

# Effects of Surfactants on the Biodegradation of Toluene in Soil

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## (Abstract)

The objective of this investigation was to observe and evaluate the effects of surfactants on the biodegradation of toluene in the subsurface. Soil microcosms containing gasoline were used to simulate conditions of an aquifer contaminated with hydrocarbons. The surfactants were the nonionics, Surfonic N-40 and N-95. Three volumes of gasoline, 0.005, 0.05, and 0.1 mL, were injected into separate microcosms in order to observe changes in toluene concentration in both adsorbed component conditions and free-phase, interstitial gasoline conditions. The data presented are the results from the microcosms containing 0.005 mL gasoline. Dissolved toluene was only partially biodegraded in the microcosms containing free-phase hydrocarbons. The lack of complete biodegradation was believed to be due to substrate toxicity.

The surfactants coated the microcosm soil with hemimicelles, which partitioned toluene from solution. The concentration of surfactant required to reach a minimum surface tension in microcosms increased two orders of magnitude over that required to reach the critical micelle concentration in distilled water. The results of partitioned toluene into surfactants on soil was a reduced toluene concentration in solution, which, in turn, decreased the biodegradation rate.

Surfactants may be beneficial for the mobilization of free phase hydrocarbons in the subsurface. The cost to satisfy the surfactant demand imparted by the soil must be considered. In this investigation, predominantly adsorbed hydrocarbons; which may be associated with smaller volume, more volatile hydrocarbons; dominated the system. When surfactants were applied physicochemical mobility was not enhanced and biological degradation was reduced.

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## Chapter 1

### INTRODUCTION

Groundwater comprises approximately two-thirds of the world's freshwater supply including that which is not readily available in ice caps and glaciers (Freeze and Cherry, 1979). When only readily available, freshwater resources are considered, groundwater represents 95 percent of the world's supply (Freeze and Cherry, 1979). It is the drinking water source for over half of the population in the United States. The average residence times of groundwater in the subsurface are on the order of tens to thousands of years including shallow and deep circulations. It is imperative that protective and remedial measures are taken to preserve this slowly renewable resource.

Groundwater resources are frequently contaminated with organic and inorganic pollutants from surface activities. Leaking underground storage tanks, pipelines, waste disposal sites, and spills represent a large proportion of the sources of groundwater contamination. Immiscible organic fluids, like liquid hydrocarbons, that are lost to the subsurface present a wide-spread threat to groundwater resources. Even though some of the lost fluids may be recovered, residual, immobile organics in the subsurface can act as a long-term source of groundwater contamination (Barker and Mayfield, 1988).

Some of the methods used to clean-up or control contaminated subsurface sites, including sites stricken with hydrocarbon contamination are; excavation of the contaminated soil and disposal in



landfills, manipulation of the hydraulic gradient in an area while extracting groundwater for treatment at the surface, isolation of the area with relatively impermeable barriers, enhanced volatilization, in-situ biodegradation, and enhanced organic mobility through the use of surfactant solutions (Wilson et al., 1985).

Each of these techniques suffers from certain limitations. Soil excavation can be very expensive. In addition, one must assume perpetual liability for the landfilled soil. Pumping and treating techniques are not efficient for removing the residual organic liquids which are associated with liquid hydrocarbon contamination. Isolating the contaminated site involves the risk of fluctuating water tables perpetuating the likelihood of increased contamination. Enhanced volatilization is insufficient for non-volatile compounds. Biological degradation of subsurface contamination has proven successful for contaminant reduction, but is relatively slow for large amounts of liquid hydrocarbons in residuum.

Surfactants have proven successful in the mobilizations of liquid hydrocarbons in residuum as demonstrated by Tuck et al. (1988) and others (Vignon and Rubin, 1989). Adsorbed organic components, however, react differently to surfactant mobilization techniques than separate phase, organic liquids. Adsorbed components may persist in the aquifer and could continue to contaminate groundwater.

A combination of both soil-washing and biological degradation techniques could prove to be an effective and economical remedial measure. Rapid removal of large amounts of liquid organics could be

achieved through surfactant-amended, purging solutions followed by the near-complete degradation of the remaining components biologically. Literature, however, addressing the potential of applying both techniques together is lacking.

The objective of this investigation was to observe and evaluate the effectiveness of applying the surfactant washing technique together with bioremedial methods on contaminant reduction in the subsurface. Changes in dissolved toluene concentration were used to monitor gasoline biodegradation in soil microcosms both with and without surfactant amendments. Biodegradation rates, initial and final toluene concentrations, and microbial growth rates in microcosms with and without surfactants were compared.

Initial results suggested that the fate of the surfactant in soil was important in determining its effects on toluene biodegradation. An additional objective was added to determine the fate of the surfactant in soil in which critical micelle concentrations of the surfactants were measured.

## Chapter 2

### LITERATURE REVIEW

#### INTRODUCTION

Historically, groundwater was considered to be relatively unaffected by surface activities. Recently, however, it has been shown that groundwaters are contaminated by organic and inorganic constituents with alarming frequency. Liquid hydrocarbons represent a major source of all groundwater contamination. It was estimated that 10 to 30 percent of some 3.5 million reported underground storage tanks in the United States may be leaking (Dowd, 1984). In addition to the reported incidents numerous unreported and undetected losses occur.

Gasoline and oils in the subsurface pose challenging problems to remediation scientists. The complexities inherent in the subsurface like aquifer heterogeneity and the characteristics of multiphase flow in porous media require innovative remedial technologies. In order to effectively improve the quality of aquifers and groundwaters contaminated with oil or gasoline, one must understand the behavior and fate of immiscible fluids in the subsurface, and be familiar with the remediation technologies available.

#### LIQUID HYDROCARBONS IN THE SUBSURFACE

The behavior of liquid hydrocarbons in the subsurface is controlled physically and chemically by the intrinsic properties of the invading fluid and the geologic materials receiving it. Fluid density and viscosity, subsurface permeability, pore size, and degree of wetness

drastically affect the flow of liquid hydrocarbons. Fractured rock passes oil in a manner quite unlike unconsolidated material, therefore, this discussion of the behavior of hydrocarbons in the subsurface will center around flow through porous media.

Oil released into the zone of aeration, which is that part of the aquifer above the water table, is actively drawn by capillary attraction into the soil pore spaces (Wilson and Ward, 1987) under oil-wet conditions. In the case of water-wet conditions, gravity plays an important role in the vertical migration of the hydrocarbon. The bulk of the oil migrates downward toward wetter regions of the aquifer. The capillary effect on the hydrocarbon becomes less prevalent as the amount of water in the soil increases. Provided that sufficient quantities of the liquid hydrocarbon are present to saturate the soil pore spaces and allow bulk flow, the oil will continue toward the water table. Smaller volumes of oil may not reach the water table, but instead may be held interstitially by capillary attraction and surface tension.

The relatively thin aquifer zone immediately above the water table in which water is held by capillary forces is called the capillary fringe. As the migrating mass of hydrocarbon reaches the capillary fringe it begins to move preferentially through channels of lower water saturation by displacing air (Wilson and Ward, 1987). This phenomenon is known as interfingering. If the bulk density of the hydrocarbon is less than that of water, the downward migrating mass of oil physically depresses the capillary fringe and the water table. The result of this type of migration is a pancake-shaped lens of immiscible fluid on the

water table. The oil tends to slowly flow in the direction of water flow until residual saturation is reached. In the case of a large volume, denser than water hydrocarbon, the bulk flow of oil is downward beyond the water table, displacing water, until residual saturation conditions with respect to the oil prevail or until an impermeable boundary is reached. In each case, components dissolve from the whole liquid hydrocarbon into groundwater. Groundwater may transport the organic components, which are retarded to varying degrees along the way by interactions with the surfaces and films of the soil matrix.

An important aspect of the above is that left in the trail of the migrating bulk fluid is a residuum of interstitially trapped oil, held by capillary action and surface tension, which will not flow. This interstitially trapped oil, or oil remaining in pore spaces, may occur both above and below the water table. While substantial volumes of the immiscible bulk fluid may be removed by pumping techniques, the oil in residuum persists in major proportions in the soil pore spaces as a source of contamination.

#### FATE OF CONTAMINANTS IN THE SUBSURFACE

Contaminants in the subsurface are subjected to several natural physical, chemical and biological processes. The pertinent processes as described by Keely et al. (1986) include as physical processes; advection, hydrodynamic dispersion, molecular diffusion, and density stratification, as chemical processes; redox reactions, ion-exchange, complexation, co-solvation, partitioning, and sorption, and as

biological processes; substrate mineralization, biotransformation, and co-metabolism. Further discussion will focus mainly on the partitioning and sorption chemical processes and the biological processes. Limited discussion of other processes will be presented as necessary.

Hydrocarbon contamination may reach the groundwater either dissolved in infiltrating water and other fluids, or as immiscible organic phases (MacKay et al., 1984). The former can result from the residual saturation of oil. In addition, the immiscible organic phases lose water soluble constituents to the groundwater. Co-solvation can drastically increase the concentration of poorly soluble organics in water. The result of co-solvation may be concentrations of organic compounds in solution in excess of their respective solubility limits in water alone.

Organic contaminants tend to adsorb onto and partition into organic films in the porous media. Adsorption, as defined by Mingelgrin and Gerstl (1983), is an excess of solute concentration at an interface over the concentration in the bulk solution, whereas, partition is defined as the ratio between the activities of a solute in two bulk phases in equilibrium. Adsorption on solids is a function of a competition for surface sites, while partitioning is a function of solute solubility in the phases present. Describing the phenomenon of surface uptake Mingelgrin and Gerstl (1983) state that "there is a continuum of possible interactions, starting with fixed site adsorption and ending with a true partition between three-dimensional phases." Thus, surface uptake cannot simply be described as adsorption or

partition. Karickhoff and Morris (1985) suggest that the length of time a compound has to adsorb at a surface decreases its ability to desorb. The fate of organic contaminants in the subsurface is affected by adsorption, partition and the length of time they are subjected to these processes.

In the subsurface there are diverse populations of microorganisms which are capable of degrading natural and xenobiotic organic compounds (Howard and Banerjee, 1984). An organic chemical introduced into the subsurface is subject, in addition to the physical and chemical processes, to enzymatic or non-enzymatic biotransformations by the microbes present in the soil (Alexander, 1981). Non-enzymatic reactions rarely lead to appreciable changes in the chemical structure of the organic compound (Alexander, 1981). The mineralization, or complete biodegradation, of the organic molecules is almost always the result of microbial activity (Alexander, 1981). Organic compounds in the subsurface may be biodegraded if microbial populations are present with the required enzyme systems or if the microbes can acclimate to the organic substrate by enzyme induction, gene transfer or mutation (Howard and Banerjee, 1984). In some instances microbial populations capable of utilizing a particular substrate are initially absent from a specific site and biodegradation of that substrate is therefore very slow. Biodegradation of organic substrates may also be reduced if the substrate is held from microbe availability on the surfaces of or in the organic phases of the porous media by adsorption and partition. In some instances, chemicals may be biologically transformed through co-

metabolism (Alexander, 1981; Dalton and Stirling, 1982). In this case, the chemical is not used for growth by the microbes, but is incidentally oxidized during the biooxidation of some other growth-sustaining substrate.

#### CONTAMINATED AQUIFER AND GROUND WATER REMEDIAL ALTERNATIVES

A number of techniques are either currently practiced or are being investigated as potential alternatives to clean-up subsurface sites and groundwaters contaminated by a diverse array of compounds. In addition to economics, often the particular site conditions dictate the remedial schemes which can be employed. Each site and type of contamination must be considered individually in order to judiciously select the optimum treatment technique.

The characteristics of the contaminant and the site govern the degree of effort that must be employed in order to achieve the desired results. The physicochemical properties of the contaminant in part determine its fate in the environment. The components of gasoline, for example, tend to dissolve into water, volatilize, adsorb onto and partition into interfaces, to some degree hydrolyze and chemically degrade, and biologically degrade. In the subsurface the soil matrix provides sites for adsorption and organic films for partition. Groundwater acts as a solvent which transports the components. Gasoline in soil and groundwater can be difficult to treat.

The volatility of the lighter hydrocarbons is exploited in some cases to aid removal of the petroleum contaminants from soils. Soil



vapor extraction is employed to physically weather (Wilson and Ward, 1987) and carry hydrocarbons as vapor from soil pore spaces. The vapor extraction method is most effective in soils of higher permeability (Krishnayya et al., 1988) and lower organic content. High permeability allows a substantial flux of contaminant carrying gases while low organic content provides fewer opportunities for the partition of contaminants into organic matter.

The vapor extraction method is effective in the zone of aeration for removing hydrocarbon vapors. However, one must consider that this method of removal is simply a transfer of contaminants from the soil to the atmosphere and is not an actual reduction in the mass of the contaminants. In addition, only the compounds which readily vaporize can be removed by the volatilization method. Contaminant vapors should be contained and removed from the venting gases or prevented from entering the atmosphere via carbon adsorption or some other means of treatment. Transfer of contamination to any location other than an appropriate waste containment or treatment facility is not in keeping with the goals of environmental quality remediation and should be discouraged.

Soil excavation is a simple method of removing hydrocarbon contamination from sites where the depth of contamination is shallow. Excavation is most applicable when the contamination has not reached the groundwater. The cost of excavating and properly disposing of the contaminated soil must be weighed against alternate methods of remediation and their effectiveness.

Groundwater extraction and treatment at the surface is successfully employed to treat some types of subsurface contamination. Pump and treat methods are particularly useful for contaminants which are readily soluble in water. Retardation of components in the soil matrix limits this technique in effectiveness. Petroleum hydrocarbons that are retained in the zone of aeration are not typically recoverable by conventional pumping methods (Testa and Paczkowski, 1989). Additionally, hydrocarbons interfingering with the capillary fringe are not mobile and are not recoverable (Testa and Paczkowski, 1989). However, several methods of oil emulsification have been employed.

Synthetic surfactants and biological emulsification are two proven methods of enhanced oil recovery. Surfactants have been employed for enhanced or tertiary oil recovery by the petroleum industry. The effect of surfactants is to emulsify oil trapped interstitially in oil producing strata which promotes mobilization. Tertiary methods attempt to recover the oil remaining in oil-bearing strata, which may be as much as 60 percent of the original amount (Ling et al., 1987) after primary and secondary methods have been exhausted. Bioemulsification (Jenneman et al., 1979; Vanloock et al., 1979) has been investigated as a tertiary oil recovery technique. In the latter, microbes are stimulated to produce natural surfactants through nutrient addition to achieve the emulsification.

## GASOLINE BIODEGRADATION

Biological degradation of contaminants in the subsurface is currently a topic of much interest. Several researchers have determined that in-situ biodegradation is the most effective means of hydrocarbon contaminant reduction. Numerous bacterial cultures have been isolated from groundwater contaminated by gasoline. Among those capable of degrading gasoline are species of the genera Pseudomonas, Nocardia, Acinetobacter, Micrococcus, and Flavobacterium (Perry and Shell, 1968). As a group these microbes degrade gasoline, but no single isolate can utilize all of the gasoline components. Gasoline is a complex mixture of saturated, straight-chain and branched alkanes, olefins, cycloalkanes and aromatics (Britton and TRI, Inc., 1985). Single bacterial species are not likely to be able to utilize all of the organic substrates available in whole gasoline. The common gasoline constituents and their relative proportions of the total mixture are listed in Table 1.

Gasoline is immiscible with water. However, the individual constituents of gasoline may dissolve into water at different concentrations from whole gasoline based on their partition coefficients. Octanol-water partition coefficients ( $K_{ow}$ ) of selected aromatics present in gasoline are listed in Table 2. The  $K_{ow}$  of compounds provides a qualitative, relative measure of their solubility in water in the presence of gasoline.

Gasoline constituents dissolved in groundwater are metabolically available to soil microbes. Microorganisms in the subsurface grow as colonies on the porous media and colloidal material transported with

Table 1  
Gasoline Composition

Hydrocarbons	Typical composition percentage
Alkanes	52.6
Monocycloalkanes	34.6
Dicycloalkanes	5.2
Alkylbenzenes:	
Benzene	up to 5.0
Toluene	up to 20.0
xylenes	up to 20.0
Indanes and Tetralins	0.9
Naphthalenes	0.3

(after McDuffie, 1982)

Table 2  
Physical Properties of Selected Gasoline Constituents

Compound	Molecular Weight	Vapor Pressure (25 °C, mm Hg)	Aqueous Solubility (25 °C, mg/L)	Henry's Law Constant (Atm/mM)	Partition Coefficient (log Kow)
<u>Aromatics</u>					
Benzene	78	95	1780	7	2.11
Toluene	92	29	535	6.7	2.69
O-xylene	106		175		
M-xylene	106		200		
P-xylene	106		200		3.15
Ethylbenzene	106	7	152	6.6	3.15
Propylbenzene	120		60		
Naphthalene	128		32	10	3.36
Biphenyl	154		7.5		4.09
<u>Alkanes</u>					
Butane	58		79		
Pentane	72		2		
Hexane	86				
Cyclohexane	84		130		
Heptane	100		50		
Octane	114		16		
Iso-octane	114				

(after McDuffie, 1982)

groundwater (Molts et al., 1986; Rittman and McCarty, 1980).

Microorganisms attached to subsurface media obtain nutrients from passing groundwater (MacKay et al., 1984). Energy for growth in the subsurface results from the oxidation of organic substrates or inorganic compounds such as hydrogen or reduced forms of iron, nitrogen or sulfur (MacKay et al., 1984).

Biodegradation of any substrate is dependent upon several factors. The substrate must be available in sufficient quantities to provide energy for growth. At low concentrations the compound may still be biodegraded provided another energy source is available (MacKay et al., 1984). Other factors affecting the biodegradation of compounds include ambient temperature and pH, presence and density of a population of microbes capable of degrading the compound, presence of toxic compounds, availability of appropriate nutrients, and availability of appropriate external electron acceptors where needed for the microbial electron transport system (MacKay et al., 1984). Biodegradation may also occur in the absence of external electron acceptors by anaerobic fermentation. Aerobic processes in general tend to proceed at much higher rates, even though some compounds are not biodegradable aerobically.

A literature survey indicated that biodegradation of gasoline products tends to occur readily in the subsurface. Studies have shown increases in microbial populations and decreases in the concentration of gasoline components in groundwater near gasoline spills. McDuffie (1982) states that microbial breakdown is the most important degradation process for gasoline components in that chemical reactions, including

hydrolysis and redox reactions, are slow. In the case of gasoline, McDuffie may have given the physicochemical process of volatilization too little consideration. Barker and Mayfield (1988) stated that the most significant aromatic hydrocarbon attenuation mechanism in soil appears to be biodegradation.

Provided the site of gasoline contamination remains aerobic, populations of bacteria including the genus Pseudomonas grow "luxuriantly" and may eventually degrade the residual oil, including benzene and toluene (McDuffie, 1982). Pseudomonas and Arthrobacter were isolated from the site of a large gasoline spill in the Los Angeles-Glendale, California area (Britton and TRI, Inc., 1985; McKee et. al., 1972). The bacteria degraded the gasoline components under aerobic conditions. Studies have also shown that biodegradation in the subsurface can be stimulated by the addition of appropriate external electron acceptors. At the site of a gasoline spill in Ambler, Pennsylvania nitrogen, phosphate, and oxygen were supplied through forced aeration into the subsurface (Raymond et al., 1976). As a result of the nutrient addition, gasoline-utilizing bacteria multiplied and gasoline degradation was accelerated. Similar results have been noted for gasoline contaminated sites near leaking storage tanks and other spills.

The concentration of dissolved gasoline components in the subsurface may be reduced biologically, however, organic compounds can continue to leach into the groundwater from gasoline in residuum. Biological methods of remediation alone may require extended operating

periods to satisfactorily remove gasoline components. In the case of gasoline, the greatest task involved with biological treatment is solubilizing the components from the soil matrix and immiscible phases in order to make them available to the microbes. Surfactants represent a potential method of achieving this solubility.

The effect of synthetic surfactants in combination with in-situ biodegradation has not been well documented. The potential for employing enhanced oil mobilization techniques in combination with in-situ biodegradation seems promising in the remediation of hydrocarbon-contaminated soils and groundwater.

#### SURFACTANTS FOR AQUIFER RESTORATION

Recently, surfactants have been used for in-situ treatment of soils contaminated with organic compounds (Vignon and Rubin, 1989; Vanlooche et al., 1979). Vignon and Rubin (1989) citing Ellis et al. (1985) stated that by trial and error a mixture of nonionic surfactants was found which proved effective in removing 91 percent of the crude oil distillates contaminating soil of low organic content. The mixtures that had proven effective for the petroleum industry application of enhanced oil recovery, however, were completely ineffective in the soil restoration application (Vignon and Rubin, 1984 citing Ellis et al., 1985). This finding suggests that the effectiveness of surfactants depends upon the surfactant type, concentration and specific conditions of the application. Vignon and Rubin citing Olfenbuttel (1987) from personal communication stated that a full scale aquifer restoration



project was undertaken in which a nonionic surfactant was injected into a contaminated aquifer and which successfully removed chlorinated hydrocarbons from sandy soil. Indigenous bacteria plugged the soil pores via excretion of extracellular polysaccharides and reduced the effectiveness of the remediation effort.

#### SURFACTANT CHARACTERISTICS

A surfactant or surface active agent as defined by Rosen (1978) is a substance that, when present at low concentrations in a system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces (interfaces). The interfacial free energy is the minimum amount of work required to create a particular interface (Rosen, 1978). The reduction of surface free energy, or surface tension, is commonly used as the measure of surfactant effectiveness. Often the terms surface tension and surface free energy are used interchangeably. Surface tension seems to be the more descriptive term as applied to the liberation of contaminants from surfaces.

A surfactant has a characteristic molecular structure consisting of a structural group that has very little attraction for the solvent, known as a lyophobic group, together with a group that has a strong attraction for the solvent called the lyophilic group (Rosen, 1978). Surfactants are classified based on the nature of the lyophilic group as follows:

Anionic:	The surface active portion of the molecule bears a negative charge.
Cationic:	The surface active portion of the molecule bears a positive charge.
Zwitterionic:	The surface active portion of the molecule may contain both a positive and a negative charge.
Nonionic:	The surface active portion of the molecule bears no apparent charge.

In order to choose a surfactant for a specific task with minimal trial and error experimentation one must consider the physical and chemical properties of the surfactant, the interfacial phenomena involved, the intended role of the surfactant, and the relationship of the interfacial properties of the surfactant to those of the surfaces concerned (Rosen, 1978). "In spite of a wealth of experience in the field, the utilization of surfactants for a particular purpose remains more of an art than a science" (Rosen, 1978).

A unique property of surfactants is the formation of pseudophase aggregates through self-association in the bulk solution called micelles (Kile and Chiou, 1989). Stated simply micellization occurs at a specific minimum surfactant concentration which results from overcrowding of the interfacial surfaces by monomeric molecules (Atwood and Florence, 1983). The minimum surfactant concentration which allows the formation of micelles is called the critical micelle concentration (CMC). Surfactant molecules exist in the monomeric state below the CMC. Above the CMC the concentration of individual molecules is thought to remain constant while the concentration of micelles may increase (Kile and Chiou, 1989). One of the most important consequences of

micellization is that micelles are capable of solubilizing organic compounds of limited solubility in a particular solvent (Atwood and Florence, 1983). The lyophobic tails of the molecules compose the core of the micelle, which is shielded from the surrounding solvent by a shell consisting of the lyophilic head groups (Atwood and Florence, 1983). Experimental evidence suggests that the mobility of hydrocarbon chains in the micellar interior resembles that in a liquid hydrocarbon (Atwood and Florence, 1983). Micellization represents a method by which nonpolar hydrocarbons may gain solubility in a polar solution.

Micelles have been described as possessing several morphologies. Some of the shapes documented include spheres, cylinder, discs, middle phases, and neat phases (Atwood and Florence, 1983). Middle phases are hexagonal arrays of indefinitely long, parallel rods. Neat phases exhibit a lamellar structure. The latter two phases result from crowding of micelles in high concentration solutions.

The CMC of surfactants may be affected by several factors. Increasing the hydrocarbon chain length reduces the CMC. This is particularly pronounced in the nonionic surfactants. Increasing the ionized groups in the surfactant increases the CMC. Nonionic surfactants tend to have low CMC's due to the lack of electrical work necessary to form micelles (Atwood and Florence, 1983). Addition of electrolytes to solution changes the CMC. Organic solutes tend to affect the CMC in both directions. Temperature and pressure also affect the CMC to varying degrees.

The effect of surfactants below the CMC on solute solubility has received little attention (Kile and Chiou, 1989). Kile and Chiou (1989) showed that surfactants below the CMC significantly enhanced the solubility of some extremely water insoluble organic solutes. The solutes studied included DDT and trichlorobenzene. For organic solutes of relatively high solubility enhancement below the CMC was indiscernible (Kile and Chiou, 1989). The solubility enhancement was attributed to a partition-like interaction of solutes with the microscopic organic environment of the dissolved monomers of surfactant. The determinations were based on a ratio of solute concentration in the solvent containing monomeric surfactant and an organic immiscible phase. No soil was present in the experiments.

#### **SURFACTANT EFFECTS IN SOIL AND ON MICROBES**

Literature concerning the pharmaceutical aspects of surfactants suggests that surfactants may act both favorably and detrimentally to microbes. In some instances the surfactant acts synergistically with antimicrobial agents to destroy bacteria. The surfactant may alter the permeability of the microbial cell to external compounds, which may increase or decrease the susceptibility of bacteria to external agents. Surfactants may also affect the enzymes produced by the microbes for external compound degradation. Surfactants have been shown to shield microbes from antimicrobial agents by surficial adsorption onto the microbial surface.

Urano and Saito (1984) showed that nonionic surfactants adsorbed onto the surfaces of microbes in activated sludge at greater rates than ionic surfactants. They also found, in a separate study (Urano and Saito, 1984), that the nonionic surfactants adsorbed onto soil surfaces at the fastest rates. Urano and Saito (1984) stated that the effect of the adsorbed ionic surfactants on the biological activity of the microbes was minute. No discussion of the effect of the nonionic surfactant on the viability of the activated sludge was presented.

Vignon and Rubin (1989) showed that at nonionic surfactant doses below 0.1 percent by weight, adsorption of the surfactant onto soils prevented the optimal solubilization of anthracene and biphenyl. Surfactant concentrations of 0.1 percent appeared to be a threshold in effectiveness.

The surfactants used in this study are biodegradable nonionics. Studies (Texaco Chemical Company, 1987) have shown that conventional activated sludge degraded more than 90 percent of the nonionic surfactants Surfonic N-95 and N-40.

## Chapter 3

### METHODS AND MATERIALS

#### EXPERIMENTAL APPROACH

Soil microcosms were used in a study designed to simulate the effects of synthetic surfactants on the biodegradation of gasoline components in the subsurface. Table 3 outlines the matrix of synthetic surfactants and gasoline volumes used in this study. Gasoline was injected onto 5.0 grams (g) of soil with a 10-microliter (uL) syringe or pipet prior to the addition of a known volume of solution containing bacteria. The actual concentrations of the gasoline components which dissolved into the microcosm solution were measured and recorded. Volatilization and component solubility affected the final concentrations of the solution components.

Surfactant critical micelle concentrations were determined in the presence and absence of soil to help explain differences observed between microcosms containing and without surfactants. Soil surface area plays an important role in the action of surfactants in solution.

Measured toluene concentrations and applied surfactant doses in each phase of this investigation were reported in milligrams per liter (mg/L) to facilitate comparisons between experiments with and without soil. The mass of soil in each microcosm was 5.0 g. Sixty-nine grams of soil were used in the critical micelle concentration (CMC) experiments. In the cases involving surfactants and soil, a more appropriate unit of measure may have been "concentration per unit area

Table 3  
Experimental Matrix

Gasoline Volume Injected (mL)	Surfactant Concentrations (mg/L)				
	30	60	120	1000	3000
	N-40+N-95 1:6	N-40+N-95 1:6	N-95	N-95	N-95
0.005	x	x	x	x	x
0.05		xx	x		
0.1		x	x		

of soil," but soil surface area was not estimated in this study. The solution concentration of surfactants within vessels containing soil after adsorption onto the soil was not determined. The concentration of surfactant was, therefore, reported as if all of the applied surfactant remained in solution.

All glassware used in the experiments was washed and cleaned with 10 percent hydrochloric acid. Aseptic techniques were practiced to the degree possible during all stages of the experiments. Non-biological toluene losses were determined through the use of sterile microcosms that were identical to the conditions of the inoculated microcosms. Differences in gasoline component concentrations observed with time between active and control microcosms, therefore, were attributed to biological activity.

Each experiment was designed to provide a comparison of microcosms which were identical in all respects except either the presence or absence of surfactants. As results were obtained, additional investigations, including toluene leaching and surfactant adsorption observations, were incorporated into the study. A detailed discussion of the measures taken to provide a controlled comparison of the effects of synthetic surfactants on the biodegradation of gasoline components in the subsurface and additional experiments is presented in the following sections.



## BIOKINETICS

Pseudomonas putida, obligately aerobic, acclimated to toluene as the sole carbon and energy sources was obtained from Sybron Chemicals, Inc. of Salem, Virginia. P. putida was selected for its ability to degrade gasoline components. In addition, acclimated bacteria tend to degrade the desired substrate preferentially over additional substrate such as the surfactant. The acclimation period believed to be required by the microbes provided maximum time for the synthetic surfactants to produce any effects on the gasoline components in the soil.

The rate of population doubling time was measured for P. putida both in the presence and absence of the surfactants. The experiment was designed to determine the effect on the ability of the microbes to metabolize toluene and to observe any changes in growth rate that might be attributable to the presence of the surfactants. The first-order, growth-rate constants were calculated from these data.

Bacteria were grown in a buffered, minimal-salts medium to provide the basic inorganic nutrients required for microbial metabolism. The medium is a combination of salts and Sorensen's phosphate buffer solution (Table 4). The Sorensen's Buffer and the salts solution were autoclaved separately before they were mixed. The formulation for the minimal salts medium was supplied by Sybron Chemicals, Inc.

Four, identical, two-liter jars were filled with one-liter each of the minimal salts medium. Two of the jars contained 60 mg/L of the surfactant Surfonic N-95. In sets of two, the jars were then inoculated with 20 mg/L and 40 mg/L of a bacterial suspension in minimal-salts

Table 4  
Minimal Salts Medium

Sorensen's Buffer	Salts Solution
3.8 g $\text{KH}_2\text{PO}_4$ 12.5 g $\text{K}_2\text{HPO}_4$ 1.0 g $(\text{NH}_4)_2\text{PO}_4$ ----- Add 1.0 mL Salts Solution to the above dissolved in 0.99 L distilled water	4.0 g $\text{MgSO}_4$ 0.2 g $\text{NaCl}$ 0.2 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g $\text{MnSO}_4 \cdot 14\text{H}_2\text{O}$ 0.2 g $\text{CaCl}$ ----- Above dissolved in 0.1 L distilled water

medium. The jars were placed on a Rotary Agitator (Analytical Testing and Consulting Services, Inc. Model DC-18) for mixing and aeration. Changes in solution turbidity with time were monitored with a Hach Model 2100-A Turbidimeter. Two initial levels of bacterial inocula were studied to observe any dependence of growth rate on initial population size.

### MICROCOSMS

Microcosms are small-scale, batch reactors used, in this case, to simulate to some extent the subsurface environment. Microcosms were constructed from glass test tubes (13 by 100 mm) equipped with threaded caps. The closures were lined with Teflon septa in an attempt to provide an inert, leak-proof seal. Each active microcosm contained  $5.0 \pm 0.1$  g of soil, predetermined volumes of gasoline, known volumes of the minimal salts medium, microbes at a concentration of 50 mg/L, and predetermined concentrations of synthetic surfactants. Control microcosms were constructed exactly as above, but contained no bacteria.

The soil used in this investigation was collected during a previous study from an excavation at an uncontaminated site in Newport News, Virginia (Morris, 1988). Soil components govern the permeability of the material and the number and nature of active sites within the matrix. This particular soil was selected because it possessed a reasonably equitable mix of sand and silt and only moderate amounts of clay. The soil was collected aseptically from two to three feet below the surface and stored at 4 degrees Celsius ( $^{\circ}\text{C}$ ) in clean, glass jars.

The amount of organic matter in the soil used in this study was 1.8 percent by weight. The mass of organic matter in the soil was determined by differences in weight of dried samples ignited at 420 °C. The ignition temperature of 420 °C was selected to preserve calcium carbonate and waters associated with mineral structures.

The soil was autoclave sterilized prior to use in a Barnstead Laboratory Sterilizer a total of seven times at 120 °C for 30 minutes each and 15 pounds of pressure per square inch above ambient. The first five times, the soil was autoclave sterilized in a clean, enamel-coated pan. Then it was transferred to the microcosm test tubes and sterilized twice again.

Sterilization was necessary to prevent indigenous microbes from degrading the synthetic surfactants preferentially over the gasoline components. Had this occurred, little information could have been collected concerning the effects of surfactants on the gasoline component concentrations in the microcosms. In addition, the soils were inoculated with a fixed population of microbes acclimated to the substrate to prevent unequal numbers of microbes in test tubes, which would affect the rate of substrate removal and render a comparative study useless.

The gasoline used in this study was purchased from Campus Exxon in Blacksburg, Virginia. Either 0.005, 0.05 or 0.1 mL of gasoline (89 octane, unleaded regular) was injected directly onto the soil with either a microliter syringe or pipet. Following the addition of gasoline, the microcosms were sealed and stored for 24 hours at 10 °C

for equilibration. The equilibration period allowed volatilization, sorption, and partition of the gasoline components out of the organic liquid and into the soil and head space within the tubes. It also prevented the formation of an immiscible phase of gasoline at the surface of the microcosm when the bacterial solution was added.

Separate phase gasoline was desired only in the soil pore spaces so that the effect of surfactants on interstitially trapped gasoline could be observed. Attempts were made to ensure the presence of interstitial gasoline by increasing the amounts of gasoline added initially to soil in three different experiments. Following the equilibration period, a solution of the minimal salts medium containing appropriate amounts of bacteria and surfactant was added with a self-filling buret. A covered, 1-liter (L) aspirator bottle was used as the buret reservoir. The reservoir was attached to the buret with flexible plastic tubing and was stirred continuously during filling operations.

The bacteria were grown and maintained in sealed glass flasks containing the minimal salts medium. They were placed on a mechanical shaker for aeration. The cultures were rejuvenated every week and 24 hours prior to use in microcosms by inoculating 100 mL of minimal salts medium with approximately 25 mL of bacterial solution from the previous week. Renewing the culture prior to use in the experiments provided viable populations for timely responses in the microcosms. Toluene, which was the sole carbon and energy sources, was added at concentrations of approximately 250 mg/L every 3 to 4 days.

Microbial densities in stock cultures were determined by calculating the difference in weight of dry, Gelman filters (0.2 micrometer pore size) before and after filtering a known volume of bacterial solution. These procedures are outlined as Physical Examination Method 209C entitled "Total Suspended Solids Dried at 103-105 °C" in Standard Methods for the Examination of Water and Wastewater (1985). This information was used to calculate the dilution factor necessary to deliver the desired number of microbes to microcosms. In every case, a suspension of minimal salts medium containing 50 mg/L of bacteria was supplied to each active microcosm at the appropriate time.

Once all of the ingredients had been added, the tubes were mixed vigorously with a Fisher Scientific Vortex Genie. Each tube then received enough of the appropriate solution to minimize headspace. The microcosms were incubated in darkness at 20 °C for the duration of the study.

#### OXYGEN SUPPLY

Hydrogen peroxide was added to the microcosms to provide aerobic conditions without allowing the escape of the volatile components of the gasoline. Farmer (1989) devised a scheme for providing optimum hydrogen peroxide doses sufficient for P. putida. Five microliters of 31 percent hydrogen peroxide solution were injected into each microcosm. The microcosms were then inverted several times to provide mixing. Microcosms were injected with hydrogen peroxide at the beginning of each experiment. The initial injection was usually sufficient to provide

oxygen for the complete biological degradation of toluene in the 0.005 mL gasoline studies. In the microcosms receiving 0.05 mL gasoline, hydrogen peroxide was supplied following each 5 to 10 mg/L reduction in the dissolved toluene concentration. In microcosms receiving 0.1 mL gasoline, hydrogen peroxide was provided every three to five days.

#### ANALYTICAL METHODS

Toluene degradation was used as an indicator of the biological removal of the gasoline components in the microcosms, even though toluene is an aromatic compound and most gasoline components are aliphatics, because it is a major constituent in gasoline and is commonly detected in gasoline-contaminated groundwaters. Each microcosm was sampled by rapidly removing the lid and withdrawing the liquid with a microliter syringe. The lids were quickly replaced to prevent toluene evaporation even though the microcosms were designed to be sacrificial.

The concentration of toluene was measured in a Hewlett Packard Model 5880-A gas chromatograph using flame ionization. The toluene peak was isolated from the gasoline spectrum on a 6 foot by 1/8 inch stainless steel column packed with 0.2 percent Carbowax 1500 on 80/100 mesh Carbopack-C with nitrogen as the carrier gas at a flow rate of 30 mL/min. A 2.0 uL sample was injected into the 150 °C injector port. The oven temperature was programmed for 17 minutes at 130 °C then at 175 °C for 20 minutes to first separate the toluene peak and then to clear the column of residual sample. The detector temperature was 225 °C.

To determine that the surfactants were not affecting the detection of toluene with packed-column gas chromatography, comparisons were made between analyses of toluene solutions with and without both 60 mg/L and 1000 mg/L surfactant. No differences in toluene concentrations were noted.

Following the biodegradation of toluene initially dissolved in the microcosm solution, the microcosms were allowed to remain undisturbed for extended periods of time. Limited oxygen and lack of substrate reduced biological activity, and, as a consequence, toluene concentrations increased as it leached from soil. The amount of toluene remaining in the soil in selected microcosms was determined by extraction into methylene chloride and subsequent GC analyses. Farmer (1989) found that the extraction efficiencies using this technique ranged from 50.5 percent to 72.1 percent. A mass balance of toluene was performed by determining the difference in the total amount of toluene present in the soil after the equilibration period and subsequent concentrations. The total amount of toluene left in the soil following the equilibration period was determined through methylene chloride extractions of toluene from the soil in microcosms immediately without further additions.

#### **SURFACTANTS AND CRITICAL MICELLE CONCENTRATION**

The surfactants used in this investigation included the nonionic surfactants, Surfonic N-40 and Surfonic N-95, both supplied by the



Texaco Chemical Company. The physical properties of the surfactants are listed in Table 5.

The critical micelle concentrations (CMC) of Surfonic N-95 and the combination of Surfonic N-95 and N-40 were measured as changes in surface tension at the liquid surface of microcosm solutions. A Fisher Scientific Model 20 Surface Tensiometer was used to measure the surface tension. The instrument operates on the principles described by Dr. Pierre Lecomte du Nouy, in which the force necessary to pull a platinum-iridium ring of known dimensions free from a liquid surface film is measured.

Scaled-up versions of the microcosms used in the degradation studies were approximated by adding 69.0 g of soil, 69 uL of gasoline, and 100 mL of minimal salts medium with 50 mg/L bacteria to acid cleaned glass beakers. The surfactants were incrementally added from concentrated stock solutions with an automatic pipet, and the resulting suspensions were stirred vigorously with a glass rod after each surfactant addition. Two measurements of surface tension were made after each addition. The first measurement was made immediately after mixing and the second approximately 5 minutes later after the soil settled. Each of these experiments was performed twice.

The CMC of Surfonic N-95 and a 1:6 mixture of N-40 and N-95 were measured in distilled water for comparison with the CMC in the microcosm solution (reported as if all surfactant added remained in solution). The comparison was intended to show the effects on the CMC of the

Table 5  
Physical Properties of Surfonic N-40 and N-95

Characteristic	Surfonic N-40	Surfonic N-95
Chemical Family	alkylphenol ethoxylate	alkylphenol ethoxylate
Molecular Weight	396	632
Specific Gravity	1.026	1.061
Solubility	slightly soluble	soluble
Vapor Pressure	<1 mm Hg	<1 mm Hg
pH	7.0	7.0
Evaporation	nil	nil
HLB*	8.9	12.9

\* HLB = Hydrophile-Lipophile Balance  
(after Texaco Chemical Company, 1987)

additional surface area supplied by the soil, the salt ions, and the presence of the bacteria and the gasoline.

## Chapter 4

### RESULTS

#### INTRODUCTION

Satisfactory results were obtained only in microcosms that initially were dosed with 0.005 mg/L gasoline. In the experiments containing the larger volumes of gasoline, biodegradation either did not occur or proceeded for only short periods. Toxicity of the gasoline was suspected, so the study was limited to a single, lower gasoline concentration. The elimination of higher gasoline dosages resulted in the elimination of microcosms containing interstitial gasoline in the soil. In this study, all gasoline was thought to be adsorbed to the soil. Further discussion will focus on the initial gasoline experiments in which 0.005 mL gasoline were added.

#### BACTERIAL GROWTH AND SUBSTRATE UTILIZATION

The population doubling time for P. putida was not affected by the presence of 60 mg/L of Surfonic N-95, as is indicated in Figure 1. Figure 1 illustrates the linearized growth curves for P. putida when turbidity was plotted as a function of time on a log scale. A lag period of approximately 120 minutes was observed. The growth rates were identical when two, different, initial inoculum levels were added. The slopes of the growth curves in microcosms with and without surfactants were also identical, which indicated that the growth rates were not affected by surfactant additions. Population doubling times and growth

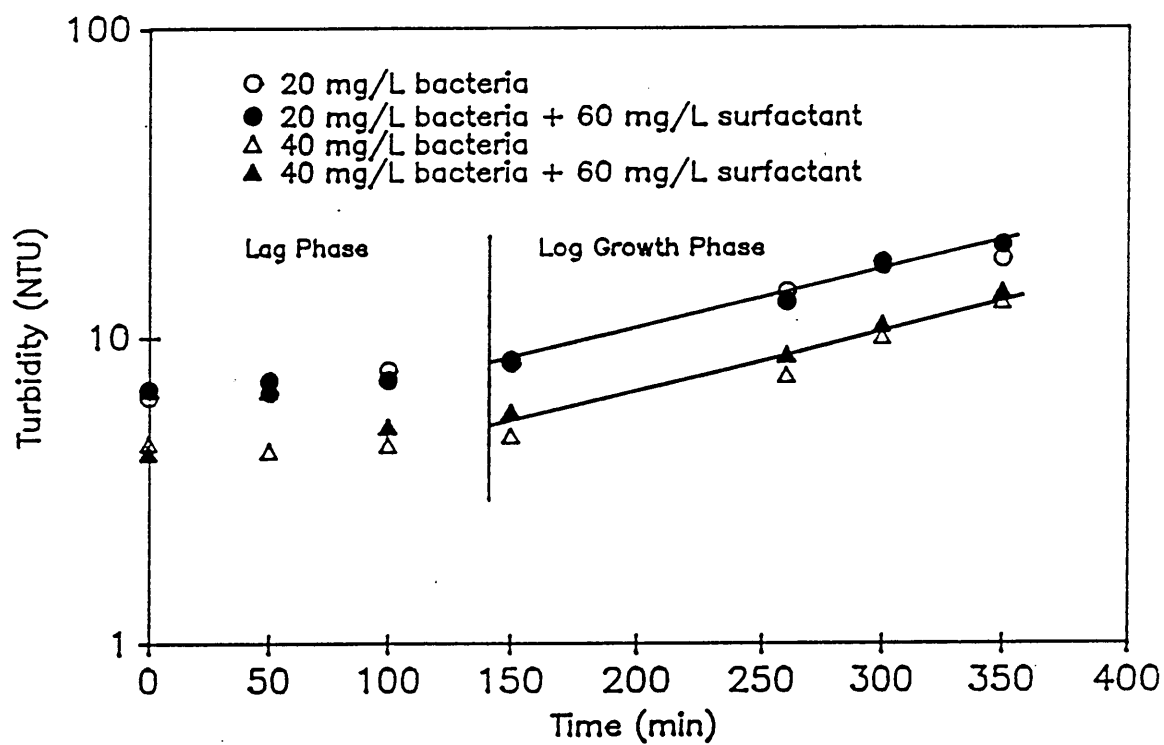


Figure 1 Growth of *P. putida* with and without surfactant (400 mg/L initial toluene dose, 25 °C)

rate coefficients,  $k_1$ , for all situations studied are listed in Table 6. The growth rate coefficients were calculated as  $k_1 = \ln 2/t_d$ , where  $t_d$  = population doubling time in days.

Figure 2 represents typical data generated from observations of biodegradation at the various surfactant concentrations studied. Figure 2 compares the changes in dissolved toluene concentration with time in microcosms with and without surfactant. The toluene concentration in control or sterile microcosms was consistently greater than that in the active microcosms during the observation period, which suggests that the removal of dissolved toluene was the result of biological activity.

Toluene volatilized in control microcosms over extended periods of time, but the amount lost by volatilization during the biodegradation period of approximately five days was negligible in most cases. In the microcosms containing 120 mg/L surfactant, nearly all of the toluene was lost from the surfactant control microcosms by the seventh day, probably either because sterilization was incomplete or because the caps were not properly sealed. Toluene persisted at appreciable concentrations for longer periods of time in other experiments.

In every case, biodegradation of dissolved toluene occurred after a short lag period and continued steadily to completion following an initial injection of hydrogen peroxide for oxygen supply. Biodegradation failed to occur in microcosms unless hydrogen peroxide doses were added.

Table 6  
Microbial Growth Parameters

Initial Bacterial Inocula (mg/L)	Population Doubling Time (hr)	First Order Growth Rate Constant, $k_1$ (hr <sup>-1</sup> )
20 without surfactant	2.3	0.30
20 with 60 mg/L surfactant	2.4	0.29
40 without surfactant	2.4	0.29
40 with 60 mg/L surfactant	2.4	0.29

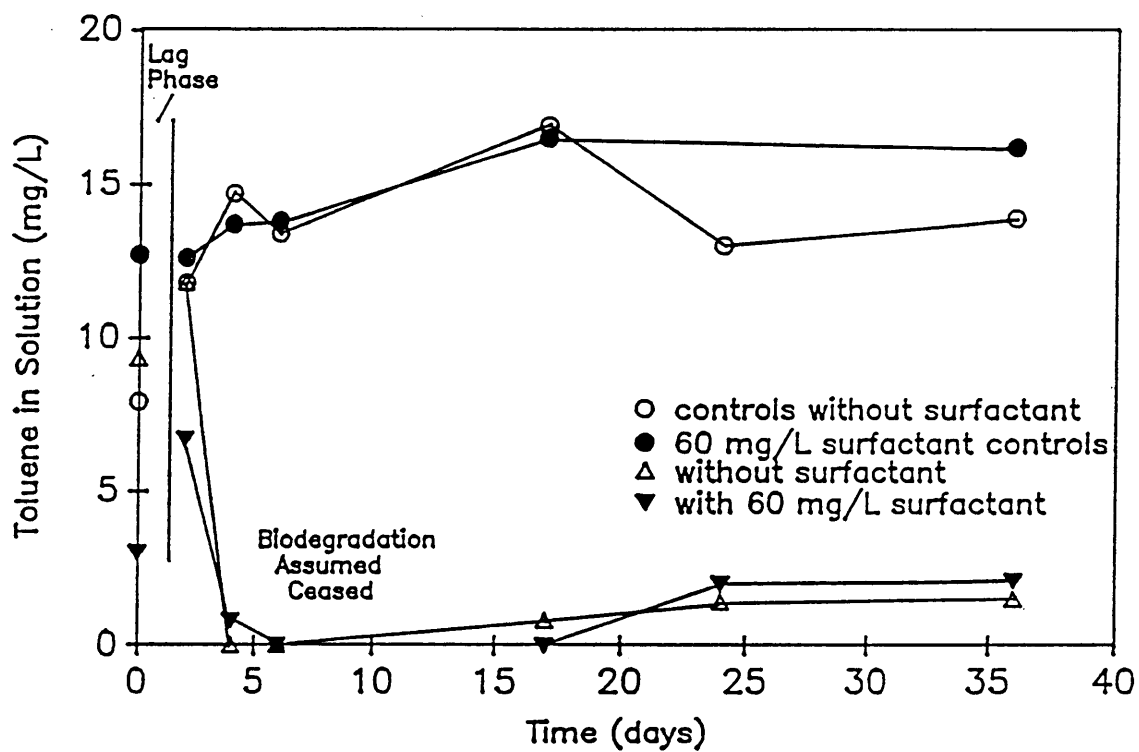


Figure 2 Typical data indicating toluene biodegradation followed by toluene leaching from soil



Maximum biodegradation rates for each experiment were calculated as the average slope of the most linear portion of each degradation curve. Figure 3 is typical of the biodegradation data used to determine the maximum biodegradation rates. This approach was adopted to eliminate the effects of initial lag periods and subsequent substrate limitations on the maximum rate. The changes in biomass were not identified. A comparison of biodegradation rates, however, is meaningful since each active microcosm received organism additions of equal concentrations. The maximum biodegradation rates calculated for each curve are shown in Figure 4 as a function of the initial dissolved toluene concentration. The first-order regression is shown for the data from microcosms containing surfactants.

Substrate utilization rates are dependent upon the concentration of available substrate when substrate is limiting and the concentration of microbes. For this reason, every effort was made to ensure that initial concentrations of toluene and P. putida were the same in each microcosm, but in spite of the effort, the initial amounts of dissolved toluene in the microcosms containing surfactants varied. Figure 4 also indicates that initial substrate concentrations are suppressed in microcosms containing surfactants compared to tubes without surfactants.

Figure 5 indicates the initial dissolved toluene concentration measured in each microcosm experiment. The solid line in Figure 5 represents the average initial concentration of dissolved toluene in the microcosms without surfactant amendments. The microcosms containing 30

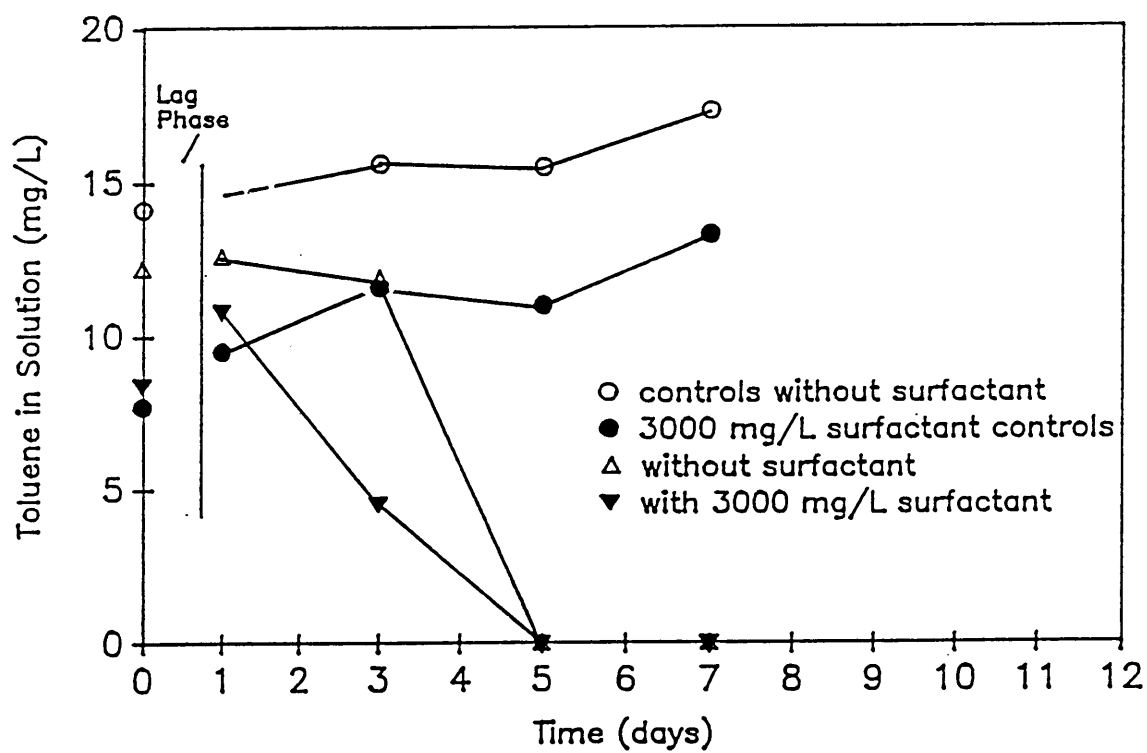


Figure 3 Typical data used to determine toluene biodegradation rates

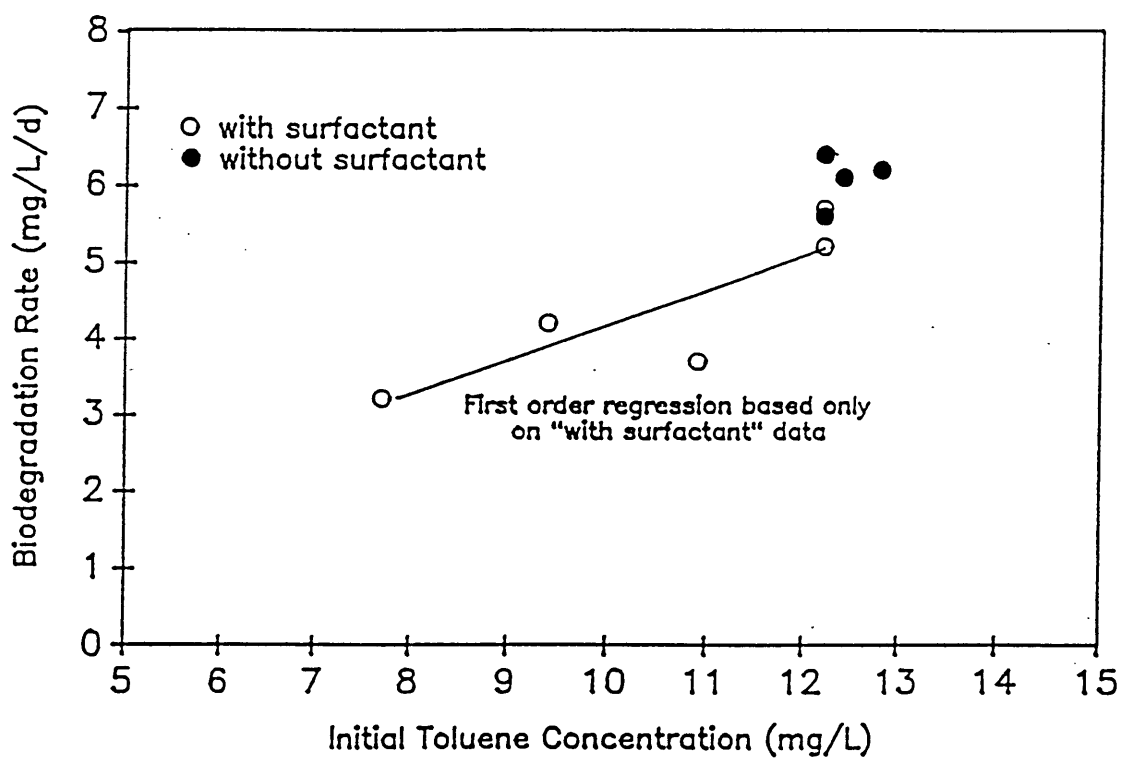


Figure 4 Toluene biodegradation rates as a function of initial dissolved toluene concentration

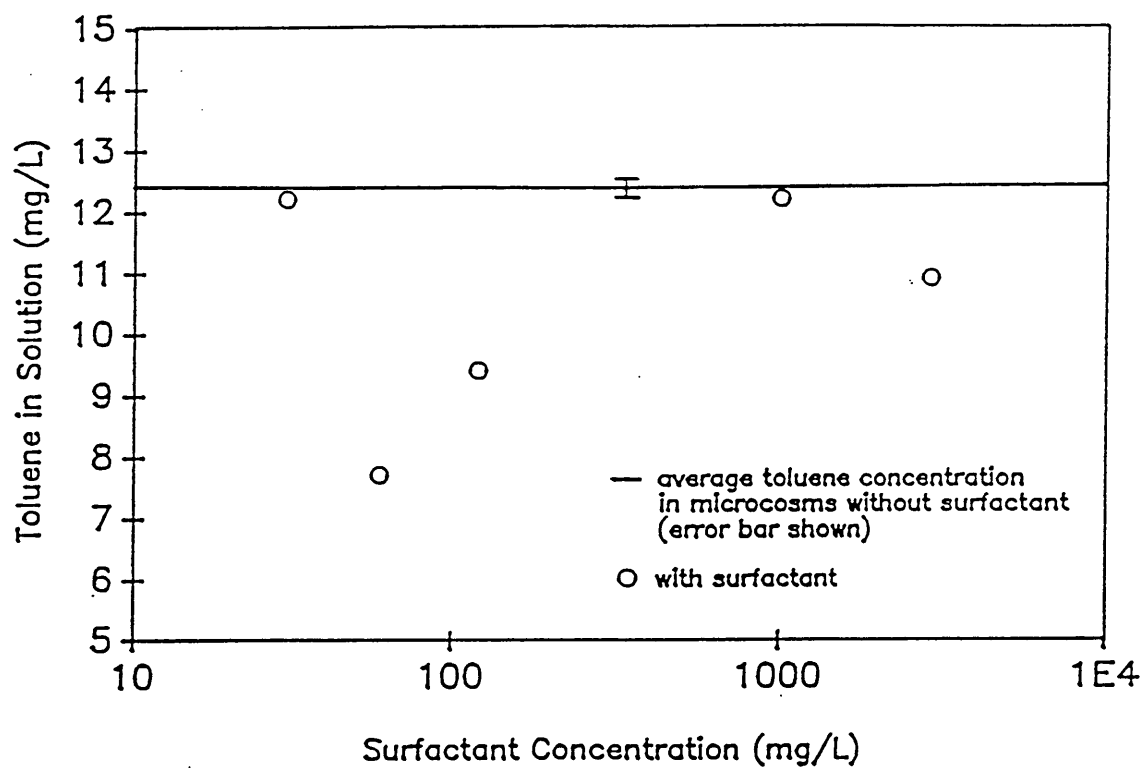


Figure 5 Comparison of initial dissolved toluene concentrations between microcosms with and without surfactants

and 1000 mg/L surfactant contained effectively the same amount of toluene as their counterparts without surfactants. In every other case, however, the initial dissolved toluene concentration is lower in tubes containing surfactant solutions than in tubes without surfactant amendments. Among the microcosms containing surfactants, the minimum toluene concentration was observed in the 60 mg/L surfactant solution, while the maximum concentration was observed in the 30 and 1000 mg/L surfactant solutions.

#### SURFACTANT SOLUTIONS IN MICROCOSMS

The critical micelle concentration of Surfonic N-95 and the 1:6 mixture of Surfonic N-40 and N-95 were determined in distilled water for comparison to their respective CMC's in the microcosm solutions. Figures 6 and 7 show the determination of the CMC's by surface tension reduction of N-95 and the mixture of N-40 and N-95 in distilled water, respectively. The CMC of N-95 in distilled water was 21 mg/L. The CMC of the surfactant mixture in distilled water was 13 mg/L. When applied to the microcosm solution, however the CMC of the surfactants increased markedly. The CMC of both N-95 and the mixture of N-40 and N-95 in the microcosm solution is approximately 1000 mg/L (reported as though all surfactant added remained in solution). Figures 8 and 9 show the determination of the respective CMC's for N-95 and the surfactant mixture in the microcosm solution.

Note the changes in the surface tension curves in Figures 6 through 9. In distilled water, the surface tension was sharply

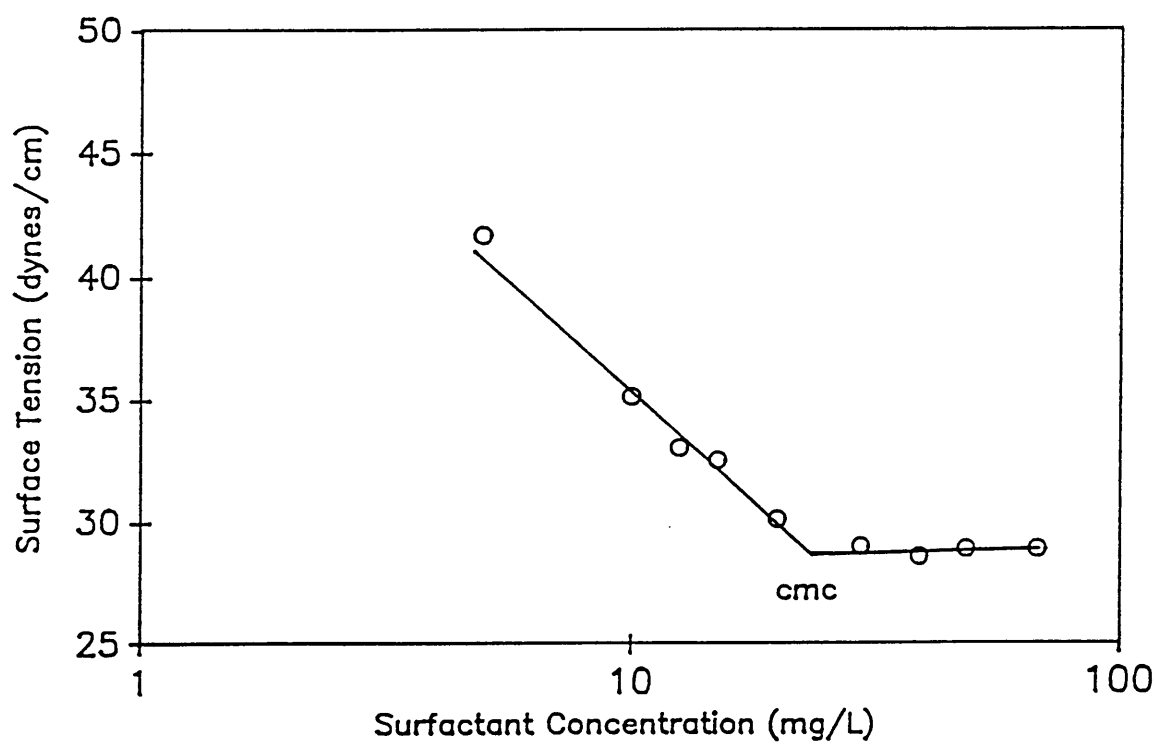


Figure 6 CMC determination of Surfonic N-95 in distilled water

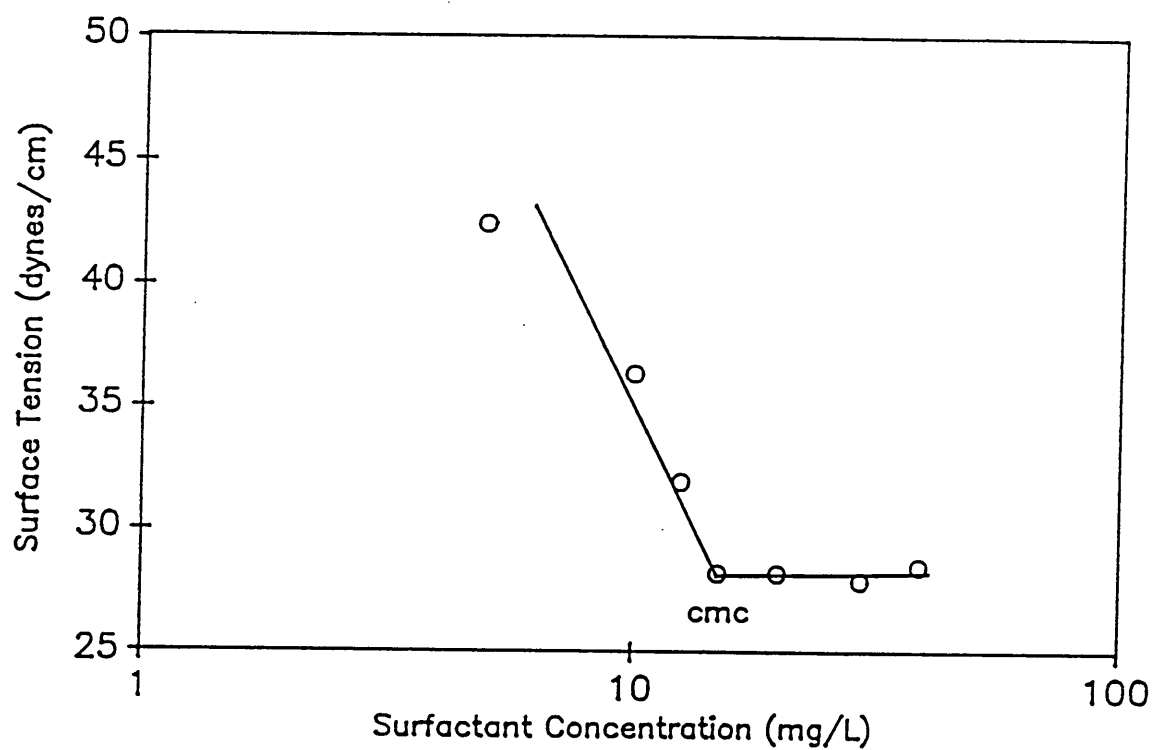


Figure 7 CMC determination of the 1:6 mixture of Surfonic N-40 and N-95 in distilled water

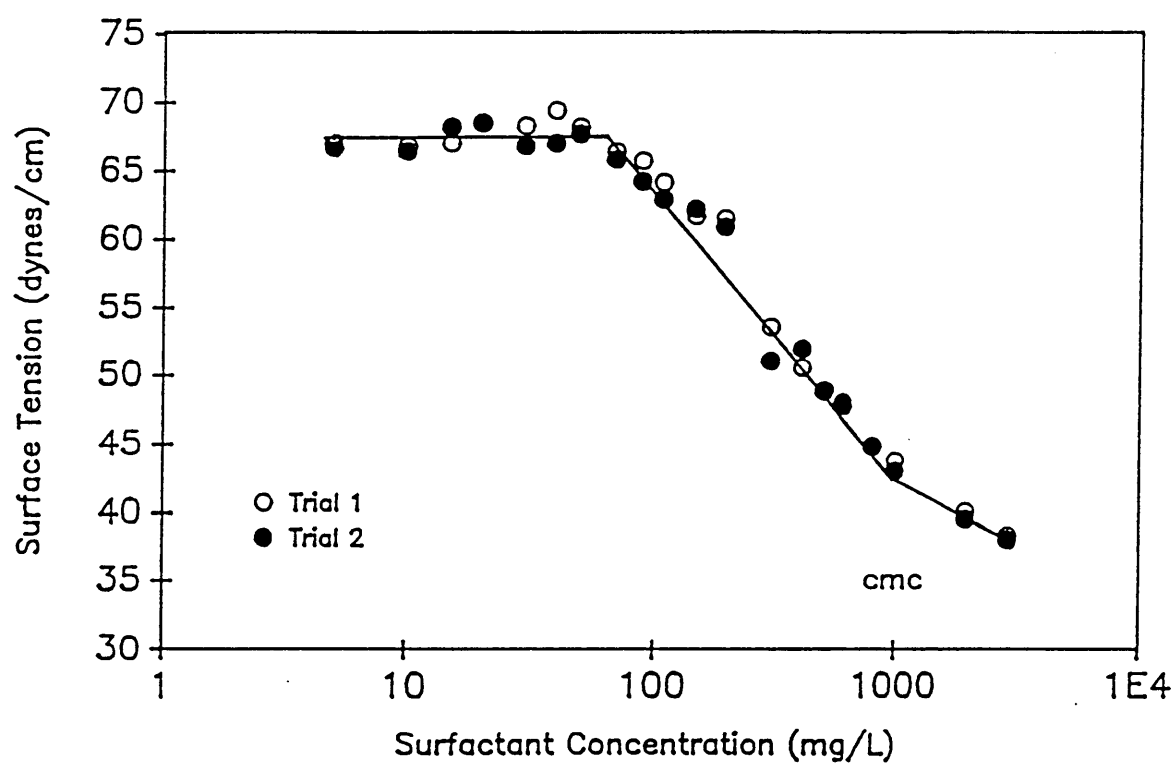


Figure 8 CMC determination of Surfonic N-95 in microcosm solution (data shown were collected after 5 minute settling period)



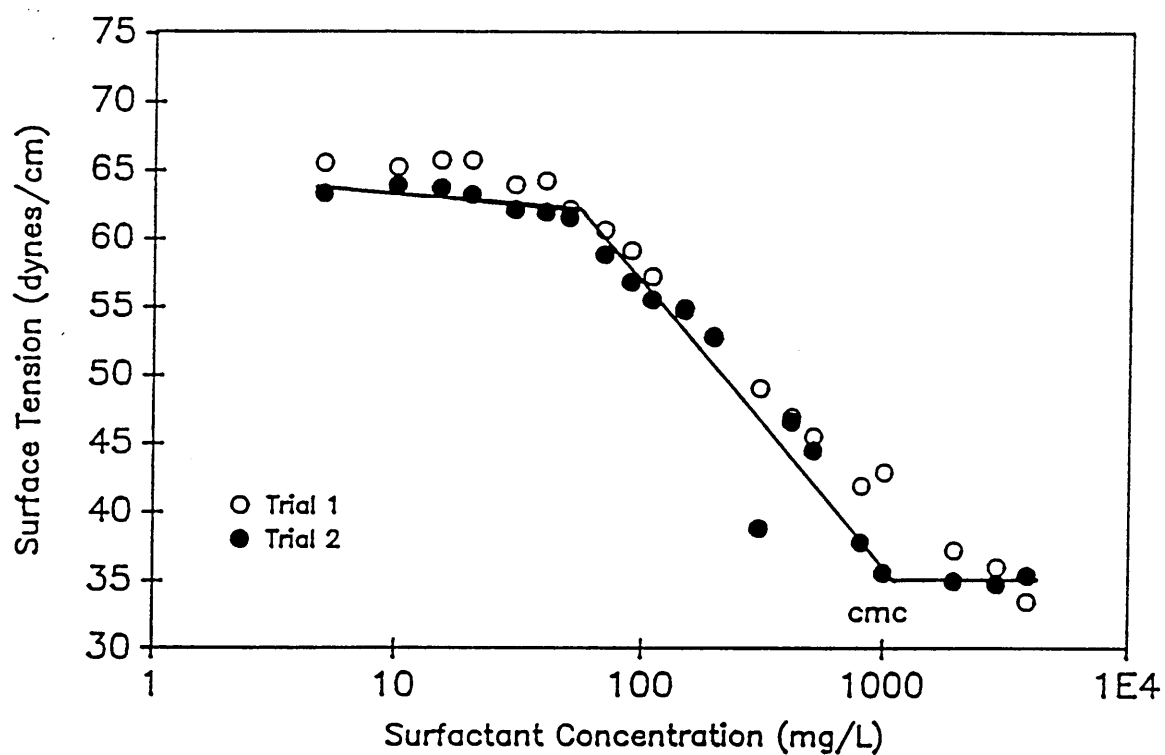


Figure 9 CMC determination of the 1:6 mixture of Surfonic N-40 and N-95 in microcosm solution (data shown were collected after 5 minute settling period)

reduced to the CMC without any lag by both surfactant solutions (Figures 6 and 7). In the microcosm solutions, on the other hand, the surface tension remained essentially unaffected until the surfactant concentration reached about 100 mg/L. The surface tension then slowly decreased with increasing surfactant concentration, relative to the sharp reduction that occurred in distilled water, until the CMC was reached at about 1000 mg/L. Surface tension in these studies pertains to the surface tension at the interface of the aqueous solution and air.

#### LEACHING OBSERVATIONS

Each set of microcosms used in this study was monitored over extended periods subsequent to the complete biological utilization of dissolved toluene. The activity of P. putida slowed shortly after the initial concentration of toluene was depleted, and it was believed oxygen limited conditions prevailed. Figure 2 (previously presented) shows, in addition to the biological removal of toluene, subsequent increases in the dissolved toluene concentration with time in previously active microcosms.

In each case, more toluene dissolved from the soil in microcosms containing surfactant. The data in Figure 10 show the maximum concentrations of toluene redissolved from the soil which contained surfactant as a function of surfactant concentration. The solid line represents the average amount of toluene leached from soil without surfactant in three trials. In a fourth trial, a larger amount (3.2 mg/L) of toluene was leached into solution from soil containing no

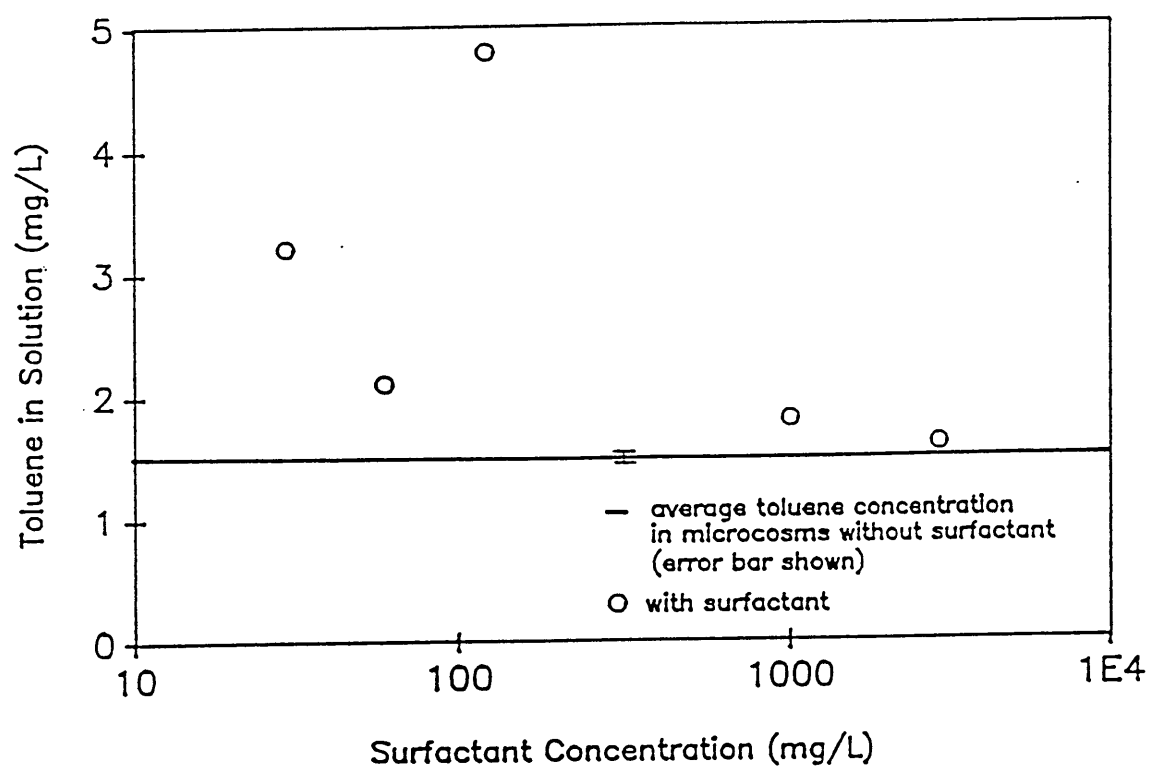


Figure 10 Comparison of average concentrations of toluene leached from soils containing surfactants and those without surfactants

surfactant. In this trial, 30 mg/L surfactant was added. The large concentration of leached toluene is not included in the average.

#### METHYLENE CHLORIDE EXTRACTIONS

The initial amount of toluene originally transferred to the microcosm soil and which remained after the 24-hour equilibration period, as determined from multiple extractions of the microcosms with methylene chloride, ranged from 47.9 mg/L to 74.2 mg/L with a mean of 61.0 mg/L. Table 7 lists the initial extractable amounts of toluene determined in each experiment. These data were considered to be the final amounts of toluene in mass balance calculations.

The mass balance of toluene was described as: the change in the amount of toluene due to biodegradation, leaching, methylene chloride extractions, and volatilization equals the original amount of toluene applied to and remaining in the soil after the equilibration period, normalized to the microcosm volume. Volatile losses were approximated by analysis of control microcosms. Table 8 lists the mass balance values calculated for the experiments with and without surfactant. Approximately half of the original amount of toluene remained unaccounted for in both cases.

Figure 11 shows the toluene concentration in methylene chloride after extractions of either three or four separate trials in each surfactant experiment. These data represent the amount of toluene extracted from microcosm soil containing surfactants after both the biodegradation and leaching periods.

Table 7  
Total Amount of Toluene in Soils  
Determined from Multiple Methylene Chloride Extractions

Experiment Identification	Average Initial Amount of Toluene Extracted (mg/L in methylene chloride)
30 mg/L Surfactant	56
60 mg/L Surfactant	48
120 mg/L Surfactant	74
1000 mg/L and 3000 mg/L Surfactant	64

Table 8  
Mass Balance of Toluene Based on Average Values  
Normalized to Equal Solution Volumes

Toluene (mg/L)						
	Biodegraded	Leached	Extracted	Volatile Losses	Total Accounted For	Total Unaccounted For
Without Surfactant	12.6	3.0	8.7	11.6	35.9	25.1
With Surfactant	10.5	2.3	7.9	11.6	32.0	29.0

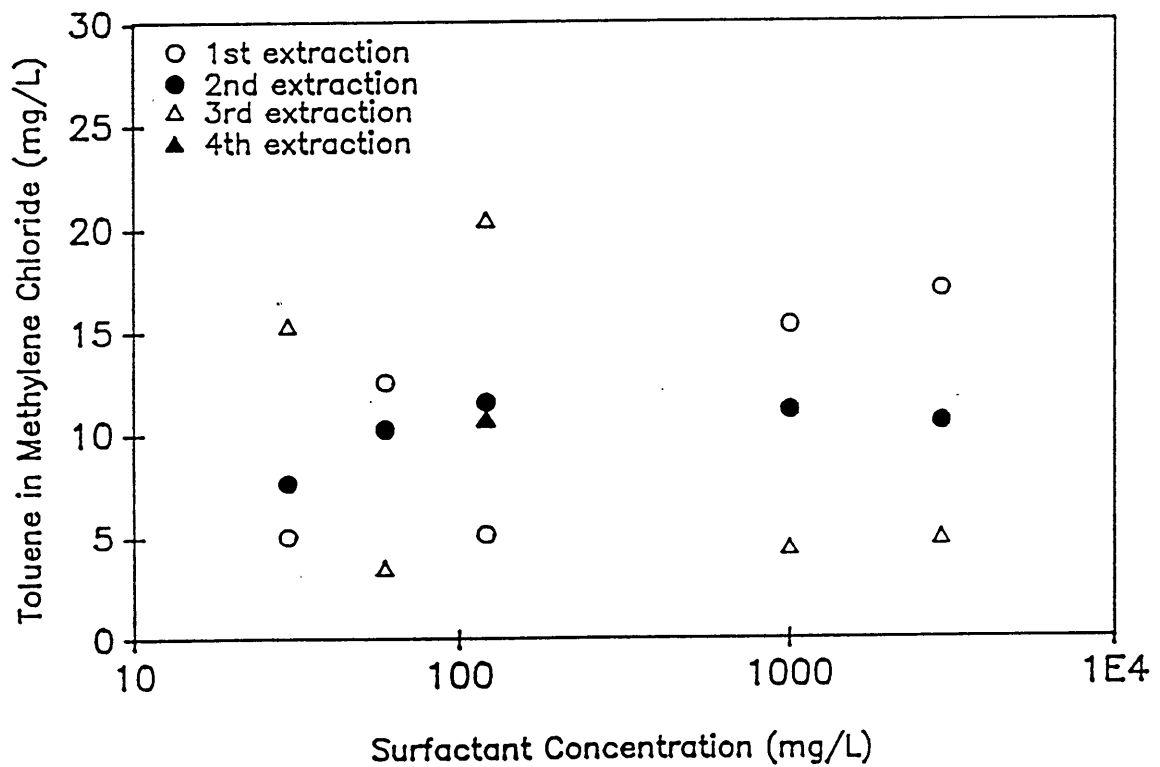


Figure 11 Data representing multiple extractions of toluene from soil containing surfactants with methylene chloride

The average amounts of toluene extracted from soil in microcosms both with and without surfactants were nearly equal. The broad range of data suggests that no difference existed between the amount of toluene which could be extracted from microcosms with and microcosms without surfactants. Figure 12 shows the average amounts of toluene extracted into methylene chloride from soils both with and without surfactants. The solid line in Figure 12 represents the average amount of toluene extracted from soil in microcosms that did not contain surfactant. Neither surfactant presence nor concentration affected the extractable amounts of toluene.



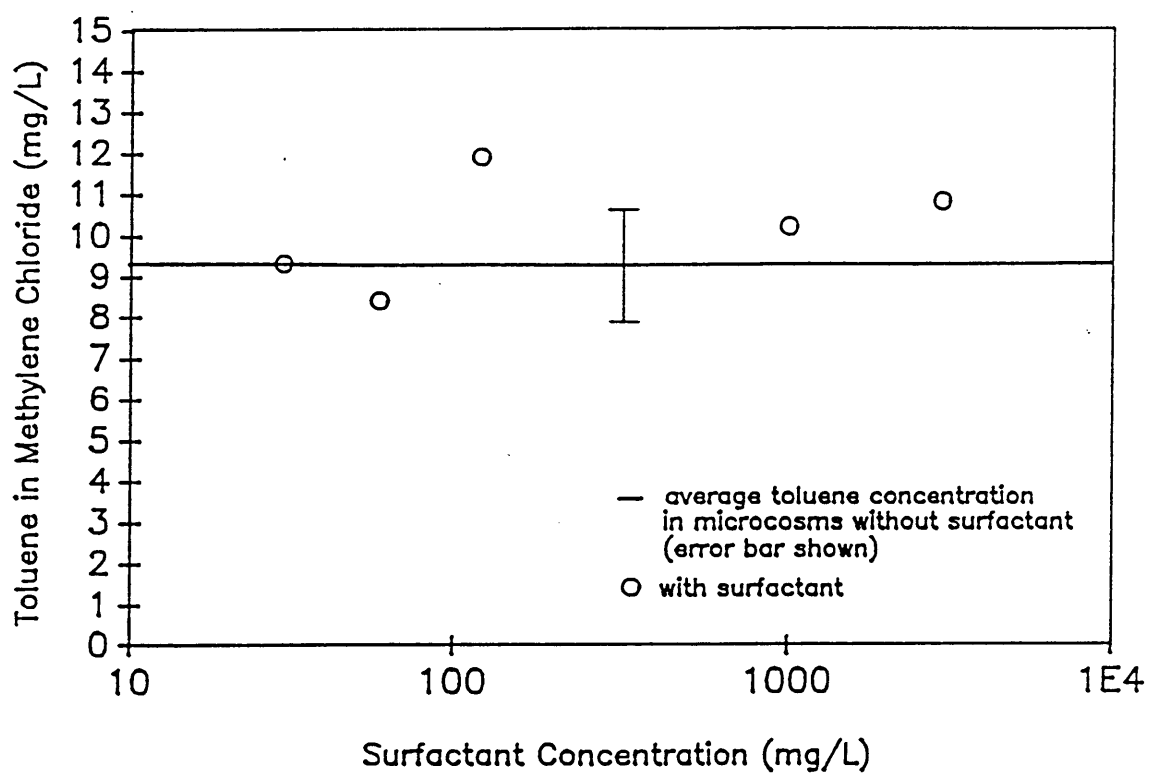


Figure 12 Comparison of average amounts of toluene extracted from soils containing surfactants and soils without surfactants with methylene chloride

## Chapter 5

### DISCUSSION

The objectives of this study were to observe and evaluate the effects of surfactant solutions on the biodegradability of gasoline, as indicated by toluene degradation. Efforts were made to establish a range of gasoline volumes in soil in order to evaluate the effects of two contrasting conditions on the biodegradation of toluene, those two conditions being 1) large-volume, immiscible, organic liquids in pore spaces and 2) lower volume, adsorbed components on soil. In the batch microcosms, larger volumes of gasoline proved detrimental to P. putida, probably because the gasoline concentrations were toxic. Only the microcosms containing 0.005 mL initial gasoline were used to fulfill the objectives of this investigation. The 0.005 mL initial gasoline injection was believed to have effectively simulated conditions which would exist in aquifers contaminated with small volumes of gasoline.

An investigation of the effects of surfactant solutions on adsorbed hydrocarbons is valuable. Gasoline evaporates readily and, as a result, enhanced volatilization techniques are commonly successful for removing residual gasoline from soils. The components which remain, however, are sorbed in soils and in organic films on soil particles. Biodegradation, in combination with surfactant emulsification, is potentially a viable remedial technique. Remediation techniques that involve solubilizing residual oil to remove whole organic fluids, then biologically degrading the remaining organics could prove to be a

relatively fast and economical means of effectively reducing the concentration of contaminants in the subsurface. The results of this study and the results of emulsification experiments practiced on a jet fuel spill (Vignon and Rubin, 1989) demonstrated that the potential for employing both techniques in combination is promising. In the case of the jet fuel spill, bacteria proliferated in the presence of surfactant solutions. In this investigation, P. putida multiplied at equal rates in the presence and absence of surfactants when toluene concentrations were not limiting. The equal growth rates are demonstrated in Figure 1. P. putida also biodegraded toluene in soil microcosms containing and without surfactants. Biodegradation rates, however, were reduced in microcosms containing surfactant amendments, which was related to reduced initial dissolved concentrations.

The literature states that surfactant additions have proven successful as a method to remove oil and organic contaminants from the subsurface in enhanced oil recovery applications and in remedial efforts (Ling et al., 1987; Tuck et al., 1988). The results of this investigation illuminated an important consideration regarding the application of surfactant solutions for the removal of hydrocarbons in soil. Small, but significant, amounts of contaminants may be temporarily rendered unavailable to microbes when they are dissolved in surfactant coatings on soil particles. Reduced availability of substrate reduces the rate of biodegradation of that compound. In time, the trapped organics may dissolve from the surfactant films into the aqueous solution and be liberated as the surfactant is degraded.

Organic release through surfactant biodegradation could be rapid in a mixed bacterial population. Once the organic is released from the surfactant, it may again become biologically available. Therefore, the advantages of emulsifying large amounts of residual oils to aid recovery and speed remedial efforts may not be substantially negated by reduced bioavailability.

The mobilization of immiscible, organic liquids trapped in soil pore spaces may be biologically beneficial. Emulsified or mobile organic fluids may be removed to a large degree through pumping techniques. In addition, mobile organic contaminants may disperse in the subsurface to the degree that potentially detrimental concentrations may be substantially reduced. In either case, soil organisms may provide the final contaminant reductions subsequent to the surfactant amendments, which suggests that surfactants may be very useful in certain situations.

In order to gain insight into circumstances in which surfactants may prove beneficial for subsurface hydrocarbon remediation, the results of this investigation are evaluated in the following sections.

#### **EFFECTS OF SURFACTANTS ON DISSOLVED TOLUENE CONCENTRATION**

As noted in Figures 6 through 9, the shapes of the curves representing the reduction in surface tension with increasing surfactant concentration are noticeably different when soil is introduced into the system. The initial lag period in surface tension reduction up to 100 mg/L in the microcosm solutions is caused by surfactant films partially

coating soil particles. Surfactant coatings in soils represent a surfactant demand by the soil which must be satisfied before surfactant molecules are capable of reducing the surface tension in the microcosm solution. A similar surfactant demand, or sink, also was noted by other researchers (Tuck et al., 1988; Vignon and Rubin, 1989). The demand is a result of hemimicelle formation in the soil matrix.

Hemimicelles (Atwood and Florence, 1983 citing Fuerstenau et al., 1956) are surfactant layers formed on solid surfaces. Atwood and Florence (1983), citing Fuerstenau et al. (1956), stated that at some minimum concentration at the solid/liquid interfaces, well below the CMC, surfactant molecules begin to associate at interfaces in two-dimensional patches. The formation of hemimicelles in patches suggests that some soil surfaces are still in contact with the aqueous solution while others are coated with surfactant. Ionic surfactants may form bilayers in which a layer of surfactant molecules adsorbs onto solid surfaces with the surface-active "heads" oriented toward the active sites at the interface and the hydrophobic "tails" oriented toward the liquid, which is joined in tail-to-tail fashion by a second layer of surfactant molecules (Naylor, 1989). The hydrophobic tail region of the hemimicelle is protected from the aqueous solution by the surface-active or hydrophilic heads of the second layer. Nonionic surfactants probably form patches by adsorption at the interface with the hydrophobic "tails" orient towards the solid surface and the hydrophilic "heads" toward the solution. The hemimicellar structure was believed to have played an

important role in determining dissolved toluene concentrations in this investigation.

Tuck et al. (1988) described the adsorption of surfactant at solid surfaces as a reduction in the surfactant concentration which is capable of mobilizing tetrachloroethylene. The surfactant which adsorbed at the solid surfaces in their experimental columns was no longer available to mobilize the immiscible liquid PCE. A similar demand was demonstrated in this investigation by measuring the changes in surface tension of a microcosm solution with increasing surfactant concentrations.

In Figures 8 and 9 an initial "lag period" is evident in which no reduction of surface tension was realized, even though surfactant concentrations increased well above their respective CMC's in distilled water (Figures 6 and 7). The lag period, or surfactant demand, which had to be satisfied prior to surface tension reduction, indicated that the soil surfaces were coated with hemimicelles. The surfactant demand noted in this work, in all probability, was greater than that which would actually be seen in undisturbed, compacted soil. Soil-surface areas were increased in this study by thoroughly mixing the microcosm solutions after each surfactant addition in order to duplicate the mixing that occurred in each microcosm experiment. A smaller surface area would be available in compacted soil pore spaces.

In Figures 8 and 9, at concentrations above 100 mg/L surfactant, the slope of the surface tension curves sharply decreased, which suggests that hemimicelles had been formed to some degree on the soil surfaces and excess surfactant molecules had begun to adsorb at the

solution surface, thus lowering the surface tension. The surface tension continued to decrease until the critical micelle concentration was reached. Above the CMC, additional surfactant had much less effect on the surface tension.

The formation of hemimicelles on soil surfaces probably served to partition toluene between the solution and the hydrophobic regions of the surfactant. This explanation is supported by a comparison of the data shown in Figure 5 with those shown in Figures 8 and 9. Figure 5 indicates that the initial concentrations of dissolved toluene were lower in microcosms containing surfactant than in those without surfactants except when they contained 30 and 1000 mg/L surfactant. It appears that surfactant had little effect on the concentration of the dissolved toluene in microcosms containing 30 mg/L surfactant, because very few, if any, hemimicelles had been formed. In the microcosms containing 1000 mg/L, sufficient micelles had formed in solution to solubilize toluene from the hemimicelles. Figures 8 and 9 indicate that 30 mg/L surfactant is far to the left of the surface tension curve in the lag period, which suggests that only part of the surfactant demand had been filled, whereas the 1000 mg/L concentration is coincident with the CMC.

Sixty mg/L surfactant reduced to a greater degree the toluene concentration as more hemimicelles formed. Sixty mg/L surfactant occurs at a later point (further to the right) in the surfactant demand period, which suggests a greater number of hemimicelles existed. At 120 mg/L surfactant, an equilibrium was reached between a large, perhaps maximum,

number of hemimicelles and an increasing number of surfactant molecules in solution. This is evidenced by the sharp change in slope of the surface tension curve in Figures 8 and 9. Excess surfactant, i.e. amounts greater than that required to form the hemimicelles, in the bulk solution increased the concentration of toluene in the aqueous solution above that noted when the surfactant demand was nearly satisfied.

In the microcosms containing 1000 mg/L surfactant the initial concentration of dissolved toluene was expected to be greater than in the microcosms containing 120 mg/L because a greater concentration of surfactant molecules were assumed to be present in the bulk solution. The assumption was based on the observed reduction in surface tension. Figure 5 in fact shows increased dissolved toluene concentrations at the 1000 mg/L surfactant concentration, while Figures 8 and 9 show a near minimum in solution surface tension at 1000 mg/L surfactant.

The concentration of toluene decreased to 10.9 mg/L in the microcosms containing 3000 mg/L surfactant from 12.2 mg/L in the microcosms containing 1000 mg/L surfactant, but the solution surface tension remained at a minimum. An important factor which may have contributed to the change in toluene concentration was that 3000 mg/L surfactant was above the CMC in the microcosm solution while 1000 mg/L was at or slightly below the CMC.

The decrease in toluene concentration from microcosms containing 1000 mg/L surfactant to the microcosms containing 3000 mg/L surfactant may have been the result of toluene adsorbing and partitioning into a greater abundance of micelles in the solution which were not



representatively sampled with the microsyringe. The micelles and surfactant monomers may have concentrated at the solution surface and interfaces such that the concentration of toluene in the center of the microcosm tube may have been considerably lower than the actual concentration of toluene in the total solution. A difference in toluene concentration between the bulk solution and the solution surface was noted in control microcosms. Samples collected at the surface tended to contain greater concentrations of toluene than samples collected near the center of the tube. Adsorption is expected when a non-polar solute is present in a polar solvent. Therefore, the reduction in dissolved toluene concentration in the microcosm solution containing 3000 mg/l surfactant may be the result of experimental error.

To help prevent unrepresentatively sampling the microcosms, the syringe was filled and emptied several times to mix the solution. The tubes were not rigorously mixed to avoid injecting soil particles into the GC column and to avoid mechanically releasing toluene from the soil and the surfactant. The mixing technique was probably insufficient.

The fact that substantially more micelles existed in the 3000 mg/L solution suggests another potential point of concern. Solution micelles may concentrate at interfaces, therefore, small amounts of the organic desired to be removed may actually be delayed in removal. This suggests that there may be a minimum dose of surfactant required to accomplish a specific remedial task. Note, however, that the system employed in this investigation was a static, batch system. A flow-through system may

have enhanced the solubility of the micelles by mechanical action such that the interfacial adsorption would be minimized.

#### BIOLOGICAL CONSIDERATIONS

The batch microcosm system employed in this investigation limited the scope of the study to smaller initial amounts of gasoline. Appreciable biodegradation did not occur in every experiment attempted in which greater than 0.005 mL gasoline was injected. Since the objective of the study was to investigate the effects of surfactant solutions on biodegradation of gasoline components, these experiments were deemed to be of little use. The most likely explanation for the lack of biodegradation in the microcosms containing higher gasoline concentrations is that the gasoline was toxic. A flow-through system would probably have eliminated the toxic conditions.

Biodegradation rates were calculated from changes in the dissolved toluene concentration for each set of microcosms observed. The rates were plotted as a function of the initial dissolved toluene concentration in Figure 4. Biodegradation rates are directly dependent on the initial concentration of the substrate. Figure 5 indicates that lower, initial, dissolved-toluene concentrations were present in microcosms containing surfactant solutions. Data from control microcosms containing surfactant amendments indicated lower initial toluene concentrations than control microcosms without surfactants. Reduced toluene concentrations in microcosms containing surfactants is in agreement with the data gathered from active microcosms and probably

occurs because surfactant molecules adsorbed on soils dissolved toluene from the aqueous solution. Figure 4 indicates that biodegradation rates were lower when the microcosms contained surfactant solutions. The lower rates observed during this investigation were directly correlated with the suppression of the initial dissolved toluene concentration.

Toluene binding in hemimicelles likely caused reductions in dissolved toluene concentrations, which, in turn, reduced the amount available for immediate biodegradation. As a result, biodegradation rates were lower in the microcosms with surfactant solutions than in those without them.

Suppressed biodegradation rates were not evident in the growth rate experiment indicated in Figure 1. Even though the surfactant probably adsorbed at the glass-liquid interface in the jars and at the surface of the solution, thus concentrating the dissolved toluene there, the initial concentration of toluene was sufficiently high that it was not rate-limiting, even when some was held in surfactant layers. More importantly, the surface area available for surfactant adsorption was much smaller in the jars without soil than in the microcosms containing soil. Therefore, the effects of toluene partitioning into hemimicelles was drastically reduced in the jar.

These observations suggest that certain surfactants may not be deleterious to microbial populations or microbial degradation, provided the concentration of the organic substrate being degraded is never limiting. Substrate limitations are probably not of concern initially when hydrocarbon-mobilization by surfactants is practiced, even though,

surfactants may affect the concentration of hydrocarbons in solution. These results demonstrated that P. putida can degrade toluene in the presence of several concentrations of surfactants.

#### TOLUENE REMAINING IN SOILS

The results of this investigation also indicate that surfactants may affect the biological availability of organic compounds. Figure 10 indicates that a greater concentration of toluene leached from soils containing surfactant solutions than soils without surfactants. A possible explanation is that an equilibrium existed between the high toluene concentration in the hemimicelles and the absence of toluene in the aqueous solution following biodegradation, which resulted in greater amounts of toluene leaching into solution in microcosms containing surfactant. Biodegradation rates were assumed to be very low after the initial substrate was utilized.

According to the simplified equation for the mineralization of toluene used to model the biodegradation of hydrocarbons, ( $C_7H_8 + 9O_2 = 7CO_2 + 4H_2O$ ) each 1 mg/L of toluene degraded requires 1.6 mg/L of  $O_2$  (Barker and Mayfield, 1988). The stoichiometry may vary for a single, acclimated bacterial species, but the relationship suggests that conditions in the microcosms were probably dissolved oxygen deficient. The initial amount of oxygen supplied to the microcosms through hydrogen peroxide additions was 2.6 mg/L in addition to the approximately 8.0 mg/L of dissolved oxygen resulting from equilibration with the atmosphere. Assuming some variance in the stoichiometric relationship,

10.6 mg/L of  $O_2$  was probably sufficient to provide aerobic conditions for the biodegradation of 10 to 12 mg/L of toluene.

P. putida are obligate aerobes and were probably detrimentally impacted by the lack of oxygen. Some of the aerobic microbes, however, probably survived in the reduced oxygen conditions and some indigenous, facultative bacteria may have survived the initial sterilization of the soil. Active microbes, in addition to volatilization, may explain why initially dissolved toluene was reduced in control microcosms and why leached toluene disappeared at extended periods of time. The rates of toluene reduction in control microcosms appeared to be equal between microcosms containing and without surfactants.

The data in Figure 12 represent the average amount of toluene extracted from soils containing surfactants. The solid line in the figure represents the average amount of toluene extracted from soil containing no surfactant. The amounts of toluene extracted from the soil after the biodegradation and leaching periods was equal whether or not surfactants were present. The broad range covered by the data in Figure 11, representing individual extractions in microcosms containing surfactants, is indicative of the variability of the methylene chloride extraction technique.

Farmer (1989) measured 50.5 percent to 72.1 percent efficiency for the methylene chloride extraction technique. The extraction efficiency in this investigation may have been lower than 50 percent due to soil clogging. The wet soil became very tacky and clumped upon addition of methylene chloride.

Using a mass balance approach, those microcosms with the greatest amount of initially dissolved toluene should exhibit the smallest amount of toluene remaining the soil, assuming that volatilization rates and the amounts of toluene remaining in the soil prior to the methylene chloride extraction stage were equal between the microcosms with and without surfactants. The trend of higher initial dissolved toluene concentrations corresponding to lower concentrations leached from soil is evident from a comparison of Figures 5 and 10.

Variability in the amounts of toluene extracted from the soil aggravated the mass balance calculations. The amount of toluene listed as unaccounted for in Table 8 was most likely lost through volatilization and continued biodegradation. As toluene was removed from solution by volatilization and degradation, an undetermined and varying amount of toluene continually leached into solution from the soil.

#### IMPLICATIONS FROM THE DATA

The results of this investigation of the effects of surfactants on the biodegradation of gasoline in the subsurface favor the application of surfactants for some subsurface remedial uses, but not for others. The results suggest that surfactants are not beneficial as a means of aquifer and groundwater remediation for adsorbed hydrocarbons. Their ability to mobilize organic liquids in the subsurface, which is potentially beneficial, was not observed in this study, however, it was noted in the literature review from other investigations.

The literature indicates that surfactants are useful as a means of mobilizing interstitial oil in the subsurface. Surfactant amended purging solutions reduce the interfacial tension between the immiscible fluid and water which promotes mobilization. In the case in which no interstitial organic fluid exists, however, which were the conditions simulated in this study, oil emulsification is not the mechanism required for remediation. Instead, increased solubility of the organics is the result desired from the surfactant solutions. In this investigation, no increase in toluene solubility was achieved with the surfactants employed above the solubility in solutions without surfactants. In fact, a measurable decrease in dissolved toluene concentration was noted at surfactant concentrations below 1000 mg/L in microcosm solutions. This was attributed to the formation of hemimicelles on soil surfaces into which toluene dissolved.

Toluene dissolved into the surfactant films in the soil matrix reduced the concentrations of toluene dissolved in solution, which in turn resulted in reduced toluene biodegradation rates. Even though, the concentrations of toluene in the aqueous solution were initially reduced in microcosms containing surfactants, increased concentrations of toluene were subsequently released from the soil. These observations suggest that toluene is not available to soil microbes while bound in the surfactant hemimicelles, but that it is only temporarily bound and easily released. The latter indicates that toluene is eventually available for biodegradation.

The fact that surfactants adsorb onto soil surfaces is important to note. Not only may the organic chemicals targeted for removal be retained in surfactant layers in the soil, but also great expense may be incurred to first supply surfactant to satisfy the demand in the soil, then to add the amount necessary to achieve organic mobilization. Surfactants may prove to be too expensive for remedial situations where volatile hydrocarbon removal is required in which adsorbed components in soil may be the dominant form of contamination.

Surfactant additions appear to be a practicable method of removing trapped, free phase organics from some subsurface conditions. Relatively rapid removal of relatively large amounts of organic contaminants serves to effectively improve the quality of the aquifer. In addition, surfactants may promote biological activity by reducing toxic concentrations of organics through mobilization. In the case of free phase organics in the subsurface, the advantages of contaminant mobilization probably outweigh the disadvantages of reduced biodegradation rates, assuming that organics dissolved in surfactants are released from the surfactant films and are eventually biodegraded. Perhaps the best application of surfactants for subsurface restoration is in the case of large volumes of separate phase organic liquids trapped in permeable soils. On the other hand, perhaps the least desirable circumstances for the use of surfactants as a remedial tool are predominantly adsorbed organics of moderate to high aqueous solubility, which might be found with smaller volumes of gasoline in soil.



## Chapter 6

### SUMMARY AND CONCLUSIONS

The effects of surfactant solutions, applied as a means of mobilizing gasoline components in the subsurface, on the biodegradation of toluene was observed in this investigation. Gasoline hydrocarbons adsorbed in soils was simulated in this study. The results indicated that the surfactants employed in the soil microcosms decreased the biological availability of toluene and therefore the rate of biodegradation. The biodegradable, nonionic surfactants did not impair the ability of P. putida to degrade toluene when toluene was not limiting.

Hemimicelles or surfactant films are believed to have formed on the heterogenous soil surfaces. Hemimicelles constitute a surfactant demand which limits the amount of surfactant available to mobilize hydrocarbons. The amount of surfactant required to reduce surface tension to a minimum increased two orders of magnitude when applied to microcosm solutions containing soil over the amount required in distilled water. The aqueous surfactant concentration was not measured after the formation of hemimicelles, therefore, the amount of surfactant is stated as the concentration which would exist if all of the surfactant remained in solution for comparison between systems containing soil and those without soil.

The surfactant films provided sites into which organic contaminants may have dissolved, which decreased their biological

availability. Initial, dissolved toluene concentrations were reduced in microcosms containing surfactants and as a result biodegradation rates were reduced in those microcosms. The toluene which was believed to be dissolved into hemimicelles, however, slowly redissolved into solution. This suggested that the once unavailable hydrocarbons became biologically available.

Provided the amounts of surfactants needed to satisfy the demand in the soil is not cost prohibitive, surfactant mobilization in combination with biodegradation could prove to be a viable practice for remediation of aquifers contaminated with separate phase liquid hydrocarbons. Surfactants can emulsify immiscible liquids in the subsurface and promote mobility.

In the case of single-phase, smaller-volume, volatile-hydrocarbon contamination, like gasoline, surfactants may diminish the remediation effort by restricting the availability of organic compounds to microbes. A fluid composed largely of volatile compounds may, if spilled or leaked in small quantities, be retained in soils only as adsorbed components. Surfactants may promote adsorption of compounds at soil surfaces in hemimicelles. Those compounds in hemimicelles may be biologically unavailable.

#### SPECIFIC CONCLUSIONS

-P. putida, acclimated to toluene, multiplied at equal rates in solutions with and without 60 mg/L Surfonic N-95 when toluene was not limiting.

-Surfactants formed coatings, called hemimicelles, in soils which resulted in a surfactant demand or additional amounts of surfactant required to achieve surface tension reduction.

-The hemimicelles in soils provided a favorable environment into which toluene dissolved, which reduced the aqueous toluene concentration.

-The reduced aqueous concentration of toluene resulted in reduced toluene biodegradation rates, which occurred in microcosms containing surfactants.

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