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THE ROLE OF (BIO)SURFACTANT SORPTION IN PROMOTING THE BIOAVAILABILITY OF NUTRIENTS LOCALIZED AT THE SOLID–WATER INTERFACE

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ABSTRACT

Bioavailability is herein defined as the accessibility of a substrate by a microorganism. Further, bioavailability is governed by (1) the substrate concentration that the cell membrane "sees," (i.e., the "directly bioavailable" pool) as well as (2) the rate of mass transfer from potentially bioavailable (e.g., nonaqueous) phases to the directly bioavailable (e.g., aqueous) phase. Mechanisms by which sorbed (bio)surfactants influence these two processes are discussed. We propose the hypothesis that the sorption of (bio)surfactants at the solid–liquid interface is partially responsible for the increased bioavailability of surface-bound nutrients, and offer this as a basis for suggesting the development of engineered in-situ bioremediation technologies that take advantage of low (bio)surfactant concentrations. In addition, other industrial systems where bioavailability phenomena should be considered are addressed. © 1999 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved

KEYWORDS

Bioavailability; biofilms; bioremediation; biosurfactants; oligotrophic; surfactants.

INTRODUCTION

Many environmental systems are considered oligotrophic (from *oligo-*, "scant" and *-trophic*, "nutrition") because of the lack of assimilable organic carbon and/or other important nutrients present in the bulk fluid stream. Representative examples of such systems include industrial and domestic "clean" water process and distribution systems, aquifers, and the sediments of an alpine lake or stream. Even soils that have been subjected to contamination by hydrophobic pollutants often contain pore water chemical concentrations in parts per billion or lower. However, it is well known that microbial biofilms persevere in such systems. Thus, how can this significant biofilm activity be explained in environments of such apparent malnutrition?

The proliferation of biofilms in oligotrophic environments likely results from microbial response to nutrient availability at the solid–water interface (Kefford *et al.*, 1982; Bright and Fletcher, 1983; Remberger *et al.*, 1986; Griffith and Fletcher, 1991; Mueller, 1996). Nutrient accumulation at this interface occurs by a variety

of mechanisms that include physical processes (e.g., intraparticle diffusion, bound water partitioning, physiosorption), chemical processes (e.g., electrostatic interactions, intramineral and intraorganic partitioning, ion exchange, ligand exchange, and covalent bonding), and biological processes (e.g., complexation with biofilm matrix components). However, the degree to which biofilms thrive in these specialized microniches depends on the bioavailability of those nutrients and the strategies employed by biofilm bacteria to scavenge them. Thus, these environments may not be oligotrophic in the strictest sense because of the potentially high nutrient concentrations localized at the solid–water interface. We will thus refer to environs where little carbon is available in the aqueous phase but significant carbon is accumulated at the solid–water interface as "pseudo-oligotrophic". The existence of biofilms in many pseudo-oligotrophic environments, including industrial "clean" water systems (Gillis and Gillis, 1996), drinking water distribution systems (Camper, 1996), and soils contaminated with hydrophobic chemicals (Mihelcic *et al.*, 1993) emphasizes the need to understand and quantify the bioavailability of sorbed nutrients in these systems.

It is traditionally believed that bacteria can only assimilate aqueous phase nutrients (Wodzinkski and Coyle, 1974; Mihelcic *et al.*, 1993). However, recent research suggests that some microorganisms invoke effective scavenging strategies (Korber *et al.*, 1995; Stone, 1997; Tang *et al.*, 1998), including biosurfactant production (Haferburg *et al.*, 1986; Georgiou *et al.*, 1992), which enhance the bioavailability of nonaqueous phase-, and in particular, surface-(ad)sorbed nutrients. Thus, armed with the capability to facilitate bioavailability, microbes may thrive on a surface where the limiting nutrient may not be present in the aqueous phase.

The objectives of this discussion are (1) to identify processes that influence bioavailability of sorbed nutrients, (2) to summarize key interactions between (bio)surfactants, microbial cells, the solid–water interface, and hydrophobic nutrients, and (3) to address the impact of these processes on the use of engineered bioremediation technologies that take advantage of (bio)surfactant sorption.

BIOAVAILABILITY DEFIND

Bioavailability is loosely defined as the accessibility of a nutrient either by an organism capable of transporting the substrate across its outer membrane or by an extracellular enzyme. More specifically, bioavailability describes nutrient concentration *in,* and rate of mass transfer (e.g., desorption or dissolution) *to,* the *directly bioavailable* (e.g., aqueous) phase. "Directly bioavailable" (as opposed to *potentially bioavailable,* e.g., nonaqueous or sorbed) implies that a nutrient is in a phase (e.g., aqueous or "free dissolved") amenable to membrane transport or reaction with an exoenzyme.

Bioavailability vs. Bioreactivity. Understanding the difference between a nutrient's bioavailability and its bioreactivity is essential in order to predict its impact on biofilm activity. In short, bioavailability describes the physico-chemical phenomena that govern phase partitioning, speciation, mass transfer, and mass transport of a nutrient to the site of biological uptake (i.e., the microbial cell membrane) or extracellular transformation. Bioreactivity, on the other hand, governs whether or not the nutrient will be assimilated into a cell or transformed by an exoenzyme if *it is bioavailable.* Thus, while a nutrient may indeed be bioavailable, it may not necessarily be bioreactive (i.e., if the enzymatic and/or physiological machinery required for uptake and/or transformation are not present or inactive). Thus, the activity of a biofilm may be controlled by either bioavailability limitations or bioreactivity limitations (i.e., recalcitrance) (Huesemann, 1997).

Bioavailability Phenomena. A number of processes influence bioavailability in systems where a solid–water interface is present. Perhaps two of the most important phenomena are (1) reduction in the directly bioavailable nutrient concentration resulting from solid phase partitioning (e.g. sorption or precipitation) and speciation (e.g., ligand exchange), and (2) slow mass transfer rates from the potentially bioavailable (e.g. sorbed) to the directly bioavailable (e.g. aqueous) phase. These two phenomena are discussed in more detail.

Concentration-Limited Bioavailability. Concentration-limited bioavailability refers to the case by which the concentration of directly bioavailable species is reduced in response to phase partitioning phenomena. The effect of concentration on cell enzyme activity and growth is not difficult to conceptualize when one

considers the dependence of enzyme kinetics (e.g., as described by the Michaelis-Menten model) on substrate concentration. In short, concentration-limited bioavailability may be quite important for nutrients having a significant affinity for the solid surface.

Mass Transfer-Limited Bioavailability. Microorganisms tend to utilize nutrients that are present in the aqueous phase, and the assumption that *only* aqueous phase nutrient is *directly* bioavailable is a common one (Wodzinkski and Coyle, 1974; Mihelcic *et al.*, 1993). Depletion of the aqueous nutrient pool by microbes results in disequilibrium of aqueous-nonaqueous partitioning, thus promoting mass transfer (e.g., desorption, dissolution) of nutrients from nonaqueous (i.e., potentially bioavailable) to aqueous (i.e., directly bioavailable) phases. Further, when partitioning kinetics are *slower* than uptake/biotransformation kinetics, then bioavailability is *mass-transfer limited* (indicating the rate limitation of a partitioning process that results from slow boundary layer diffusion). The degree to which partitioning kinetics reduce nutrient bioavailability depends on the characteristic time for mass transfer (T_m^*) relative to the characteristic time for uptake/biotransformation (T_b^*) (Bouwer *et al.*, 1997). Thus, concentration-limited bioavailability is defined by the case where $T_m^* < \cong T_b^*$, while mass transfer-limited bioavailability is defined by the case where $T_m^* >> T_b^*$. The degree of concentration-limited bioavailability further depends on the affinity of the nutrient for the solid phase (e.g., the nutrient's equilibrium partitioning coefficient).

The following discussion will focus on how (bio)surfactant sorption influences (1) the directly bioavailable nutrient concentration, and (2) the kinetics of nutrient mass transfer to the directly bioavailable phase in response to nutrient assimilation by microorganisms. In addition, the effects of (bio)surfactant sorption on cell adhesion and cell membrane phenomena will be discussed briefly.

INFLUENCE OF (BIO)SURFACTANT SORPTION ON NUTRIENT BIOAVAILABILITY

Surfactants are amphiphilic molecules (i.e., having both hydrophilic and hydrophobic moeities) that tend to (1) form stable aggregates ("micelles") in solution above a critical concentration (i.e., the critical micelle concentration, or "CMC"); (2) solubilize hydrophobic compounds into their micellar core; (3) participate in speciation reactions with charged compounds; and (4) sorb to solid–water interfaces (Rosen, 1978). In addition, surfactants may influence the activity of membrane-bound proteins, alter cell adhesion properties, and influence the fundamental mechanisms of nutrient uptake in cells. Biosurfactants simply define those classes of surfactants that are synthesized by microorganisms. Since both chemical and biological surfactants possess many common characteristics with respect to their behavior in solution, we will hereinafter refer to them collectively as (bio)surfactants. Because different classes of nutrients react differently with (bio)surfactants, the following discussion will focus only on the reactions involving low-polarity hydrophobic organic compounds (HOC) that tend to associate with the hydrophobic moeities of (bio)surfactant monomers and the hydrophobic cores of their micelles.

We will highlight one particular feature of (bio)surfactants that has a direct consequence in evaluating nutrient bioavailability: accumulation of (bio)surfactant at the solid–liquid interface (i.e., sorption). (Bio)surfactant sorption may influence equilibrium partitioning stoichiometry and mass transfer kinetics of HOC between nonaqueous and aqueous phases. These phenomena will be investigated with a model that has been developed specifically for assessing the bioavailability of HOC in response to both concentration and mass transfer limitations during surfactant enhanced HOC biotransformation (Nichols *et al.*, 1998; Jordan *et al.*, 1998). For the purposes of this discussion, we have used the model to investigate the effects of (bio)surfactant sorption on HOC bioavailability in a solid (mineral surface)-water system, quantitatively describing the HOC and (bio)surfactant partitioning equilibria and kinetics and the kinetics of HOC biodegradation (Figure 1).

Model formulation. The model describes HOC speciation as linear, nonequilibrium (first-order mass transfer) partitioning between sorbed, sorbed micellar, aqueous, and aqueous micellar phases. Equilibrium (bio)surfactant speciation is governed by monomer sorption and micelle formation both at the surface and in solution. HOC biotransformation is modeled by first-order transformation kinetics, assuming that only free dissolved HOC is directly bioavailable. These processes are described by a coupled set of partial differential

equations and solved simultaneously with respect to both time and HOC concentrations. Detailed model formulation, implementation, and validation are described elsewhere (Nichols *et al.*, 1998).

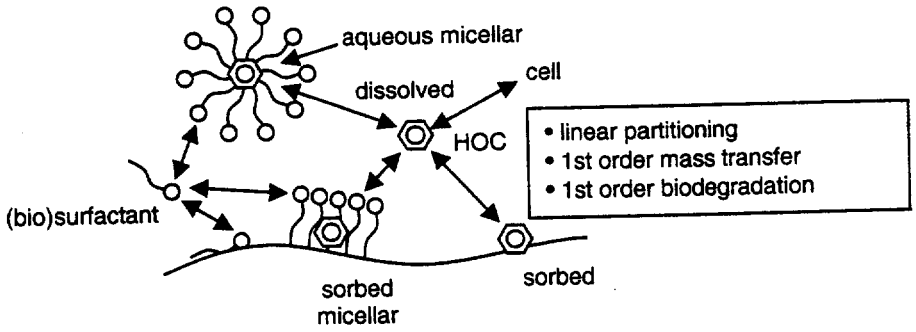

Figure 1. Bioavailability model for surfactant-enhanced biotransformation.

CASE 1: Influence of (bio)surfactant sorption on concentration-limited bioavailability

HOC partitioning in soil–water systems in response to (bio)surfactant addition has been studied extensively (Jafvert, 1991; Edwards *et al.*, 1991; Liu *et al.*, 1991; Sun and Boyd, 1993). Two primary conceptual models describe the influence of (bio)surfactant sorption on HOC speciation: one describes the reactions on mineral surfaces (e.g., metal oxides) and the other describes the reactions on organic surfaces (e.g., natural organic matter).

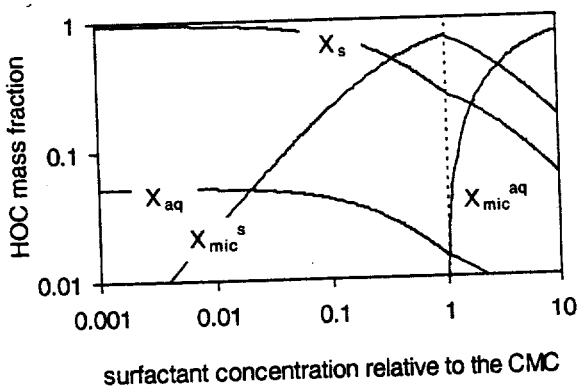

Figure 2. Influence of (bio)surfactant concentration relative to the CMC on *equilibrium HOC partitioning in the absence of biological activity* at the mineral–water interface. HOC mass distributes among mineral-sorbed (X_s), sorbed micellar (X_{mic}^s), aqueous micellar ($x_{mic}aq$), and free aqueous (X_{aq}) phases.

HOC partitioning in response to (bio)surfactant sorption on a mineral surface. (Bio)surfactants tend to form ordered aggregates on mineral surfaces (Rosen, 1978; Mannhardt *et al.*, 1992; Manne and Gaub, 1995), thus resulting in the development of surface hydrophobicity. As a result, HOCs, which normally have a low affinity for adsorption to the "clean," hydrophilic mineral surface (although they may still absorb into the intramineral porosity), may tend to partition into the micellar core of surface-bound (bio)surfactant aggregates. The formation of sorbed micelles on a mineral surface can have a profound impact on HOC partitioning. In particular, association of HOC with the hydrophobic core region of sorbed micelles results in a decrease in the free aqueous phase HOC mass fraction at low surfactant concentrations (Figure 2). If it is assumed that only free dissolved HOC (X_{aq}) is directly bioavailable, then (bio)surfactant sorption will tend to *decrease* bioavailability at low (bio)surfactant concentrations if HOC metabolism is indeed governed by the concentration of free dissolved HOC (e.g., Michaelis-Menton or nonzero-order kinetics). Figure 2 also shows that further increase in (bio)surfactant concentration beyond the CMC (indicating that the mineral's sorption capacity for (bio)surfactant has been reached and aqueous micelles begin to form) tends to further decrease the free aqueous phase HOC concentration via partitioning into aqueous phase micelles. In

summary, if cells do not have access to HOC partitioned into the hydrophobic core of either sorbed or aqueous phase micelles, then HOC bioavailability will likely decrease in the presence of (bio)surfactants at any concentration. This is illustrated in Figure 3, which shows that increasing (bio)surfactant concentration increases the biodegradation time required for a 2-log reduction in total HOC in a solid–water system.

HOC partitioning in response to (bio)surfactant sorption on an organic surface. Luthy and coworkers (Edwards *et al.*, 1994, 1994a, 1994b) have proposed a model for HOC partitioning at the soil-water interface in the presence of (bio)surfactants where HOC sorption is dominated by partitioning into natural organic matter (NOM). HOC phase distributions as a function of (bio)surfactant concentration are qualitatively similar to those observed for HOC{bio)surfactant partitioning at the mineral-water interface (cf. Figure 2). The primary difference between these two cases is that (bio)surfactant sorption to organic matter is governed by partitioning of (bio)surfactant monomers into the NOM matrix as opposed to monomer (ad)sorption and micelle formation at the mineral–water interface.

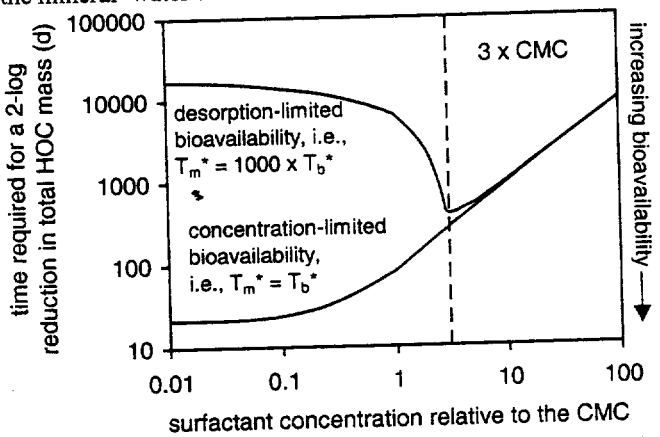

Figure 3. Influence of (bio)surfactant sorption on concentration vs. mass transfer-limited bioavailability.

It should be emphasized that a decrease in HOC bioavailability in response to increasing (bio)surfactant concentration is valid only if (1) microbial cells do not have direct access to HOC partitioned into the core of sorbed and aqueous phase micelles, and (2) partitioning equilibrium exists. While assumption (1) is likely to species-dependent (Guha and Jaffe, 1996, 1997) and is certainly valid in some pure-culture systems (and probably partially valid in mixed environmental cultures), assumption (2) is not likely to be valid in a biological system where the dissolution or desorption rate of nonaqueous phase HOC govern cell activity (e.g., as is often the case in soils contaminated with pure-phase or soil-sorbed HOC).

CASE 2: Influence of (bio)surfactant sorption on mass transfer-limited bioavailability

As mentioned earlier, microbial activity depletes the nutrient pool in the directly bioavailable (e.g., aqueous) phase. Consequently, microbial degradation of HOC should stimulate HOC mass transfer from sorbed (i.e., potentially bioavailable) to aqueous (i.e., directly bioavailable) phases. (Bio)surfactant sorption and micelle formation results in the introduction of additional pseudophases (sorbed and aqueous micelles) that are also involved in mass transfer processes, potentially altering the magnitudes of concentration gradients that drive mass transfer. Since the rate of mass transfer is governed by HOC concentration gradients across an interfacial boundary layer, then it is expected that the presence of (bio)surfactants alters bioavailability when bioavailability is mass transfer-limited.

Figure 3 illustrates the effects on bioavailability of (bio)surfactant sorption to a mineral surface where HOC desorption is limited by a slow mass transfer (desorption) rate. Increasing (bio)surfactant concentration up to the CMC increases the rate of HOC biotransformation (i.e., where rate is defined by the time (days) required to reduce the total system HOC mass by 100x) by over an order of magnitude. These results show that bioavailability is enhanced near the (bio)surfactant's CMC, which indicates the concentration at which the sorbent's (bio)surfactant sorption capacity is reached (and aqueous phase micelles form and begin to compete with sorbed phases for HOC partitioning). The primary mechanism by which (bio)surfactant

sorption enhances bioavallability is by increasing the HOC flux out of the soil phase (a more detailed description of this phenomena can be found in Jordan *et al.*, 1998). Increased sorbed-to-aqueous HOC flux is a result of a steeper concentration gradient for solid-water mass transfer that exists in the presence of (bio)surfactant phases. Well above the CMC, bioavailability decreases as a result of sequestration of HOC into aqueous (bio)surfactant micelles (cf. Figure 2) and the primary system control mechanism changes from mass transfer limited to concentration-limited bioavailability.

When slow HOC desorption from natural organic matter (NOM) limits bioavailability, the mechanisms by which (bio)surfactants influence HOC bioavailability are not well understood. However, because NOM itself has both amphiphilic and micellar characteristics, we propose the hypothesis that (bio)surfactant sorption to NOM results in a chemical and/or conformational change of the NOM aggregate in response to a "micellemicelle" interaction (Rosen, 1978). We are currently investigating this hypothesis in our laboratory.

Influence of (bio)surfactant sorption on microbiological phenomena

This discussion has focused on the effects of (bio)surfactant sorption on physico-chemical processes, i.e., phase partitioning equilibria and mass transfer. However, one should keep in mind that (bio)surfactant sorption may also influence microbiological processes. Most notably, it is well known that (bio)surfactant association with surfaces and outer membranes alters cell adhesion phenomena (Doyle, 1990; Neu, 1996; Velraeds *et al.*, 1996; Fletcher, 1996). In addition, the interaction of cell membranes with (bio)surfactants may result in changes in cell membrane structure and function (Schnaitman, 1971), affecting the physico-chemical (membrane structure) and biochemical (enzyme/protein conformation and/or activity) processes that are responsible for nutrient uptake and transformation (Breuil and Kushner, 1980; Lang *et al.*, 1989). Finally, interaction of the cell membrane with (bio)surfactant micelles may provide a mechanism for direct microbial access to hydrophobic nutrients accumulated within the micellar core (Reddy *et al.*, 1982; Guha and Jaffe, 1996, 1997). The importance of these mechanisms cannot be underestimated, as illustrated by the inability of a recent review to explain the efficacy of (bio)surfactant-enhanced bioremediation in terms of physicochemical phenomena alone (Rouse *et al.*, 1994). These authors particularly emphasized the inhibition of biotransformation when (bio)surfactants were present at supra-CMC concentrations, citing micellar toxicity as a possible suspect in inhibiting biodegradation. This observation was further supported in a later review that also showed evidence for inhibited HOC biotransformation near and above the CMC (Jordan and Cunningham, 1998). Inhibited biodegradation near the CMC could possibly suggest that even sorbed phase micellar structures could inhibit membrane function of bacteria attached to a micelle-coated surface.

CONCLUSION

Industrial water systems prone to bioavailability-influenced cell activity are primarily pseudo-oligotrophic environments where limiting carbon sources are either hydrophobic or otherwise concentrated at the solid–water interface. For example, water process and distribution systems containing humic substances, and soil/aquifer material contaminated with hydrophobic organic chemicals (e.g., high molecular weight alkanes, PAHs, and PCBs) are two primary candidates for the study of bioavailability phenomena.

We are currently investigating bioavailability limitations in soil contaminated with hydrophobic organic chemicals. We propose that using low (bio)surfactant concentrations may be the basis for a viable technology to promote the cleanup of contaminated soils based on the results presented in the previous discussion as well as recent literature (Aronstein *et al.*, 1991; Aronstein and Alexander, 1992; Rouse *et al.*, 1994; Jordan and Cunningham, 1998). The use of low (bio)surfactant concentrations (resulting in most of the (bio)surfactant existing in a sorbed phase) has a distinct advantage as an *in-situ* treatment technology (vs. the use of high surfactant concentrations that promote chemical solubilization) because bioavailability of chemicals from soil can be promoted via instantaneous desorption without increasing ecological and human health risk due to chemical solubilization, mobilization, and transport.

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