Influence of biosurfactant and non-biosurfactant producing bacteria on phenanthrene removal from model soils

by Julie Ann Eyre

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
Polycyclic aromatic hydrocarbons (PAHs, e.g. phenanthrene) may occur in the environment as a result of fossil fuel combustion or as by-products from industrial processes, or from natural processes such as forest fires. PAHs include mutagenic and carcinogenic compounds, emphasizing the need for efficiently controlling and predicting their fate and transport in the subsurface.

Some PAH remediation technologies that are currently in use include pump and treat systems, soil vapor extraction, and excavation. Each of these technologies are expensive and time consuming treatments. Bioremediation offers a cost effective and efficient remediation option.

Because PAHs are hydrophobic, they tend to sorb strongly to soil particles. PAH biotransformation in soil is often limited by the rate at which PAHs can desorb from the soil. Previous research has shown that surfactant or biosurfactant addition increases the rate and extent of desorption and biotransformation of phenanthrene in soil. In this research, the expression “biosurfactant” is a surfactant produced by bacteria and “surfactant” is used for a synthetically produced surfactant.

Few studies have examined how in-situ biosurfactant production effects desorption and biotransformation. The general goal of this research project was to examine the effects of in-situ biosurfactant production on desorption and biotransformation of phenanthrene from two different types of model poly-tetra-fluoro-ethylene (PTFE) particles, porous and non-porous.

Results indicate that phenanthrene initially adsorbed to PTFE particles can be biotransformed by biosurfactant and non-biosurfactant producing bacteria. Instantaneous desorption was increased in the presence of both strains of bacteria. Biosurfactant producing bacteria were just as effective at phenanthrene biotransformation per cell mass than non-biosurfaptant producing bacteria. It was determined that the maximum concentration in the aqueous phase was a factor of the mass of phenanthrene initially adsorbed and the partition coefficient. Most likely once phenanthrene desorbs from the surface of the particles, it can re-adsorb either to the particle surface or on biomass present in the column, before it is carried put of the column.

INFLUENCE OF BIOSURFACTANT AND NON-BIOSURFACTANT PRODUCING BACTERIA ON PHENANTHRENE REMOVAL FROM MODEL SOILS

by

Julie Ann Eyre

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana
December, 1999

APPROVAL

of a thesis submitted by

Julie Eyre

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. A. Cunningham (co-chair)	(Signature)	Dec 14, 1999 (Date)
Dr. J. Sears (co-chair)	John T. Sears (Signature)	Dec 14, 1999 (Date)

Approved for the Department of Chemical Engineering

Dr. J. Sears (Dept. Head)	John T. Sears (Signature)	Dec 14, 1999 (Date)

Approved for the College of Graduate Studies

Dr. B. McLeod (Graduate Dean)	Bruce R. McLeod (Signature)	12-17-99 (Date)

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University-Bozeman, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature Cynthia Agee

Date 12/17/99

ACKNOWLEDGEMENTS

I would like to thank my committee members: Al Cunningham, John Sears, Anne Camper, and Phil Stewart for their support, guidance, and interest in this research project. Their ability and patience in answering my numerous questions was greatly appreciated.

Thanks also to Ryan Jordan, acting as my mentor throughout this research project. His insight and knowledge was a significant help to me. Finally thanks to the Bioremediation Lab group, for many hours of in-sight in the lab as well as on presentations and writing.

Completion of this, and almost every project at the Center for Biofilm Engineering, would be nearly impossible without the assistance of the finest laboratory manager: John Neuman. John's attention to detail and desire to help students, at all hours of the night, including weekends, was an invaluble asset.

This research was supported by the Center for Biofilm Engineering at Montana State University, a National Science Foundation-sponsored engineering research center.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs, e.g. phenanthrene) may occur in the environment as a result of fossil fuel combustion or as by-products from industrial processes, or from natural processes such as forest fires. PAHs include mutagenic and carcinogenic compounds, emphasizing the need for efficiently controlling and predicting their fate and transport in the subsurface.

Some PAH remediation technologies that are currently in use include pump and treat systems, soil vapor extraction, and excavation. Each of these technologies are expensive and time consuming treatments. Bioremediation offers a cost effective and efficient remediation option.

Because PAHs are hydrophobic, they tend to sorb strongly to soil particles. PAH biotransformation in soil is often limited by the rate at which PAHs can desorb from the soil. Previous research has shown that surfactant or biosurfactant addition increases the rate and extent of desorption and biotransformation of phenanthrene in soil. In this research, the expression "biosurfactant" is a surfactant produced by bacteria and "surfactant" is used for a synthetically produced surfactant.

Few studies have examined how in-situ biosurfactant production effects desorption and biotransformation. The general goal of this research project was to examine the effects of in-situ biosurfactant production on desorption and biotransformation of phenanthrene from two different types of model poly-tetra-fluoro-ethylene (PTFE) particles, porous and non-porous.

Results indicate that phenanthrene initially adsorbed to PTFE particles can be biotransformed by biosurfactant and non-biosurfactant producing bacteria. Instantaneous desorption was increased in the presence of both strains of bacteria. Biosurfactant producing bacteria were just as effective at phenanthrene biotransformation per cell mass than non-biosurfactant producing bacteria. It was determined that the maximum concentration in the aqueous phase was a factor of the mass of phenanthrene initially adsorbed and the partition coefficient. Most likely once phenanthrene desorbs from the surface of the particles, it can re-adsorb either to the particle surface or on biomass present in the column, before it is carried out of the column.

CHAPTER 1

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs, e.g. phenanthrene) are hydrophobic and they tend to sorb strongly to soil particles. PAHs include mutagenic and carcinogenic compounds, emphasizing the need for efficiently controlling and predicting their fate and transport in the subsurface. Current remediation technologies include pump and treat systems, soil vapor extraction, and excavation. These technologies are expensive and time consuming. Bioremediation potentially offers a cost effective and efficient remediation option. Previous research has shown that surfactant or biosurfactant addition enhances bioremediation (Alexander *et al.*, 1991; Falatko *et al.*, 1992; Alexander *et al.*, 1993; Alexander *et al.*, 1992; Michelic, 1993). In this research, the expression "biosurfactant" is a surfactant produced by bacteria and "surfactant" is used for a synthetically produced surfactant.

Purpose

The purpose of this research was to examine the effects of in-situ biosurfactant production on desorption and biotransformation of phenanthrene from two different types of model poly-tetra-fluoro-ethylene (PTFE) particles, porous and non-porous.

Two different strains of bacteria, *Pseudomonas saccharophilia* P15, a non-biosurfactant producing bacteria, and *Pseudomonas aeruginosa* 19SJ, a biosurfactant

producing bacteria, were used in this research project to determine if initially adsorbed phenanthrene could be biotransformed. The effects that these bacteria have on desorption of initially adsorbed phenanthrene was also examined. The mass of phenanthrene removed by desorption and biotransformation was combined together to determine the apparent cumulative removal. Apparent cumulative removal is equal to the mass of phenanthrene removed by desorption plus the mass removed by biotransformation.

Physical morphology of subsurface soils can also affect the bioavailability of sorbed hydrocarbons. Naturally occurring soil particles contain pores of many different sizes, many of which are smaller than the size of most microorganisms. Bioavailability of a PAH may be limited by desorption and diffusion out of these soil pores. Two different types of PTFE particles were used in this research to examine the effects of porosity on bioavailability of initially adsorbed phenanthrene, one without pores and one with pores unavailable to microorganisms.

Previous research has shown that the addition of surfactants or biosurfactants can increase the rate of desorption (DiVincenzo, 1996), however if applied above the critical micelle concentration (CMC), biotransformation is inhibited (Michelic, 1993). If surfactants or biosurfactants are applied below the CMC, biotransformation can be enhanced (Alexander *et al.*, 1991; Falatko *et al.*, 1992; Alexander *et al.*, 1993; Alexander *et al.*, 1992).

Background

Polycyclic aromatic hydrocarbons (PAHs, e.g. phenanthrene) may occur in the environment as a result of fossil fuel combustion, as by-products from industrial

processes, or from natural processes such as forest fires. PAHs include mutagenic and carcinogenic compounds, emphasizing the need for efficiently controlling and predicting their fate and transport in the subsurface. Figure 1.1 shows the chemical structure of

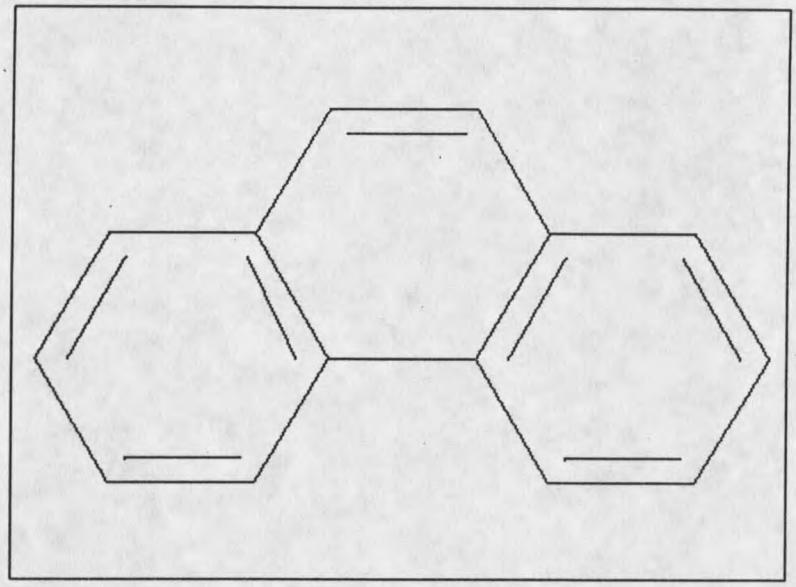

Figure 1.1 – Chemical structure of a phenanthrene molecule.

phenanthrene.

Once phenanthrene is present in the environment, it can partition into four phases. Phenanthrene can adsorb to soil surfaces, dissolve in the bulk aqueous fluid, vaporize into vapor, or remain among a non-aqueous phase liquid (NAPL). Since phenanthrene is hydrophobic, most phenanthrene will be present adsorbed to the soil surface. This research focuses on the saturated zone, where vapors are not present.

Previous research has shown that the addition of surfactants or biosurfactants can increase the rate of desorption (DiVincenzo, 1996), however if applied above the critical micelle concentration (CMC), biotransformation is inhibited (Michelic, 1993). If surfactants or biosurfactants are applied below the CMC, biotransformation can be

enhanced (Alexander *et al.*, 1991; Falatko *et al.*, 1992; Alexander *et al.*, 1993; Alexander *et al.*, 1992).

A surfactant molecule, or monomer, has a hydrophobic tail and a hydrophilic head. An example of the chemical structure of a synthetic surfactant molecule, Triton X-100 is shown in Figure 1.2. One surfactant molecule is known as a monomer. In

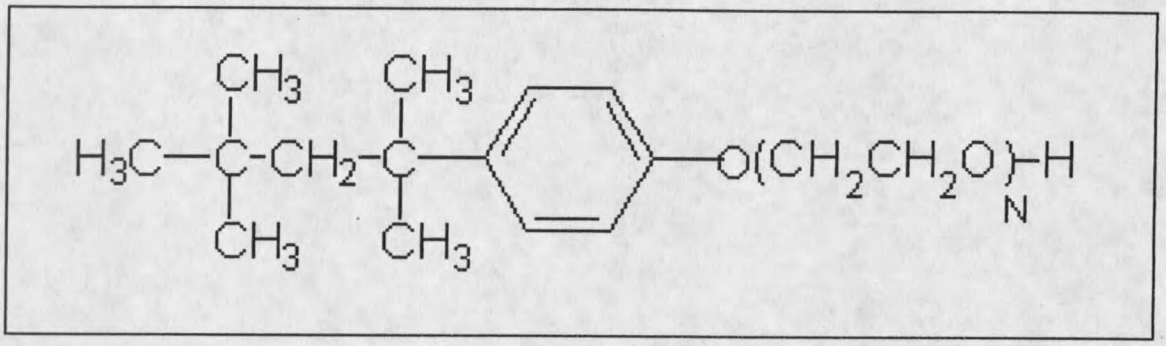

Figure 1.2 – Example of chemical structure of a synthetic surfactant molecule, Triton X-100. N is approximately equal to 10. The hydrophobic tail is the carbon chain on the right side of the structure.

soil/aqueous environments, the hydrophobic tail will most likely sorb to the soil surface (as long as the surface is hydrophobic), while the hydrophilic head will be associated with the aqueous phase (Figure 1.3). As surfactant concentration increases, binding sites are filled on the soil surface, causing sorbed monomers to form hemicelles. With

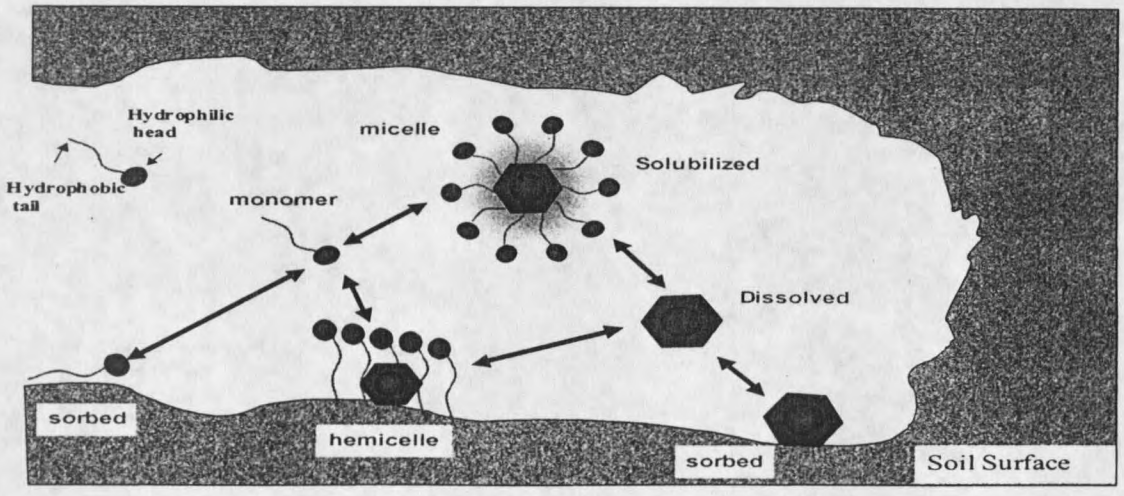

Figure 1.3 – Interactions of biosurfactant with soil and PAH

increasing surfactant concentration, the binding sites become occupied and micelles will form in the aqueous phase. The concentration at which this happens is referred to as the critical micelle concentration (CMC). When hemicelles and micelles are present, the tails form hydrophobic regions that will promote the redistribution of PAHs from the aqueous phase (Figure 1.3).

Physical morphology of subsurface soils can also affect the bioavailability of sorbed PAHs. Naturally occurring soil particles contain pores of many different sizes, many of which are smaller than the size of most microorganisms but large enough to allow diffusion of PAHs into the pores. Bioavailability of a PAH may be limited by adsorption, desorption, and diffusion into and out of these soil pores.

Prior Research

Biotransformation of Initially Adsorbed PAHs. Experimental evidence has shown that microorganisms are most effective at biotransforming dissolved organic chemicals, and that the concentration in the bulk water determines the rate of uptake (Bouwer *et al.*, 1998). Even if a bacterial cell is in direct contact with sorbed materials, there are theoretical arguments and experimental evidence suggesting that direct biotransformation of sorbed organic molecules will be insignificant. Van Loosdrecht *et al.* (1990) have described how geometric analysis suggests that only a small fraction of the bacterial surface can be in direct contact with the adsorbed materials on the soil surface. Most of the bacterial surface cannot be in direct contact with the solid and diffusion directly from the solid particle into the microbial cell is slow. The direct transfer of substrate between

bacterial cell and soil surface would be of limited capacity compared to transfer through the aqueous phase (van Loosdrecht *et al.*, 1990).

Alexander *et al.* (1986) suggested that microorganisms may biotransform a substrate that has minimal aqueous solubility by one or a combination of these mechanisms. It could be biotransformed the instant it dissolves in water, after its aqueous solubility has been biologically enhanced, or by mechanisms involving physical contact with the solid phase of the substrate. Growth of pure cultures of bacteria on naphthalene, phenanthrene, and anthracene was faster on those solid substrates having higher water solubility (Alexander *et al.*, 1986). This research only compared the rate of dissolution and degradation, and did not address the mechanisms by which microorganisms biotransformed substrates with minimal aqueous solubility. Additional work must be performed which assess these mechanisms (Alexander *et al.*, 1986).

Studies have been performed which demonstrate that the biotransformation of an organic substrate can occur when it is initially adsorbed. Alexander *et al.* (1995) performed a column study measuring the biotransformation of phenanthrene initially adsorbed in column studies under constant, intermittent, or no water flow conditions. In all three flow conditions phenanthrene was biotransformed; however, less was biotransformed under intermittent or no water flow conditions. The authors concluded that this result was due to lack of oxygen present in the column under intermittent or no water flow conditions.

Cornelissen *et al.* (1998) examined the biotransformation and desorption rates of 15 PAHs initially adsorbed to sediments. Biphasic biotransformation profiles were observed: an initial phase of rapid biotransformation, followed by a later phase of slow

biotransformation. Desorption profiles were also biphasic, indicating that a large fraction desorbs fast, while a smaller fraction desorbs much slower. The authors concluded from the biphasic biotransformation profiles that the initially rapid biotransformation is limited by microbial factors, while the slower biotransformation is limited by mass transfer.

Carmichael *et al.* (1997) examined the rates of biotransformation of soils impacted with aged PAHs. The rates of desorption were much slower than biotransformation, suggesting that desorption may control biotransformation. By keeping the bulk liquid concentration essentially zero, this study also determined that bacterial cells were able to biotransform the PAHs the instant they desorbed.

The rate of substrate dissolution or desorption may limit bacterial growth. Linear biotransformation trends can be explained through the use of a first order mass transfer equation (Volkering *et al.*, 1992). Volkering *et al.* (1992) compared theoretical biotransformation curves, derived using a first order mass transfer model, to experimental curves obtained from biotransformation of solid naphthalene. The results indicated that the biotransformation of solid naphthalene could be described using the first order mass transfer equation. These results indicate that biotransformation depends on desorption or mass transfer from the sorbed or solid phase to the aqueous phase.

Michelcic *et al.* (1993) developed a model which assumed that sorbed substrate was not biotransformed. It assumed that both suspended and attached cells could biotransform soluble substrate and that desorption of sorbed compounds was instantaneous. In other words the soil-water distribution was always at equilibrium. This model best fits experimental data of biotransformation of a sorbed substrate (Michelcic *et al.*, 1993).

Other studies suggest that sorbed compounds are available to microorganisms without prior desorption (Crocker, 1995). Remberger *et al.* (1986) suggested that sorbed substrates are available for biotransformation, though it was not proven whether biotransfomation occurred in the sorbed state or after desorption.

Apparent Cumulative Removal (Desorption Plus Biotransformation). For a remediation technology to be viewed as a viable option, it must be efficient at removing both dissolved PAHs in the groundwater and PAHs sorbed to soil, while minimizing the extent of the PAH contamination of groundwater and soil. When bioremediation is applied correctly it has the potential to biotransform the dissolved PAHs, increase the mass transfer from the soil to the groundwater, and minimize the extent of the PAH contamination in the groundwater and soil.

One field application of bioremediation is natural attenuation. A large amount of research has been performed on the effects that natural attenuation has on the size of dissolved plumes in the environment. Natural attenuation is the natural degradation of compounds in the environment via indigenous microorganisms, volatilization, or abiotic reactions (i.e. hydrolysis) (Borden 1994). If indigenous organisms that have the capability of biotransforming the constituent of interest, and electron acceptors are available (oxygen, nitrate, etc.) then natural attenuation of the dissolved plume through biotransformation processes is possible. Biotransformation usually occurs at the edge of the plume where dissolved oxygen and other electron acceptors are plentiful in the groundwater. Biotransformation on the edge of the plume causes the plume to shrink, decreasing the mobility of the contaminants.

Laboratory batch experiments have demonstrated that the presence of bacteria can enhance the rate of dissolution. Alexander *et al.* (1986) determined in a batch experiment with octadecane that the biotransformation rate was 200 times faster than its spontaneous dissolution. This result was consistent with the findings of Goma *et al.* (1974).

Alexander *et al.* (1991) performed batch experiments with phenanthrene-impacted high organic and low organic content soils. In these experiments it was found that even when non-detectable concentrations of phenanthrene desorbed from the surface, phenanthrene was still biotransformed. Phenanthrene was biotransformed from the high organic soils at a much slower rate than from low organic soils, or soils that phenanthrene can readily desorbed from. The results in this study indicate that even if a PAH will not readily desorb from soil, bacteria still have the capability of biotransforming it.

Rijnaarts *et al.* (1990) performed an experiment to investigate the effects of desorption, from soil aggregates, on the biotransformation kinetics of α-hexachlorocyclohexane (α–HCH). Desorption and biotransformation was shown to be controlled by intraparticle mass transfer processes. This was determined by applying two different models to the experimental data. A general first order model and sorption-retarded radial diffusion model were created. When applied to experimental data, the sorption-retarded radial diffusion model fit the data best, indicating that intraparticle mass transfer controlled desorption and biotransformation rates (Rijnaarts *et al.*, 1990).

Harms (1996) found similar results as Rijnaarts *et al.* (1990). Harms examined the effect of substrate separation from bacteria on biotransformation rates, and concluded that biotransformation rates can be enhanced by promoting the effective diffusivity of a substrate or by decreasing the average distance between the cells and the substrate.

Bosma *et al.* (1997) created a model for biotransformation that takes into account the biochemical activity of microorganisms and mass transfer of a chemical to the microorganism. Through the application of this model to experimental data, it was again found that mass transfer of the substrate to the microorganism was the most critical component of biotransformation and not the biochemical activity of the microorganism.

Michelic *et al.* (1993) reported that high substrate concentration and fast mass transfer rate to the cell surface of the microorganism are two benefits to a microorganism growing on a solid surface with a desorbing substrate. The enhanced mass transfer from the solid surface to the microorganism is due to a shorter diffusion distance. Furthermore, it is believed that facilitated nutrient uptake at a solid surface may enhance biotransformation, especially in systems where nutrient concentrations are low (Michelic *et al.*, 1993).

Bellin *et al.* (1993) investigated the effects of bacterial biomass on the sorption and transport of naphthalene in soils. It was determined that the presence of bacteria actually decreased the sorption of naphthalene in the soils. Bacteria were first grown on the soil and then naphthalene was flushed through the column. The study does not mention if the bacteria were hydrophobic or hydrophilic, but it does indicate that once the substrate enters the bulk aqueous phase, it is unlikely to sorb to the biomass (Bellin *et al.*, 1993).

Surfactant Enhanced Bioremediation. In recent years the field of bioremediation has been investigating the application of biosurfactants to hydrocarbon impacted sites. Most of this research has been investigating the effects of surfactant addition as opposed

to stimulating bacteria *in-situ* to produce biosurfactant, or injecting bacteria capable of producing biosurfactant. For this review "biosurfactant" is a surfactant produced by bacteria, while "surfactant" is a surfactant produced synthetically.

Research has been performed which shows that surfactants must be applied above the critical micellar concentration (CMC) to promote mobilization of hydrocarbons from soil surfaces (DiVincenzo, 1996). However, surfactants applied above the CMC prohibit biotransformation of adsorbed hydrocarbons (Michelic, 1993).

Alexander *et al.* (1991) found that non-ionic surfactants applied below the CMC increased phenanthrene biotransformation even though it did not enhance the extent of desorption. The authors believe that the rate of instantaneous desorption was increased by the addition of surfactant, perhaps by altering the strength of sorption or complexation of the substrate in some way that the compound becomes more available for microorganisms without appearing in the bulk solution (Alexander *et al.* 1991).

Alexander *et al.* (1993) examined whether surfactants applied at a distance from phenanthrene could still stimulate phenanthrene biotransformation. Surfactants were applied at concentrations above the CMC and below the CMC. In both cases phenanthrene biotransformation was increased: however, the addition of surfactant below the CMC had the greater effect on phenanthrene biotransformation. Again the authors concluded that the rate of instantaneous desorption was increased by the addition of surfactant, perhaps by altering the strength of sorption or complexation of the substrate in some way that the compound becomes more available for microorganisms without appearing in the bulk solution (Alexander *et al.* 1993).

The availability of hydrocarbons associated with the micellar phase of surfactants has been in question. Jaffe *et al.* (1996) created a mathematical model which examined the effective bioavailability of phenanthrene in the micellar phase. The model simulated experimental data well, indicating that a fraction of the micellar-phase hydrocarbons were directly bioavailable. For three different surfactants tested, the bioavailable micellar phase decreased with increasing surfactant concentration. The authors concluded that the optimum surfactant concentrations for biotransfomation are below the CMC (Alexander *et al.* 1996).

Falatko *et al.* (1992) examined the effects of biosurfactants on the solubility and biodegradation of petroleum hydrocarbons. Biosurfactants used for this study were produced on two different types of substrates, gasoline and a mixture of glucose with vegetable oil. It was determined that both types of biosurfactant increased the solubility, but the gasoline grown biosurfactants did not inhibit biotransformation, whereas biotransformation was inhibited by biosurfactants produced on the glucose and vegetable oil mixture (Falatko *et al.* 1992).

Bioavailability. Bioavailability is generally defined as the availability of a chemical to biotransformation, and it is determined by the extent to which a chemical is exposed to organisms (Hamelink *et al.* 1994). It is generally believed that bioavailability of hydrocarbons is limited by their low aqueous solubility, assuming that hydrocarbons can only be biotransformed in the aqueous phase. This is best explained by noting that intrinsic microbial kinetics are often best described by models that incorporate the dependence of substrate concentration on the rate of biotransformation

(i.e. Michaelis-Menton or first-order kinetics). Biotransformation rates should be faster when substrate concentrations are higher (up to a point, where the substrate concentration may either inhibit microbial activity via toxicity or saturate the enzymes responsible for biotransformation). Since hydrocarbon concentrations in water are limited by their solubility, one would expect that their bioavailability would be limited as well. Partitioning into non-aqueous phases (non aqueous phase liquids, soil particles, etc.) may decrease the aqueous phase concentration of the hydrocarbon to values well below its solubility limit, further limiting its bioavailability and resulting in slow biotransformation rates (Jordan *et al.* 1999).

Bioavailability is not necessarily limited by solubility alone. In multiphase systems, where hydrocarbons may partition among aqueous and non-aqueous phases, biotransformation may be limited by partition rates. If the characteristic time for mass transfer, or partitioning, to the aqueous phase is slower than the characteristic time for biotransformation, then mass transfer may limit bioavailability (Jordan *et al.* 1999).

Examining the physical morphology of subsurface solids can provide insight into to how subsurface solids can impact the biotransformation of sorbed hydrocarbons. Naturally occurring particles contain pores of many different sizes, many of which are smaller than the sizes of most microorganisms. For example, analysis of one of the coarser sands size from the Borden Aquifer in Ontario, Canada, indicated that roughly 50 percent of the intraparticle pore volume resides in pores that are less than 0.1 μm in diameter. Pores with a diameter larger than 1 μm comprised about 12 percent of the total pore space, while only about 5 percent of the pore volume was attributed to pores larger than 2 μm. Most indigenous bacteria range from 0.5 to 1.0 μm, therefore most bacteria

will be physically excluded from most of the intraparticle pores of these grains. The mean diameter of intraparticle pores occupied by bacteria has been estimated to be typically larger than 2 μm. This is likely to be larger than the intraparticle pore spaces in most natural soils (Bouwer *et al.* 1998).

Even if bacteria are in direct contact with sorbed hydrocarbons, there are theoretical arguments and experimental evidence to suggest that direct biotransformation of sorbed hydrocarbons will be insignificant. Most experimental evidence collected to date shows that bacteria are indeed most effective in using dissolved hydrocarbons, and the concentration in the aqueous fluid determines the rate of biotransformation and bioavailability (Bouwer *et al.* 1998).

The impact of adsorption on biotransformation is still not fully understood. Complex models have been developed, which attempt to fully describe the effect of adsorption on biotransformation. These models are most often dependent on the conditions of the model simulation (i.e. numerical calculations of the models are needed in order to estimate the overall effect of adsorption/desorption on the rate and extent of biotransformation).