrated in earlier work with P. stutzeri P16 that partitioning of phenanthrene between micelles and the aqueous phase inhibited phenanthrene degradation when all of the phenanthrene was solubilized by the surfactant Tergitol NP-10 (Grimberg and Aitken, 1995; see Figure 2.6). A simple equilibrium partitioning model was confirmed in the present study to simulate phenanthrene disappearance in a batch assay (Figure 4.1). The two surfactant concentrations tested were well over the CMC for Tergitol NP-10 (82 mg/L), and the initial phenanthrene concentration in the liquid phase was well over the aqueous solubility of 1.2 mg/L. Also included in Figure 4.1 is a simulation illustrating that, for the kinetic parameters used, hypothetical phenanthrene disappearance would have been substantially faster in the absence of surfactant if it were soluble at the starting concentration of 6.8 mg/L.

![Figure 4.1](image_url)

**Figure 4.1.** Biodegradation of micelle-solubilized phenanthrene at Tergitol NP-10 concentrations of 500 mg/L (●, solid line) or 1,000 mg/L (○, long dashed line). Open squares are for uninoculated control flasks containing 500 mg/L surfactant. Symbols and error bars are means and standard deviations, respectively, of triplicate samples. Lines depict model simulations based on instantaneous partitioning of phenanthrene between micelles and the aqueous phase and independently measured kinetic parameters. The short dashed line indicates hypothetical removal of phenanthrene in the absence of surfactant if it were completely soluble at an initial concentration of 6.8 mg/L. Biodegradation parameters for the culture used in this experiment were $q_{\text{max}} = 2.57 \pm 0.52$ mg phenanthrene (g biomass)$^{-1}$ min$^{-1}$ and $K_S = 0.27 \pm 0.13$ mg L$^{-1}$ ($r^2=0.992$, n=9).
Dissolution-limited Growth of *P. stutzeri* P16 in the Presence of Solid Phenanthrene

The growth of *P. stutzeri* P16 was observed to be limited by phenanthrene dissolution in a batch system containing solid phenanthrene with a known and constant surface area (Figure 4.2). P16 was inoculated in this system after the phenanthrene concentration reached equilibrium in the aqueous phase (1.2 mg/L). Shortly after inoculation there was a relatively rapid decline in the aqueous phase phenanthrene concentration, indicating that phenanthrene was being consumed faster than it could be replenished by dissolution. A linear growth rate was clearly observed as the aqueous phenanthrene concentration approached zero. Linear growth continued and aqueous phenanthrene concentrations stayed low for the remainder of the experiment. Simulations based on independently measured Monod kinetic parameters and phenanthrene dissolution kinetics accurately followed both the aqueous phase phenanthrene and biomass concentrations.

![Graph showing the growth of *P. stutzeri* P16 and aqueous phase phenanthrene concentration](image)

**Figure 4.2.** Batch growth of *P. stutzeri* P16 (●) and aqueous phase phenanthrene concentration (○) in flasks containing solid phenanthrene. Symbols and error bars represent means and standard deviations, respectively, of triplicate flasks. Lines depict model predictions based on biodegradation of aqueous phase phenanthrene only and independently measured kinetic parameters (Table 3.1).

The rate of phenanthrene dissolution in a well-mixed batch system has been shown to increase in the presence of surfactants at concentrations above the CMC (Grimberg et al., 1995). The conceptual and mathematical models described in Section 2 would predict that the rate of growth of a phenanthrene-degrading bacterium would increase proportionally with the...
dissolution rate. Results (Figures 4.3a-c) from experiments with two different surfactant concentrations indicate that the growth of strain P16 was significantly faster than the control without surfactant. In all three cases, growth rates (Figure 4.3a) eventually became linear as the liquid phase phenanthrene concentration (Figure 4.3b) was depleted to near-zero. The slopes of the linear portions of the growth curves in Figure 4.3a were very close to the slopes predicted by dissolution-limited growth for each of the surfactant concentrations and the control (not shown).

![Graph showing biomass concentration over time for different surfactant concentrations.](image)

**Figure 4.3a.** Batch growth of *P. stutzeri* P16 in the presence of Tergitol NP-10 at 500 mg/L (●) or 1,000 mg/L (△), and in the absence of surfactant (O), in flasks containing solid phenanthrene. Symbols and error bars represent means and standard deviations, respectively, of triplicate flasks for 500 mg/L surfactant concentration and duplicates for 1,000 mg/L surfactant concentration. The control with no surfactant was a single flask. All flasks were inoculated as soon as liquid was added (i.e., the initial phenanthrene concentration in all cases was zero).

The initial liquid phase phenanthrene concentration was zero at the time of inoculation in this experiment, but phenanthrene accumulated rapidly in the flasks containing surfactant (Figure 4.3b). As the culture grew in these flasks, the phenanthrene concentration steadily declined and eventually approached zero; phenanthrene in the liquid phase was depleted faster in the presence of 500 mg/L surfactant than with 1,000 mg/L surfactant. Relatively minor losses of surfactant seemed to occur in the first 40 hours of the experiment, but surfactant concentrations were essentially constant for the remainder of the experiment (Figure 4.3c).
Figure 4.3b. Liquid phase phenanthrene concentrations corresponding to growth data shown in figure 4.3a. Symbols and conditions are as described in Figure 4.3a.

Figure 4.3c. Surfactant concentrations corresponding to batch growth data in Figure 4.3a. Symbols and conditions are as in Figure 4.3a.
Except for the linear growth phase, the data shown in Figures 4.3a-c could not be simulated well using published phenanthrene degradation kinetic coefficients for *P. stutzeri* P16 (Stringfellow and Aitken, 1994b). Therefore, a second experiment was conducted in which kinetic coefficients were determined from rate measurements at the end of the experiment. Results (Figure 4.4) were closely simulated by the biodegradation model (eqs. 2.8 and 2.9).

![Graph](image)

**Figure 4.4.** Biomass (o) and liquid phase phenanthrene (●) concentrations during batch growth of *P. stutzeri* P16 in the presence of solid phenanthrene and 500 mg/L Tergitol NP-10. Phenanthrene was at saturation (13.3 mg/L) when the flasks were inoculated. Symbols and error bars represent means and standard deviations, respectively, of triplicate growth flasks. Lines depict simulations based on biokinetic parameters determined at the end of the experiment (Table 3.2).

**Discussion**

Because PAH are naturally occurring compounds and widespread in the environment (Guerin, 1978; Evans et al., 1990; Christensen and Zhang, 1993), indigenous PAH-degrading bacteria are readily found in environmental samples (Foght et al., 1990; Foght and Westlake, 1991; Aitken et al., 1996). The mere presence of PAH degraders in contaminated soils or sediments does not, however, ensure that PAH contamination can be remediated quickly or easily. In some cases it has been observed that compounds which have persisted for many years in contaminated soils are readily degraded when added exogenously to the same soils (Weissenfels et al., 1992; Erickson et al., 1993). It has been proposed that such limited bioavailability of PAH and other hydrophobic compounds can be overcome by adding surfactants to these systems (reviewed in Rouse et al., 1994). However, a number of reports
have indicated that adding surfactants can actually inhibit biodegradation in some cases. Many of these experiments have been conducted in complex systems and with a wide range of initial conditions, so that it is difficult to establish cause-and-effect relationships or to compare different studies directly.

Various explanations have been proposed for the negative effects of surfactants on biodegradation (Rouse et al., 1994; Liu et al., 1995), but few of these have been verified experimentally. Among the proposed effects is the partitioning of hydrophobic substances into surfactant micelles (Laha and Luthy, 1991; Volkering et al., 1995), with the assumption that microorganisms do not have direct access to compounds inside micelles. The dynamics of exchange of hydrophobic compounds between micelles and water has been studied extensively in the recent past (Gehlen and De Schryver, 1993; Chen et al., 1994). Rate coefficients for the exit of hydrocarbons from micelles are very high but have been observed in general to be lower than rate coefficients for their entrance (Vermunicht et al., 1991; Gehlen and De Schryver, 1993; Chen et al., 1994). This difference in mass transfer rates has been suggested as an explanation for the apparent inhibition of hydrocarbon biodegradation by surfactants (Laha and Luthy, 1991; Volkering et al., 1995).

While potential differences in entrance and exit rates for partitioning of hydrocarbons in micelles could explain decreased biodegradation rates in the presence of surfactants, models which assume rapid equilibrium between micelles and the aqueous phase lead to the same conclusion in systems containing completely solubilized hydrocarbon (no excess hydrocarbon phase). Results published earlier (Grimberg and Aitken, 1995) and those presented in Figure 4.1 illustrate that simulations based on this assumption matched experimental observations for phenanthrene biodegradation by P. stutzeri P16. In effect, partitioning of phenanthrene into micelles reduces the concentration in the aqueous phase "seen" by the bacteria at any instant in time, and effectively increases the Monod half-saturation coefficient, \( K_s \), when expressed in terms of the total liquid phase concentration (eq. 2.7).

An experimental system containing solid phenanthrene with a constant surface area was developed to study the influences of surfactants on mass transfer processes (Grimberg et al., 1995) and on biodegradation. When strain P16 was grown in the presence of solid phenanthrene and in the absence of surfactants, the phenanthrene was rapidly depleted from the aqueous phase and linear growth was observed as the aqueous phenanthrene concentration approached zero (Figure 4.2). The linear growth rate clearly indicated that growth was limited by the dissolution of solid phenanthrene. A model accounting for simultaneous dissolution and biodegradation simulated the experimental data well when published kinetic coefficients for dissolution (Grimberg et al., 1994; Grimberg et al., 1995) and phenanthrene biodegradation by P16 (Stringfellow and Aitken, 1994b) were used. Similar results have been reported for solid naphthalene biodegradation (Volkering et al., 1992; Volkering et al., 1993).

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. The rate of phenanthrene dissolution has been shown to increase in the presence of surfactants in a manner that could be simulated with a model based on mass
transfer mechanisms (Grimberg et al., 1995). Therefore, the growth of *P. stutzeri* P16 under dissolution-limited conditions also would be expected to increase with increasing surfactant concentration. Increased rates of growth were observed in the presence of the surfactant Tergitol NP-10 (Figure 4.3a), although an apparent lag in removal of phenanthrene from the liquid phase (Figure 4.3b) could not be explained with a model that used previously established Monod kinetic coefficients ($q_{max}$ and $K_s$). The apparent lag in depletion of phenanthrene from the liquid phase was not likely due to the use of the surfactant as an alternative carbon source by P16, since the surfactant concentration remained high and was essentially constant after about 40 hours of incubation (Figure 4.3c). Minor initial losses of surfactant may have been due to sorptive processes. Previous experiments also indicated that strain P16 could not use Tergitol NP-10 as a carbon source, and that Tergitol did not influence the growth of the organism on peptone (Grimberg and Aitken, 1995).

An experiment similar to that reported in Figure 4.3 was conducted in which the required phenanthrene degradation coefficients were determined at the end of the experiment. The maximum specific phenanthrene degradation rate ($q_{max}$; Table 3.2) was lower than that reported earlier for strain P16 (Stringfellow and Aitken, 1994b), suggesting that the inoculum was less active than in previous experiments with this organism. However, the model predictions in this experiment matched both the growth and phenanthrene concentration data quite well (Figure 4.4). The assumptions used in the model - that phenanthrene present in micelles or in the solid phase is not directly available for biodegradation by strain P16, and that partitioning between micelles and the aqueous phase is always at equilibrium - therefore seem to be justified by the results of this study. Equilibrium partitioning of phenanthrene between micelles and the aqueous phase has also been assumed in a model which accurately describes the effects of surfactants on rates of phenanthrene dissolution (Grimberg et al., 1995). Volkering et al. (1995) developed a model to quantify the biodegradation of naphthalene and phenanthrene in the presence of surfactants, in which it was assumed that the exit of PAH from micelles was the rate-limiting step. This model was unable to simulate microbial growth or liquid phase substrate concentrations in their experimental system.

For the microorganism and surfactant used in this study, it appears that micelles can be treated as a distinct phase with respect to the influence of surfactants on biodegradation of a hydrophobic compound. While the conceptual and quantitative models agree well with the experimental observations, this might not be the case for different combinations of hydrocarbon, surfactant and microorganism. For example, bacteria with an affinity for hydrophobic surfaces might alter mass transfer processes, which could influence the effects of surfactants. It has also been suggested that bacteria which produce biosurfactants might gain direct access to hydrocarbons within micelles or microemulsions via complex transport mechanisms (Hommel and Ratledge, 1993). Interactions between the bacterial cell and micelles were ignored in developing the model proposed in this study. Such interactions would result in higher rates of degradation of solubilized compounds than would be predicted if only the amount present in the aqueous phase were bioavailable. We did not observe significant stimulation of phenanthrene degradation by P16 when phenanthrene was completely solubilized and, in fact, observed an inhibitory effect that could be explained with a simple partitioning model (Figure 4.1; Grimberg and Aitken, 1995).
The proposed biodegradation model provides a benchmark case to describe biodegradation of hydrophobic compounds in the presence of surfactants. It accounts only for the physical effects of surfactants on partitioning of the compound between the aqueous and micellar phases, and on the dynamics of exchange between the nonaqueous, aqueous and micellar phases. More complex mechanisms involving direct interactions of microorganisms with micelles or with nonaqueous phases would require extension of the proposed model. An implication of this work is that surfactants will stimulate biodegradation processes whenever biodegradation is limited by mass transfer processes and for as long as the hydrophobic substrate is present in excess. Unless a surfactant has deleterious effects on metabolic processes within an organism, the extent of stimulation by a surfactant at a given concentration should be proportional to its effect on the mass transfer process(es). Overdosing surfactant to the point of solubilizing all of the hydrophobic substrate could, however, have a negative effect on the rate of biodegradation, particularly if the amount of the compound solubilized is well below its saturation 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.
5. Notation

\[ C_a \] aqueous phase phenanthrene concentration, M L^{-3}

\[ C_{a,sat} \] aqueous phase saturation concentration, M L^{-3}

\[ C_T \] total bulk liquid (aqueous + micellar) phenanthrene concentration, M L^{-3}

\[ C_{T,0} \] initial bulk liquid phenanthrene concentration, M L^{-3}

\[ C_{T,sat} \] bulk liquid saturation concentration, M L^{-3}

\[ C_{surf} \] surfactant concentration, M L^{-3}

\[ CMC \] critical micelle concentration, M L^{-3}

\[ k_{L,a} \] mass transfer coefficient for phenanthrene dissolution into aqueous phase, L t^{-1}

\[ k_{L,m} \] mass transfer coefficient for micelle transport away from solid/liquid interface, L t^{-1}

\[ k_{L,obs} \] observed mass transfer coefficient for phenanthrene dissolution, L t^{-1}

\[ K_S \] Monod half saturation coefficient, M L^{-3}

\[ q \] specific phenanthrene degradation rate, t^{-1}

\[ q_{max} \] maximum specific phenanthrene degradation rate, t^{-1}

\[ r_{bio} \] rate of phenanthrene biodegradation, M L^{-3} t^{-1}

\[ r_{diss} \] rate of phenanthrene dissolution into water, M L^{-3} t^{-1}

\[ SC \] solubilization capacity, M M^{-1} (phenanthrene/surfactant)

\[ X \] biomass concentration, M L^{-3}

\[ X_0 \] initial biomass concentration, M L^{-3}

\[ Y \] yield coefficient, M M^{-1} (biomass/phenanthrene)
6. References


