



Surfactant-enhanced bioremediation: a review of the effects of surfactants on the bioavailability of hydrophobic organic chemicals in soils

Authors: Ryan N. Jordan and Alfred B. Cunningham

This is a postprint of a book chapter that originally appeared in Bioavailability of Organic Xenobiotics in the Environment in 1999. DOI: [10.1007/978-94-015-9235-2_24](https://doi.org/10.1007/978-94-015-9235-2_24)

Jordan, R.N., and A.B. Cunningham, "Surfactant-enhanced Bioremediation: A Review of the Effects of Surfactants on the Bioavailability of Hydrophobic Organic Chemicals in Soils," In Ph. Baveye et al. (eds.), Bioavailability of Organic Xenobiotics in the Environment, Kluwer Academic Publishers, the Netherlands, pp. 463-496 (1999).

SURFACTANT-ENHANCED BIOREMEDIATION:

A Review of the Effects of Surfactants on the Bioavailability of Hydrophobic Organic Chemicals in Soils

Ryan N. Jordan

Alfred B. Cunningham

Introduction

Hydrophobic organic chemicals (HOCs) often exhibit limited bioavailability to microorganisms and can persist in the subsurface for long periods of time. The use of surfactants has been proposed to enhance the effectiveness of both in-situ bioremediation and ex-situ slurry-reactor bioremediation by increasing HOC bioavailability. However, the fate of HOCs in response to surfactant addition at both the laboratory and field scale is difficult to predict; in some cases, surfactants sometimes even inhibit HOC biodegradation. The objective of this review is to identify factors that influence the effectiveness of surfactant-enhanced bioremediation (SEB) of soils contaminated with HOCs.

We propose that the effectiveness of SEB can be interpreted based on determination of an SEB effectiveness factor, η_{fs} , defined as the ratio of the characteristic time for HOC biodegradation in the absence of surfactants to the characteristic time for HOC biodegradation in the presence of surfactants. Observed effectiveness factors were calculated from literature reports of SEB in soil systems to identify trends in η_{fs} as a function of total surfactant concentration in the system (S_T). We observed that in approximately 2/3 of the data, the presence of surfactants at concentrations both below and above their critical micelle concentrations (CMCs) resulted in η_{fs} values greater than 1, indicating that surfactant amendment enhanced HOC biodegradation in these cases. In addition, the data showed that supra-CMC surfactant doses were inhibitory in a higher percentage of cases than when surfactants were applied below their CMC. Still, no distinct correlation between η_{fs} and S_T was observed, emphasizing the need to (a) identify the fundamental mechanisms by which surfactants influence bioavailability in soil-water systems, and (b) incorporate key processes into a predictive model for η_{fs} .

Mechanisms by which surfactants influence HOC bioavailability in soil water systems are reviewed herein. These mechanisms can be classified in one of three categories: (1) mechanisms influencing HOC soil-water partitioning equilibria, (2) mechanisms influencing HOC mass transfer (desorption) rates, and (3) mechanisms influencing microbial processes involved in HOC uptake and degradation. A critical review of SEB in soil-water systems is then undertaken, followed by an interpretation of observed values of η_{fs} from literature data.

Bioavailability, in a general sense, is defined as the accessibility of a substrate to a microbe or enzyme that facilitates the transformation of that substrate. When the substrate of concern is a hydrophobic organic chemical (HOC), bioavailability can be limited by low aqueous concentration (or solubility) of HOC, slow mass transfer of HOC from nonaqueous (e.g., soil or NAPL) to aqueous phases, or slow uptake of HOC across the cell membrane. (However, one must keep in mind that limited bioavailability of HOC does not imply its recalcitrance [1]).

The bioavailability of HOCs is often thought to be limited by their low aqueous solubility (i.e., assuming that HOC can only be degraded in the aqueous phase). This can be explained by noting that intrinsic microbial kinetics are often best described by models that incorporate the dependence of substrate concentration on the rate of biodegradation (e.g., Michaelis–Menton or first-order kinetics). Thus, biodegradation rates are faster when substrate concentrations are higher (up to a point where the substrate concentration may either inhibit microbial activity via toxicity or degradation enzyme saturation). Partitioning of an HOC into nonaqueous phases (NAPLs, soil particles, etc.) may decrease the aqueous phase concentration of the HOC to values well below its solubility limit, further limiting its bioavailability and resulting in slow biodegradation rates.

However, bioavailability is not necessarily limited by solubility alone. In multiphase systems, where HOC may partition among aqueous and nonaqueous compartments, biodegradation of HOC in the aqueous phase may promote dynamic partitioning of HOC (mass transfer from a nonaqueous to the aqueous phase in response to disequilibrium). Partitioning processes take time, and if characteristic times for mass transfer of HOC to the aqueous phase are slower than characteristic times for HOC biodegradation, then mass transfer phenomena may limit bioavailability.

Finally, bioavailability also accounts for limitations of HOC uptake across the cell membrane. Cells may interact differently with dissolved (free aqueous), solubilized (e.g., in amphiphilic macromolecular aggregates such as surfactant micelles), or nonaqueous (NAPL or crystalline) HOC when in direct contact with these phases, yet still be able to facilitate uptake by direct mechanisms. Consequently, intrinsic rates of uptake and degradation of HOC may vary as a function of the HOC phase and a microorganism's ability to interact with HOC in a nonaqueous phase.

Thus, bioavailability should be viewed as being governed by the concentration of HOC available to the degrading population (typically, that concentration in the aqueous phase), the mass transfer of HOC to the degrading population from nonavailable phases, and uptake mechanisms during the direct contact of cells with various HOC phases. Surfactants can have profound effects on any of these three mechanisms, and these effects are the focus of this review.

2. Interpretation of SEB Using an Effectiveness Factor Approach

2.1. AN EFFECTIVENESS FACTOR FOR EVALUATING SURFACTANT-ENHANCED BIOREMEDIATION

The family of technologies by which surfactants are employed to increase the bioavailability of contaminants in the environment is called surfactant-enhanced bioremediation (SEB). Surfactants have the potential to either enhance or inhibit observed rates of biodegradation [2,3]. To evaluate the performance of SEB in a contaminated soil, it is useful to define an effectiveness factor, η_{is} , that describes the degree of influence of the surfactant relative to a surfactant-free control:

$$\eta_{fs}(S_T) = \frac{t^*(S_T = 0)}{t^*(S_T)} \quad (1)$$

where $t^*(S_T = 0)$ is the characteristic time for HOC biodegradation in the *absence* of surfactant treatment [time], $t^*(S_T)$ is the characteristic time for HOC biodegradation in the *presence* of surfactants [time], and S_T is the total concentration of surfactant in the system.

HOC degradation can sometimes be approximated by first order kinetics,

$$\frac{dC_{aq}}{dt} = -k_{soil} C_{aq} \quad (2)$$

where k_{soil} is the first order rate coefficient describing degradation of aqueous phase HOC in the presence of a soil phase [time^{-1}], and C_{aq} is the aqueous phase HOC concentration [mg L^{-1}]. Assuming first-order kinetics are a valid approximation, it is useful to note that the characteristic time for biodegradation can be defined as [4]

$$t^* = \frac{1}{k_{soil}} \quad (3).$$

Consequently, Equation 1 can be rewritten as:

$$\eta_{fs}(S_T) = \frac{k_{soil}(S_T)}{k_{soil}(S_T = 0)} \quad (4)$$

where $k_{soil}(S_T)$ is the rate coefficient observed for biodegradation in the *presence* of surfactant treatment [time^{-1}], and $k_{soil}(S_T = 0)$ is the rate coefficient observed for biodegradation in the *absence* of surfactant treatment [time^{-1}]. It is apparent from Equation 4 that for values of $\eta_{fs} < 1$, the presence of surfactants inhibits bioavailability, while for values of $\eta_{fs} > 1$, the presence of surfactants enhances bioavailability.

We will first discuss some of the fundamental mechanisms important in SEB, and explain how surfactants influence key bioavailability processes. We will then describe the relative effectiveness of surfactants from a variety of SEB studies in our survey of recent literature. Finally, we will interpret our findings based on the relationship between η_{fs} values observed in literature (based on Equation 1) and key processes that may be influenced by surfactant addition.

2.2. INFLUENCE OF SURFACTANTS ON KEY BIOAVAILABILITY PROCESSES

Identification of the surfactant-impacted processes that influence HOC bioavailability is required to formulate a successful model for predicting $\eta_{fs}(S_T)$. Although this review will not define an explicit function for $\eta_{fs}(S_T)$ in terms of key system parameters, it does identify key processes that may eventually be incorporated into a predictive bioavailability model based on $\eta_{fs}(S_T)$. The mathematical formulation of this model will be the subject of a future paper.

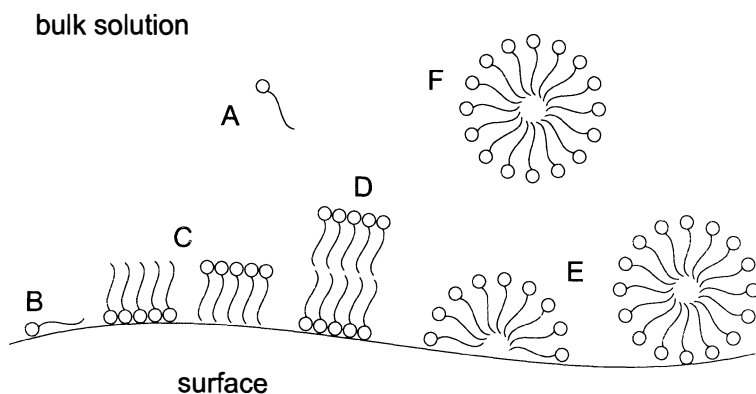


Figure 1. Surfactant structures at solid–liquid interfaces. Monomers may be found in solution (A) or adsorbed at the interface (B). Increasing surfactant concentration results in monomer aggregation into hemimicelles (C), admicelles or bilayers (D), half–cylinders or hemispheres (E, left), and cylinders or spheres (E, right). Micelle formation in solution consists may consist of spheres, cylinders, or planar bilayer sheets (F).

In modeling bioavailability, a number of researchers [4–9] have identified the processes of HOC partitioning and soil–water mass transfer as potential limiting processes in HOC biodegradation. Surfactants can influence HOC bioavailability by altering both HOC partitioning equilibria and interphase mass transfer rates. In addition, whether or not the micellar phase of HOC is directly bioavailable is a key issue in characterizing bioavailability [10]. Finally, the interaction of surfactants with cell membranes and membrane–bound proteins may influence intrinsic rates of HOC uptake and degradation [11].

2.2.1. Physico–Chemical Influences

Surfactant Sorption to Soil. One of the fundamental properties of surfactants is their association at the solid–aqueous interface [12], resulting in the formation of interfacial aggregates (Figure 1) having a variety of structures.

The orientation of surfactants at an interface depends on (1) the charge at the interface, (2) the charge of the hydrophilic moiety of the surfactant, and (3) for uncharged interfaces, the relative hydrophobicities of the surfactant’s hydrophobic tail and the surface [13,14]. Figure 2 shows the various orientations of surfactant micelles at a soil–water interface as a function of surface chemical characteristics.

Figure 2A illustrates the influence of the type of adsorbed surfactant structure on surface characteristics. Organization of adsorbed micellar structures on a surface progresses from a hemimicellar structure (Figure 2A, left) at low surfactant concentrations (thus making the surface more hydrophobic) to an admicellar structure (Figure 2A, center) at higher surfactant concentrations (possibly altering the surface

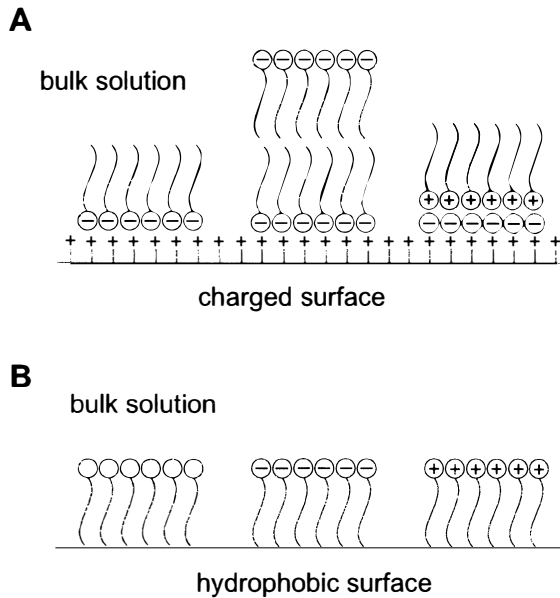


Figure 2. Examples of surfactant organization soil–water interfaces. (A) Surfactant micelle formation may be governed by the charge of the hydrophilic head of the surfactant. The presence of counterions (right) may result in adsorption of surfactant that has a charge similar to the surface. (B) Reversal of surface hydrophobicity by surfactant adsorption.

charge or polarity). This progression with increasing surfactant concentration can result in a reversal in surface charge or hydrophobicity. For example, consider the case illustrated in Figure 2A for the adsorption of an anionic surfactant on a surface containing positively charged functional groups (e.g., a mineral oxide at a pH above its isoelectric point). Surface charge progresses from positive (for $S_T = 0$) to uncharged (for S_T at a concentration that favors hemimicellar formation) to negative (for S_T at a concentration that favors admicellar formation). This example also illustrates a reversal in surface hydrophobicity from the case where no surfactant is present (hydrophilic surface) to a surfactant concentration that favors hemimicellar formation (hydrophobic surface). Alternatively, surfactant addition may change the hydrophobic surface to a charged, hydrophilic surface (Figure 2B) via aggregation of hemimicelle, hemispherical, or half-cylindrical structures orienting at the surface with their hydrophilic groups pointed towards the bulk solution.

The ability of surfactants to modify surface chemical features may have a profound impact on bioavailability. Surfactants may alter (1) the surface charge or hydrophobicity that governs the degree of HOC partitioning, (2) the surface free energy that influences the kinetics of the adsorption reaction between an HOC and a soil surface, and (3) the association of microbial cell attachment to the surface.

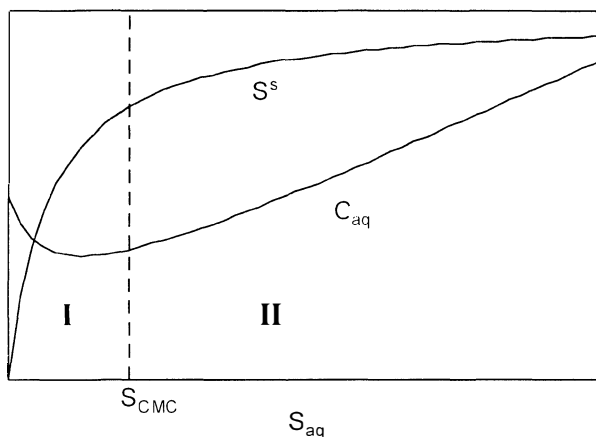


Figure 3. Solubilization plot showing the reduction in the apparent HOC solubility (C_{aq}^s) resulting from sorption of surfactant onto soil (Region I), and the increase in HOC solubility resulting from surfactant micelle formation in the aqueous phase (Region II). S_s = sorbed surfactant concentration; S_{aq} = aqueous surfactant concentration; S_{CMC} = surfactant critical micelle concentration in the presence of a soil phase.

Surfactant sorption to soil is a well-known [15,16], but little understood process. Soils harbor surfaces that are both charged and hydrophilic (e.g., mineral oxides), as well as surfaces that have a mixture of nonpolar and polar functionalities (e.g., natural organic matter). Because of the heterogeneous distribution of these surfaces in soil, surfactant–soil sorption is a complex process that makes interpretation of isotherms extremely difficult.

However, one effect of surfactants at sub-CMC concentrations is their ability to decrease solubility of HOCs, a result of the formation of sorbed micellar structures that promote partitioning of HOC out of the aqueous phase. At higher surfactant concentrations, the formation of surfactant micelles in solution competes for HOC partitioning with immobile phases (i.e., mineral oxides, natural organic matter, and sorbed surfactant), resulting in a corresponding increase in HOC solubility above the surfactant's critical micelle concentration in the bulk liquid. Figure 3 illustrates the relationship between the surfactant sorption isotherm and HOC apparent solubility in a *solubilization plot*.

It is generally believed that surfactant monomers do not interact with HOC [17], except for extremely hydrophobic chemicals [18]. Thus, in the following discussion, we will assume that surfactant speciation in response to increasing surfactant concentration progresses as shown in Figure 4. Below a surfactant's CMC in the presence of soil, the primary structure that influences HOC partitioning is the sorbed micellar aggregate, while above the CMC, the concentration of sorbed micelles remains constant and competes for HOC partitioning with an aqueous micellar phase.

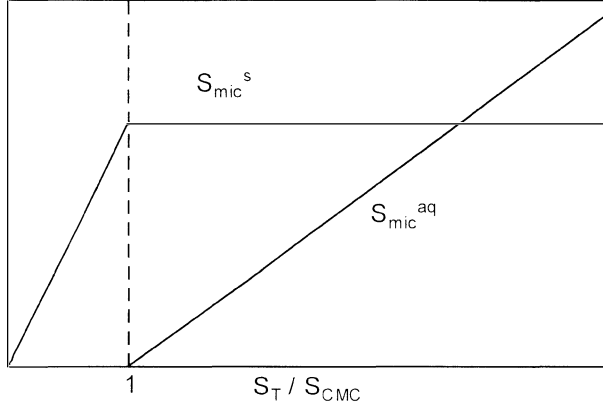


Figure 4. Model for surfactant partitioning in a soil–water system. S_{mic}^s represents the concentration of surfactant in the sorbed, micellar phase, while S_{mic}^{aq} represents the aqueous micellar surfactant concentration. S_{CMC} is the concentration of surfactant in the presence of soil at which micelles form in solution, and also indicates the total surfactant concentration at which the surface's sorption capacity for surfactant is reached. Distribution of surfactant monomers has been ignored in this model.

HOC' Soil–Water Partitioning. Surfactant addition results in a redistribution of HOC from the soil and water phases to the soil, water, and surfactant (sorbed and mobile) phases (Figure 5).

If it is assumed that partitioning isotherms that govern the ratio of HOC between nonaqueous and aqueous phases are linear, then HOC speciation among phases can be described by a series of linear partitioning coefficients:

$$K_{p,s} = \frac{C_s}{C_{aq}} \quad (5)$$

$$K_{p,mic}^s = \frac{C_{mic}^s}{C_{aq}} \quad (6)$$

$$K_{p,mic}^{aq} = \frac{C_{mic}^{aq}}{C_{aq}} \quad (7)$$

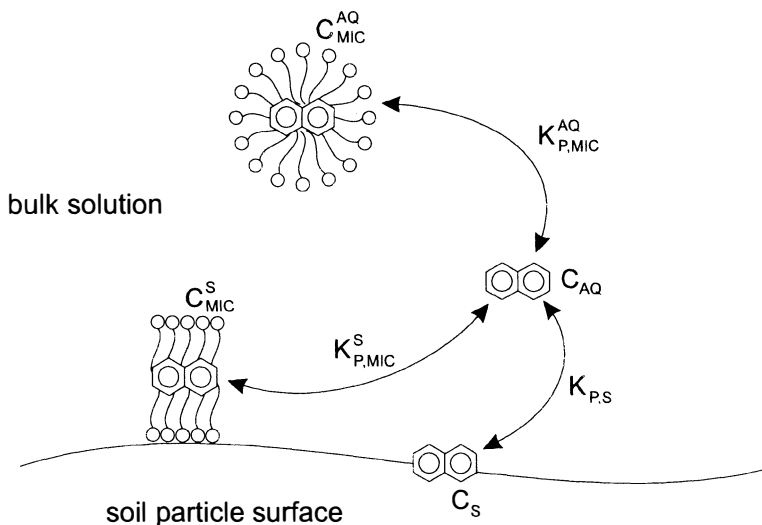


Figure 5. HOC Equilibria in a soil–water system containing surfactants. The influence of surfactant monomers is neglected for illustrative clarity. C represents the concentration of HOC in the indicated phase, and K_p indicates linear partitioning coefficients between the nonaqueous and aqueous phases.

where C_s is the sorbed HOC concentration [mg HOC kg^{-1} soil], C_{mic}^s is the HOC concentration in sorbed micelles [mg HOC mg^{-1} surfactant in the sorbed micellar pseudophase], C_{mic}^{aq} is the HOC concentration in aqueous phase micelles [mg HOC mg^{-1} surfactant in the aqueous micellar pseudophase], and K_p 's are linear distribution coefficients for HOC partitioning between the aqueous phase and the sorbed [L kg^{-1}], sorbed micellar [L mg^{-1}], and aqueous micellar phases [L mg^{-1}], respectively (Equations 5-7). Surfactant addition has two primary effects on the redistribution of HOC:

- C_s , concentration of HOC adsorbed to soil, decreases as previously sorbed HOC redistributes itself among sorbed and mobile surfactant micelles, and
- C_{aq} , the dissolved HOC concentration, decreases in response to maintaining equilibrium by additional nonaqueous (surfactant) phases.

The effect of surfactant addition on HOC distribution among the four compartments is illustrated in Figure 6.

Figure 6 emphasizes that in the presence of sub-CMC surfactant concentrations, the aqueous and sorbed phase HOC concentrations may decrease significantly as HOC partitions into a sorbed surfactant phase (Region II). As aqueous phase micelles form, HOC partitioning between sorbed and aqueous phase surfactant is in competition (Region III).

Surfactant alteration of HOC distribution can have significant effects on HOC bioavailability. Since surfactants promote desorption of HOC (resulting in a decrease in C_s), and HOC in the aqueous and micellar phase is assumed to be more bioavailable

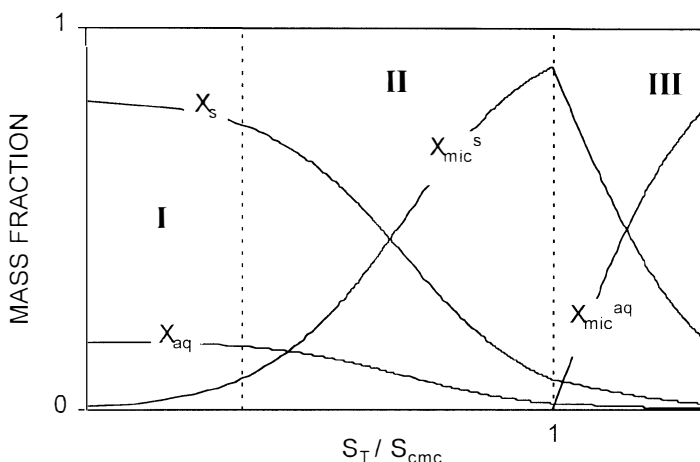


Figure 6. Influence of total surfactant concentration relative to the CMC (S_T/S_{CMC}) on the equilibrium partitioning of HOC in a soil–water system (in terms of the fraction of total HOC mass in the system present in the aqueous (X_{aq}), sorbed (X_s), sorbed surfactant–associated (X_{mic}^s), and aqueous surfactant–associated (X_{mic}^{aq}) HOC phases. Surfactant sorption is modeled by the formulation in Figure 4; HOC partitioning between aqueous and nonaqueous phases is modeled via linear isotherms as described in Figure 5 and Equations 5–7. The X axis is a log scale.

than HOC in the sorbed phase, then surfactant addition should result in an increase in HOC bioavailability. However, if bioavailability of micellar–phase HOC is limited, then the reduction of aqueous phase HOC (C_{aq}) may result in a decrease in HOC bioavailability in response to surfactant addition (particularly if the kinetics of HOC mass transfer from the micellar to the aqueous phase are slower than the kinetics of the biotransformation process). Bioavailability of micellar–phase HOC will be addressed in more detail in section 2.2.2.

Soil–Water Mass Transfer. Slow desorption has been identified as a process that influences the bioavailability of HOCs in soil–water systems [1,2,19]. HOC entrapped in soil organic matter within intraparticle porosity can result in characteristic times of biodegradation on the order of years or decades. HOC mass transfer out of a soil particle can be characterized by a dual–process reaction–transport model (Figure 7).

HOC sequestered in soil organic matter must first desorb and enter the aqueous solution occupying the nanoporosity. This process can be modeled by using first order kinetics, described by a rate coefficient, k . Following desorption and entry into the nanoporosity, the HOC must diffuse out of the particle into the bulk solution. This process is governed by the diffusion coefficient, D , of the HOC molecule. Because diffusion and adsorption/desorption will likely occur throughout the nanopore, the desorption flux of HOC from a particle can be described as a retarded diffusion process [21]. In addition to diffusion through intraparticle pores, HOC may also diffuse in nonaqueous soil phases, such as soil organic matter or mineral matrices [19].

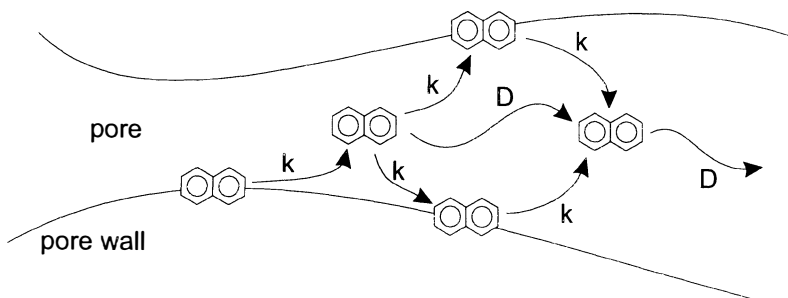


Figure 7. Conceptual model for retarded diffusion of HOC within a representative pore of a soil particle. Sorption/desorption is approximated by two key processes: (1) the sorption reaction between the HOC and the chemically reactive phases present on the pore wall surfaces, and (2) the diffusive transport of HOC through the pore [7,20]. The coefficients k and D represent the first-order reaction coefficients describing sorption-desorption kinetics and the diffusion coefficient, respectively.

The heterogeneous nature of the distribution of surface chemical features and of the physical shapes and sizes of nanopores within a single particle makes it impractical, if not impossible, to characterize HOC desorption in terms of adsorption-desorption kinetic coefficients and diffusion parameters. Thus, a simple approach that has been successful in describing HOC desorption rates [4,22] is the first-order mass transfer model:

$$\frac{dC_s}{dt} = -k_{m,s}(C_{\text{pore}} - C_{\text{aq}}) \quad (8)$$

where dC_s/dt is the HOC flux between the soil and bulk aqueous phases [$\text{mg L}^{-1} \text{ time}^{-1}$], C_s is the sorbed HOC concentration that includes (a) HOC that is truly adsorbed to natural organic matter or mineral surfaces distributed throughout the soil particle as well as (b) HOC dissolved in the aqueous phase occupying intraparticle porosity [mg kg^{-1}], C_{pore} is the average HOC concentration in the aqueous phase occupying the intraparticle porosity [mg L^{-1}], and $k_{m,s}$ is the first order mass transfer coefficient that governs the rate at which HOC in the aqueous phase approaches equilibrium with sorbed HOC [time^{-1}]. This model incorporates the intrinsic kinetics of the sorption-desorption and intraparticle diffusion processes (defined by k and D in Figure 7) into the mass transfer coefficient, $k_{m,s}$. Thus, the HOC flux out of the particle is also influenced by the concentration gradient between the pore water HOC and the bulk aqueous HOC concentrations ($C_{\text{pore}} - C_{\text{aq}}$).

This model illustrates that as the gradient between the soil and bulk aqueous phases increases (i.e., in response to a change in C_{pore} or C_{aq} from the equilibrium condition defined by $K_{p,s}$), HOC flux in response to disequilibrium increases. At equilibrium, $C_{\text{aq}} = C_{\text{pore}}$ and HOC flux between the two phases is zero. Thus, any process that decreases the bulk aqueous phase HOC concentration (C_{aq}) over a time scale that is

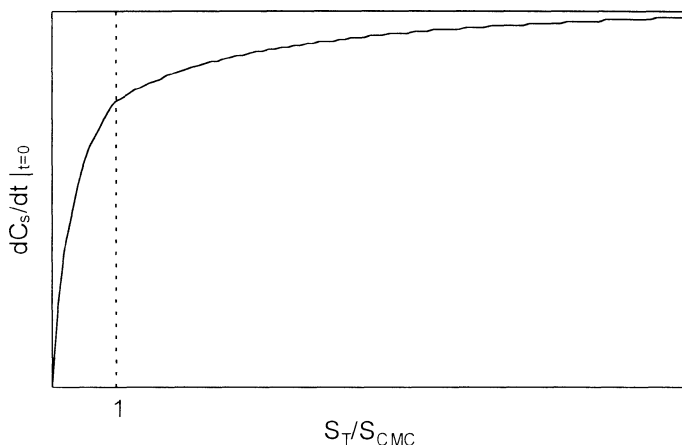


Figure 8. Influence of surfactant concentration relative to the CMC (S_T/S_{CMC}) on the initial flux ($dC_s/dt|_{t=0}$) of HOC from the soil phase (C_s) to the bulk aqueous phase (C_{aq}) at $t = 0^+$ following surfactant addition. It is assumed that the system is at equilibrium at $t = 0^-$ (immediately prior to surfactant addition) and that HOC–surfactant and surfactant–soil partitioning equilibrium exists immediately following surfactant addition. Thus, the flux represents the response to disequilibrium resulting from the concentration gradient between the sorbed and aqueous phase HOC as defined in Equation 8. Surfactant sorption is modeled by the formulation in Figure 4; HOC partitioning between aqueous and nonaqueous phases is modeled via linear isotherms as described in Figure 5 and Equations 5–7.

faster than the characteristic time for desorption (e.g., biodegradation of aqueous phase HOC or surfactant addition) will promote desorption of HOC. This concept is illustrated in Figure 8, which shows the influence of surfactant addition at various concentrations on the initial rate of HOC flux out of the soil phase.

Of particular interest from Figure 8 is the effect of sub-CMC surfactant concentrations on the mass transfer rate. The sorption of surfactant to soil surfaces at low surfactant concentrations results in a dramatic decrease in the aqueous phase HOC concentration (as indicated by the X_{aq} line of Figure 6). Thus, low surfactant concentrations may have a profound impact on bioavailability (cf. Figure 12), although it is traditionally thought that the greatest benefit of surfactants to enhancing bioavailability is achieved at supra-CMC concentrations that promote HOC solubilization. Increased mass transfer rates, as implied in Figure 8, have been implicated as being responsible for bioavailability enhancement at low surfactant concentrations [23–25].

Thus, surfactant addition can alter HOC desorption rates by decreasing the bulk aqueous phase HOC concentration (C_{aq}), steepening the concentration gradient for mass transfer. Surfactants may also have an effect on the nature and magnitude of $k_{m,s}$ by influencing (1) HOC diffusion within the intraparticle porosity, (2) HOC concentration

gradients that drive HOC adsorption–desorption reactions on pore wall surfaces, and (3) the intrinsic nature of the sorption–desorption reaction. These processes are described in more detail:

a. HOC intraparticle diffusion. In addition to dissolved aqueous phase HOC diffusing through nanopores, surfactant micelles containing HOC may also be diffusing. The formation of aqueous micelles within a nanopore will depend on the size of the pore relative to the micellar radius. However, there is little information about micelle–assisted or –retarded diffusion of solutes in nanopores.

It would be a fair generalization to say that the presence of micellar phase HOC diffusing in a nanopore would increase HOC flux out of the particle if the micelle diffused at a faster rate than aqueous phase HOC. The relative fluxes of solubilized (micellar phase) HOC vs. dissolved HOC would depend on the relative degree of association of each phase with sorption sites (retarded diffusion) as well as the relative rates of diffusion of each of those phases. Micelles are likely to be more mobile than aqueous phase HOC, suggesting that they may diffuse faster. However, their large molecular weights also may result in relatively slow aqueous diffusion rates.

In a pore that is physically inaccessible to surfactant micelles, but accessible to surfactant monomers, HOC partitioning and transport would rely only on the presence of a monomer phase, with the potential for formation of adsorbed micellar structures on the pore walls. The presence of adsorbed micelles may retard HOC diffusion. However, they may also increase the kinetics of HOC desorption from pore wall surfaces.

b. HOC concentration gradients driving desorption. Reduction of C_{pore} (by partitioning into surfactant present in the nanopores as discussed above) promotes the desorption reaction by increasing the concentration gradient at the surface–liquid boundary layer. The net effect would be an increase in the flux of HOC into the pore water, and possibly, out of the particle.

c. Intrinsic sorption–desorption properties. The value of a kinetic coefficient (e.g., first order) that describes the intrinsic nature of a sorption–desorption reaction may be governed by the nature of the sorbent (soil surface), the sorbate (HOC), and solvent composition (pore water). Surfactants are well known for their ability to alter the chemistry of a surface, e.g., by promoting charge reversal or altering hydrophobicity [12]. In addition, this alteration in surface chemistry often corresponds to a decrease in the surface energy. Thus, it would be expected that the intrinsic nature of the sorption reaction (i.e., binding energy, reaction kinetics, and partitioning equilibria) may indeed be changed in the presence of surfactants.

Summary. This discussion emphasizes the complexity involved, even within a single intraparticle pore, in interpreting the influence of surfactants on HOC partitioning and mass transfer phenomena. However, it does not prevent us from recognizing that surfactant concentration may indeed have a predictable influence on the value of the mass transfer coefficient, $k_{m,s}$, that describes HOC desorption rates. Further, the mass transfer model is a valuable tool for evaluating complex diffusion–reaction scenarios in a system that is difficult to characterize at the microscale.

2.2.2. Microbiological Influences

Knowledge of the physico–chemical reactions between surfactants, soil, and HOC in a soil system allows one to reasonably design an SEB technology (i.e., appropriately control the surfactant dose) to maximize HOC bioavailability (i.e., based on principles of partitioning behavior, mass transfer, and the assumption that only aqueous phase HOC is directly bioavailable). More complicated and more difficult to predict, however, are the microbiological processes that are influenced by surfactants, including cell–

interface interactions, interactions between surfactants and cell membranes, and micellar-phase bioavailability, all of which may have an important bearing on rates of HOC uptake.

By definition, surfactants are compounds that associate at interfaces. In soil systems, important interfaces are the soil–water interface and the cell–water interface (i.e., the exterior of the cell membrane). The chemical nature (charge and hydrophobicity) of the soil, the cell, and either end of the surfactant may have a significant impact on the adhesion of bacteria to soil surfaces. Since bacterial adhesion to nonaqueous substrates may be required for efficient metabolism of HOC [26–32], then the influence of surfactants on cell adhesion may significantly alter bioavailability. Further, surfactants may have either a positive (enhanced adhesion to the interface) or negative (de-adhesion) effect on cell adhesion [33].

Rouse *et al.* [3], in their review of the effects of surfactants on biodegradation, investigated the effects of surfactant concentration, surfactant charge, and micro-organism genus on HOC biodegradation. They noted that no single underlying factor could predict the influence of surfactants on bioavailability. However, they recognized that for systems containing mixed bacterial cultures, supra-CMC surfactant concentrations were often associated with inhibition. They implicated micelle toxicity and limited bioavailability of micellar-phase HOC as potential reasons for this correlation.

Influence of Surfactants on the Interaction of Bacteria with Interfaces. Cell adhesion to interfaces is a complex phenomenon governed by a wide range of mechanisms. Foremost among these mechanisms are the deposition of amphiphilic molecules at interfaces [12,33] that may be of either microbial or anthropogenic origin.

Recall from Figure 2 that the chemical nature of surfactants and surface functional groups governs the type and orientation of micellar structures at the soil–water interface. Further, surfactant addition can result in alteration of surface properties such as surface charge or hydrophobicity. This is an important result because the interaction of cells with surface functionalities is an important factor influencing cell adhesion [34,35]. Further, cell adhesion may be an important process governing the ability of a cell to facilitate the uptake of nonaqueous phase HOC [26–32].

For example, cell hydrophobicity may be an important factor to consider when attempting to predict the influence of surfactants on cell adhesion. Figure 9A illustrates how cell surface hydrophobicity may influence a cell's ability to adhere to surfaces coated with a variety of surfactant types. Figure 9B shows the influence of surfactant coatings on the adhesion of hydrophilic (negatively charged) cells. Figure 9C illustrates similar reactions when the surfactant adheres to the cell and alters cell surface properties.

Although Figure 9 illustrates a few generalities based on the relative hydrophobicity or charge between a surface, an adsorbed surfactant layer, and a cell wall, the reader should keep in mind that cell adhesion is a complex phenomenon that may be governed by mechanisms other than hydrophobic or electrostatic interactions (e.g., specific adhesin–receptor interactions [36]). However, the more simply characterized electrostatic and hydrophobic interactions have been implicated in the ability of cells to adhere to nonaqueous phase substrates [29,37–39]. Thus, surfactant addition may influence surface or cell hydrophobicity, alter cell adhesion (and thus, the proximity of the cell to the substrate), and effect bioavailability.

A recent review by Neu [33] discusses in detail the interactions between microbial cells, surfaces, and surface-active agents. Although the focus of this work is on the influence of biosurfactants on cell adhesion, the basic concepts are applicable for any surface-active molecule, and we highly recommend this review for further information on surfactant–cell–surface interactions.

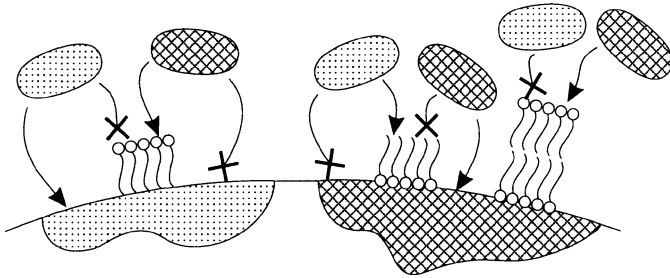
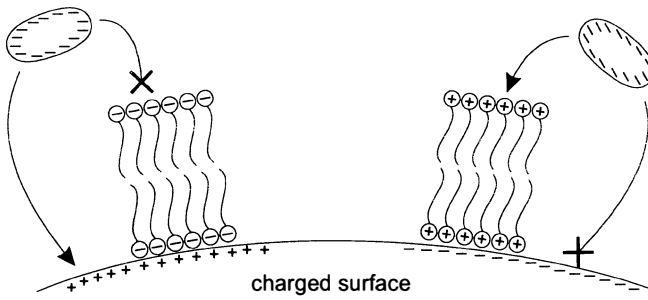
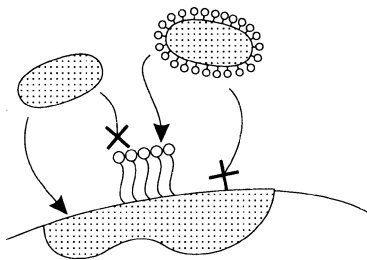
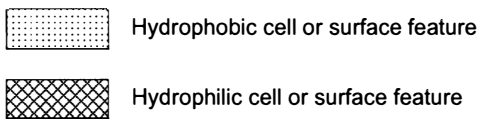
A**B****C**

Figure 9. Influence of surfactants on cell adhesion (adapted from Neu [33]). See text for discussion.

Influence of Surfactants on HOC Uptake and Degradation. The mechanisms that govern the uptake of HOC across the cell membrane are not well understood. It is often hypothesized that HOC must be dissolved in the aqueous phase in order to be transported into the cell membrane. However, other researchers have suggested that hydrocarbons emulsified in the droplets of oil-in-water emulsions are transported into the cell membrane [31,40]. Recent work has focused on the potential for a direct interaction between the cell membrane and HOC solubilized in a surfactant micelle [10,41–43]. This research suggests that direct bioavailability of micellar phase HOC depends on the compatibility between the surfactant micelle and the cell membrane, a phenomenon that is governed by the structure of the surfactant and the species- or genus-specific nature of the cell membrane [43–48].

Jaffe and coworkers modeled the influence of surfactant structure on the degree of cell-micelle interaction and bioavailability of solubilized HOC [41]. However, reports describing the influence of species- or genus-specific cell membrane properties that govern the relationship between cell-surfactant compatibility and bioavailability of micellar phase HOC are virtually nonexistent. Much of the research involving surfactant-enhanced biodegradation of environmentally significant contaminants involves the use of undefined, uncharacterized microbial populations. Thus, the influence of surfactant-cell membrane compatibility on bioavailability cannot usually be explained, although it has often been implicated as both inhibiting [44,46] and enhancing [43–45,47,48] bioavailability. In fact, membrane-surfactant interactions may be responsible for the apparent toxicity of surfactants at supra-CMC concentrations [24,29,49–53]; it is well-known that surfactants are capable of fluidizing the cell membrane, resulting in solubilization of key membrane proteins responsible for HOC uptake and degradation [11]. Conversely, sub-CMC surfactant concentrations, promoting an increase in membrane permeability by surfactant monomers, may result in enhanced uptake of HOC [47,48].

In summary, important microbiological processes involved in HOC uptake in the presence of surfactants are (1) bioavailability of micellar phase HOC and (2) compatibility between cell membranes and surfactants. Maintaining surfactant-cell membrane compatibility is important for the following reasons [3]:

- Some surfactants may disrupt cell membranes. A moderate degree of permeabilization of the cell membrane may result in enhanced diffusion of HOC into the cell; however, more severe permeabilization may result in lysis and complete inhibition of cell function.
- Surfactants have different effects on gram positive cells than on gram negative cells; consequently, a different set of surfactant-cell compatibility requirements must be met depending on the nature of the cell wall structure of the degrading organism(s), requiring different types of surfactants.
- Surfactants may form complexes with membrane-bound proteins responsible for HOC transport or degradation; alternatively, surfactants micelles in solution could sequester extracellular enzymes responsible for HOC degradation or microbial surfactants responsible for HOC uptake, inhibiting their activity and reducing bioavailability of HOC.

There exists evidence in the literature that surfactant-cell compatibility cannot be ignored. For example, Tween 80, a commercial nonionic surfactant, exhibited an inhibitory response to the uptake of PAHs by an uncharacterized microbial population when the surfactant was present above its CMC [52]. In another study, the same surfactant enhanced PAH biodegradation by a pure culture up to a concentration of 2 g/L (150 times the CMC) without exhibiting a toxic response [54].

Rouse *et al.* [3] attempted to correlate surfactant type, microbial genus, and gram stain reaction with the degree of bioavailability inhibition or enhancement. They concluded that in general, surfactants above their CMCs resulted in inhibition of HOC biodegradation in mixed cultures, suggesting that toxicity of surfactant micelles may hinder HOC bioavailability. However, there is enough recent research that shows enhanced bioavailability at supra-CMC concentrations with mixed cultures in soil–water systems (cf. Figure 12) that may render this generalization inappropriate in soil environments.

The lack of conclusive evidence identifying the influence of surfactant–micelle compatibility places serious limitations on the ability to design an effective SEB technology based on fundamental principles. These limitations are further discussed in *Section 5. Recommendations for Future Research*.

Effects of Micellar Phase Bioavailability. The previous two sections have addressed the impact of surfactants on cell adhesion and on the interaction of cells with surfactant micelles. Surfactant–cell membrane compatibility suggests that solubilized HOC may be directly bioavailable to some organisms with some surfactants. Thus, the application of a surfactant suite that is compatible with the degrading microbial population may significantly enhance bioavailability. Further, aggregate formation at the soil–water interface results in an additional micellar phase (adsorbed) that may provide directly bioavailable HOC to cells capable of adhering to the interface. The phenomena associated with micellar phase bioavailability are the topic of this section.

For the purposes of the current discussion, a distinction must be made between HOC that is *bioavailable* and HOC that is *directly bioavailable* [10]. HOC in any phase is likely to be bioavailable, access to it by a microorganism dependent only on its mass transfer to the aqueous phase. However, for HOC to be directly bioavailable from a particular phase, it must be able to be transported directly to the cell membrane in that phase via direct contact between the cell and the phase in question.

From this definition, it is clear why aqueous phase HOC is directly bioavailable. However, the degree to which nonaqueous phase HOC (e.g., adsorbed to a surface, dissolved in a NAPL, or solubilized in a surfactant micelle) is directly bioavailable is largely unknown, and it is a common assumption that only HOC in the aqueous phase is directly bioavailable. However, recent work suggests that bacteria may have direct access to HOC solubilized in surfactant micelles [10,41–43] via a physico–chemical interaction between the micelle and the cell membrane. This supports the hypotheses of other researchers who suggest that hydrocarbons are transported across the cell membrane as emulsified (solubilized) hydrocarbon microdroplets, their formation promoted by the production of microbial surfactants [31,40]. Our aim in this section is to discuss how surfactants influence HOC bioavailability by comparing the assumptions that (1) only aqueous phase HOC is directly bioavailable, and (2) HOC solubilized in micelles is also directly bioavailable.

a. Only aqueous phase HOC is directly bioavailable. If the aqueous phase is the only directly bioavailable HOC phase, then the rate of biodegradation of HOC will depend on (a) the concentration of HOC in the aqueous phase, and (b) the mass transfer of HOC from nonaqueous to aqueous phases.

From section 2.2.1, we saw that the aqueous phase HOC concentration decreased in response to increasing surfactant concentrations (Figure 6) as a result of redistribution of aqueous phase HOC into surfactant micellar phases. Consequently, since the rate of biodegradation is controlled by the concentration of HOC in the aqueous phase, governed by a first order degradation rate coefficient k_d [time⁻¹],

$$\frac{dC_{aq}}{dt} = -k_b C_{aq} \quad (9)$$

then the resulting decrease in C_{aq} would result in a decreased biodegradation rate in the presence of surfactants.

Further, if mass transfer processes controlling the exchange of HOC between the surfactant micellar phases and the aqueous phases are governed by first order formulations,

$$\frac{dC_{mic}^s}{dt} = k_{m,mic}^s (K_{p,mic}^s C_{aq} - C_{mic}^s) \quad (10)$$

$$\frac{dC_{mic}^{aq}}{dt} = k_{m,mic}^{aq} (K_{p,mic}^{aq} C_{aq} - C_{mic}^{aq}) \quad (11)$$

then we can see that the characteristic times for mass transfer depend on the values of the mass transfer coefficients, $k_{m,mic}^s$ [time⁻¹] and $k_{m,mic}^{aq}$ [time⁻¹]. If these characteristic times are slower than characteristic times for biodegradation, then the presence of surfactants may indeed reduce bioavailability based on micelle–aqueous mass transfer resistance.

It is commonly assumed that micelle–aqueous partitioning is a rapid process [52]. However, micelle–aqueous partitioning kinetics data for environmentally significant HOCs (PAHs, PCBs, etc.) is limited [52,55–57]. Rosen discusses a variety of factors that may influence the partitioning of solutes in micelles [12], and suggests that solute hydrophobicity, hydrophobicity of the micellar core, and other factors may result in sequestration of HOCs deep in the micellar core and their slow release. Thus, the assumption that micelle–aqueous partitioning kinetics is rapid relative to biodegradation may not be appropriate in all cases involving contaminants of environmental concern. In fact, slow micellar release may be responsible for limiting the bioavailability of HOC partitioned into surfactant micelles [10,51].

h. HOC solubilized in micelles is directly bioavailable. Jaffe and coworkers proposed a mechanism by which bacteria facilitate the direct uptake of micellized HOC via contact between the micelle and the cell membrane [10,41] (Figure 10). They hypothesized that the rate of biodegradation depended on a *bioavailable* concentration that included the aqueous phase HOC as well as a fraction of micellized HOC:

$$C_{bio} = C_{aq} + f C_{mic} S_{mic} \quad (12)$$

where f represents the fraction of micellized HOC that is directly bioavailable [1], C_{mic} is the HOC concentration in the micellar phase [mg HOC mg⁻¹ surfactant in the micellar phase], and S_{mic} is the concentration of surfactant in the micellar phase [mg L⁻¹].

This expression for the bioavailable concentration was then coupled to a Monod kinetic model for microbial growth:

$$\mu = \frac{\mu_{max} C_{bio}}{K_s + C_{bio}} \quad (13)$$

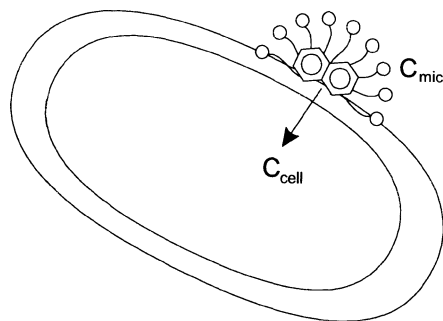


Figure 10. Interaction of cells and micelles to facilitate the direct uptake of solubilized HOC. C_{cell} represents the HOC concentration in the cell [mg HOC L^{-1} cytoplasmic fluid]. Mass transfer across the membrane is driven by the concentration gradient defined by C_{mic} and C_{cell} .

where μ is the growth rate of the microorganism [time^{-1}] and μ_{max} [time^{-1}] and K_s [mg L^{-1}] are the kinetic coefficients for the Monod model.

The results from the initial study [10] that fitted f as the only fitting parameter to a series of biodegradation experiments suggested that as surfactant concentration increased, f decreased. However, f assumed nonzero values between 0.1 and 0.9 in all cases, indicating that 10% to 90% of micellized HOC was directly bioavailable. They postulated that the mechanism of direct bioavailability was via contact between the cell membrane and the micelle (Figure 10), suggesting that the concentration gradient between the micellized HOC (C_{mic}) and the HOC concentration in the cell governed the rate of uptake. Thus, as surfactant concentration increased, the HOC mass was diluted in a larger surfactant volume (resulting in a decrease in C_{mic}) that resulted in a decline in f .

In a later study [41], Jaffe and coworkers successfully predicted the value of f for a variety of surfactants, recognizing that the degree of micellar bioavailability is governed by the structure of the surfactant and compatibility between the surfactant and the cell membrane. This study represents an important landmark in the study of surfactant-enhanced bioremediation by strongly suggesting that micellized HOC may be directly bioavailable. Their results offer insight into the mechanism of hydrocarbon uptake by microbial cells and the possible involvement of microbial surfactants and emulsifiers ("biosurfactants") in HOC degradation. Further, this may explain why microorganisms have appeared to have direct access to HOC that is sorbed to surfaces and soil [5,38,58–60]. If HOC partitioned into a sorbed micellar phase of biosurfactants was directly bioavailable, then the rate of biodegradation may be higher than predicted if it was assumed that only aqueous phase HOC was directly bioavailable.

If micellar phase HOC is directly bioavailable, then Equation 9 is invalid, and must be replaced by an expression that incorporates the dependence of biodegradation rate on the *bioavailable* HOC concentration (i.e., similar to Equation 12). Further, in the presence of soil, the availability of HOC present in either sorbed or aqueous micellar

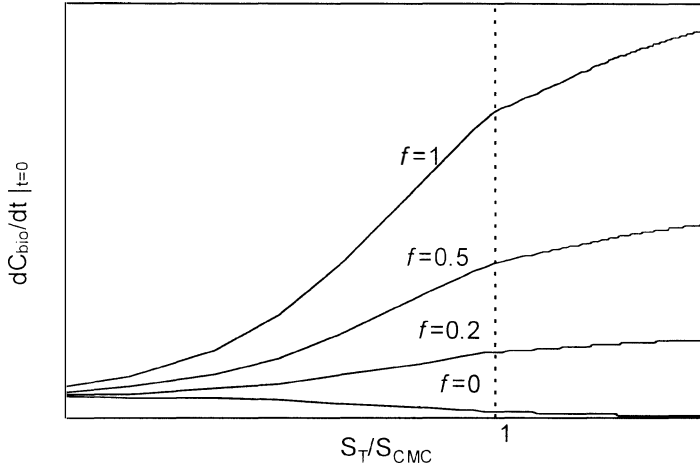


Figure 11. Influence of surfactant concentration relative to the CMC (S_T/S_{CMC}) and the bioavailable fraction of micellized HOC (f) on the initial observed rate of biodegradation ($dC_{bio}/dt |_{t=0}$) as defined by Equations 9 and 14. Partitioning equilibria of HOC is assumed. Surfactant sorption is modeled by the formulation in Figure 4; HOC partitioning between aqueous and nonaqueous phases is modeled via linear isotherms as described in Figure 5 and Equations 5–7. The X axis is a log scale.

phases may significantly alter rates of biodegradation. This has been observed in recent studies that showed that apparently soluble HOC (aqueous + micellized) governed microbial kinetics [41,61]. For example, biodegradation of HOC in a soil–water–surfactant system may be governed by Equation 9, with C_{aq} replaced by C_{bio} defined by:

$$C_{bio} = C_{aq} + f(C_{mic}^{aq} S_{mic}^{aq} + C_{mic}^s S_{mic}^s R_{s/w}) \quad (14)$$

where S_{mic}^{aq} [$mg L^{-1}$] and S_{mic}^s [mg surfactant kg^{-1} soil] represent the aqueous and sorbed micellar surfactant concentrations, respectively, and $R_{s/w}$ [$kg L^{-1}$] is the soil:water ratio. Equations 9 and 14 imply that no mass transfer limitations between phases exist. Thus, we can observe the effect of surfactant addition on the initial ($t = 0$) observed rate of biodegradation in response to surfactant addition as a function of surfactant concentration (Figure 11).

Figure 11 shows that, in the absence of mass transfer limitations, the degree of micellar phase bioavailability has a significant impact on biodegradation rates. If micellar phase is directly bioavailable then surfactant addition should markedly improve bioavailability. The curves for $f = 0$ and $f = 1$ represent the extreme cases where none and all of the micellar phase is directly bioavailable, respectively.

3. A Review of the Influence of Surfactants on Bioavailability in Contaminated Soils

Other reviews have addressed the influence of surfactants on HOC solubilization and transport [62], microbial adhesion [33], surfactant–cell interactions [3], and surfactant chemistry at solid–liquid interfaces [12]. It will be the focus of this review to address studies that have investigated the complex interactions between microbial cells, surfactants, HOCs, and soils. Thus, only research that meets the criteria that HOC must be originally present in a sorbed soil phase is emphasized. In addition, a new body of research is emerging that investigates the potential for SEB of soils where a NAPL phase is present, and key studies in this area will also be discussed.

3.1. SOIL–SORBED HOC

Surfactant-enhanced bioremediation at low surfactant concentrations has some key advantages, particularly when applied to in-situ bioremediation: (1) it is cost effective; (2) there is less risk for off-site mobility of the contaminant; and (3) sub-CMC surfactant concentrations may be less toxic to the microbial populations responsible for contaminant degradation. A cost-effective SEB scheme can be designed by considering the surfactant concentration that yields the maximum benefit in achieving cleanup goals. Undesirable contaminant mobility can be controlled via application of more frequent but smaller surfactant dosages, and by careful monitoring of the rate and extent of HOC degradation [63]. Finally, minimizing the toxic effects of surfactants on indigenous microbes will not only result in a more efficient remediation strategy, but gain favor with regulators when applying the technology.

Alexander and coworkers [23–25] investigated the influence of nonionic surfactants on the desorption and mineralization of phenanthrene and biphenyl in low, medium, and high-organic carbon soils. The first study [23] compared the relative influences of surfactant addition on mineralization in high and medium organic carbon soils in non-slurried soil reactors. The second study [24] examined similar effects in low organic carbon aquifer sediments in slurry systems. The final study in this series [25] examined the potential for low concentrations of surfactants to enhance the mobilization and mineralization of HOCs in a soil column.

In the initial study [23], the comparison between a medium organic carbon soil (Lima silt loam, $f_{oc} = 7.6\%$) and a high organic carbon soil (Edwards muck, $f_{oc} = 32.9\%$) was made. They determined both desorption extents and mineralization rates/extents as a function of surfactant concentrations below and above their CMCs, but observed no correlation between the extent of desorption and extent of mineralization. However, it was observed that mineralization was enhanced when surfactants were present at sub-CMC concentrations, and mineralization was inhibited when surfactants were present above their CMCs. They hypothesized that the extent of desorption (i.e., the solubilization process) was not as important in enhancing bioavailability as was the instantaneous desorption rate of HOC from the soil. They speculated that surfactant addition may have promoted instantaneous desorption, thus enhancing the rate of supply of HOC to the degrading microbial population.

The mechanism for this enhancement is unknown. However, some possibilities include: (1) an increase in the rate of diffusion of HOC out of soil particles resulting from solubilization of intraparticle HOC; (2) an increase in the instantaneous rate of desorption from soil surfaces resulting from a decrease in the surface energy promoted by surfactant adsorption; or (3) partitioning of HOC into sorbed micellar structures that are either directly bioavailable or allow for the rapid partitioning of HOC to the aqueous phase in response to degradation. Unfortunately, it is unclear from this research whether or not the HOC present was initially sorbed to soil or in a pure crystalline form. Thus,

the low surfactant concentrations could have either enhanced desorption of HOC from soil or dissolution of HOC from its crystalline phase.

Another unique finding from this research is that surfactants had a greater ability to enhance mineralization when HOC was sorbed to organic-rich soil. The authors did not suggest a particular mechanism for this behavior. However, because soil organic matter (SOM) itself has an amphiphilic molecular structure [64], interaction of surfactants with SOM may promote solubilization of HOC into surfactant micelles, similar to the ability of surfactants to solubilize proteins originally bound in a cell membrane [11].

Another important difference observed between the medium (loam) and high (muck) organic carbon soils was the relative degree of inhibition of phenanthrene mineralization at the higher surfactant concentration (100 $\mu\text{g/g}$ soil). A greater degree of inhibition was observed in the loam. One explanation for this is that surfactant concentrations (in terms of mass of surfactant per mass of pore water) in this soil were higher than those in the Edwards muck. Thus, there may have been a greater degree of micelle formation in the loam soil that could have exhibited toxic effects to the degrading population. In addition, surfactant sorption may have been greater in the muck, resulting in less influence from aqueous phase micelles that could interact with cell membranes. Finally, the indigenous population of HOC-degrading organisms in the loam may have been more susceptible to surfactant toxicity than in those in the muck.

This study showed that surfactants more effectively promoted the mineralization of phenanthrene than biphenyl. The authors suggested that this implies that the identity of the compound is important in the design of an effective SEB scheme. However, compound properties cannot be implicated as being solely responsible for this difference. It is likely that different microbial populations may be responsible for the degradation of the different compounds, and surfactants may have markedly different cell-specific effects on these different populations that could result in different relative rates and extents of mineralization in the presence of surfactants.

In a similar study, the influence of surfactants on the mineralization of HOCs from low-organic carbon aquifer sediments ($f_{oc} = 0.4\%$) was investigated [24]. Both desorption and mineralization extents for phenanthrene and biphenyl were enhanced in the presence of surfactants. The authors suggested that the enhancement was due primarily to the enhanced partitioning of the chemical to the aqueous phase. However, it was observed in the same study that similar surfactant concentrations inhibited mineralization when the aquifer sand was supplemented with an enrichment culture specific to the compound being degraded. Thus, there appear to be population- (or more appropriately, genus- or species-) specific cell-surfactant interactions that result in different relative degrees of mineralization by nonenriched and enriched cultures, and surfactant enhanced mineralization may not be a result of partitioning phenomena alone.

A key observation in this research is that although low surfactant concentrations resulted in enhancement of phenanthrene mineralization, higher surfactant concentrations offered no additional benefit. This indicates that there is a point of diminishing returns above which the benefits of additional surfactant are not realized. What makes these observations unique is that the point of diminishing returns likely occurred below the CMC of the surfactants, a result that conflicts with the traditional paradigm of SEB by which increased solubilization (resulting from greater surfactant addition) results in enhanced biodegradation.

The final study [25] investigated the potential for SEB under field conditions. Surfactant was applied at the soil surface of a column of Lima silt loam that was contaminated with HOC. Surfactant concentrations were very low, with aqueous phase concentrations likely spanning two to four orders of magnitude below the CMC. Consequently, there was little evidence of inhibition resulting from toxicity. However, consistent with the authors' earlier hypothesis [23,24] that surfactants enhance

instantaneous desorption rates, both the rate and extent of phenanthrene mineralization, and the extent of biphenyl mineralization, were enhanced in the presence of surfactants. Further, there was no evidence of parent contaminant leaching through the bottom of the column. Thus, application of low concentrations of surfactant may be a viable technology for the cleanup of HOC-contaminated soil without risk of either apparent toxicity to indigenous microbial populations or surfactant-promoted mobilization of HOC.

One alternative to avoiding the risk of off-site HOC migration is the use of cationic surfactants to enhance HOC bioavailability. Cationic surfactants are relatively immobile in soils dominated by negatively charged clays and other mineral surfaces. Crocker *et al.* [65] examined the bioavailability of naphthalene sorbed to smectite clay coated with a cationic surfactant (hexadecyltrimethylammonium, HDTMA). One of the primary concerns with HDTMA, as well as many other cationic surfactants, is its toxicity to microorganisms [21], even at sub-CMC concentrations. The results of this study showed that HDTMA-modified smectite was not toxic to microorganisms, suggesting that HDTMA was irreversibly-bound to the clay, an important feature for minimizing off-site migration of solubilized HOC.

Their results further showed desorption rates of naphthalene from the HDTMA-modified clay that were very rapid, and there was no evidence of desorption-limited biodegradation induced by the modification of the clay by surfactant coating. Further, the rate of mineralization of sorbed naphthalene to a *Pseudomonas putida* strain was faster than the rate predicted by assuming that sorbed HOC was not directly bioavailable, indicating that this strain had direct access to sorbed naphthalene. This direct access may be facilitated by the reported hydrophobic nature of the cell and/or its ability to interact directly with sorbed micellar structures containing solubilized HOC.

One promising SEB technology employs the use of microbially produced surface active compounds ("biosurfactants" or "bioemulsifiers") [66–69]. Biosurfactants offer some advantages over chemical surfactants, including their biodegradability, compatibility with the microorganism that produces them, and potential to be produced in-situ. Biosurfactants may be particularly useful at low concentrations because of their high surface activities and solubilization potentials. However, biosurfactants in SEB have been studied comparatively little relative to their synthetic analogs. Although they exhibit similar physico-chemical behavior, they may interact with microbial populations in unpredictable manners. Biosurfactants are a highly diverse group of molecules [68], and there is much research potential for their application in SEB.

Among the most well-studied biosurfactants are the rhamnolipids of *Pseudomonas aeruginosa* [70–76]. Jain *et al.* [63] examined the effects of adding biosurfactants isolated from a culture of *Pseudomonas aeruginosa* UG2 or UG2 inocula to soil microcosms containing tetradecane, hexadecane, pristane, and 2-methylnaphthalene. Biosurfactant concentrations were added over a range of 10–100 µg/g soil (which corresponded to aqueous concentrations far less than the probable CMC of the surfactant). Results indicated that when present at a concentration of 100 µg/g, the biosurfactant enhanced the degradation of the alkanes, but not 2-methylnaphthalene, the most soluble contaminant, over a 2-month degradation period. Addition of UG2 inocula had no effect on biodegradation, resulting in either (1) failure of UG2 to compete with the degrading population, (2) toxicity of HOC present in the mixture to UG2, or (3) failure of UG2 to produce an adequate quantity of biosurfactant to effect degradation.

These authors noted that surfactant addition might realize the greatest benefit when degradation of more insoluble compounds is considered. However, this may not be generally applicable, particularly if micellar phase HOC is not directly bioavailable, since more insoluble compounds may partition into micelles and be rendered less available for degradation (cf. section 2.2.1). Thus, biosurfactants will probably realize the greatest benefit to microbes that have membranes similar to those that produced the

surfactant. This feature may be unique to a particular strain, species, or genus. The study of rhamnolipids from *Pseudomonads* is particularly useful, since these organisms are ubiquitous in the soil environment [77] and are known to be important in the degradation of a wide variety of organic contaminants [78].

Thibault *et al.* [79] investigated the influence of a chemical surfactant, Witconol SN70, in combination with bacterial isolates (*Pseudomonads*) K-12 and B-24, on the mineralization of pyrene in low- and medium-organic carbon soils ($f_{oc} = 0.33\%$ and 2.92% , respectively). Addition of the surfactant alone (at 20X CMC) in the presence of the indigenous microbial population resulted in either no mineralization enhancement or slight inhibition. However, addition of the surfactant with either strain enhanced pyrene mineralization in the low-organic carbon soil under unsaturated conditions (similar results were shown for the medium-organic carbon soil). The mechanism of enhancement is unknown, but it is likely attributed to enhanced solubilization of pyrene and/or an increase in its desorption rate from the soil. Under unsaturated conditions, the high soil:water ratio may have resulted in a significant, if not predominant, fraction of the surfactant being sorbed to the soil. Thus, it is likely that the surfactant could have increased the rate of pyrene desorption from the soil.

When the soil was saturated, addition of the surfactant with or without the additional inoculum inhibited mineralization. The authors speculated that saturated conditions (which were agitated) in the presence of surfactant could have promoted pyrene solubilization and subsequent toxicity to microbes. Inhibition of pyrene mineralization in the presence of strain K-12 and surfactant under saturated conditions may have also resulted from preferential use of the surfactant as a carbon source (although this alternative was ruled out for strain B-24). Finally, the authors suggested that the surfactant could have altered the physiology of pyrene uptake and degradation. This effect may have been magnified under saturated slurry conditions, since it would be expected that the lower soil:water ratio would have resulted in a greater aqueous micellar phase surfactant concentration than under unsaturated conditions, and greater mixing may have enhanced cell-micelle contact.

Laha and Luthy [51] investigated the effect of nonionic surfactants on phenanthrene mineralization in soil-water systems amended with a PAH-degrading inoculum. Surfactant doses that resulted in supra-CMC concentrations in the presence of soil completely inhibited phenanthrene mineralization, and doses resulting in sub-CMC surfactant concentrations partially inhibited mineralization or had no effect. These authors performed independent tests to rule out the possibilities of slow HOC exit rates from micelles, PAH toxicity, or use of the surfactant as a preferred substrate. This phenomenon may appear to be a toxic effect; however, dilution of the surfactant to sub-CMC concentrations restored phenanthrene mineralization capability. Thus, it was suggested that the surfactants had an inhibitory, but reversible effect, on cell membrane processes that influence uptake and/or degradation of phenanthrene, indicative of a physico-chemical interference with the cell membrane. In another study [80], surfactant inhibition was not observed when naphthalene was the carbon source. Thus, surfactants may have targeted compound- or cell species-specific mechanisms of uptake or degradation that resulted in the inhibition of phenanthrene mineralization, but not naphthalene mineralization.

A follow-up study by Laha and Luthy [52] expanded the suite of surfactants in a similar series of experiments. Similar inhibition was observed for all surfactants tested, which included several surfactants with diverse molecular structures, CMCs, and aggregation numbers. Again, supra-CMC surfactant concentrations completely inhibited phenanthrene mineralization, and sub-CMC doses resulted in partial inhibition or no enhancement. These studies are relatively unique in that the authors measured the effect of a soil phase on the CMC of the surfactant. Thus, it was easy to show the relationship between the aqueous phase surfactant concentration (relative to the CMC) to the degree of mineralization. The results from this study show that micelle formation by a variety

of surfactants is involved in the inhibition of phenanthrene mineralization in soil–water systems.

For one surfactant investigated (octylglucoside), sub-CMC surfactant doses partially inhibited mineralization. This surfactant was known to be toxic in aqueous cultures above the CMC, but not below the CMC. Those results suggest that sorbed surfactant may play a role in inhibition, and gives rise to the possibility that sorbed micellar structures may interact with cell membranes.

This work emphasizes the need to investigate specific cell–surfactant interactions. The authors suggested that reversible physico–chemical interactions between surfactant micelles and cell membranes were responsible for the inhibition of HOC degradation. Proposed mechanisms of inhibition included partial complexation of membrane-bound proteins and/or phospholipids without dissociation of the lamellar membrane structure.

3.2. NAPL-ASSOCIATED HOC

Falatko and Novak [81] investigated the potential for uncharacterized mixtures of biosurfactants to increase solubility and promote degradation of a gasoline pool at the top of a column of clean sand. Biosurfactants were produced by growth of an activated sludge consortium on glucose and vegetable oil, or by a gasoline-degrading consortium during growth on gasoline. Biosurfactants were applied to the column at a concentration of 600 mg TOC/L. It is unknown whether or not this is either above or below the surfactants' CMC, particularly when adsorption of the surfactant to the sand is considered. However, biosurfactants tend to exhibit low CMCs [68], and this concentration likely resulted in micelle formation in solution.

The glucose–and–vegetable oil mixture markedly inhibited gasoline degradation in the soil column, while the gasoline-based biosurfactant mixture either promoted degradation or had no effect. These results suggest that the microbial origin and degree of compatibility of biosurfactants with the HOC degrading population may play a key role in their effectiveness in promoting HOC degradation. The authors noted that sorption of biosurfactant to the sand may have further inhibited degradation, but offered no supporting evidence for this claim other than the biosurfactant exhibited a retardation factor of 3.13 in the column system. Also, it should be noted that the “soil” in this system consisted of acid-washed silica sand, and gasoline components were present primarily in a nonaqueous phase. Thus, the effect of these biosurfactants on desorption of HOC from a reactive soil phase in this study is unknown.

Herman *et al.* [82] examined the potential of rhamnolipid biosurfactant at concentrations of either 0.1 mM (~ 1X CMC) or 1.0 mM (~ 10X CMC) to promote the mobilization and mineralization of residual hexadecane in a sand column. At 0.1 mM, the biosurfactant did not directly promote mineralization of hexadecane. At 1.0 mM, however, rhamnolipid increased transitory biodegradation rates by factors of 3–12. These increased apparent removal rates were a combination of both HOC mobilization and biodegradation. The authors noted that one feature of surfactant application that may indirectly promote biodegradation of NAPLs is the redistribution of the NAPL throughout the soil. This side effect may increase the surface area of the NAPL, indirectly supporting a biodegradation enhancement.

Fu and Alexander [83] examined the effects of nonionic and anionic chemical surfactants on the degradation of HOCs initially present in NAPLs in soil microcosms. The degree of bioavailability enhancement of NAPL-associated HOCs was highly variable. For example, low (sub-CMC) concentrations of some surfactants increased the rate and extent of phenanthrene mineralization present in heptamethylnonane, but enhancement was not observed for other surfactants. One surfactant promoted the mineralization of biphenyl in hexadecane but not in several other NAPLs. Surfactants

also promoted the mineralization of di-(2-ethylhexyl) phthalate in dibutyl phthalate, when little degradation occurred in the absence of surfactant.

Potential factors influencing the bioavailability of HOCs in NAPLs included the HOC partitioning equilibria and rates between the NAPL and water, NAPL viscosity, surface-area to volume ratio of the NAPL:water interface, and competition for micronutrients by organisms utilizing the NAPL as a substrate. The presence of surfactant can effect any one of these processes. Thus, predicting SEB in soils with a NAPL phase present becomes a daunting task. Their results indicated that SEB of NAPL-bound HOCs in soils is an unpredictable and complex process that is governed by the identity of the surfactant, the HOC, and the NAPL, as well as the other phenomena that are addressed in this review.

4. Discussion

One should recognize by now that SEB is influenced by surfactant concentration. Surfactants may exhibit markedly different effects as a function of their concentration in the presence of soil. It is believed that surfactant adsorption and micellar phase toxicity/bioavailability are two key processes (both of which are influenced by the surfactant concentration in the system) influencing HOC bioavailability in the soil environment. Consequently, we have tried to select studies that examine a broad range of surfactant concentrations to compare the effects of sub-CMC and supra-CMC surfactant doses. Unfortunately, doses required to achieve the CMC in the presence of soil are often significantly higher than doses required to achieve the CMC in aqueous solution [16,52], and many studies fail to report the aqueous phase surfactant concentrations in the presence of soil. Thus, characterization of sub-CMC and supra-CMC surfactant concentrations may only be rough estimate.

Application of surfactants above the CMC is a common practice in the technology known as "surfactant enhanced aquifer remediation" (SEAR), by which contaminant desorption and solubilization is promoted in a soil flushing scheme that is combined with ex-situ treatment [84-86]. However, solubilization of contaminant may result in enhanced mobility and toxicity of HOC to microbes, inhibiting the effectiveness of in-situ bioremediation. In addition, supra-CMC surfactant concentrations have been known to inhibit uptake and/or degradation of HOCs in soil-water systems [52,79]. However, if micelles are "compatible" with cell membranes (cf. section 2.2.2) and micellar phase HOC is directly bioavailable, then supra-CMC surfactant application may have real benefits in a successful SEB design.

We will summarize data reported in literature by plotting SEB effectiveness factors, η_{fs} , as a function of normalized surfactant concentration (relative to the surfactants' CMC values, i.e., xCMC). The method for determining η_{fs} , the plotted η_{fs} values, and interpretation of the η_{fs} vs. xCMC graph are the subjects of this section.

4.1. METHOD FOR ESTIMATING η_{fs} FROM LITERATURE DATA

Recall from section 1 that η_{fs} defines the ratio of the characteristic time for HOC biodegradation in the absence of surfactant to the characteristic time for HOC biodegradation in the presence of surfactant (Equation 1). Further, it was shown that η_{fs} can be defined in terms of first order biodegradation rate coefficients (Equation 4). Alternatively, η_{fs} as defined in Equation 1 may be a function of treatability criteria other than first order rate coefficients that reflect cleanup goals (e.g., the extent of degradation (endpoint) or maximum observed rate).

Because degradation data reported in literature is highly variable, measures of biodegradation are inconsistent. Consequently, we determined values of η_{fs} that were

based on measures most appropriate to each study. Thus, η_{fs} may reflect characteristic times for HOC biodegradation based on HOC mineralization rates (e.g., reflected by $^{14}\text{CO}_2$ evolution), HOC parent compound disappearance rates, or even bacterial growth rates (further recognizing that any of these rates may be controlled by zero, first-order, or even Monod-style kinetic formulations). In most cases, η_{fs} values were calculated from zero-order estimates of % mineralization over the time course of the experiment.

In conclusion, the reader should keep in mind that it might not be appropriate to quantitatively compare values of η_{fs} from one study to another. However, the data does illustrate the impact of surfactant concentration relative to the CMC on the potential for enhancing or inhibiting bioremediation. Finally, the database represents a unique survey of the literature that emphasizes the need to better understand factors influencing SEB.

The criteria for selection of appropriate data that appears on the survey plot (Figure 12) are as follows:

- A surfactant-free control must have been performed, in order to obtain a value for t^* ($S_T = 0$)
- The experiment must have monitored HOC degradation where HOC was present as a soil-sorbed phase in the absence of NAPL.
- The surfactant's CMC must be known, so the surfactant concentration in the system relative to its CMC can be ascertained.

4.2. SUMMARY OF REPORTED SEB EFFECTIVENESS FACTORS

Figure 12 shows the plot of reported SEB effectiveness factors (η_{fs}) as a function of total surfactant concentration in the system. Each of the points on the graph indicates the result of a single experiment. Values of $\eta_{fs} > 1$ indicate enhancement of biodegradation resulting from surfactant addition, while values of $\eta_{fs} < 1$ indicate surfactant-induced inhibition. Biodegradation in these studies was monitored via $^{14}\text{CO}_2$ mineralization except in ref. [63], in which biodegradation was monitored via disappearance of the parent compound. η_{fs} values were calculated by determining the ratio of characteristic times (Equation 1) based on average zero-order biodegradation rates (e.g., % mineralization over the time course of the experiment).

One should keep in mind that most of the research fails to report the surfactant's CMC in the presence of a soil phase, only its CMC in aqueous solution. Thus, the surfactant concentrations in Figure 12 have been normalized only to a surfactant's reported aqueous CMC. A more accurate depiction of this data would consider the influence of surfactant sorption to soil and a plot of η_{fs} as a function of surfactant concentration relative to the CMC in the presence of soil, thus shifting the $x\text{CMC} = 1$ line to the right. Because of the lack of available data to perform this, the reader should consider that the $x\text{CMC}$ values shown in Figure 12 likely underestimate the true values of the aqueous surfactant concentrations in the presence of soil.

4.3. INTERPRETATION OF SEB EFFECTIVENESS FACTORS DERIVED FROM LITERATURE DATA

It is important to recognize that Figure 12 consists of all points meeting the criteria outlined in Section 4.1, regardless of whether or not they are statistically significant values when compared to their surfactant-free controls. As shown on Figure 12, there is a large body of data that falls near the $\eta_{fs} = 1$ line, indicating that surfactants play a relatively minor role in either promoting or inhibiting biodegradation. This emphasizes the need to understand how surfactants influence bioavailability, allowing the practitioner to avoid treatments that have little or no effect on achieving cleanup goals.

Figure 12 can be interpreted by classifying the four quadrants identified by the $x\text{CMC} = 1$ and $\eta_{fs} = 1$ lines. Although the exact mechanisms influencing the location of

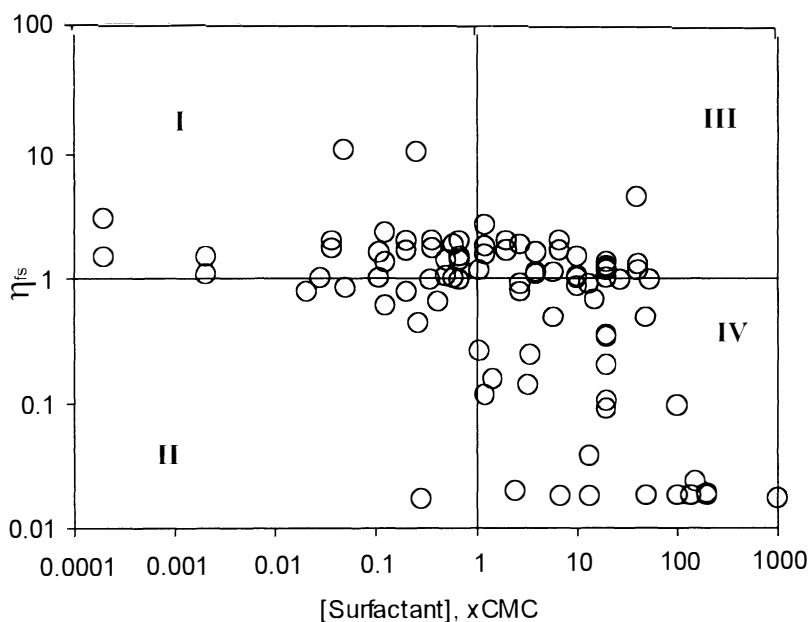


Figure 12. Summary of reported SEB effectiveness factors ($\eta_{f,s}$) calculated from selected literature. xCMC defines the total concentration of surfactant in the system relative to its reported CMC value. Data points were calculated from results presented in refs. [23–25,51,52,63,79].

these points are not always apparent from careful examination of their literature source, a summary of processes that could influence the points' locations relative to the (1,1) origin can be made. These processes are summarized in Table I.

Although the benefits of low (sub-CMC) surfactant concentrations have been recognized (cf. Section 4.1), neither low nor high surfactant concentrations seem to afford distinct advantages when considering their general applicability to SEB. Figure 12 emphasizes that there seems to be little general correlation between surfactant concentration and the effectiveness of SEB, thus emphasizing the need to understand the key interactions that affect HOC bioavailability in soil systems.

However, it is clear from Figure 12 that in experiments where surfactants were applied above the CMC (quadrants III and IV), a greater percentage resulted in some degree of inhibition of bioavailability ($> 50\%$) than in experiments where surfactants were applied below the CMC ($< 25\%$). This result is consistent with a similar data set that was constructed for SEB in aqueous or aqueous–NAPL systems (data not shown). In addition, at supra-CMC surfactant doses, bioavailability was completely inhibited (exhibited by points that fall below the $\eta_{f,s} = 0.1$ line) in a larger number of cases than at sub-CMC surfactant doses.

Table 1. Summary of processes influencing the locations of η_{fs} vs. xCMC points relative to the (1,1) origin of Figure 12.

Quadrant I: *Enhanced Bioavailability at Low Surfactant Concentrations*

- Direct bioavailability of HOC solubilized in sorbed micellar structures
- Enhanced cell–soil contact resulting from surfactant sorption to soil or cells
- Enhanced HOC desorption rates resulting from decreased surface energy promoted by surfactant sorption
- Enhanced intraparticle diffusion of HOC

Quadrant II: *Inhibited Bioavailability at Low Surfactant Concentrations*

- Toxicity or cell membrane interference exhibited by surfactant monomers or sorbed micellar structures
- Inhibited cell–soil contact resulting from surfactant sorption to soil or cells
- Reduced HOC bioavailability resulting from solubilization into sorbed micelles and corresponding slow HOC exit rates
- Inhibited intraparticle diffusion of HOC

Quadrant III: *Enhanced Bioavailability at High Surfactant Concentrations*

In addition to Quadrant I processes:

- Enhanced solubility of HOC via solubilization into aqueous micelles and a rapid rate of supply of HOC to the aqueous phase in response to biodegradation
- Enhanced HOC uptake via direct bioavailability of micellized HOC

Quadrant IV: *Inhibited Bioavailability at High Surfactant Concentrations*

In addition to Quadrant II Processes:

- Toxicity or cell membrane interference exhibited by surfactant monomers or aqueous (non-sorbed) micellar structures
 - Dilution of HOC among a large micellar mass resulting in slow HOC exit rates from micelles
-

5. Recommendations for Future Research

This literature survey has recognized a number of key areas in which little is known about processes influencing SEB:

1. The existence of sorbed surfactant micellar structures and their effects on the physiology of attached microbial cells;
2. The interactions of surfactant monomers and micelles with cell membrane processes that are important in the uptake and degradation of HOCs of environmental interest;
3. The mechanism(s) by which cells facilitate the direct uptake of micellar phase contaminant;
4. Identification of cell and/or surfactant-specific properties that define the degree of toxicity and/or compatibility with a particular microbial species;
5. Influence of surfactants on individual species within a mixed consortia;
6. Influence of surfactants on HOC desorption rates from soil and identification of the specific mechanisms by which HOC desorption is facilitated by surfactants.

Examination of items 1–5 in the above list emphasizes the need to investigate the interactions between surfactants and microbial cells. Item 6 has been recognized as the key physico-chemical influence by which surfactants promote bioavailability of soil-sorbed HOC. However, a specific description of the mechanism(s) involved has not yet been identified. Learning more about items 1–6 in this list can only enhance the effectiveness of SEB designs from the ground up. Selection of an appropriate suite of surfactants that are compatible with the degrading population as well as the selection of an appropriate dosage regime that optimizes the benefits of SEB at a minimum of cost are the key components in designing an effective SEB strategy.

Finally, what is markedly absent from this body of research are published studies of field applications of SEB. This is largely a result of the failure to understand the technology and apply it at larger scales. However, as this field of research matures, understanding this technology across scales will require an iterative approach between the field and the laboratory.

6. Conclusion

The foregoing review highlights a variety of key processes that seem to be important in SEB. In particular, the relationships between (1) surfactant-cell membrane interactions, (2) micellar phase bioavailability, (3) surfactant sorption, and (4) HOC solubilization appear to be key features that must be considered in the design and application of a successful SEB technology.

Further, it should be emphasized that a well-designed SEB strategy must consider the coupled mechanisms that influence the complex physical, chemical, and microbiological processes that can be affected by surfactant amendment. Finally, it is apparent from the literature that, although SEB in aqueous and aqueous-NAPL systems has been studied extensively, more data is needed from soil-water and soil-NAPL-water systems to continue to address the feasibility of this technology under laboratory conditions more appropriate to soil cleanup in the field.

7. Acknowledgments

The authors wish to express their acknowledgment to Pacific Northwest National Laboratory for providing support for this research. This research was performed at the

Center for Biofilm Engineering at Montana State University–Bozeman, a National Science Foundation Engineering Research Center supported under cooperative agreement #EEC–8907039. In addition, we recognize Rebecca Stone and Eric Nichols, students in the Montana State University Undergraduate Scholars Program, for their valuable contributions to this review.

8. References

1. Huesemann, M.H. (1997) Incomplete hydrocarbon biodegradation in contaminated soils: limitations in bioavailability or inherent recalcitrance? *Bioremediation Journal*, **1**, 27–39.
2. Mihelcic, J.R., Lueking, D.R., Mitzel, R. and Stapleton, J.M. (1993) Bioavailability of sorbed– and separate–phase organic chemicals. *Biodegradation*, **4**, 141–153.
3. Rouse, J.D., Sabatini, D.A., Suflita, J.M. and Harwell, J.H. (1994) Influence of surfactants on microbial degradation of organic compounds. *Crit. Rev. Environ. Sci. Technol.* **24**, 325–370.
4. Bouwer, E.J., Zhang, W., Wilson, L.P. and Durant, N.D. (1997) Biodegradation of coal tar constituents in aquifer sediments, in H. Rubin, N. Narkis and J. Carberry (eds.), *Soil and Aquifer Pollution: Non–Aqueous Phase Liquids—Contamination and Reclamation*, Springer–Verlag, Heidelberg, Germany.
5. Guerin, W.F. and Boyd, S.A. (1992) Differential bioavailability of soil–sorbed naphthalene to two bacterial species. *Appl. Environ. Microbiol.* **58**, 1142–1152.
6. Miller, M.E. and Alexander, M. (1991) Kinetics of bacterial degradation of benzylamine in a montmorillonite suspension. *Environ. Sci. Technol.* **25**, 240–245.
7. Mihelcic, J.R. and Luthy, R.G. (1991) Sorption and microbial degradation of naphthalene in soil–water systems under denitrification conditions. *Environ. Sci. Technol.* **25**, 169–177.
8. Scow, K.M. and Hudson, J. (1992) Effect of diffusion and sorption on the kinetics of biodegradation: theoretical considerations. *Soil Sci. Soc. Am. J.* **56**, 119–127.
9. Scow, K.M. and Alexander, M. (1992) Effect of diffusion on the kinetics of biodegradation: experimental results with synthetic aggregates. *Soil Sci. Soc. Am. J.* **56**, 128–134.
10. Guha, S. and Jaffe, P.R. (1997) Biodegradation kinetics of phenanthrene partitioned into the micellar phase of nonionic surfactants. *Environ. Sci. Technol.* **30**, 605–611.
11. Schnaitman, C.A. (1971) Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X–100. *J. Bacteriol.* **108**, 545–552.
12. Rosen, M.J. (1978) *Surfactants and Interfacial Phenomena*, Wiley, New York.
13. Mannhardt, K., Schramm, L.L. and Novosad, J.J. (1992) Adsorption of anionic and amphoteric foam–forming surfactants on different rock types. *Colloids Surf.* **68**, 37–53.
14. Manne, S. and Gaub, H.E. (1995) Molecular organization of surfactants at solid–liquid interfaces. *Science*, **270**, 1480.
15. Edwards, D.A., Adeel, Z. and Luthy, R.G. (1994) Distribution of nonionic surfactant and phenanthrene in a sediment/aqueous system. *Environ. Sci. Technol.* **28**, 1550–1560.
16. Liu, Z., Edwards, D.A. and Luthy, R.G. (1992) Sorption of non–ionic surfactants onto soil. *Wat. Res.* **26**, 1337–1345.
17. Edwards, D.A., Luthy, R.G. and Liu, Z. (1991) Solubilization of polycyclic aromatic hydrocarbons in micellar nonionic surfactant solutions. *Environ. Sci. Technol.* **25**, 127–133.

18. Kile, D.E. and Chiou, C.T. (1989) Water solubility enhancements of DDT and trichlorobenzene by some surfactants below and above the critical micelle concentration. *Environ. Sci. Technol.* **23**, 832–838.
19. Pignatello, J.J. and Xing, B. (1996) Mechanisms of slow sorption of organic chemicals to natural particles. *Environ. Sci. Technol.* **30**, 1–11.
20. Wu, S.-c. and Gschwend, P.M. (1986) Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. *Environ. Sci. Technol.* **20**, 717–725.
21. Nye, J.V., Guerin, W.F. and Boyd, S.A. (1994) *Environ. Sci. Technol.* **28**, 144.
22. Zhang, W.-X. (1995) Effect of sorption on bioavailability of hydrophobic organic contaminants: experimental and model studies, Ph.D. Dissertation, Johns Hopkins University, Baltimore, Maryland.
23. Aronstein, B.N., Calvillo, Y.M. and Alexander, M. (1991) Effect of surfactants at low concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. *Environ. Sci. Technol.* **25**, 1728–1731.
24. Aronstein, B.N. and Alexander, M. (1992) Surfactants at low concentrations stimulate biodegradation of sorbed hydrocarbons in samples of aquifer sands and soil slurries. *Environ. Toxicol. Chem.* **11**, 1227–1233.
25. Aronstein, B.N. and Alexander, M. (1993) Effect of a non-ionic surfactant added to the soil surface on the biodegradation of aromatic hydrocarbons within the soil. *Appl. Microbiol. Biotechnol.* **39**, 386–390.
26. Aiba, S., Moritz, V., Someya, J. and Huang, K.L. (1969) Cultivation of yeast cells by using *n*-alkanes as the sole carbon source. *J. Ferment. Technol.* **47**, 203.
27. Efroymsen, R.A. and Alexander, M. (1991) Biodegradation by an *Arthrobacter* species of hydrocarbons partitioned into an organic solvent. *Appl. Environ. Microbiol.* **57**, 1441.
28. Liu, D. (1980) Enhancement of PCBs biodegradation by sodium lignosulfonate. *Wat. Res.* **14**, 1467.
29. Nakahara, T., Hisatsuka, K.-i. and Minoda, Y. (1981) Effect of hydrocarbon emulsification on growth and respiration of microorganisms in hydrocarbon media. *J. Ferment. Technol.* **59**, 415.
30. Mimura, A., Watanabe, S. and Takeda, I. (1971) Biochemical engineering analysis of hydrocarbon fermentation (III). *J. Ferment. Technol.* **49**, 255.
31. Reddy, P.G., Singh, D.K., Roy, P.K. and Baruah, J.N. (1982) Predominant role of hydrocarbon solubilization in the microbial uptake of hydrocarbons. *Biotech. Bioeng.* **24**, 1241.
32. Ramsay, B., McCarthy, J., Guerra-Santos, L.H., Kappeli, O. and Fiechter, A. (1988) Biosurfactant production and diauxic growth of *Rhodococcus aurantiacus* when using *n*-alkanes as the carbon source. *Can. J. Microbiol.* **34**, 1209.
33. Neu, T. (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. *Microbiol. Rev.* **60**, 151–166.
34. Doyle, R.J. and Rosenberg, M. (1990) *Microbial Cell Surface Hydrophobicity*, American Society for Microbiology, Washington, D.C.
35. Rosenberg, M. and Kjelleberg, S. (1986) Hydrophobic interactions in bacterial adhesion. *Adv. Microb. Ecol.* **9**, 353–393.
36. Beachey, E.H. (1981) Bacterial adherence: adhesin–receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J. Infect. Dis.* **143**, 325–345.
37. Bright, J.J. and Fletcher, M. (1983) Amino acid assimilation and electron transport system activity in attached and free-living marine bacteria. *Appl. Environ. Microbiol.* **45**, 818–825.
38. Griffith, P.C. and Fletcher, M. (1991) Hydrolysis of protein and model dipeptide substrates by attached and nonattached marine *Pseudomonas* sp. strain NCIMB 2021. *Appl. Environ. Microbiol.* **57**, 2186–2191.

39. Zhang, Y. and Miller, R.M. (1994) Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. *Appl. Environ. Microbiol.* **60**, 2101–2106.
40. Yoshida, F., Yamane, T. and Nakamoto, K.-i. (1973) Fed-batch hydrocarbon fermentation with colloidal emulsion feed. *Biotech. Bioeng.* **15**, 257–270.
41. Guha, S. and Jaffe, P.R. (1996) Bioavailability of hydrophobic compounds partitioned into the micellar phase of nonionic surfactants. *Environ. Sci. Technol.* **30**, 1382–1391.
42. Bury, S.J. and Miller, C.A. (1993) Effect of micellar solubilization on biodegradation rates of hydrocarbons. *Environ. Sci. Technol.* **27**, 104–110.
43. Miller, R.M. and Bartha, R. (1989) Evidence from liposome encapsulation for transport-limited microbial metabolism of solid alkanes. *Appl. Environ. Microbiol.* **55**, 269–274.
44. Itoh, S. and Suzuki, T. (1972) Effect of rhamnolipids on growth of *Pseudomonas aeruginosa* mutant deficient in *n*-paraffin-utilizing ability. *Agr. Biol. Chem.* **36**, 2233–2235.
45. Guerin, W.F. and Jones, G.E. (1988) Mineralization of phenanthrene by a *Mycobacterium* sp. *Appl. Environ. Microbiol.* **54**, 937.
46. Cserhati, T., Illes, Z. and Nemes, I. (1991) Effect of non-ionic tensides on the growth of some soil bacteria. *Appl. Microbiol. Biotechnol.* **35**, 115–118.
47. Van Hoof, P.L. and Rogers, J.E. (1992) Influence of low levels of nonionic surfactants on the anaerobic dechlorination of hexachlorobenzene, in *Biosystems Technology Development Program. Bioremediation of Hazardous Wastes*, U.S. Environmental Protection Agency, Washington, D.C. pp. 105.
48. Breuil, C. and Kushner, D.J. (1980) Effects of lipids, fatty acids, and other detergents on bacterial utilization of hexadecane. *Can. J. Microbiol.* **26**, 223–231.
49. Robichaux, T.J. and Myrick, H.N. (1972) Chemical enhancement of the biodegradation of crude-oil pollutants. *J. Pet. Technol.* **24**, 16.
50. Tanaka, A. and Fukui, S. (1971) Studies on the utilization of hydrocarbons by microorganisms (XIII). *J. Ferment. Technol.* **49**, 809.
51. Laha, S. and Luthy, R.G. (1991) Inhibition of phenanthrene mineralization by nonionic surfactants in soil–water systems. *Environ. Sci. Technol.* **25**, 1920–1930.
52. Laha, S. and Luthy, R.G. (1992) Effects of nonionic surfactants on the solubilization and mineralization of phenanthrene in soil–water systems. *Biotech. Bioeng.* **40**, 1367–1380.
53. Tiehm, A. (1994) Degradation of polycyclic aromatic hydrocarbons in the presence of synthetic surfactants. *Appl. Environ. Microbiol.* **60**, 258–263.
54. Mueller, J.G., Chapman, P.J., Blattman, B.O. and Pritchard, P.H. (1990) Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. *Appl. Environ. Microbiol.* **56**, 1079.
55. Almgren, M., Grieser, F. and Thomas, J.K. (1979) Dynamic and static aspects of solubilization of neutral arenes in ionic micellar solutions. *J. Am. Chem. Soc.* **101**, 279–291.
56. Turro, N.J., Zimmt, M.B., Lei, X.G., Gould, I.R., Nitsche, K.S. and Cha, Y. (1987) Additive effects of the CIDNP, cage effect, and exit rate of micellized radical pairs. *J. Phys. Chem.* **91**, 4544–4548.
57. Attwood, D. and Florence, A.T. (1983) *Surfactant Systems: Their Chemistry, Pharmacy, and Biology*, Chapman and Hall, London.
58. Shimp, R.J. and Young, R.L. (1988) Availability of organic chemicals for biodegradation in settled bottom sediments. *Ecotox. Environ. Safety*, **15**, 31–45.
59. Gordon, A.S. and Millero, F.J. (1985) Adsorption mediated decrease in the biodegradation rate of organic compounds. *Microb. Ecol.* **11**, 289–298.

60. Remberger, M., Allard, A.S. and Neilson, A.H. (1986) Biotransformation of chloroquacols, chlorocatechols, and chloroveratroles in sediments. *Appl. Environ. Microbiol.* **51**, 552–558.
61. Robinson, K.G., Ghosh, M.M. and Shi, Z. (1996) Mineralization enhancement of non-aqueous phase and soil-bound PCB using biosurfactant. *Wat. Sci. Tech.* **34**, 303.
62. West, C.C. and Harwell, J.H. (1992) Surfactants and subsurface remediation. *Environ. Sci. Technol.* **26**, 2324–2330.
63. Jain, D.K., Lee, H. and Trevors, J.T. (1992) Effect of addition of *Pseudomonas aeruginosa* UG2 inocula or biosurfactants on biodegradation of selected hydrocarbons in soil. *J. Ind. Microbiol.* **10**, 87–93.
64. Wershaw, R.L. (1993) Model for humus in soils and sediments. *Environ. Sci. Technol.* **27**, 814–816.
65. Crocker, F.H., Guerin, W.F. and Boyd, S.A. (1995) Bioavailability of naphthalene sorbed to cationic surfactant-modified smectite clay. *Environ. Sci. Technol.* **29**, 2953–2958.
66. Cooper, D.G. (1986) Biosurfactants. *Microbiol. Sci.* **3**, 145–149.
67. Zajic, J.E. and Seffens, W. (1984) Biosurfactants. *Crit. Rev. Biotechnol.* **1**, 87–107.
68. Kosaric, N. (1993) *Biosurfactants: Production, Properties, Applications*. M. Dekker, New York.
69. Georgiou, G., Lin, S.-C. and Sharma, M.M. (1992) Surface-active compounds from microorganisms. *Bio/Technology*, **10**, 60–65.
70. Zhang, Y. and Miller, R.M. (1992) Enhanced octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant). *Appl. Environ. Microbiol.* **58**, 3276–3282.
71. Van Dyke, M.I., Couture, P., Brauer, M., Lee, H. and Trevors, J.T. (1993) *Pseudomonas aeruginosa* UG2 rhamnolipid biosurfactants: structural characterization and their use in removing hydrophobic compounds from soil. *Can. J. Microbiol.* **39**, 1071–1078.
72. Arino, S., Marchal, R. and Vandecasteele, J.-P. (1997) Identification and production of a rhamnolipid biosurfactant by a *Pseudomonas* species. *Appl. Microbiol. Biotechnol.* **45**, 162–168.
73. Ochsner, U.A., Koch, A.K., Fiechter, A. and Reiser, J. (1994) Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.* **176**, 2044–2054.
74. Burger, M.M., Glaser, L. and Burton, R.M. (1963) Formation of rhamnolipids of *Pseudomonas aeruginosa*. *J. Biol. Chem.* **238**, 2595.
75. Koch, A.K., Kappeli, O., Fiechter, A. and Reiser, J. (1991) Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. *J. Bacteriol.* **173**, 4212–4219.
76. Guerra-Santos, L.H., Kappeli, O. and Fiechter, A. (1986) Dependence of *Pseudomonas aeruginosa* continuous culture biosurfactant production on nutritional and environmental factors. *Appl. Microbiol. Biotechnol.* **24**, 443–448.
77. Brock, T.D., Madigan, M.T., Martinko, J.M. and Parker, J. (1994) *Biology of Microorganisms*. Prentice-Hall, Inc. Englewood Cliffs, NJ.
78. Hinchee, R.E., Brockman, F.J. and Vogel, C.M. (1995) *Microbial Processes for Bioremediation*. Battelle Press, Columbus, OH.
79. Thibault, S.L., Anderson, M. and Frankenberger Jr., W.T. (1996) Influence of surfactants on pyrene desorption and degradation in soils. *Appl. Environ. Microbiol.* **62**, 283–287.
80. Liu, Z., Jacobsen, A.M. and Luthy, R.G. (1995) Biodegradation of naphthalene in aqueous nonionic surfactant systems. *Appl. Environ. Microbiol.* **61**, 145–151.

81. Falatko, D.M. and Novak, J.T. (1992) Effects of biologically produced surfactants on the mobility and biodegradation of petroleum hydrocarbons. *Wat. Environ. Res.* **64**, 163–169.
82. Herman, D.C., Lenhard, R.J. and Miller, R.M. (1997) Formation and removal of hydrocarbon residual in porous media: effects of attached bacteria and biosurfactants. *Environ. Sci. Technol.* **31**, 1290.
83. Fu, M.H. and Alexander, M. (1995) Use of surfactants and slurring to enhance the biodegradation in soil of compounds initially dissolved in nonaqueous-phase liquids. *Appl. Microbiol. Biotechnol.* **43**, 551–558.
84. Fountain, J.C. (1997) The role of field trials in development and feasibility assessment of surfactant-enhanced aquifer remediation. *Wat. Environ. Res.* **69**, 188.
85. Fountain, J.C., Klimek, A., Beikirch, M.G. and Middleton, T.M. (1991) The use of surfactants for *in situ* extraction of organic pollutants from a contaminated aquifer. *J. Hazard. Mater.* **28**, 295–311.
86. Pennell, K.D., Abriola, L.M. and Weber Jr., W.J. (1993) Surfactant-enhanced solubilization of residual dodecane in soil columns. I. Experimental investigation. *Environ. Sci. Technol.* **27**, 2332–2340.