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BIOSURFACTANT ENHANCED NONAQUEOUS PHASE LIQUID (NAPL) REMOVAL AND BACTERIAL TRANSPORT IN POROUS MEDIA

by

Guiyun Bai

A Dissertation Submitted to the Faculty of the
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1997
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Guiyun Bai entitled Biosurfactant Enhanced Nonaqueous Phase Liquid (NAPL) Removal and Bacterial Transport in Porous Media and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director Raina M. Miller
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>6</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>7</td>
</tr>
<tr>
<td>CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW</td>
<td>9</td>
</tr>
<tr>
<td>Introduction</td>
<td>9</td>
</tr>
<tr>
<td>Literature Review</td>
<td>12</td>
</tr>
<tr>
<td>Entrapment And Transport of NAPL</td>
<td>12</td>
</tr>
<tr>
<td>Residual Saturation</td>
<td>14</td>
</tr>
<tr>
<td>NAPL Size Distribution</td>
<td>16</td>
</tr>
<tr>
<td>Surfactant Enhanced NAPL Remediation Technology</td>
<td>18</td>
</tr>
<tr>
<td>Laboratory Experiments</td>
<td>22</td>
</tr>
<tr>
<td>Field Studies</td>
<td>26</td>
</tr>
<tr>
<td>Microbially Produced Surfactants (Biosurfactants)</td>
<td>29</td>
</tr>
<tr>
<td>Dissertation Format</td>
<td>31</td>
</tr>
<tr>
<td>CHAPTER 2: PRESENT STUDY</td>
<td>33</td>
</tr>
<tr>
<td>APPENDIX A: BIOSURFACTANT ENHANCED REMOVAL OF RESIDUAL HYDROCARBONS FROM SOIL</td>
<td>37</td>
</tr>
<tr>
<td>APPENDIX B: INFLUENCE OF CATION TYPE, IONIC STRENGTH AND PH ON SOLUBILITY AND MOBILIZATION OF RESIDUAL HYDROCARBON BY A BIOSURFACTANT</td>
<td>65</td>
</tr>
<tr>
<td>APPENDIX C: THE INFLUENCE OF RHAMNOLIPID BIOSURFACTANT ON THE TRANSPORT OF BACTERIA THROUGH A SANDY SOIL</td>
<td>93</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>130</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>(A) Schematic of liquid-water interface in porous medium.</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(B) Interface in capillary tube.</td>
<td>13</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Blob entrapment mechanisms.</td>
<td>17</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Structures of selected glycolipids from microorganisms.</td>
<td>32</td>
</tr>
</tbody>
</table>
ABSTRACT

The well documented ineffectiveness of traditional pump-and-treat technology on the cleanup of non aqueous phase liquid (NAPL) contaminated sites has incurred an intensive research activities in improving the efficiency of NAPL removal from subsurface. Surfactant enhanced subsurface remediation has been proposed as one such option. In this dissertation, a series laboratory experiments were conducted to investigate the potential application of a microbially produced surfactant (biosurfactant) on NAPL removal and the effect on bacteria transport. Monorhamnolipid biosurfactant, produced by Pseudomonas aeruginosa ATCC 9027, was used in all the studies. Hexadecane was used as model NAPL to represent petroleum based products which are common NAPLs detected in contaminated sites.

Results showed that rhamnolipid biosurfactant is effective in removing residual hexadecane from sandy soil. In the surfactant concentration tested in this study (40 to 1500 mg/L), mobilization of hexadecane is the main mechanism of the removal. In addition to displacement of hexadecane droplets from subsurface porous matrixes, dispersion or emulsification of hexadecane into surfactant solution also played an important role in hexadecane removal. The performance of this anionic rhamnolipid surfactant is greatly affected by the addition of electrolytes and the change of pH. Addition of Na\(^+\) and Mg\(^{2+}\) can significantly increase the solubilization capacity of rhamnolipid and reduce the interfacial tension between hexadecane and surfactant solution, while addition of Ca\(^{2+}\) has a competing effects of enhanced solubilization and
Ca\(^{2+}\) induced rhamnolipid precipitation. Control of ionic strength and pH can be used to optimize surfactant systems to enhance the NAPL removal depending on the nature of NAPL (LNAPL or DNAPL).

Addition of rhamnolipid can also enhance the transport of three bacterial cells with varying hydrophobicity, *P. Aeruginosa* ATCC 9027, 27853, and 15442, by decreasing cell adsorption. This is because the adsorption of surfactant to the porous medium surface increases the surface negative charge density, hence the adsorption of bacteria to the surface is reduced. No significant influence of rhamnolipid on the bacteria surface properties is observed. The measured bacteria breakthrough curves were simulated by an advection-dispersion transport model incorporating two domain reversible sorption (instantaneous and rate-limited) and with two first order sink terms for irreversible sorption. Model simulation suggests that rhamnolipid mainly affects the irreversible sorption of cells.
CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Introduction

The widespread detection of organic solvents and other petroleum based products in ground water has prompted intensive study of non-aqueous phase liquid (NAPL) transport and dissolution in the subsurface environment. NAPL contaminated sites are often associated with leaking underground storage tank, pipeline ruptures and illegal disposal of waste materials (Feenstra and Coburn, 1986; Mercer and Cohen, 1990). Typical NAPLs of concern include transformer oils that contain polychlorinated biphenyls (Schwartz et al., 1982), trichloroethylene and related chlorinated hydrocarbons (Palumbo and Jacobs, 1982), petroleum based products including gasoline or diesel (Pfannkuch, 1983), coal tar from gas manufacturing plants (Villaume, 1982, 1984), and solvents from wood treating operations (Hickok et al., 1982). U.S. Environmental Protection Agency (USEPA) (1979) has estimated that in 1974 alone, about 310,200 tons of waste solvents were produced by degreasing operation. In addition, there are an estimated 796,000 motor fuel storage tanks in the U.S.A (USEPA, 1986), most of which are considered to be leaking.

Following a spill or leak, NAPLs generally migrate downward through the vadose zone due to gravitational forces. If a large enough volume is spilled, NAPLs may reach the water table. If the NAPL is less dense than water (LNAPL) (e.g., most petroleum hydrocarbons), it will tend to spread laterally along the top of water table, forming a pool
of free product. However, fluctuations in the water table will result in vertical displacement of LNAPLs and their subsequent redistribution in the saturated zone. In contrast, NAPLs that are more dense than water (DNAPLs) (e.g. most chlorinated solvents) will continue to migrate vertically through the saturated zone until they reach a confined boundary such as rock layer (Mackay et al., 1985; Schwille, 1988). In either cases, as the NAPLs are transported through the subsurface, a portion of the NAPL will be retained within soil pores as immobilized “ganglia” or “globules” due to the interfacial forces between the water phase and the NAPL. Such entrapment is a complex interaction of soil and NAPL fluid characteristics. Eventually, NAPL migration will cease when all the NAPL is trapped as discontinuous and essentially immobile ganglia. The trapped NAPL, known as the residual NAPL saturation, may occupy from 5 to 40% of the pore volume (Schwille, 1984; Hunt et al., 1988).

Present knowledge of the behavior of NAPLs in porous media comes largely from investigations conducted by petroleum engineers with the aim of improving oil recovery from petroleum reservoirs. Numerous studies have been conducted to examine the entrapment and mobilization of residual organic liquids such as crude oil and refined mineral oils in the porous media (Chatzis et al., 1983; Anderson, 1987). The general conclusion of these studies is that under conditions of stable immiscible displacement, the amount of the NAPL trapped within a porous medium and the “blob” size distribution within the porous medium are strongly dependent on several factors. These include the pore geometry, NAPL phase fluid properties (interfacial tension, viscosity, and density),
system wettability, and Darcy velocity of the ambient aqueous phase flow (Wardlaw, 1982; Mohanty et al., 1987).

NAPLs in saturated subsurface zones create a long term source of pollution as they slowly partition into the aqueous phase by interphase mass transfer processes (Mackay et al., 1985; Mackay and Cherry, 1989; Mercer and Cohen, 1990). Organic species resulting from dissolution of NAPL sources are found in an alarming number of municipal drinking water supplies (Feenstra and Cohurn, 1986). Many of these species are classified as potential carcinogens and have recommended maximum contaminant levels of only a few parts per billion in drinking water (Federal Register, 1987).

The solubility of most of the NAPL is usually considerably low in terms of molar fraction (usually $< 10^{-3}$). Thus, when contacted with ground water, a NAPL of moderate solubility (such as PCE) can contaminate as much as 10,000 times of its own volume to its solubility limit. Yet, organic compounds in the subsurface are only rarely found at concentrations approaching their solubility limits. Usually the observed concentration is more than 10 times lower than the equilibrium solubility (Mackay et al., 1985). This phenomenon is attributed to irregular NAPL distribution, nonuniform flow patterns, dilution effects, and rate-limited mass transfer between NAPL and aqueous phase (Hunt et al., 1988; Powers et al., 1992). This implies that the volume of ground water that could be contaminated by an NAPL is much larger than that calculated by assuming dissolution to the solubility limit. Thus, it is evident that what might once have been considered as a small spill or leak (for example, tens of gallons of pure industrial solvent
considered as a small spill or leak (for example, tens of gallons of pure industrial solvent every time a tank is filled or a transfer line is flushed) may actually constitute a significant source of contamination if the spills reaches the groundwater zone.

Literature Review

Entrapment and Transport of NAPLs

Migration of NAPLs in subsurface environment is a complicated process. Immiscible displacement of NAPLs by water, or vice versa, is influenced by the viscosity and the density difference between the two fluids, by interfacial tension, as well as by local pore geometry (Kueper and Frind, 1988).

The entrapment process and resulting general shape of residual NAPL can be described by the capillary phenomena. Most natural sandy aquifers are mainly composed of materials such as quartz, feldspar, and calcite. In such systems, organic fluids usually behave as the non-wetting phase and water as the wetting phase (Mercer and Cohen, 1990). As shown in Figure 1a, a discontinuity in pressure exists across the interface between the two immiscible liquids in the pore space. The difference between the pressure of the two side of the interface is defined as the capillary pressure ($P_c$). The magnitude of $P_c$ depends on the interfacial curvature as well as interfacial tension, as given by Laplace Equation:

$$P_c = \gamma_{ow} \left( \frac{1}{r_1} + \frac{1}{r_2} \right)$$
Figure 1a. Schematic of liquid-water interface in porous medium

Figure 1b. Interface in a capillary tube
where $\gamma_{ow}$ is the interfacial tension between the NAPL phase and water, and $r_1$ and $r_2$ are the principle radii of the curvature of the interface. Since it is difficult to evaluate the radius of curvature in an irregular pore space, the pores are frequently assumed to be cylindrical and the menisci are spherical (Figure 1b). With this assumption, the Laplace Equation reduces to:

$$p_c = \frac{2\gamma_{ow} \cos \theta}{r}$$

where $\theta$ is the contact angle of water phase with solid phase, and $r$ is the radius of a cylindrical pore. The Laplace equation also describes the interfacial forces controlling the entry and entrapment of non-wetting phase (NAPL) in the porous media. Accessible pores with radius larger than $r$ will be filled with NAPL at a capillary pressure defined by this equation (Anderson, 1987). Accordingly, the NAPL enters large pores preferentially during gravitational drainage. And eventually, blobs can become detached from the continuous NAPL as the interface between water and NAPL phase becomes unstable. Analysis of the Laplace equation leads to the conclusion that the interfacial tension, contact angle, and the pore size distribution all influence the immiscible displacement process during NAPL and aqueous phase migration in porous media.

**Residual Saturation.** The extent of the NAPL entrapped in the porous media depends on three forces: viscous, buoyant, and capillary forces. The viscous force ($F_v$), arises from fractional forces when water passes a NAPL blob, and is proportional to the velocity and viscosity of the flushing solution. The buoyant force ($F_b$) is proportional to
the density difference between the NAPL and water. The capillary force \( F_c \), is proportional to the interfacial tension between the NAPL and aqueous phase.

For horizontal displacement of a NAPL, mobilization of a globule requires that the viscous force plus the buoyant force exceed the net restraining capillary force:

\[ F_v + F_b > F_c \]

This relation can be characterized by two dimensionless numbers: The Capillary number \( (N_c) \) and the Bond number \( (N_B) \). The Capillary Number is the ratio of viscous forces to capillary forces. The Bond Number is the ratio of gravitational forces to capillary forces. Morrow and Songkran (1981) give the following definition for the Capillary Number and Bond Number:

\[
N_c = \frac{q \mu_w}{\sigma_{ow}}
\]

\[
N_B = \frac{\nabla \rho g (k / \phi)}{\sigma_{ow}}
\]

Where: 
- \( q \) = flow rate(cm/sec)
- \( \mu_w \) = water viscosity(g/cm-sec)
- \( \sigma_{ow} \) = interfacial tension(dynes/cm)
- \( \nabla \rho \) = Fluid-density difference(g/cm^3)
- \( k \) = absolute permeability(cm^2)
- \( \rho \) = porosity
- \( g \) = gravity acceleration(cm/sec^2)
The correlation between $N_T (N_C + M_B)$ and the residual NAPL saturation has been extensively investigated in the petroleum industry (Morrow and Songkran, 1981; Wilhite, 1985; Lake, 1989). It has been found that there is a critical value of the $N_T (N_T^*)$ at which residual NAPL begins to be released from porous media. Further increase of $N_T$ from this critical value ($N_T^*$) causes the residual NAPL saturation decrease rapidly. Results show that for a well sorted sand, the $N_T^*$ value is around $10^{-5}$.

The majority of the studies of oil recovery in petroleum industry have focused on the Capillary number. This is probably because in the petroleum industry, the density difference between oil and water is relatively insignificant. Recent studies, however, show that the Bond number is also important due to the significant density difference between most NAPLs (for example, gasoline, TCE, etc.) and water.

**NAPL Size Distribution.** Chatzis et al. (1983) have shown that pore geometry controls the distribution of residual NAPL blob size when the interfacial tension between the NAPL and the aqueous phase is high during immiscible displacement. In a solid porous medium with a high pore-body-to-throat ratio, NAPL tends to “snap-off” in individual pore bodies (Chatzis et al., 1983; Morrow et al., 1988) (Figure 2). Snap-off occurs when interfaces within a pore throat become highly curved and thus unstable as wetting phase enter the pore throat. When all the throat menisci connected to NAPL in the pore body are broken, a blob of NAPL becomes isolated. Thus, the number of pore throats connected to each pore body is an important factor in determining whether a NAPL blob will become entrapped in a given pore body by the snap-off mechanism.
Figure 2. Blob entrapment mechanisms
This characteristic is referred to as pore connectivity or pore topology. Single blobs, that exist within one pore body and are formed by the snap-off mechanism, are the predominant type of blob in well sorted unconsolidated sands (Wilson and Conrad, 1988; Conrad et al., 1989).

Another mechanism for the residual NAPL formation is “bypassing”. Bypassing occurs in regions with several adjacent large pore bodies that are connected by relatively large throats and surrounded by smaller throats, such as would be found in a lens of coarse sand within a finer sand. The actual geometry of blobs generated by this mechanism is very complex, often encompassing several adjacent pore bodies and throats. Chatzis et al., (1983) have provided a thorough discussion of NAPL entrapment by this mechanism.

Surfactant-Enhanced NAPL Remediation Technology

Remediation of NAPL contaminated sites has traditionally involved the initial step of recovering as much of the NAPL free product from the aquifer as possible. This is followed by extracting contaminated ground water to the surface, treating the ground water, and recharging it, a process often referred to as pump-and-treat technology. Due to the large residual amount of the contaminant present (5 to 40 % residual saturation after removal of free product) and the low water solubility of many NAPL, the amount of NAPL dissolved in water is relatively insignificant. In addition, concentrations of NAPL in ground water rarely exceed 10% of their aqueous solubility as discussed above. Thus, the conventional pump-and-treat technologies, which are based on NAPL dissolution,
have been proven to be an ineffective and costly means for aquifer restoration (Mackay and Cherry, 1989; Haley et al., 1991; and Macdonald and Kavanaugh, 1994).

Surfactant-enhanced aquifer remediation has been proposed as an alternative method for remediating residual NAPL contaminated aquifers. Two very different approaches are being involved for achieving enhancement: i) Solubilization of NAPL components in surfactant micelles and ii) Mobilization of residual NAPL by reducing the interfacial tension between the NAPL and aqueous phases.

The first approach is based on the ability of micellar surfactant solution to increase the aqueous solubility of hydrophobic organic compounds. Surfactants are molecules that have both hydrophilic and hydrophobic moieties. A unique characteristic of surfactant molecules is their ability to self-assemble into dynamic aggregates known as micelles when the surfactant concentration reaches a critical micelle concentration (CMC). Below the CMC, surfactants exist solely as monomers in solution. These surfactant monomers have a minimal effect on the solubility of hydrocarbons. For example, Kile and Chiou (1989) conducted a solubilization enhancement experiment with DDT and trichlorobenzene (TCB) in petroleum sulfonates, which are a commercial mixture of emulsified sulfonated hydrocarbons and free mineral oils. They found that solubility was enhanced by 4-5 fold. These surfactant mixtures do not form micelles as evidenced by the linear decrease in surface tension for concentrations up to 500 mg/L. It was concluded that the solubility enhancement by surfactant solutions below CMC is due to the association of hydrophobic compound molecules and surfactant monomers. This
mechanism of solubilization can be significant for compounds that have extremely low water solubility such as DDT.

When surfactant concentrations approach the CMC, the lipophilic (hydrophobic) moieties of the surfactant monomers associated with one another to form micelles. Thus, in aqueous solution, the polar or ionic portion of the molecule is pointed towards the bulk solution and the non-polar tails are oriented inside the hydrophobic mantle forming a hydrophobic core. The organic interior (core) of micelles represents a volume of hydrophobic "pseudophase" which serves as an oil sink into which hydrophobic contaminants can partition. As a result, the apparent NAPL solubility is dramatically enhanced when the surfactant concentration exceeds CMC. Thus, aqueous micellar solutions have been proposed for use in pump-and-treat systems to enhance the recovery of residual NAPLs.

The second approach to surfactant-enhanced subsurface remediation, mobilization, is based on the reduction of interfacial tension between the NAPL and the aqueous phase. As described above, the capillary forces which act to retain the residual NAPL droplets within a porous medium is proportional to the interfacial tension. When a surfactant is added to the aqueous solution, the amphiphilic nature of surfactant molecules causes them to accumulate at interfaces (e.g. air-water, oil-water, and solid-water). At the oil-water interface the hydrophilic moieties (polar or ionic heads) of the surfactant molecules are partitioned in the aqueous phase, while the hydrophobic moieties (nonpolar tails) are partitioned into the NAPL phase; thus, both moieties of the
surfactant molecule are in their preferred phase and the free energy of the system is minimized. In other words, the interfacial tension between the aqueous phase and the NAPL is reduced. As a result, capillary forces are reduced and the residual NAPL droplets are mobilized and released from the porous medium under normal flow regimes.

Surfactant-enhanced mobilization of oil from the subsurface has been extensively investigated by the petroleum industry for enhanced oil recovery (EOR). Thousands of papers have been published on the use of surfactants to improve oil recovery and hundreds of field tests of this process have been conducted during the past 30 years. Many of these field tests, particular those done after the 1980s, were technically successful. In such systems, surfactant solutions containing cosurfactant and brine are used to promote the formation of middle phase microemulsions which lead to ultralow interfacial tensions (<10^{-3} dyn/cm) between the oil and aqueous phases. Hundreds of surfactants and surfactant mixtures have been identified for use under a variety of very harsh subsurface conditions. Extensive reviews of this field can be found in Shah (1981) and Lake (1989). However, due to the fundamental differences between EOR and enhanced subsurface remediation with respect to the goal of the application and the subsurface environments, several issues must be resolved prior to successful transfer of such systems to aquifer remediation. Two major concerns are the potential downward migration of DNAPL, and the spread of NAPL into finely texture materials and uncontaminated aquifers.
Laboratory Experiments. The first systematic study using a surfactant to remove hydrophobic chemicals from a contaminated soil was conducted by Ellis et al. (1985). Laboratory studies were conducted to develop an improved in situ treatment methodology and were designed to determine whether a significant enhancement of flushing efficiency could obtained by adding surfactant to the flushing solution. The surfactant used was a mixture of 2% of each of two nonionic surfactants, Adsee 799 and Hydronic NP90. The contaminants were Murban crude-oil, PCBs and chlorophenols, which were prespiked to the soil. Column experiments showed that 70% of polychlorinated biphenyls (PCBs) and 90% of the crude oil were removed by flushing with this surfactant mixture. The more hydrophilic chlorophenols were readily removed from the soil by flushing with water alone.

Following this study, several surfactant or surfactant mixtures were tested for their efficiency on removing sorbed or deposited hydrophobic contaminants. Gannon et al. (1989) tested the use of sodium dodecyl sulfate (SDS) to flush p-dichlorobenzene (DCB), naphthalene, and biphenyl from soil. Abdul and Gibson (1991) conducted an experiment using a nonionic surfactant, alkyl polyoxyethlene glycol, commercially known as Witconol SN 70, to remove sorbed PCBs from a soil column. Three surfactant concentrations, 0.5%, 1%, and 2% were tested. Results showed that after 20 pore volumes, 66%, 86%, and 56% of PCBs were removed by 0.5%, 1% and 2% surfactant solutions, respectively. Of the surfactant concentrations tested, 1% was found to be most effective. This was attributed to soil clogging at the high surfactant concentration.
Although these results are promising, the recovery of hydrocarbons existing as NAPLs at residual saturation has proven to be far more difficult. Initial success was achieved by the Texas Research Institute (Texas Research Institute, 1985), which reported approximately 80% recovery of the gasoline present in Ottawa sand using a mixture of 2% Richonate YLA, an alkyl benzene sulfonate, and 2% Hyonic PE-90, an ethoxylated nonylphenol. After several years, Ang and Abdul conducted another study in which the residual level of an oil [automotive transmission fluid (ATF)] was flushed from a sandy soil column by an Witconol SN 70 solution at different concentrations. Results showed that the aqueous surfactant solution was at least twice as efficient as water in removing residual ATF from sandy soil, and the rate and extent of ATF removal was greater for higher surfactant concentrations. Three mechanisms were identified as being involved in the flushing process: displacement, emulsification and enhanced solubilization of the oil.

For all of the above studies, the NAPLs involved were less dense than water (LNAPL). In such cases, the NAPL can be recovered as a free product when the interfacial tension is lowered by addition of surfactant. While this strategy works well for LNAPL, for NAPLs that are more dense than water (DNAPL), a reduction in interfacial tension may cause downward migration of released DNAPL. This concern was first addressed by Fountain et al. (1991). In their study, tetrachloroethylene (PCE) was injected into a sand column to form a pool of PCE in the center of the column, then 18 different surfactant solutions, each at a concentration of 5%, were used to flush the column. The 18 surfactants had differing capacities for solubilization of PCE and
reducing the interfacial tension. Results showed that i) solubilization capacity was the most important factor in determining the recovery efficiency, ii) emulsification also played an important role in the recovery efficiency, iii) there was no correlation between the reduction in interfacial tension and flushing efficiency, and iv) when the interfacial tension was lowered to 5 dyn/cm, rapid downward PCE migration was observed. Based on these results, It was concluded that i) attainment of ultralow interfacial tension is not only unnecessary, but, in the case of DNAPL, may be undesirable, ii) surfactant systems should be designed to have maximum solubilization capacity for the recovery of DNAPL.

The concern of downward migration of DNAPL was later confirmed by Pennell et al. (1994). Different surfactant mixtures at a concentration of 4% were used to flush residual PCE from a sandy column. The interfacial tension between surfactant solutions and PCE ranged from 5 to 0.1 dyn/cm. When the interfacial tension reached 0.6 dyn/cm, 99% of the PCE was released from the column. They concluded that a ultra-low interfacial tension (< 0.001 dyn/cm) was not necessary for significant mobilization of PCE from the sandy column. In addition, it was concluded that buoyancy forces are important in evaluating the mobilization of a DNAPL such as PCE.

Abriola et al. (1995) have reviewed published researches related to surfactant enhanced recovery of entrapped DNAPLs in porous media. Three issues of particular importance to the design of a successful field remediation scheme were discussed: i) rate limited micellar solubilization, ii) control of downward mobilization, and iii) the influence of physical heterogeneity on NAPL distribution and recovery. Experimental and
modeling investigations which explored each of the issues were presented and discussed.
The conclusions were that i) the micellar solubilization process is substantially rate-
limited under flow conditions anticipated in engineered recovery schemes, ii) buoyancy
forces may play an important role in DNAPL mobilization, and iii) the entrapment of
DNAPL in low permeability zones will strongly influence the performance of surfactant-
enhanced operations at the field scale. In a separate study, Pennell et al. (1993) also
observed rate- limited mass transfer phenomena when residual dodecane was flushed by a
4% Tween 80 surfactant solution in a column experiment. They reported that the
concentration of dodecane in the column effluent was considerably less than the
equilibrium value measured in batch experiments. This discrepancy was attributed to
rate-limited solubilization based on observation that i) the increase in effluent dodecane
concentration following flow interruption and ii) the reduction of the dodecane
concentration in steady-state flow effluent as the pore water velocity was increased.

Although NAPL solubility in surfactant solutions is usually one or two orders of
magnitude greater than the aqueous solubility, this may still not high enough for field
application. In cases where rate-limited solubilization occurs, the NAPL concentration in
effluent removed from subsurface could be one order of magnitude less than the
equilibrium solubility. To enhance the solubilization capacity of the surfactant or
surfactant mixtures even further, Shiau et al. (1995) proposed a method using a middle
phase microemulsion process to "microemulsify" DNAPLs into the aqueous phase. The
formation of middle phase microemulsions can reduce the interfacial tension between
NAPL and surfactant solution to ultralow values, so that the two phases are actually "miscible". Hence the NAPL will be easily flushed out with surfactant solution. The apparent "solubility" of NAPL in the aqueous phase in the form of microemulsion can be an additional one or two orders of magnitude higher than the solubility in the surfactant solution without a microemulsion. Again, in such an application, the concern of downward migration of DNAPLs still needs to be addressed.

Field Studies. While the reported laboratory experiments were generally successful, the results from field tests were mixed. The first field test was conducted by Nash and Traver (1987) at Volk Air National Guard Base, Camp Douglas, WI, in 1987. The contaminated site was historically used as a fire training area from World War II. The area had received waste solvents including used lubrication oil, and JP-4 fuel oil. It was estimated that about 52,000 gallons of solvents had leached into the soil. The subsurface soil was determined to be 85-95% sand and 5-15% fines. Before the field experiment, soil samples from the contaminated site were taken to the laboratory and packed into glass columns creating a simulated in situ environment. Then 12 pore volumes of surfactant solutions, a mixture of 2% of Adsee 799 and 2% Hyonic PE90, were passed through the column. The laboratory results were very encouraging. Among the columns tested, 75% to 90% of the contaminants were removed, with no significant decrease in permeability noted. However, when the same surfactant solutions were applied to the field, the soil permeability was dramatically reduced which resulted in complete clogging of the two wells. Results showed that no significant reduction in the
level of contamination was observed. This has been regarded as a typical example of how simple laboratory experiments failed to simulate a complex field environment.

After the failure of the first field test, Fountain et al. (1992, 1993) conducted two separate field trials on the surfactant-enhanced removal of DNAPLs. The first trial was conducted at the Borden Canadian Air Force Base from June 1990 to August 1991. The subsurface lithology in this site was clean sand with <1 % clay and < 1,000 mg/kg organic content. The test occurred in a 3m x 3m x3m cell which had been contaminated with 271 liters of PCE in a controlled release. Before applying surfactant solutions, direct pump and water flooding was used to remove the PCE free product until no significant volume of free product was further recovered. This process recovered about 60 liters of injected PCE. Then 14 pore volumes of surfactant solution were flushed through the cell. Results showed that: i) after surfactant flushing, 90 % of the PCE was removed from subsurface, ii) no downward migration of was observed, iii) remediation was incomplete because NAPL remained in zones of low hydraulic conductivity.

The second trial was conducted from June 1991 to February 1993 at chlorocarbons manufacturing plant in Corpus Christi, Texas. In this site, the subsurface lithology was a fine grained sand with variable Semitic clay content (1-15%), little organic carbon (250-310 mg/kg) and highly saline ground water. The test area was contaminated with carbon tetrachloride. In the test, 12.5 pore volumes of surfactant solution was flushed through the area. Results showed that: i) the contaminant concentration in effluent was 20 times higher than its aqueous solubility, indicating the
surfactant was an effective method of increasing the efficiency of the pump-and-treat operation, ii) while significant removal of the contamination was achieved in the high conductivity zone (such as sand with 1-15% clay), the contaminants in low conductivity zones (such as a clay zone) was not reduced at all, and 3) there was no downward migration. Based on the results from these two trials, Fountain et al. (1995) concluded that surfactant flushing could be successful only under the following conditions: 1) residual NAPL is present, 2) the hydraulic conductivity is moderate to high ($>10^{-3}$ cm/s), and 3) an aquitard is present below the targeted zone to act as a barrier to vertical migration. They also concluded that hydrogeological parameters (aquifer heterogeneity) and the contamination distribution, not the surfactant performance, are the variables that determine the ultimate level of remediation of NAPL-contaminated sites.

In summary, surfactants can effectively enhance the efficiency of pump and treat operations in NAPL-contaminated sites. For LNAPLs, mobilization is an effective way to recover the contaminants, while for DNAPLs, downward migration should be prevented by controlling the reduction in interfacial tension. In this case, solubilization of the DNAPL becomes the primary mechanism for NAPL removal. The ultimate level of remediation is governed by the subsurface hydrogeology of the contaminated site. The performance of a surfactant will only affect the rate at which the contaminant mass is removed from the aquifer. Since aquifer heterogeneity is the primary factor responsible for reduced efficiency, a thorough understanding of the hydrogeology/lithology of the contaminated site is required to ascertain the success of an operation. Determination of
the best surfactant for use in remediation is dependent upon the following criteria: i) the capacity of the surfactant to increase contaminant solubility or reduce interfacial tension, depending on the nature of the NAPL (dense or light) requiring remediation, ii) minimization of surfactant adsorption and precipitation, iii) toxicity and biodegradability of the surfactant.

Microbially Produced Surfactants (Biosurfactants)

All the research discussed above has been focused on the use of synthetic surfactants. One class of surfactants that is less well studied are biologically produced surfactants (biosurfactants). A biosurfactant is defined as a surface active substance produced by living cells - in the majority of the cases, by microorganisms. Compared with chemically synthesized surfactants, biosurfactants have several potential advantages: i) microorganisms are able to produce surfactants with a naturally wide diversity of structures, each of which may have unique properties. ii) it may be possible to induce in situ production. iii) Biosurfactants are natural biodegradable products whose use may be more acceptable than use of synthetic surfactants in environmental application. Thus, the release of biosurfactants may be easier to justify to regulatory agencies than the release of synthetic surfactants.

Although they are naturally occurring products, the role of biosurfactant in nature is still unclear. Because biosurfactants are often produced under nutrient limiting conditions (Syldatk and Wagner, 1987), it is generally agreed that the production of biosurfactants is closely associated with the availability of nutrients by microorganisms.
The enhancement of the uptake of slightly water soluble carbon sources such as hydrocarbons by microorganisms is believed to be one of the major functions of biosurfactants in environment. Rosenberg (1986) has suggested three possible roles of surfactants in environment: adhesion of microorganisms to interfaces, emulsification of slightly soluble substrates in aqueous solution, and desorption of microorganisms from interfaces. The suggested roles were based on the fact that microbial adhesion is an important physiological mechanism for growth and survival in the natural environment. A special case for adhesion is growth of bacteria on slightly soluble hydrocarbons. Biosurfactant might be produced for this purpose to help bacteria adhere or/and to aid in solubilization of substrate. At some time following the attachment, growth conditions may become unfavorable. For example, nutrients may be depleted or toxins may accumulate. Biosurfactants may then play a role in deadhesion by accumulating and forming a hydrophilic film at the attachment interface. This may allow the desorption of cells so they can find a new habitat. While these suggested roles are clearly provocative, no evidence was presented to substantiate the hypothesis presented.

Biosurfactants can be classified into several groups based on their chemical structure: glycolipids, lipopeptides, lipopolysaccharides, phospholipid, and fatty acids/neutral lipids. Among them, the glycolipid and lipopeptide surfactants are commonly isolated and best investigated. In glycolipids surfactants, carbohydrates are combined with long chain aliphatic acids or hydroxyl-aliphatic acids. Glycolipids usually have molecular weight ranging from 400 to 1000. The biosurfactant used in this study,
rhamnolipid of *Pseudomonas* sp., belongs to this category. Figure 3 shows the structure of several glycolipids produced by different of microorganisms. A thorough review of the biosurfactant classification and their properties can be found in Zajic and Panchal (1976) and Zajic and Seffens (1984).

**Dissertation Format**

This dissertation consists of three manuscripts that were submitted to peer-reviewed journals; two of them have been accepted for publication (appendix A and C) and another one (appendix B) is in the review progress. I designed and conducted all of the experiments reported in each appendix. My major adviser, Dr. Raina Miller, helped me in choosing the area of research and in interpreting the results. She has also provided numerous detailed editorial comments after I prepared each of the manuscript drafts. Dr. Mark Brusseau, as the co-author of the papers, has helped me in the interpretation of the results, especially in the model simulation section in Appendix C.
Figure 3. Structure of selected glycolipids from microorganism

(A) Monorhamnolipid of *P. aeruginosa*
(B) Lactonic sophorose lipid of *T. bombicola*
(C) Trehalose-6- monocynomycolate of *R. erythropolis*
(D) Cellobioselipid of *U. zeae*
(E) Mannosylerythritol lipid of *S. melanogramma*
CHAPTER 2. PRESENT STUDY

Due to the ineffectiveness of classical pump-and-treat technology on the cleanup of NAPL-contaminated sites, it is imperative that new technologies to be developed to enhance the removal of NAPL from subsurface. Surfactant-enhanced subsurface remediation has been proposed as one such technology. Currently, this technology is being developed in several laboratories, and it is clear that a better understanding of the fundamental rules governing the interaction between surfactant, NAPL, and soil matrix is critical to the successful field implementation of this technology.

In the studies presented in this dissertation, a biological surfactant, *P. aeruginosa* rhamnolipid, was evaluated for its potential application in subsurface remediation using a series of laboratory experiments. The research work presented in Appendix A was conducted to investigate the potential for use of rhamnolipid in the removal of a model NAPL, hexadecane, from sandy soil, and to determine the relative roles of solubilization and mobilization in NAPL removal. The purpose of the second manuscript (Appendix B) was to study the effect of common groundwater cations on rhamnolipid solubilization capacity and the ability of rhamnolipid to reduce interfacial tension, and the resulting effect on NAPL removal. The study presented in Appendix C was conducted to investigate the effect of rhamnolipid on the transport of bacteria in the subsurface. Bacterial transport is a key issue in bioremediation of NAPL-contaminated sites where *in situ* biosurfactant production is preferred or the addition of bacteria may be required.
The major findings derived from these studies can be summarized as follows:

1. **Rhamnolipid is effective in removing a model NAPL, hexadecane, from sandy soil.**
   
   Efficiency of removal is highly dependent on the pore size. Mobilization of hexadecane is the main mechanism of NAPL removal, and the removal is dependent on surfactant concentration.

2. **Two processes, displacement of NAPL droplets from subsurface porous matrices and micellar solubilization of NAPL, have been recognized as the two mechanisms in surfactant-enhanced NAPL removal.** This study showed that in addition to these two mechanisms, a third mechanism dispersion or emulsification, is also common and important. The extent of dispersion is dependent on the nature of both NAPL and surfactant, as well as on the surfactant concentration.

3. **The performance of an anionic surfactant in the subsurface is greatly impacted by the presence of ions in the matrix solution.** The type of ion, ionic strength and pH have a significant influence on both the surfactant solubilization capacity and the interfacial tension between surfactant solution and NAPL. By adjusting the ionic strength and the pH, the surfactant system can be optimized to enhance the solubilization of NAPL or the reduction in the interfacial tension between the NAPL and the surfactant solution depending on the specific situation.

4. **Rhamnolipid also has an influence on the transport of bacteria in porous media.**
   
   Addition of rhamnolipid can enhance the transport of bacteria in subsurface, or in other words, reduce the adsorption of bacteria to the porous media. This is because
the adsorption of surfactant to the porous medium increases the surface negative charge density. As a result, the adsorption of bacteria to the surface is reduced. No significant influence of rhamnolipid on the bacteria surface properties was found.

5. The transport of bacteria in porous media can be closely simulated by the advection-dispersion solute transport model incorporating two-domain reversible adsorption and two first-order sink terms for irreversible sorption.

Based on the findings from this research, the following recommendations are made for the further research on biosurfactant-enhanced subsurface remediation:

1. The properties and the performance of a surfactant is primarily dependent on their chemical structure. So biosurfactants with different molecular structure should be evaluated for their potential application in surfactant-enhanced subsurface remediation.

2. Addition of a co-surfactant to the surfactant system can have a significant influence on surfactant behavior. Depending on their composition, a biosurfactant mixture could be more effective than a single surfactant in the removal of NAPL from the subsurface.

3. The efficiency of surfactant-enhanced NAPL recovery from an aquifer is controlled by the subsurface heterogeneity, especially the soil permeability and soil clay content. This is because surfactants can cause dispersal of clay particles and subsequent reduction of soil permeability. A better understanding of the interaction between the
surfactant and the soil matrix, particularly clay soil, is needed prior to a successful transfer of this technology to a full scale field application.
APPENDIX A

BIOSURFACTANT ENHANCED REMOVAL OF RESIDUAL HYDROCARBON FROM SOIL

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Abstract

An anionic monorhamnolipid biosurfactant produced by Pseudomonas aeruginosa was investigated for its potential to remove residual hexadecane from sand columns. In a series of column experiments, residual hexadecane saturation was established by pumping $^{14}$C-hexadecane into water-saturated sand columns and then flushing with water at a velocity of 25 cm hr$^{-1}$. Monorhamnolipid solutions of varying concentration were then applied to the columns at a velocity of 15 cm hr$^{-1}$ to remove the residual hexadecane. Of the rhamnolipid concentrations tested, which ranged from 40 to 1500 mg L$^{-1}$, the optimal concentration for residual removal was 500 mg L$^{-1}$, approximately 10 x critical micelle concentration. Approximately 84% of the residual was removed from the column packed with 20/30 mesh sand and 22% was removed from the 40/50 mesh column. The primary mechanism for residual removal was mobilization (displacement and dispersion), while solubilization was found to be insignificant. The performance of monorhamnolipid was compared with that of two synthetic surfactant solutions on a mass basis (500 mg L$^{-1}$) for the 40/50 mesh sand. Sodium dodecyl sulfate (0.2 x cmc), and polyoxyethylene (20) sorbitan monooleate (38 x cmc), removed 0% and 6.1% of the residual saturation, respectively.
Introduction

The widespread detection of organic solvents and other petroleum based products in ground water has prompted intensive study of non-aqueous phase liquid (NAPL) transport and dissipation in subsurface environments. Entrapped NAPL creates a long-term source of pollution since the NAPL will slowly dissolve into the aqueous phase. Low solubility and rate-limited mass transfer of NAPLs cause traditional pump-and-treat remediation methods to be inefficient (Mackay and Cherry, 1989; Keely 1989). A modification of this technique that has been proposed for enhancing NAPL removal is the addition of surfactants to pump-and-treat systems. One class of surfactants that have received relatively little attention in remediation are microbially produced surfactants (biosurfactants). Biosurfactants are of growing interest in remediation for several reasons: a) they have unique chemical structures which may have unusual properties beneficial for remediation, b) biosurfactants are a naturally-occurring, biodegradable product that may be acceptable for addition to contaminated sites, c) ex situ production may be cost-effective in comparison to synthetic surfactants, and d) it may be possible to stimulate in situ production of biosurfactants at the site of contamination.

Addition of surfactants can be expected to enhance NAPL removal in two ways: a) solubilization and b) mobilization due to a reduction in interfacial tension. The solubilization power of any surfactant is dependent on the ability of the surfactant to increase the aqueous-phase solubility of NAPL constituents. A dramatic enhancement in NAPL constituent solubility is commonly observed above the critical micelle
concentration (cmc), which is attributed to partitioning into the hydrophobic core of surfactant micelles (Edwards et al., 1991; Kile and Chiou, 1989). In this process, high surfactant concentrations are generally required because the solubility of NAPL constituents in surfactant solutions depends entirely on the surfactant concentration.

Mobilization can be divided into displacement and dispersion. Displacement is the release of NAPL droplets from porous media due to a reduction in interfacial tension (Ang and Abdul, 1991; Abdul and Gibson, 1991). From a theoretical perspective, entrapped NAPL will undergo displacement if the interfacial tension between the aqueous and NAPL phase is reduced sufficiently to overcome the capillary forces that caused the formation of residual saturation. Displacement is analogous to the process used in enhanced oil recovery (EOR) operations in which an optimized system is designed to reduce the interfacial tension between the NAPL and aqueous phase. In such applications, the surfactant solution often contains cosurfactant and brine to promote the formation of a middle-phase microemulsion, which is characterized by ultralow interfacial tensions (Bourrel and Schecter, 1988; Lake, 1989). While such surfactant mixtures are suitable for petroleum recovery, modifications, e.g. lower salt concentrations and lower surfactant concentrations, must be made to successfully extend their application to environmental remediation technologies.

Dispersion is the process in which the NAPL is dispersed into aqueous phase as very small emulsions (Ang and Abdul, 1991; Abdul and Gibson, 1991). Emulsions are generally not thermodynamically stable. However, due to kinetic constraints, they may
remain stable for significant time periods. Dispersion is related to both the interfacial tension and the surfactant concentration, and is different from displacement in that the displacement process is only related to the interfacial tension between aqueous and NAPL phases and no emulsion forms.

Several laboratory studies have shown that surfactants are effective in the washing of soils contaminated with various hydrocarbons (Ang and Abdul, 1991; Abdul and Gibson, 1991; Gannon, 1989; Pennell et al., 1993). In these studies, solubilization was the major process for removal of hydrocarbons. It has been argued that the surfactant should be chosen to promote the solubilization process, and that attainment of ultralow interfacial tension is unnecessary and may be undesirable in the case of NAPLs which are more dense than water (DNAPLs) to avoid further downward migration of mobilized NAPLs (Fountain et al., 1991). While mobilization may be a drawback for DNAPLs, mobilization may prove to be the most efficient approach for removal of NAPLs which are less dense than water (LNAPLs) since the mobilized NAPL can be extracted from the subsurface.

The objectives of this study were to evaluate the potential of a monorhamnolipid biosurfactant to remove residual LNAPL saturation from sandy porous media, and to determine the role of solubilization and mobilization in residual removal. Surfactant concentrations used in this study ranged from 40 mg L\(^{-1}\) to 1500 mg L\(^{-1}\) (0.8 to 30 x cmc). These concentrations are much lower than those used in other environmentally
related studies, generally ranging from 0.5% to 4% (5,000 mg L\(^{-1}\) to 40,000 mg L\(^{-1}\)) (Ang and Abdul, 1991; Pennell et al., 1993; Pennell et al., 1994).

**Materials And Methods**

**Surfactants**  The production and purification of the monorhamnolipid used in this study (Figure 1) has been described previously (Zhang and Miller, 1994). Analysis by high performance liquid chromatography (HPLC) and fast atom bombardment-mass spectroscopy has shown that there are four rhamnolipid peaks, the dominant peak (approximately 82%) being a C\(_{20}\) rhamnolipid. The molecular weight of the dominant rhamnolipid (MW=504) was used to calculate rhamnolipid concentration in this study. All surfactant solutions were prepared with deionized, distilled water and adjusted to pH 7.0, at which the predominant surfactant structure is micellar and the surface tension of rhamnolipid solutions at the minimum (Zhang and Miller, 1992; Champion et al., 1995). The cmc of the monorhamnolipid is 50 mg L\(^{-1}\). This value was determined from a plot of surface tension versus surfactant concentration. Surface tension measurements were made using a Surface Tensiometer 21 (Fisher Scientific, Pittsburgh, PA)

The two synthetic surfactants used in this study, sodium dodecyl sulfate (SDS) and polyoxyethylene (20) sorbitan monooleate (Tween 80), were obtained from Aldrich Chemical Co. (St. Louis, MO). The cmc values for SDS and Tween are 2,360 and 13 mg L\(^{-1}\), respectively (Pennell et al., 1993; Rosen, 1989).
n-Hexadecane was selected to represent LNAPLs with low volatility such as jet fuel. It is representative because the interaction between NAPLs and surfactants depends on the Alkane Carbon Number (ACN) of the NAPLs. Most LNAPLs detected in contaminated sites have an equivalent ACN ranging from 10 to 22. For example, the equivalent ACN of JP4 jet fuel is 14. The ACN of hexadecane is 16. Hexadecane was obtained from Aldrich (Milwaukee, WI, 99% purity). $^{14}$C-Hexadecane, (specific activity 2.2 mCi mmol$^{-1}$) was obtained from Sigma (St. Louis, MO). The density of hexadecane is 0.773 g cm$^{-3}$, its aqueous solubility has been reported as 0.0063 mg L$^{-1}$ (Singer and Finnerty, 1984), and the interfacial tension between hexadecane and water has been reported as 53.3 dyne cm$^{-1}$ (Johnson and Dettre, 1966). The measured value in this laboratory was 49.5 dyne cm$^{-1}$.

**Porous Medium** Accusand (North Kato Supply, Mankato, MN) was used as the solid phase for the experiments. Analysis by the University of Arizona Soil, Water, and Plant Analysis Laboratory showed that total organic carbon was 0.04%, iron oxide content was 0.03%, and cation exchange capacity was 0.57 meq 100g$^{-1}$. A 40/50 mesh (0.3 - 0.42 mm) fraction was used for surfactant sorption tests, and both 40/50 mesh and 20/30 mesh (0.6 - 0.85 mm) fractions were used in separate column tests. Before each experiment, the sand was rinsed with distilled, deionized water.

**Solubilization Tests** The solubility of hexadecane in rhamnolipid solutions of varying concentration was determined using a mixture of unlabeled and $^{14}$C-hexadecane. In each experiment the hexadecane mixture in chloroform was added to a 20 ml test tube (final
The chloroform was allowed to evaporate and 5 ml of rhamnolipid solution was added to achieve a final rhamnolipid concentration ranging from 20 mg L\(^{-1}\) to 2000 mg L\(^{-1}\). Test tubes were shaken at 200 rpm on a rotary shaker at 22 °C for 36 hr, and then allowed to settle for 24 hr to allow phase separation. After this initial separation, 4.5 ml of the solution were transferred to a clean test tube and the solution was allowed to settle for another 24 hr. This procedure was repeated once more (4.0 ml were transferred) and then duplicate 500 μl samples were taken from each test tube using a GC syringe and transferred to scintillation vials containing 5 ml Scintiverse BD scintillation cocktail (Fisher Scientific, Tustin CA). The radioactivity in each sample was determined by liquid scintillation using a 1600TR Tri-Carb scintillation counter (Packard, Meridian, Conn.). Each experiment was performed in triplicate at room temperature.

**Sorption of rhamnolipid** A sorption isotherm was obtained using rhamnolipid and 40/50 mesh Accusand. Sterile sand (10 g) and 20-ml aliquots of filter-sterilized rhamnolipid solutions of varying concentration (50 to 3000 mg L\(^{-1}\)) were shaken at room temperature for 72 hrs. Samples were centrifuged at 6,000 rpm for 30 min. The concentration of rhamnolipid in the supernatant was determined using a Waters Associates LCM-1 HPLC system operating with a UV detector at 214 nm. The column was Nova-Pak C\(_{18}\), 3.9 X 150 mm, obtained from Millipore (Milford, MA). Elution was isocratic and the mobile phase used was acetonitrile-water (40:60 v/v) at a flow rate of 1 ml min\(^{-1}\).
**Residual saturation column experiments** The effect of rhamnolipid on removal of residual hexadecane saturation was determined using column experiments. All experiments were performed at room temperature. A stainless steel column (length = 7 cm, i.d. = 2.12 cm) fitted with filters (0.2 μm) at each end was obtained from Alltech (Deerfield, IL). The column was packed under vibration with air-dried Accusand, and then flushed with approximately 100 pore volumes of deaired, deionized, distilled water introduced from the bottom of the column at a pore water velocity of 25 cm hr\(^{-1}\). To ensure complete saturation, flushing continued until the mass of the column remained stable. Hydrodynamic properties of the column were determined using a conservative tracer, pentafluorobenzoic acid (PFBA). \(^{14}\)C-Hexadecane (0.06 mCi mmol\(^{-1}\)) was then introduced at the top of the column. To maximize relative saturation, three incremental flow rates were used: 0.1 ml min\(^{-1}\) for 30 min, 0.5 ml min\(^{-1}\) for 30 min, and 1.2 ml min\(^{-1}\) for 25 min. To establish a residual hexadecane saturation, the column was flushed with water (from the bottom) for 10 pore volumes at 5 cm hr\(^{-1}\), followed by 40 pore volumes at 25 cm hr\(^{-1}\). During the hexadecane loading and the water flushing process, the column effluent was collected in a large separatory funnel. The hexadecane was transferred into a 50 ml volumetric flask and the volume was adjusted to 50 ml using unlabeled hexadecane.

The radioactivity of the hexadecane was determined by liquid scintillation and the residual saturation was computed using the formula:

\[
\text{Volume of residual saturation} = \frac{(\text{Influent radioactivity} - \text{Effluent radioactivity}) \times (\text{MW})}{(\text{Influent specific radioactivity}) \times d}
\]
where: MW = molecular weight, and d = density of hexadecane. After the residual saturation was established, each column was flushed with a solution containing varying concentrations of rhamnolipid at 15 cm hr⁻¹. Effluent samples were collected in 20-ml scintillation vials. To differentiate between free product and solubilized (including microemulsion) hexadecane in each effluent fraction, a syringe was immersed into each sample and used to withdraw a 0.5 ml sample of the aqueous phase solution. This was added to 5 ml scintillation cocktail and assayed by liquid scintillation to determine solubilized hexadecane. To determine free product in each fraction, the majority of the remaining aqueous phase was removed from each 20-ml scintillation vial leaving approximately 1 ml of water and free product. Scintillation cocktail was added (5 ml) and radioactivity was determined.

After the column had been flushed with 120 pore volumes of surfactant solution, the remaining hexadecane in the sand was removed by flushing with 10 pore volumes of acetone, 20 pore volumes of chloroform, and finally 10 pore volumes of acetone. The stripped column was then reequilibrated with water for 40 pore volumes and was used for subsequent experiments.

**Results and Discussion**

**Rhamnolipid sorption**

Sorption of monorhamnolipid to Accusand was nonlinear as shown in Figure 2. A cmc value of 50 mg L⁻¹ is indicated on the Figure. The sorption isotherm was fitted with the Freundlich equation:
\[ q = KC^n \]

where \( q \) is the concentration of rhamnolipid sorbed (mg Kg\(^{-1}\)), \( K \) is the partition coefficient (mg\(^{1-n}\) L\(^n\) Kg\(^{-1}\)), and \( n \) is the exponent coefficient. The fitted values for \( K \) and \( n \) were 39.8 mg\(^{1-n}\) L\(^n\) Kg\(^{-1}\) and 0.375, respectively (see Figure 2, insert).

**Solubilization of hexadecane**  The methodology used to measure hexadecane solubility was designed to measure solubility in the sense that all solubilized hexadecane was partitioned into surfactant micelles that were homogeneously distributed throughout the aqueous solution. The reported hexadecane solubility in water is 0.0063 mg L\(^{-1}\) (Singer and Finnerty, 1984). Using our methodology, the measured solubility of hexadecane in water was 0.029 ± 0.009 mg L\(^{-1}\), a 4.6-fold increase over the reported value. Impurities of the \(^{14}\)C[hexadecane] are approximately 2% of the material. It is likely that impurities are oxidized products of hexadecane, all of which would be expected to have relatively high water solubility. In fact, if it is assumed that 2% of the radioactive material is water soluble, the measured water solubility would be predicted to be 0.0263 mg L\(^{-1}\) (0.02 mg L\(^{-1}\) from impurities and 0.0063 mg L\(^{-1}\) from true hexadecane solubility). This is very close to the actual water solubility measured in the absence of biosurfactant.

The solubility of hexadecane was found to increase in the presence of varying concentrations of rhamnolipid (Figure 3). The molar solubilization capacity (MSR), which is number of moles of organic compound solubilized in micelle per number of moles of surfactant used, was calculated to be 0.054 by the regression of the data from the cmc value to 2000 mg L\(^{-1}\) (Edwards et al., 1991). The micelle-aqueous phase
partition coefficient ($K_m$), which is the ratio of molar fraction of organic compound in the micelle phase over the molar fraction of organic compound in aqueous phase, was calculated to be 8.01 by the method described by Edwards et al., (1991).

**Removal of residual saturation** A breakthrough curve for pentafluorobenzoic acid (PFBA) was symmetrical, sharp, and the midpoint of the breakthrough curve ($C_{Co}^{-1}$) occurred at 1 pore volume (data not shown). Such behavior is expected for a homogeneously packed sand column. After performing this experiment, a residual hexadecane saturation was established in the column. Values for residual hexadecane saturation and other column properties are listed in Table 1.

Removal of residual hexadecane by rhamnolipid (500 mg L$^{-1}$) from both 20/30 and 40/50 mesh columns were determined in duplicate and the results are shown in Figure 4. As seen in this Figure, the recovery of residual hexadecane from the column packed with larger diameter sand (20/30 mesh) was much higher (83.6% after 120 pore volumes) than recovery from the 40/50 mesh sand column (22%). Both sets of experiments showed good reproducibility. A mass balance of hexadecane residual was calculated for one of the 20/30 mesh columns. The calculated residual (hexadecane added to the column - hexadecane in the effluent, after loading) was 1.44 ml, while the measured residual (hexadecane removed during washing + hexadecane remaining in column after washing) was 1.7 ml, a difference of 0.26 ml or 15%.

Further studies were performed to examine the influence of rhamnolipid concentration on removal. These experiments were conducted with the 40/50 mesh sand
since rhamnolipid removed the majority of the residual from the 20/30 mesh sand. In these studies, removal of residual by water and 4 additional rhamnolipid concentrations was examined. There was no hexadecane detected in the effluent of the column flushed with water. Figure 5 shows removal of hexadecane residual by 5 different rhamnolipid concentrations. Two points should be emphasized in this Figure. First, the 500 mg L\(^{-1}\) rhamnolipid solution was the optimal concentration of those tested for removal of residual over the duration of the experiment (a total of 22% removed). In contrast, all of the other rhamnolipid concentrations tested (40, 300, 800, and 1500 mg L\(^{-1}\)) removed only approximately 10% of the residual. As will be discussed below, it appears that displacement due to a reduction in interfacial tension was responsible for this 10% removal. The second point of interest is that the initial rate of residual removal increased with increasing rhamnolipid concentrations (see Figure 5, inset). For example, 10% of the residual (the total removed) was removed in only 2 pore volumes at 1500 mg L\(^{-1}\), while it took 35 pore volumes at 800 mg L\(^{-1}\) to remove the same amount of the residual.

The concentrations of the rhamnolipid solutions used to remove hexadecane residual were above the cmc except for the 40 mg L\(^{-1}\) solution, which was near cmc. Thus, under the conditions used in these experiments, the interfacial tension should be similar for all systems. Hence, the magnitude of residual removal by the displacement mechanism should have been similar for all solutions. However, as shown in Figure 5, the initial rates of residual removal increased as rhamnolipid concentration increased. To determine whether this was due to nonlinear sorption of rhamnolipid by sand, the
retardation of rhamnolipid at each concentration was calculated using the batch sorption data shown in Figure 2 under the assumptions that batch conditions for sorption were similar to column conditions, and that sorption was instantaneous. Retardation was then calculated using the following equation:

\[ R = 1 + \frac{\rho}{\theta} KC_0^n \]

where: \( R \) = retardation, \( \rho \) = bulk density (Kg L\(^{-1}\)), \( \theta \) = pore water fraction, and \( C_0 \) = influent concentration of rhamnolipid solution (mg L\(^{-1}\)).

Calculated retardation values were 25.16 (40 mg L\(^{-1}\)), 7.8 (300 mg L\(^{-1}\)), 6.0 (500 mg L\(^{-1}\)), 4.7 (800 mg L\(^{-1}\)), and 3.5 (1500 mg L\(^{-1}\)). Comparing these retardation values with the removal rates indicated by the data in shown in Figure 5, suggests that at the lower concentrations of rhamnolipid, rhamnolipid transport was retarded significantly such that the effective rhamnolipid concentration throughout the column was less than cmc for the first few pore volumes. In contrast, at the highest rhamnolipid concentration (1500 mg L\(^{-1}\)), the effective rhamnolipid concentration was higher than the cmc within the first 2-3 pore volumes, causing rapid displacement.

**Mobilization (Displacement and Dispersion) vs. Solubilization**

In all of these experiments, the majority of the hexadecane removed existed as free product, indicating that mobilization was primarily responsible for removal of residual hexadecane. These results are consistent with the results of the batch solubilization experiment. For example, the solubility of hexadecane was approximately
19 mg L$^{-1}$ in a 500 mg L$^{-1}$ rhamnolipid solution (see Figure 3). Assuming a pore volume of 9.8 ml, removal of approximately 22.3 mg of hexadecane in 120 pore volumes would be predicted due to solubilization alone. However, in the experiment, a total of 390 mg of hexadecane was removed, indicating that solubilization can at most account for only 6% of the total residual removed. In fact, the hexadecane concentration in the effluent of the water-flushed column was much less than its solubility, probably due to the rate-limited mass transfer (Pennell et al., 1993). So the contribution of solubilization to the total removal may be much less than 6%.

In Figure 6, the amount of hexadecane in each pore volume of effluent is plotted against the cumulative pore volumes of flushing solution passed through the column. The surfactant concentration used in this experiment was 500 mg L$^{-1}$. The dotted line in this Figure represents the theoretical amount of dissolved hexadecane in each pore volume assuming the solubility in 500 mg L$^{-1}$ rhamnolipid solution to be 19 mg L$^{-1}$. Three phenomena are illustrated in this Figure. First, the relative recovery of hexadecane per pore volume of flushing decreased as the flushing operation progressed (also shown in Figure 4). Second, temporal fluctuations in the relative recovery were observed, and finally, the actual recovery of hexadecane in each pore volume was greater than its solubility.

Visual observation of oil droplets in column effluent coming from the columns suggested that displacement was the primary mechanism for mobilization of residual. However, dispersion also seems to be an important mechanism in mobilization of residual
at the 500 mg L\(^{-1}\) rhamnolipid concentration. This is illustrated in Figure 5, which shows that although removal of residual increased with increasing rhamnolipid concentration for the first 10 pore volumes, total removal of residual was greatest at 500 mg L\(^{-1}\) rhamnolipid. At 500 mg L\(^{-1}\) there was an initial rapid removal during the first 20 pore volumes followed by a constant rate of removal from 20 pore volumes until the experiment was terminated at 130 pore volumes. This is in contrast to the other four rhamnolipid concentrations for which removal plateaued at approximately 10%. This behavior may be due to the effect of dispersion as described by Abdul and Gibson (1991) and Abdul et al. (1990). This group showed similar concentration-dependent removal of automatic transmission fluid from a sandy soil by several surfactant solutions. In batch experiments, dispersion of transmission fluid increased to a maximum value and then decreased as surfactant concentration was increased. Visual observation of our batch solubilization experiments suggested that dispersion in the rhamnolipid-hexadecane system was greatest at the intermediate rhamnolipid concentrations of 500 and 800 mg L\(^{-1}\). In these experiments, a series of tubes were set up to measure solubility ranging from 0 to 1600 mg L\(^{-1}\) rhamnolipid. From 0 to 200 mg L\(^{-1}\), solutions were always clear, indicating no emulsion formation. However, at 400 and 800 mg L\(^{-1}\), some replicates were cloudy, indicating emulsion formation at these concentrations. At concentrations greater than 800 mg L\(^{-1}\), the solutions in the tubes were clear and no stable emulsion was observed. These observations were consistent with column experiment results.
During flushing with 500 mg L\(^{-1}\) rhamnolipid, clouds of very small, milky like particles were observed in the effluent solution. This cloudy effluent was not observed in other column experiments. This suggests that in the 500 mg L\(^{-1}\) column experiment it is likely that meta-stable emulsions formed and, therefore, that removal of hexadecane occurred by both dispersion and displacement. Furthermore, it appears that before 20 pore volumes the primary mechanism of mobilization was displacement and after 20 pore volumes, the primary mechanism of mobilization was dispersion. This phenomenon is even more apparent in the 20/30 mesh sand where a large change in the slope of the removal curve is easily seen at 20 pore volumes (Figure 4). It is also supported by the data in Figure 6 where the large spikes in removal in the first 20 pore volumes correspond to removal of oil droplets by displacement, and where after 20 pore volumes, removal is fairly uniform (dispersion) with an occasional spike (displacement) indicating removal of an oil droplet. Thus, at 500 mg/L rhamnolipid, the removal was maximum due to the formation of meta-stable emulsions (dispersion) which did not occur at either higher or lower concentrations.

**Comparison of rhamnolipid with synthetic surfactants**

Removal of residual saturation by two synthetic surfactants, SDS and Tween 80, was compared to removal by rhamnolipid in the 40/50 mesh sand column on a mass basis (in all cases a concentration of 500 mg L\(^{-1}\) was used). This concentration was above the cmc for Tween 80 (cmc = 13 mg L\(^{-1}\)) and below the cmc for SDS (cmc = 2,360 mg L\(^{-1}\)). These surfactants were chosen because they are commonly used in remediation experiments. Similar to rhamnolipid,
SDS is an anionic surfactant, while Tween 80 is nonionic. As shown in Figure 7, removal of residual by SDS was insignificant while removal by Tween 80 was 6% in 100 pore volumes compared to 20% removal by rhamnolipid in 100 pore volumes. Since the surfactant concentration tested was low (approximately 0.05%), solubilization of hexadecane was not expected to be a large factor in residual removal. Hence, the major mechanism of removal was expected to be mobilization. A comparison of interfacial tensions for the three surfactants tested helps explain the results presented in Figure 7. The interfacial tension was 1 dyn cm\(^{-1}\) in the rhamnolipid/hexadecane system, 9.6 dyn cm\(^{-1}\) in the Tween 80/hexadecane system, and 25.2 dyn cm\(^{-1}\) in the SDS/hexadecane system (all surfactants were tested at 500 mg L\(^{-1}\)). For comparison, the interfacial tension in a water/hexadecane system was 49.5 dyn cm\(^{-1}\).

**CONCLUSIONS**

This study shows that a monorhamnolipid biosurfactant was effective in removing hexadecane residual from sand columns. Removal efficiency was dependent on both the average pore size of the sand and on biosurfactant concentration. Mobilization was the predominant mechanism of removal with solubilization playing an insignificant role. Removal efficiency was greatest at 500 mg L\(^{-1}\) rhamnolipid, a concentration where both displacement and dispersion appeared to contribute to mobilization. Residual removal by monorhamnolipid was significantly greater than removal by two synthetic surfactants tested, SDS and Tween 80, suggesting that use of microbial surfactants may offer
advantages over synthetic surfactants in remediation of contaminated sites. These laboratory-scale results indicate potential for rhamnolipid in remediation; however, many other factors will have to be considered in application of rhamnolipid on a field-scale. These include ionic strength of the soil solution, the type of cations present, and porous medium properties such as clay and organic matter content.

ACKNOWLEDGMENTS

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References


Table 1. Physical parameters of columns used in this study.

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<th>pore volume</th>
<th>porosity</th>
<th>residual saturation</th>
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<td></td>
<td>cm</td>
<td>cm</td>
<td>cm^3</td>
<td>g/cm^3</td>
<td>cm^3</td>
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<td>1.81</td>
<td>8.8</td>
<td>34.8</td>
<td>19.3</td>
</tr>
</tbody>
</table>

^a Column was packed with 40/50 mesh sand.
^b Column was packed with 20/30 mesh sand.
^c Residual saturation was measured three times in the same column and ranged from 23.9% to 24.5%.
Figure 1. The structure of *P. aeruginosa* ATCC 9027 C_{20} monorhamnolipid biosurfactant.
Figure 2. Sorption isotherm for monorhamnolipid and 40/50 mesh sand. The arrow indicates the measured cmc value of 50 mg/L and the inset shows a regression of the data on a plot of log C vs. log q, with $r^2 = 0.93$. 
Figure 3. Apparent solubility of hexadecane as a function of increasing concentration of monorhamnolipid. A regression of the data (solid line) was used to calculate a molar solubilization ratio of 0.054.

\[ r^2 = 0.96 \]

\[ \text{MSR} = 0.054 \]

\[ \text{Km} = 8.01 \]
Figure 4. Comparison of removal of residual hexadecane from 40/50 mesh and 20/30 mesh sand columns. Monorhamnolipid concentration was 500 mg/L. Experiments were performed in duplicate.
Figure 5. Percentage removal of residual hexadecane from a 40/50 mesh sand column by increasing concentrations of rhamnolipid. Insert: An expanded detail of the data from 0 to 15 pore volumes
Figure 6. Mass of hexadecane in each pore volume removed from a 40/50 mesh sand column by a 500 mg/L monorhamnolipid solution.
Figure 7. Comparison of removal of residual hexadecane from a 40/50 mesh column by SDS, Tween 80, and monorhamnolipid on mass basis. Surfactant concentrations used were 500 mg/L.
APPENDIX B

INFLUENCE OF CATION TYPE, IONIC STRENGTH AND PH ON THE SOLUBILIZATION AND MOBILIZATION OF RESIDUAL HYDROCARBON BY A BIOSURFACTANT

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Abstract

This study investigated the effect of cation type, ionic strength, and pH on the performance of an anionic monorhamnolipid biosurfactant for solubilization and removal of residual hexadecane from sand. Three common soil cations, Na\(^+\), Mg\(^{2+}\), and Ca\(^{2+}\), were used in these experiments and hexadecane was chosen to represent a nonaqueous phase liquid (NAPL) less dense than water. Results showed that hexadecane solubility in rhamnolipid solution was significantly increased by the addition of Na\(^+\) and Mg\(^{2+}\). Addition of up to 0.2 mM Ca\(^{2+}\) also increased hexadecane solubility. For Ca\(^{2+}\) concentrations greater than 0.2 mM there was a little effect on hexadecane solubility due to competing effects of calcium-induced rhamnolipid precipitation and enhanced hexadecane solubilization. Efficiency of NAPL solubilization can be expressed in terms of molar solubilization ratio (MSR). The results showed that MSR value for hexadecane in rhamnolipid solutions increased 7.5-fold in the presence of 500 mM Na\(^+\), and 25-fold in the presence of 1 mM Mg\(^{2+}\). The presence of cations also reduced the interfacial tension between rhamnolipid solutions and hexadecane. For example, an increase in Na\(^+\) from 0 to 800 mM caused a decrease in interfacial tension from 2.2 to 0.89 dyn/cm. Similarly, decreasing pH caused a reduction in interfacial tension. The lowest interfacial tension value observed in this study was 0.02 dyn/cm at pH 6 in the presence of 320 mM Na\(^+\). These conditions were also found to be optimal for removal of hexadecane residual from sand columns, with 58% of residual removed within three pore volumes. The removal of
residual NAPL from the packed columns was primarily by mobilization, even though solubilization was significantly increased in the presence of Na".
Introduction

The addition of surfactants solutions to aquifers contaminated with non-aqueous phase liquid (NAPL) has been discussed as a means by which to improve pump and treat remediation efficiency (Ang and Abdul, 1991; Abdul and Gibson, 1991; Fountain et al., 1991; West and Harwell, 1992). Addition of surfactant to the pump and treat flushing solution can enhance flushing efficiency by either mobilization of the NAPL, which results from a decrease in interfacial tension between the NAPL and the surfactant solution (Pennel et al., 1994; Lake, 1989), or by an increase in solubilization of the NAPL (Ang and Abdul, 1991; Fountain et al., 1991; Pennell et al., 1993). Surfactants can also increase the rate of the biodegradation of slightly soluble contaminants by increasing their bioavailability (Thiem, 1994; Miller, 1995; Guha and Jaffe, 1996), and enhance the mobility and removal of the sorbed heavy metals from soil by complexation (Beveridge and Pickering, 1983; Herman et al., 1995).

Most research with respect to enhanced flushing removal has focused on synthetic surfactants. Relatively little information is available concerning surfactants produced by microorganism (biosurfactants). Potential advantages of biosurfactants include (1) unusual structural diversity that may lead to unique properties, (2) the possibility of cost effective ex-situ production or perhaps even stimulation of in-situ production, and (3) biodegradability. These properties make biosurfactants a promising choice for use in environmental applications. Previous work has shown that a specific anionic biosurfactant, rhamnolipid, can enhance soil flushing efficiency as well as biodegradation
of NAPL (Zhang and Miller 1992, 1994, 1995; Miller, 1995; Bai et al, 1997; Herman et al., 1997), and the desorption of heavy metal from soil (Herman et al., 1995).

The performance of anionic surfactants is strongly affected by the presence of electrolytes in the solution. Electrolytes can influence the solubilization capacity of anionic surfactants (Tucher and Christian, 1984), cause precipitation of surfactants from the aqueous phase (Stellner and Scamehorn, 1989; Jafvert and Heath, 1991), and increase the adsorption of anionic surfactants to subsurface porous media (Palmer et al., 1992). Thus, understanding the interaction between surfactant and electrolytes and the resultant effect on surfactant performance is critical to the design and implementation of surfactant-enhanced subsurface remediation. This is particularly important in field applications, since in such situations, subsurface matrix solutions contain electrolytes such as Ca$^{2+}$, Mg$^{2+}$, Na$^+$, K$^+$, and Al$^{3+}$.

The study presented in this paper builds on results presented by Bai et al., (1997) that showed that rhamnolipid, an anionic biosurfactant produced by *Pseudomonas aeruginosa* ATCC 9027, can enhance the removal of residual hexadecane from porous media. The objective of current study was to determine whether rhamnolipid application for NAPL residual removal could be improved. Therefore, we investigate the influence of common soil cations and pH on the solubilization and mobilization of hexadecane. Specifically, a series of batch experiments were conducted with Na$^+$, Mg$^{2+}$, and Ca$^{2+}$ salts to determine the effect of ionic strength on rhamnolipid solubilization of hexadecane. A second series of batch experiments were conducted to determine the combined effect of
ionic strength and pH on interfacial tension between hexadecane and rhamnolipid solutions. Finally, a series of column experiments were performed to determine whether optimal ionic strength and pH conditions identified from batch experiments could improve removal of residual hexadecane from sand.

**Theoretical Considerations** One mechanism involved in surfactant enhanced NAPL removal is the enhanced solubilization of NAPL into surfactant solution. The solubilization of a hydrocarbon such as hexadecane in surfactant solution involves partitioning of hydrocarbons into the interior of surfactant micelles. Hence, one way to increase the amount of hydrocarbon solubilized is to increase the interior volume of the micelles. The interior volume of a micelle is dependent on both the aggregation number of the micelle and the shape of the micelle. The aggregation number of the micelle is the number of monomers in the aggregate and is dependent on the structure of the surfactant monomers. The aggregation number will increase when the cross-sectional area of the hydrophilic head group is decreased. The aggregation number will also increase as the length of the hydrophobic tail is increased (Rosen 1989; Israelachvili, 1994).

The shape of the micelle is also dependent on the structure of the surfactant monomer. This can be understood by the "geometry" approach. Suppose we view the surfactant molecule as depicted in Figure 1a, where the cross sectional area of the head group ($a_h$), the length of hydrocarbon chain ($l_C$), and the volume that is occupied by the coiled hydrocarbon chains ($V_H$) are defined. The shape of the aggregate is determined by the packing parameter, $V_H/a_h l_C$, namely, spherical micelles ($V_H/a_h l_C < 1/3$), cylindrical
micelles (1/3 < \( \frac{V_H}{a_H l_c} < \frac{1}{2} \)), lamella or vesicle (\( \frac{1}{2} < \frac{V_H}{a_H l_c} < 1 \)), or "inverted" structure (\( \frac{V_H}{a_H l_c} > 1 \)) (Mitchell et al., 1981). For rhamnolipids (Figure 1b), the tail group comprises two hydrocarbon chains, so the \( V_H \) is larger than that of single tail surfactant. In such a case, it is unlikely that a spherical micelle will form (Israelachvili, 1994). The most likely form for rhamnolipid aggregates are cylindrical micelles or vesicles.

When an electrolyte like \( \ce{Na}^+ \) is added to a rhamnolipid solution, the \( \ce{Na}^+ \) will serve to screen the negative charge of the surfactant head group, effectively reducing the electrostatic repulsion between the surfactant head groups. Hence, the 'effective' head group size (\( a_h \)) is decreased. This will increase both the aggregation number of the micelle and the total interior volume of the micelle. As a result, the solubilization of nonpolar compound in the micelles or vesicle is increased.

Another mechanism in surfactant enhanced NAPL removal is the interfacial tension reduction, which result release of entrapped NAPL from subsurface. The interfacial tension reduction between two immiscible liquids in the presence of surfactant is the result of the accumulation of surfactant molecules at the interface. The reduction in interfacial tension, \( \Pi \), can be calculated from the following equation (Rosen, 1989):

\[
\Pi = 20 + 2.3RT \Gamma_m \log(\text{CMC}/C_{20})
\]

Where \( \Gamma_m \) (mol/cm\(^2\)) is the surfactant concentration at the interface when the interface is saturated by the surfactant, \( C_{20} \) is the concentration of surfactant in the bulk phase that produces a reduction of 20 dyn/cm in the interfacial tension of the system, \( R \) is the gas constant (erg/mol·°K), and \( T \) is the temperature, °K. When electrolytes are added, the
repulsive force between head groups is reduced allowing a closer packing of surfactant at
the interface. As a result, $\Gamma_m$ is increased. The value of $\text{CMC}/C_{20}$ is also increased by
increasing the ionic strength (Rosen, 1989). Thus, when the ionic strength in the aqueous
phase increases, $\Pi$ (the interfacial tension reduction) increases, and so the interfacial
tension between two liquid phases decreases.

**Materials And Methods**

**Biosurfactant**: The production and purification of the monorhamnolipid used in this
study (Figure 1b) has been described previously (Zhang and Miller, 1992; 1994). Analysis by high performance liquid chromatography (HPLC) and fast atom bombardment-mass spectroscopy has shown that there are four rhamnolipid peaks, $C_{18}$, $C_{20}$, $C_{22}$, and $C_{24}$. The formula weight of the dominant rhamnolipid, $C_{20}$ (approximately 82%, MW=504), was used to calculate rhamnolipid concentration in this study. The critical micelle concentration (cmc) of the monorhamnolipid was measured to be 50 mg/L (0.1 mM) (Zhang and Miller, 1992), and the pKa is 5.6 (Ishigami et al., 1987). All the rhamnolipid solutions were prepared in deionized, distilled water. The final pH of the solution of rhamnolipid was adjusted to 6.0 or 7.0 with KOH and was continuously monitored during the course of all experiments. No pH change greater than 0.2 was observed.

**Chemicals**  $n$-Hexadecane was selected to represent a low volatility NAPL less dense
than water. It is representative because the interaction between NAPLs and surfactants
depends, in part, on the Alkane Carbon Number (ACN) of the NAPL. Most NAPL less
dense than water detected in contaminated sites have an equivalent ACN ranging from 10
to 22. For example, the equivalent ACN of JP4 jet fuel found in Hill AFB, Utah is 14 (D.
Sabatini, personal communication). The ACN of hexadecane is 16. Hexadecane was
obtained from Aldrich (Milwaukee, WI, 99% purity). 14C-Hexadecane, (specific activity
2.2 mCi/mmol) was obtained from Sigma (St. Louis, MO). The density of hexadecane is
0.773 g/cm³, and the interfacial tension between hexadecane and water has been reported
as 53.3 dyne/cm (Johnson and Dettre, 1966). The measured value in this laboratory was
49.5 dyne/cm.

**Effect of Ionic Strength on Hydrocarbon Solubilization:** The effect of ionic strength
on the solubility of hexadecane in 0.06, 0.4, 1, 2, and 4 mM monorhamnolipid solutions at
pH of 7.0 was determined using three different salts: NaCl, MgCl₂, and CaCl₂. For each
experiment, a mixture of hexadecane and 14C-labeled hexadecane (specific activity 0.045
µCi/mg) was dissolved in chloroform and added to 20 ml acid-rinsed, autoclaved glass
vials to a final mass of 10 mg. The chloroform was allowed to evaporate and 10 ml of
rhamnolipid-electrolyte solution was added. The vials were capped using Teflon-lined
screw caps and shaken at 200 rpm on a rotary shaker at 22 °C for 36 hrs. Each vial was
then allowed to stand for 24 hrs to allow phase separation of any emulsions that may have
formed during shaking. A syringe equipped with a stainless steel needle was used to
withdraw 8 ml of the aqueous solution from the bottom of the vial. This 8 ml aliquot was
allowed to stand for 24 hrs and then a 5 ml aliquot was removed and transferred to a new
vial in the same way. After a period of 24 hrs, a 0.5 ml aliquot was taken from each vial and added to 5 ml of ScintiVerse scintillation cocktail (Fisher Scientific, Pittsburgh, PA). Each sample was assayed for radioactivity, representing solubilized hexadecane, using a 1600 TR Tri-Carb liquid scintillation analyzer (Packard, Meridian, Conn.).

Effect of Ionic Strength on the Interfacial Tension The interfacial tension between rhamnolipid solution and hexadecane was measured using a spinning drop tensiometer described by Cayias et al., (1975). Prior to the actual interfacial tension measurement, the surfactant solution was preequilibrated with hexadecane for three days. A small amount of the equilibrated hexadecane was then contacted with surfactant solution in the spinning drop tensiometer. The system was allowed to equilibrate for 20 min before the initial reading was taken. The system was then allowed to equilibrate for another 10 min before the second reading was taken. If the second reading deviated from the first one, additional readings would be taken until a stable reading was obtained.

Effect of Ionic Strength on NAPL Removal The effect of ionic strength on surfactant enhanced removal of residual hexadecane was evaluated using column experiments. The detailed experimental procedure was described previously (Bai et al., 1996). Briefly, columns packed with sand were first saturated with distilled water from the bottom, then \(^{14}\)C-labeled hexadecane was loaded from the top of the column until displacement of the water ceased. The columns were then flushed with water from the bottom to establish residual hexadecane saturation. The residual hexadecane saturation was calculated from the difference between the volume of hexadecane loaded into the column and the volume
that was flushed out. The porous medium used in this experiment was 40/50 Accusand. The porosity of the sand was 0.38, and the column pore volume was 10.6 ml.

After establishing the residual hexadecane saturation, which was 21 ± 1%, the columns were flushed with aqueous solution (from the bottom) containing 2 mM rhamnolipid and up to 800 mM Na⁺. Solution pH was adjusted to 6 or 7 depending on the experiment. The experiments were conducted using a flow rate equivalent to a pore water velocity of 10 cm/hr. Effluent fractions (2ml) were collected in 20 ml scintillation vials. Ten ml of ScintiVerse scintillation cocktail (Fisher Scientific, Pittsburgh, PA) was added, and the radioactivity in each fraction was determined using liquid scintillation.

**Results And Discussion**

The chemical properties of surfactants are principally determined by the nature of the hydrophilic head group, with the nature of the hydrophobic tail group usually being of less importance. For ionic surfactants, the charged head group is strongly affected by the ionic strength of the surfactant solution. For anionic surfactants, solution behavior is primarily determined by the counterion, or cation, in the solution, which is the focus of the following discussion.

**Effect of Ionic Strength on Rhamnolipid Solubilization Capacity.** In this study, the solubilization of hexadecane in the presence of various cations was evaluated using two parameters: apparent solubility of the hydrocarbon and solubilization capacity of the surfactant. The solubilization capacity of the surfactant is measured by the molar
solubilization ratio (MSR), which is the number of moles of hydrocarbon solubilized per mole of surfactant micellized (Edwards et al., 1991). The MSR can be calculated from the slope of a plot of hydrocarbon solubility vs surfactant concentration.

As shown in Figure 2, the solubility of hexadecane in rhamnolipid solution is directly related to the sodium concentration in the solution. For example, hexadecane solubility in 4 mM rhamnolipid solution was increased from 52.1 mg/L in the control solution (no NaCl added) to 370 mg/L in the 500 mM NaCl solution, a 7-fold increase. MSR value were determined from linear regression of the data for surfactant concentrations above cmc. MSR values ranged from 0.054 for 0 mM NaCl to 0.401 for 500 mM NaCl, a 7.5-fold increase (Table I). It should be pointed out that the control solution actually has a small amount of Na\(^+\) from the preparation of the rhamnolipid solution. The Na\(^+\) concentration in the control ranged from 0.03 mM (in 0.06 mM rhamnolipid solution) to 2 mM (in 4 mM rhamnolipid solution).

It should be noted that the measured solubility of hexadecane in 0.06 mM rhamnolipid solutions (slightly below cmc) was 0.08 ± 0.02 mg/L. This is higher than the experimentally measured hexadecane solubility in pure water, 0.029 ± 0.009 mg/L. This effect may be attributed to the sequential-micellization phenomenon associated with heterogeneous surfactant solutions, as discussed by Kile and Chiou (1989). As described in the Materials and Methods section, the rhamnolipid used in this study is a mixture of four rhamnolipids with a nominal cmc of 0.1 mM. The monomer-micelle transition of such a heterogeneous surfactant solution may be expected to be considerably less sharp.
than that of a homogeneous surfactant solution. Additionally, the cmc of a surfactant is also affected by the addition of electrolytes and will decrease with the addition of NaCl (Rosen, 1989). Given both factors, it is possible that micelles exist at the concentration of 0.06 mM, and as a result, the measured hexadecane solubility was higher than that in pure water.

The influence of MgCl₂ on the apparent solubility of hexadecane in rhamnolipid solution is more pronounced than that of NaCl (see Figure 3). In addition, the MgCl₂ concentrations used in the experiment were up to 2 orders of magnitude less than the concentration of NaCl used. MSR values (Table I) were calculated from the slope of the lines in Figure 3. As shown in Table I, the MSR increased in the presence of MgCl₂ from 0.06 (control solution) to 1.53 (1 mM MgCl₂), a 25-fold increase. These results showed that both Na⁺ and Mg²⁺ can significantly enhance the solubility of hexadecane in rhamnolipid solution. As discussed in the theoretical section, this is due to an increase in the interior volume of rhamnolipid micelles in the presence of Na⁺ or Mg²⁺. Mg²⁺ was more effective than Na⁺ at increasing hexadecane solubility because Mg²⁺ is a divalent cation and has a stronger effect on reducing the electrical repulsion forces between the anionic head groups than does Na⁺.

The impact of CaCl₂ on the apparent solubility of hexadecane in rhamnolipid solution was somewhat different from that of MgCl₂ and NaCl, as shown in Figure 4. For Ca²⁺ concentrations up to 0.2 mM, hexadecane solubility increased as a function of rhamnolipid concentration. However, Ca²⁺ concentrations greater than 0.2 mM were
found to cause precipitation of rhamnolipid. This, while Ca\(^{2+}\) increased the solubilizing power of the rhamnolipid, rhamnolipid precipitation was a competing effect. Interestingly, even when rhamnolipid precipitation became strongly apparent (≥ 0.4 mM), there was no reduction in hexadecane solubility in comparison to the control. MSR values were not calculated for these data. The effect of Ca\(^{2+}\) is an important consideration for field applications because precipitation can cause major losses of surfactant, which can in turn effect the performance of the surfactant system (Rouse et al., 1993). These results show that, at the range of surfactant concentrations and the ionic strengths tested, hexadecane solubilization was not negatively affected by the Ca\(^{2+}\) even though some precipitation occurred.

**Effect of Ionic Strength on Interfacial Tension** The influence of ionic strength on the interfacial tension between hexadecane and surfactant solution is shown in Figure 5. There are two points to emphasize in this figure. First, interfacial tension was decreased as Na\(^{-}\) concentration was increased from 0 to 800 mM. Second, for a given Na\(^{-}\) concentration, interfacial tension decreased when the pH was reduced from 7 to 6. A decrease in pH has the same qualitative effects as that of an increase in Na\(^{+}\) concentration because as the pH of the surfactant solution is reduced, the carboxyl group in the rhamnolipid head group (pKa = 5.6) becomes more protonated, thereby reducing the repulsion between the head groups. In this set of experiments, interfacial tension was minimized at pH 6 and a Na\(^{-}\) concentration of 320 mM.
Effect of Ionic Strength on Residual NAPL Removal  The batch experiments just described show that the ionic strength and pH can be adjusted to maximize biosurfactant solubilization power and minimize interfacial tension between hexadecane and rhamnolipid solution. These data were used to design a series column experiments conducted to test whether hexadecane removal from a sandy matrix could be optimized. Figure 6 shows the effect of selected ionic strength and pH combinations on the removal of hexadecane from a sand column. In this study, the residual saturation of hexadecane in the column was 21±1%, which corresponds to 2.26± 0.1 ml hexadecane in the column. As expected, an increase in Na⁺ concentration or decrease in pH increased the amount of hexadecane removed from the column. For example, at pH of 7, the removal of residual hexadecane from the soil column was increased from 9% to 14% when the Na⁺ concentration in the surfactant solution increased from 0 to 800 mM. When the pH was decreased to 6, removal of hexadecane increased from 22% to 58% when the Na⁺ concentration increased from 0 to 320 mM. These results also showed that all residual removal took place within the first three pore volumes.

Removal of hexadecane residual was primarily by mobilization, as indicated by collection of hexadecane free product in effluent fractions. Even though increasing the salt concentration increases solubilization by almost 1 order of magnitude (Fig. 2, Table I), the contribution of solubilization was still small (less than 1%). This is because low surfactant concentration used (2 mM or 1,000 mg/L) and solubilized hexadecane removed was still insignificant compared with the total removal. However, it should be pointed out
that when the applied surfactant concentration is high, solubilization can become the main mechanism of NAPL removal. For example, synthetic surfactant concentrations ranging from 0.5% to 4% (5,000 to 40,000 mg/L) have been reported to remove NAPL primarily by solubilization (Pennell et al., 1993; Ang and Abdul, 1991). When high surfactant concentrations such as these are used, an increase in ionic strength can be used to increase NAPL solubility and improve NAPL removal by solubilization.

The mobilization of residual NAPL can be evaluated by two dimensionless parameters: Capillary number ($N_C$) and Bond number ($N_B$). $N_C$ and $N_B$ characterize the relative importance of viscous and buoyancy forces, respectively. These forces act to mobilize the NAPL and are opposed by the capillary force, which acts to retain the NAPL within a porous medium (Ng et al., 1978; Morrow and Songkran, 1981). These two dimensionless parameters can be defined as follows:

\[
N_C = \frac{q \mu_w}{\sigma_{ow}}
\]

\[
N_B = \frac{\nabla \rho g (k / \Phi)}{\sigma_{ow}}
\]

where $q$ is the Darcy velocity, cm/s; $\mu_w$ is the viscosity of surfactant solution, dyn*s/cm$^2$; $\sigma_{ow}$ is the interfacial tension between the surfactant solution and the NAPL, dyn/cm; $\Delta \rho$ is the density difference between the surfactant solution and the NAPL, g/cm$^3$; $g$ is the acceleration due to the gravity, cm/s$^2$; $k$ is the absolute permeability, cm$^2$; and $\Phi$ is the porosity. In this study, the column experiments were conducted in a vertical orientation,
with displacement of hexadecane in the direction of the buoyancy force, thus the effects of the Bond number and Capillary number are additive (Morrow and Songkran, 1981).

The correlation between mobilization of a NAPL from the subsurface and the Capillary number and Bond number has been extensively investigated in the enhanced oil recovery industry (Shah and Schecter, 1977; Morrow and Songkran, 1981). It has been found that there is a critical value of $N_T (N_C + N_B)$ at which residual NAPL begins to be released from porous media. Further increase of $N_T$ from this critical value causes the residual NAPL saturation to decrease rapidly. The relationship between the measured hexadecane saturations and calculated $N_T$ values is shown in Figure 7. The $N_C$ and $N_B$ were calculated using independently measured value for interfacial tension, Darcy velocity, permeability, and density difference, and the assumption that viscosity of 2 mM surfactant solution was the same as water, 0.01 dyn s/cm².

As shown in Figure 7, when the column was flushed with water, the calculated $N_T$ ($N_C+N_B$) values were $1.03 \times 10^{-6}$ and $2.06 \times 10^{-6}$ at the Darcy velocities of 5.7 cm/hr and 11.4 cm/hr, respectively. In both of these cases, no hexadecane was mobilized under this conditions, indicating that the $N_T$ values were lower than the critical $N_T$. When surfactant solutions were applied to the column, the interfacial tension was reduced from 49.8 dyn/cm for water to 2 dyn/cm for surfactant solutions at pH 7. This interfacial tension value corresponds to an $N_T$ value of $2.58 \times 10^{-5}$, and as shown in Figure 7, 9% of the hexadecane was released from the column under this condition, indicating that the $N_T$
value was close to the critical $N_T$ value. When Na$^+$ was added to the solution or the pH decreased to 6, the interfacial tension between the rhamnolipid solution and hexadecane was further decreased, causing an increase in $N_T$ to $5.8 \times 10^{-5}$ (pH, 7, Na$^+$, 800 mM), $7.37 \times 10^{-5}$ (pH, 6, Na$^+$, 0 mM), and $2.58 \times 10^{-3}$ (pH, 6, Na$^+$, 320 mM). Correspondingly, the residual hexadecane saturation dropped rapidly. Based on these data, the critical $N_T$ number appears to fall within the range of $5.0 \times 10^{-6}$ to $2.0 \times 10^{-5}$. This is similar to the critical $N_T$ values of $2 \times 10^{-5}$ reported by Morrow and Songkran (1981) for mobilization of Soltrol-130, and $2 \times 10^{-5}$ to $5 \times 10^{-5}$ reported by Pennell et al. (1996) for mobilization of PCE in various sands. Thus, for low surfactant concentrations control of ionic strength and pH can be used to minimize interfacial tension and maximize NAPL removal by mobilization. This strategy is good for removal of LNAPLs such as fuel oils. However, it has been pointed out that mobilization of DNAPL may result in further spreading of NAPL plumes. Therefore, for DNAPLs, control of ionic strength and pH may be used to minimize interfacial tension changes so that removal occurs primarily by solubilization.

In summary, both the ionic strength and pH of the rhamnolipid biosurfactant solution has a strong effect on the performance of rhamnolipid. An increase in ionic strength of surfactant solution can increase the surfactant's solubilization capacity and reduce the interfacial tension between surfactant solution and the NAPL phase. A decrease in pH of the surfactant solution can also decrease the interfacial tension between the aqueous and the NAPL phase. Reduced interfacial tension results in mobilization of residual NAPL from soil. In this study, at a pH of 6 and Na$^+$ concentration of 320 mM,
the interfacial tension between hexadecane and 2 mM rhamnolipid solution was reduced to 0.02 dyn/cm. As a result, 58% of the residual hexadecane was removed from a 40/50 mesh sand column in 2 pore volumes, compared to essentially no removal of hexadecane by water alone. These results show that control of ionic strength and pH need to be considered in field applications to improve the performance of anionic surfactant systems.

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References


Table I  Calculated MSR in the presence of NaCl and MgCl$_2$.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Ion concentration</th>
<th>MSR</th>
<th>$r^2$</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>0</td>
<td>0.054</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>30 mM</td>
<td>0.118</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>50 mM</td>
<td>0.136</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>100 mM</td>
<td>0.203</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>200 mM</td>
<td>0.290</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>300 mM</td>
<td>0.339</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>400 mM</td>
<td>0.379</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>500 mM</td>
<td>0.401</td>
<td>0.99</td>
</tr>
<tr>
<td>MgCl$_2$</td>
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<td>0.060</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>0.1 mM</td>
<td>0.540</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.2 mM</td>
<td>0.770</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>0.4 mM</td>
<td>0.960</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.8 mM</td>
<td>1.270</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td>1.530</td>
<td>0.99</td>
</tr>
</tbody>
</table>

notes: $r^2$ is the correlation coefficient of the hexadecane solubility data at the rhamnolipid concentration above cmc
Figure 1a. Schematic representation of rhamnolipid molecule; $a_h$ is the cross sectional area of the head group; $V_H$ is the volume occupied by the hydrocarbon chains in coiled configuration; and $L_c$ is the length of the tails.

Figure 1b. Structure of *Pseudomonas aeruginosa* ATCC 9027 monorhamnolipid.
Fig. 2. Solubility of hexadecane in rhamnolipid solution in the presence of NaCl. The data are the average of two measurements. The Molar Solubilization Ratio (MSR) was calculated from the slope of solubility data at 0.4, 1, 2, and 4 mM rhamnolipid.
Figure 3. Solubility of hexadecane in rhamnolipid solution in the presence of MgCl₂. The MSR was calculated from the slope of all the data points.
Figure 4. Solubility of hexadecane in rhamnolipid solution in the presence of CaCl₂
Figure 5. The effect of pH and Na$^+$ on the interfacial tension between hexadecane and rhamnolipid solutions. The rhamnolipid concentration used in this experiment was 2 mM.
Figure 6. The effect of pH and ionic strength on removal of residual hexadecane from a sand-packed column. In both cases, the rhamnolipid concentration was 2 mM.
Figure 7. Hexadecane capillary desaturation curve from 40/50 mesh sand columns. For all of the rhamnolipid flushing, the rhamnolipid concentration was 2 mM and the q was 5.7 cm/hr.
APPENDIX C

THE INFLUENCE OF RHAMNOLIPID BIOSURFACTANT ON THE TRANSPORT OF BACTERIA THROUGH A SANDY SOIL

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Abstract

The objective of this study was to investigate the influence of an anionic rhamnolipid biosurfactant on the transport of bacterial cells through soil under saturated conditions. Three cell types with varying hydrophobicity, *Pseudomonas aeruginosa* ATCC 9027, ATCC 27853, and ATCC 15442, were used in this study. In a series of experiments, columns packed with sterile Accusand were saturated with sterile artificial ground water (AGW) for 15 hours, then three pore volumes of $^3$H-labeled bacteria suspension with varying rhamnolipid concentrations were pumped through the column. This was followed by four pore volumes of rhamnolipid solution alone. Breakthrough curves were obtained and fitted using a two-site model (equilibrium and non-equilibrium adsorption site) with two first order sink terms for irreversible cell adsorption. The influence of rhamnolipid on the surface charge density of the bacteria and the porous medium were also investigated. Results show that rhamnolipid enhanced the transport of all cell types tested. For example, rhamnolipid increased the recovery of the most hydrophilic strain, ATCC 9027, from 22.5% to 56.3%. Similarly, the recovery of ATCC 27853 increased from 36.8% to 49.4%, and recovery of ATCC 15442, the most hydrophobic strain, increased from 17.7% to 40.5% in the presence of rhamnolipid. In the presence of rhamnolipid, the negative surface charge density of the porous medium was increased, while the surface charge density of the bacteria were not changed. Model results suggest that rhamnolipid predominantly affected irreversible adsorption of cells.
Introduction

Movement of microorganisms in subsurface environments has been examined from several different perspectives including the potential for transport of pathogenic microorganisms to water supply aquifers (Gerba, 1985), microbial-enhanced oil recovery (Jang et al., 1989), the fate of genetically engineered microorganisms in the environment (Sayler, 1986), the activity of adsorbed bacteria (Harms and Zehnder, 1994), and the delivery of microorganisms for in situ remediation of contaminated sites (Thomas et al., 1989). Previous work has shown that the retention of bacteria in soil or in other porous media is controlled mainly by two factors: physical straining and sorption to surfaces (Pekdeger and Matthess, 1983). The extent to which bacteria are retained by filtration is inversely proportional to the size of the particles (Hagedorn et al., 1981) and the rate of water flow (Wollum and Cassell, 1978). Sorption of bacteria is influenced by a number of factors, including the net surface charge of both the porous medium and the bacteria (Marshall et al., 1971), cell surface hydrophobicity (Gannon et al., 1991a), the presence of extracellular polysaccharides (Humphrey et al., 1979), and the ionic strength of aqueous solution (Gannon et al., 1991b).

The transport of bacteria in the subsurface is of special importance in implementation of bioremediation where introduction of a degrader to the contaminated site is required. The bacteria must be capable of moving or being transported to the site of contamination. However, a number of studies have shown low mobility of bacteria under natural conditions. For example, Wollum and Cassel (1978) reported that 90% of
the bacteria added to a column packed with sand were recovered within 3 cm of the surface. Similarly, Harvey et al. (1989) reported that >99% of the bacteria injected into a sandy aquifer did not reach a sampling well 1.7 m from the site of injection. Although much is known about factors affecting bacterial transport in soil, little information is currently available concerning the enhanced transport of bacteria.

The objective of this study was to investigate the influence of an anionic monorhamnolipid biosurfactant on the transport of three *Pseudomonas* spp. with differing cell surface hydrophobicity. The results of these experiments were analyzed using a modified advection-dispersion transport model that incorporated reversible and irreversible retention of bacteria.

**Materials And Methods**

**Biosurfactant** The monorhamnolipid (Fig. 1) used in this study was produced and purified as described by Zhang and Miller (1994). The molecular weight of the dominant rhamnolipid (504) was used to calculate rhamnolipid concentration in this study. All surfactant stock solutions were prepared in deionized, distilled water and adjusted to pH 7.0 with 0.1 M KOH. The critical micelle concentration (cmc) of the monorhamnolipid is 50 mg/L (Zhang and Miller, 1994).

**Bacterial strains and growth conditions** *Pseudomonas aeruginosa* 9027, 27853, and 15442 were obtained from the American Type Culture Collection (Rockville, Md). The three bacterial strains with varying cell surface hydrophobicity were chosen for this study.
These strains have been characterized previously in our laboratory using the BATH (bacterial adherence to hydrocarbon) assay. The cell surfaces range from relatively hydrophilic (ATCC 9027), to intermediate (ATCC 27853), to relatively hydrophobic (ATCC 15442) as shown in Table 1. These data are consistent with reported contact angle measurements for ATCC 9027 (26.04°) and ATCC 27853 (42.61°) (Vanhaecke et al., 1990). The relative hydrophobicity of each strain was also measured by the BATH assay for this study using artificial groundwater as the aqueous phase. Under these conditions, results showed that ATCC 27853 is the most hydrophobic strain but there was no significant difference between the hydrophobicity of ATCC 9027 and ATCC 27853 (Table 1).

Bacteria were labeled with tritium (3H) for all transport experiments using the following protocol. The bacteria were grown to stationary phase (15 hours) at 37°C in 125 Erlenmeyer flasks containing 30 ml of Kay's minimal medium (NH₄H₂PO₄, 0.3%; K₂HPO₄, 0.2%; glucose, 0.2%; FeSO₄, 0.00005%; MgSO₄, 0.1%). One ml of the stationary-phase bacteria was transferred to a 10 ml glass culture tube and 3H [D-glucose] was added (36.0 µCi/mmol). The bacteria were incubated at 37°C for 2 hours to allow uptake of the 3H [D-glucose] and then were harvested by centrifugation at 12,000 x g for 5 min, and washed three times with sterile artificial groundwater (MgSO₄ 7H₂O, 35 mg/L; CaSO₄ H₂O, 12 mg/L; NaHCO₃, 12 mg/L; NaCl, 6 mg/L; KNO₃, 2 mg/L) (McCaulou et al., 1994). The washed cells were suspended in 40 ml sterile artificial groundwater and
enumerated by using the acridine orange direct count method (Hobbi et al., 1977). For each experiment, the cell concentration was adjusted to a predetermined level.

**Column and porous media** The glass chromatography column used in this study was 5 cm long and 2.5 cm in diameter (Ketone, NJ). The original Teflon filter and the fritted stainless steel disc in each end were replaced by a stainless steel screen to prevent physical filtering of bacterial cells at the inlet and outlet of the column. Each column was packed with a 40/50 mesh fraction (equivalent to 0.3-0.42 mm) of Accusand (North Kato Supply, MN) which has the following characteristics: total organic carbon, 0.04%; iron oxide content, 0.03%; cation exchange capacity, 0.57 meq/100g. Before each experiment, the sand was rinsed with deionized, distilled water and autoclaved. The sand was not treated more extensively to avoid changing its surface properties.

**The influence of rhamnolipid on the surface charge density of porous medium** Zeta potential (ζ) of the Accusand was measured to determine the effect of rhamnolipid on the Accusand surface charge density. The zeta potential of a particle is the potential difference between the plane of shear and the bulk phase and is responsible for the particle's electrokinetic properties (Stumm, 1992). The Accusand was finely ground in preparation for the zeta potential measurement, which requires solid particles to be suspended in the electrolyte solution.

For measurement of zeta potential, 5 g Accusand particles were suspended in 100 ml artificial ground water with varying concentrations of rhamnolipid (0, 250, 500, 1000 mg/L). The ionic strength of each solution was adjusted to 2 mM with NaCl. Then
the suspended particles were equilibrated in solution for seven days prior to measurement using a Model 301 Laser Zee Meter (Penkem, Inc., Bedford Hills, New York).

The influence of rhamnolipid on the surface charge density of bacteria The electrophoretic mobility of each bacterial strain was measured to determine surface charge density. Electrophoretic mobility measurements were conducted using a Beckman P/ACE system 2100 Capillary Zone Electrophoresis (CZE) unit equipped with a UV detector set at 214 nm and an uncoated, 47-cm flexible fused silica capillary column with 75-μm ID and polyamide exterior coating (Polymicro Technologies, Phoenix, AZ).

For electrophoretic mobility measurements, bacteria were grown as described above, harvested by centrifugation, and washed three times with artificial ground water. The final cell pellet was resuspended in 10 mM Tris-HCl buffer, pH 7, with varying concentrations of rhamnolipid (0, 200, 500, and 1000 mg/L). The ionic strength of each solution was adjusted to 2 mM using NaCl. Prior to measurements, the capillary was conditioned and then flushed with Tris buffer. Then 25 nL bacteria sample (corresponding to a 5 second high pressure injection) was injected to the inlet of the capillary along with a neutral molecule, mesityl oxide, which was used as a reference marker. A 212 V/cm voltage (total 10,000 V on whole capillary) was applied to the capillary. Under the conditions used in this study, the detection limit was approximately $10^8$ cells/ml, which represents about 2500 injected cells.
Electrophoretic mobility (M) is the migrational velocity of a particle per unit applied field strength. In a capillary electrophoresis instrument, M can be calculated as follows:

\[ M = \frac{L_d L_a}{V} \left( \frac{1}{t_m} - \frac{1}{t_{ref}} \right) \]

where \( L_d \) is capillary length to the detector, \( L_a \) is the total capillary length, \( V \) is applied voltage, \( t_{ref} \) is the migration time of reference marker, and the \( t_m \) is the migration time of bacteria (Zare, 1988). Bacterial surface charge density and zeta potential can be calculated from bacteria electrophoretic (Oshima et al. 1982)

**Transport experiments** Sand was dry packed into each column under vibration. The same mass of sand was used for all experiments to ensure that each column had the same porosity and pore volume. In each case, the bulk density was 1.73 g/cm³, porosity was 38%, and pore volume was 9.7 ml. The column was saturated from the bottom up with pre-sterilized artificial groundwater for 15 hours at a flow rate of 0.33 ml/min. Then a \(^3\text{H}[\text{bacterial suspension}]\) was introduced to the column from the bottom at a flow rate of 0.33 ml/min (2 pore volumes/hr). This was equivalent to a pore water velocity of 10 cm/hr. For each experiment, 3 pore volumes of the \(^3\text{H}[\text{bacterial suspension}]\) was pumped through the column followed by 4 pore volumes of artificial groundwater. In those experiments involving biosurfactant, the bacteria were suspended in a rhamnolipid solution and 3 pore volumes of this bacterial/rhamnolipid solution was pumped through the column followed by 4 pore volumes of rhamnolipid solution of the same
concentration. The effluent from the column was collected in 1 ml fractions and 10 ml ScintiVerse BD scintillation cocktail (Fisher Scientific, Pittsburgh, PA) was added to each fraction. The radioactivity in each fraction was determined using a 1600 TR Tri-Carb scintillation counter (Packard, Meridian, Conn.).

Each experiment was conducted at room temperature (20±1.5°C). Given the fact that the artificial groundwater and the porous medium were pre-sterilized and the experiment lasted only 3.5 hours, it is assumed that no change in bacterial number or size took place during the experiment.

Model simulation  Several approaches have been used to simulate the movement of bacteria in the subsurface. Corapcioglu and Haridas (1984, 1985) presented a transport model coupled with retention, growth and decay to describe bacteria movement in subsurface. Harvey and Garabedian (1991) used filtration theory to describe small-scale movement of bacteria through a sandy aquifer. Lindqvist and Enfield (1992) used equilibrium advection-dispersion-adsorption model with a sink term to simulate the movement of bacteria through a soil column. Several researchers have used modified adsorption-dispersion models with one or two-site adsorption to simulate the bacteria movement (Tan et al. 1994; Lindqvist et al. 1994; McCaulou et al. 1994; Johnson et al. 1995; and Bengtsson and Lindqvist, 1995). In our study, a one-dimensional advection-dispersion model that includes combined equilibrium and rate-limited sorption and a first-order sink term was used to simulate experimental results. In this approach, reversible sorption of bacteria is assumed to be essentially instantaneous for a fraction of the porous
medium surface and rate-limited for the remainder. In addition, terms are included to represent irreversible sorption.

The nondimensional governing equations for transport under steady-state water flow, instantaneous and rate-limited linear reversible sorption, and first-order irreversible sorption are (van Genuchten and Wagenet, 1989):

\[
\beta R \frac{\partial C^*}{\partial T} + (1 - \beta) R \frac{\partial S^*}{\partial T} = \frac{1}{P} \frac{\partial^2 C^*}{\partial X^2} - \frac{\partial C^*}{\partial X} - \xi C^* - \eta S^*
\]

\[
(1 - \beta) R \frac{\partial S^*}{\partial T} = \omega (C^* - S^*) - \eta S^*
\]

Where the following parameters are defined as:

\[ C^* = \frac{C}{C_0} \]
\[ S^* = \frac{S_2}{[(1-F)K_pC_0]} \]
\[ X = \frac{x}{l} \]
\[ T = \frac{t}{l} \]
\[ P = \frac{U}{D} \]
\[ R = 1 + (\rho/\theta)K_p \]
\[ \beta = \frac{1}{1 + (\rho/\theta)FK_pH/R} \]
\[ \omega = k_2 (1 - \beta) \frac{R}{U/v} \]
\[ \xi = (\beta R - 1) \mu_1 U/v \]
\[ \eta = (1 - \beta) R \mu_2 U/v \]
Where $C$ ($M/V$) is the bacteria concentration in solution, $S_1$ ($M/V$) is the sorbed-phase concentration in “instantaneous” sites, $S_2$ ($M/V$) is sorbed phase concentration in rate limited sites, $C_0$ ($M/V$) is the influent bacteria concentration, $t$ is time (T), $l$ is the length of column (L), $\rho$ ($M/V$) is the porous medium bulk density, $\theta$ is the porosity of the packed column, $v$ (L/T) is the average pore water velocity, $D$ ($L^2/T$) is the hydrodynamic dispersion coefficient, $K_p$ ($V/M$) is the equilibrium sorption constant, $F$ is the fraction of sorbent for which sorption is instantaneous, $k_2$ (1/T) is the reverse sorption rate constant, and $\mu_i$ (1/T) is the first order sticking rate constant which describes the irreversible cell loss occurring from the instantaneous-sorption domain ($s_2$), and rate-limited sorption ($s_2$), respectively. The dimensionless model parameters are: $P$, the Peclet number which represents the dispersive-flux contribution to transport; $R$, the retardation factor which represents the effect of sorption on transport; $\beta$, the fraction of instantaneous retardation; $\omega$, the Damkohler number which is the ratio of hydrodynamic residence time to characteristic time for sorption; and $\xi$ and $\eta$, which are dimensionless first-order cell sticking rate constants.

If $F$ is set to 1, the above model is reduced to a one-site equilibrium model (all reversible adsorption is instantaneous). In such case, $\beta=1$ and $\eta=0$. If $F$ is set to 0, the above model is reduced to the one-site non-equilibrium model (all reversible adsorption is rate-limited). In this case, $\beta=1/R$, $\xi=0$.

In all cases, $P$ was calculated using breakthrough curves of PFBA, a conservative tracer. All of the bacterial breakthrough curves were fitted with the transport model
using non-linear-least-squares curve-fitting. The model parameters, R, β, ω, ξ, η were determined from the fitted curves.

Results

The influence of rhamnolipid on the surface charge density of bacteria

The calculated electrophoretic mobilities for strains ATCC 9027, ATCC 27853, and ATCC 15442 were $2.75 \times 10^{-8}$, $2.88 \times 10^{-8}$, and $2.33 \times 10^{-8}$ m$^2$/V s, respectively. All the three strains have a negative electrophoretic mobility, indicating a negatively charged cell surface at pH 7. For all three strains, there was no detectable difference in electrophoretic mobility in the presence of rhamnolipid at the concentration of 200, 500, and 1000 mg/L. This results are consistent with previous work by Zhang and Miller (1994) that showed no detectable association between cells and $^{14}$C-labeled rhamnolipid even after incubation periods of up to 48 hr.

The influence of rhamnolipid on the surface charge density of the porous media

Figure 2 shows the effect of rhamnolipid on the zeta potential of Accusand. In the absence of rhamnolipid, the zeta potential of Accusand in artificial ground water was measured to be -14 mV. Thus, the surface of the Accusand is negatively charged at pH 7. For comparison, the zeta potential of a clean quartz surface under similar conditions was measured to be -30 mV, indicating that Accusand carries a less negative surface charge than that of clean quartz. As rhamnolipid was added to the Accusand, the zeta potential
was reduced in direct relation to the amount of rhamnolipid added. At the highest concentration of rhamnolipid, 1000 mg/L, the zeta potential was reduced to -70 mV.

**Influence of cell density on bacterial transport**  The hydrodynamic characteristics of the sand columns were characterized using a conservative tracer, pentafluorobenzoic acid (PFBA). Each PFBA breakthrough curve was sharp and symmetrical, indicating minimal dispersion in the column (data not shown). The calculated Peclet number for these columns was 36.8, indicating the column packing was relatively homogeneous.

Figure 3 shows breakthrough curves for ATCC 9027 at varying cell concentrations. As shown in this figure, even at the highest cell concentration tested, the effluent cell concentration did not reach C/C₀=1 and in all cases bacterial cell recoveries were less than 91%, indicating that irreversible adsorption of cells occurred. The relative recovery of cells was dependent on the influent cell density, with higher recoveries at higher cell densities.

The recovery of the three different strains at a same influent concentration, 5 x 10⁷ cells/ml, was not correlated to the cell hydrophobicity (Table 2). These data show that the highest cell recovery (37%) was for ATCC 27853, the strain with intermediate hydrophobicity. The lowest cell recovery (18%) was for ATCC 15442, the most hydrophobic strain.

**Effect of rhamnolipid on bacterial transport**  The transport of ATCC 9027 in the presence of 0, 250, 500, and 1000 mg/L rhamnolipid is shown in Figure 4. Bacterial cells were added in a rhamnolipid suspension for 3 pore volumes and then the column was
further flushed with 4 pore volumes of rhamnolipid solution alone. Results showed that increasing rhamnolipid concentrations increased the transport and recovery of ATCC 9027 cells from 23% in the absence of rhamnolipid to 56% in the presence of 1000 mg/L rhamnolipid (Figure 4, Table 2).

Since rhamnolipid solutions had considerably higher ionic strength than the artificial groundwater used in the absence of rhamnolipid, the effect of ionic strength on cell recovery was also examined. As shown in Figure 3, an increase in ionic strength to 2 mM using NaCl in the absence of rhamnolipid caused lower cell recoveries, indicating that bacteria transport is inhibited by the increase of ionic strength. This result is consistent with previously reported data (Fontes et al., 1991; Gannon et al., 1991). Thus, it appears that the enhanced cell transport observed is a result of the surface active properties of the rhamnolipid solutions rather than their ionic strength.

Cell recoveries in the presence of rhamnolipid were increased from 37 to 49% for ATCC 28753 and from 18 to 41% for ATCC 15442 (Figures 5 and 6, Table 2). Although rhamnolipid increased removal of cells for all three strains tested, rhamnolipid had the greatest effect on the two strains that had highest initial sorption. For example, recovery of ATCC 15442 and 9027, which had the lowest initial recoveries, were increased by 23 and 33% respectively by 1000 mg/L rhamnolipid. In contrast, recovery of ATCC 27853, which had the highest initial cell recovery, was increased by only 12%.

**Model simulations** The data in Figures 4-6 were analyzed using the one-dimensional advection-dispersion model that was presented in the Materials and Methods section.
The optimized simulations are shown in each figure as solid lines and in most cases provided a good fit to the experimental data. The five fitted model parameters, $R$, $\beta$, $\omega$, $\xi$, and $\eta$ are presented in Table 3. Among the 5 fitted parameters, three parameters are especially important. These include the retardation factor $R$, which is the effect of reversible adsorption on cell transport, and the two dimensionless first-order irreversible sticking rate constants, $\xi$ for instantaneous sorption sites, and $\eta$ for rate-limited sorption sites. These dimensionless sticking rate constants are measures of cell "irreversible" attachment to the porous medium.

Results in Table 3 show that $R$ (reversible sorption) decreased for all three strains as the rhamnolipid concentration increased with one exception, ATCC 9027. For ATCC 9027, the fitted $R$ increased from 3.13 to 4.51 when the rhamnolipid concentration was increased from 0 to 250 mg/L. Further analysis of the simulations for ATCC 9027 shows that the fit for 0 mg/L rhamnolipid was poor in comparison to rest of the fits in this study.

The dimensionless first-order sticking rate constants for irreversible sorption, $\xi$ and $\eta$, also generally decreased as the rhamnolipid concentration increased. However, it is clearly the sticking rate constant for rate-limited sorption sites, $\eta$, that was the most affected. In each case, the fitted $\eta$ value became essentially 0 when rhamnolipid was present even at the lowest concentration.

When fitting multiple parameters, there is concern whether the solutions obtained are unique. To assess the uniqueness of the optimizations reported herein, selected breakthrough curves were subjected to additional analysis. This was done by holding
some parameters constant during the optimization. The values for these fixed parameters were obtained by independent means as follows. The retardation factor was determined by moment analysis. However, this can be done accurately only for breakthrough curves for which the elution tail has returned to a relative concentration of zero. The optimized value for $\eta$ was close to zero for some data sets. In such cases, $\eta$ can be assumed to be zero with little effect on the simulated curve. When $\eta$ is zero, the value for $\xi$ can be obtained from the equation (Angley et al., 1992): 

$$\xi = -\ln \left(\frac{C}{C_0}\right)^S,$$

where $(C/C_0)^S$ is the plateau representing steady state conditions.

The above approach was applied to the ATCC 9027 experiment with $5 \times 10^{11}$ influent cell concentration without surfactant shown in Figure 3. The values for the independently determined parameters, $R$ and $\xi$, match closely the values determined by optimization (see Table 4). In addition, the optimized values for $\beta$ and $\omega$ determined for the two-parameter fit match closely the $\beta$ and $\omega$ determined for the five-parameter fit (see Table 4). This indicates that, for this case, the five-parameter optimization provided a unique solution. This suggests that the parameter values reported herein are valid.

Discussion

Retention of bacteria in porous media is dependent on several factors: physical straining or filtration at pore constrictions, diffusion into dead end pores or micropores, and sorption to the solid matrix. Herzig et al. (1970) showed that cell retention by physical straining is insignificant when the ratio between cell diameter and the mean sand
grain diameter is <0.05. In this study, the porous medium used was relatively coarse (0.3 - 0.425 mm) and homogeneous. Assuming each bacterial cell is 2 μm long and 1 μm wide, the ratio between the diameter of the cells and the porous medium used in this study is less than 0.007. Thus, it is highly unlikely that physical straining was a major factor in cell removal. Since the particle distribution of the porous medium is narrow, loss of cells in deadened pores and micropores should also be insignificant. Therefore, in this study, the major mechanism controlling the transport of bacterial cells was sorption to the porous medium surface.

The sorption of bacteria to a solid surface can be influenced by several physicochemical forces. Attractive physicochemical forces include long range forces, e.g., electrostatic forces, Van der Waals forces, and short range forces, e.g., dipole interaction, chemical binding, and hydrophobic interactions (McEldowney and Fletcher, 1986; van Loosdrecht et al., 1989). However, in this study the cell surfaces carried a net negative charge, as did the porous medium. Therefore, electrostatic repulsions between porous medium and bacteria will tend to prevent the close approach of the two surfaces. However, the ultimate interaction between bacteria and the porous media is dependent on the balance between attractive van der Waals forces and repulsive forces. This interaction has been described by Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Rutter and Vincent, 1984). According to DLVO theory, when a colloidal particle is close to a surface with like charge, the net forces can become attractive at a certain distance, which is termed the second minimum.
The sorption process can be described by a two-phase model proposed by Marshall et al. (1971). According to this model, bacteria are initially attracted to a surface and weakly held at a finite distance from that surface (second minimum) as predicted by DLVO theory. These bacteria, which under a microscope appear to be attached to a surface, are actually held loosely to the surface and can detach from the surface easily, which is referred as reversible adsorption (Fletcher, 1980). However, this reversible sorption can be followed by an irreversible phase of sorption in which the bacteria can no longer detach from the surface. This irreversible attachment is often ascribed to: 1) the synthesis of extracellular polymeric material that acts as a glue (Humphrey et al., 1979; Fletcher and Floodgate, 1973), 2) attraction to positively charged sites on the porous medium surface, and 3) short range interactions which become significant when the two surfaces are close together (< 0.4 nm) (McEldowney and Fletcher, 1986; Tadros, 1980).

Both reversible and irreversible adsorption will affect the transport of bacteria in the subsurface. Irreversible sorption causes permanent retention of bacteria which appears as a form of “mass loss”. Reversible sorption can act to retard the movement of the bacteria in the subsurface. Both reversible and irreversible adsorption were considered in this study. As described above, the retardation factor (R) was used to describe reversible adsorption, and two dimensionless first order-sticking rate constants (ξ and η) were used to describe irreversible adsorption. Results from this study showed that irreversible adsorption of bacteria to the solid surface was reduced by the addition of rhamnolipid.
The recovery of bacteria from the column was increased by increasing the rhamnolipid concentration (Table 2). This effect was also revealed by the parameters derived from model simulation, $\xi$ and $\eta$ (Table 3).

Three possible mechanisms, acting independently or collectively, could account for the reduction of irreversible adsorption of bacteria in the presence of rhamnolipid; 1) the increase in the negative surface charge density of the porous medium, 2) the solubilization of extracellular polymeric glue and/or, 3) the physical screening of the bacteria from close contact with porous medium surfaces.

Figure 2 gives direct evidence that rhamnolipid caused an increase in the negative surface charge density (see decrease in zeta potential) of the porous medium. This increased surface negative charge density will increase the repulsion between bacteria and the porous medium and, at the same time, decrease the electrostatic attraction between bacteria and the positively charged sites on the Accusand surface. Chemical analysis of Accusand showed that this sand contains 0.03% iron oxides, indicating that there are some positively charged sites in the sand surface. A second mechanism for the reduced irreversible adsorption is the solubilization of extracellular polymeric glue. As described above, one of the mechanisms responsible for irreversible adsorption is synthesis of extracellular glue. Surfactants may solubilize this glue, thereby reducing the likelihood of bacterial attachment to the solid surface. Finally, rhamnolipid adsorbed to the Accusand may prevent the bacteria from coming close to the surface. The length of the rhamnolipid molecule was calculated to be 1.2 nm using the equation $L = 0.15 + 0.126n$ (Tanford,
In this equation, $L$ is the length of the surfactant "tail", and $n$ is the number of the carbons in the surfactant hydrocarbon chain. This length of 1.2 nm is sufficient to allow the adsorbed rhamnolipid to have a steric effect, thus reducing the chance of bacteria colliding with the surface.

Reversible adsorption of the bacteria was also reduced by the addition of rhamnolipid (R decreased). This may be caused by increased porous surface charge density. According to DLVO theory, when the surface charge density in either the porous medium or the bacteria increases, the attraction force at secondary minimum decrease (Israelachvili, 1994). This means the bacteria will be held even more loosely by the porous medium. As a result, the amount of reversible sorption decrease.

Results of this research indicate that rhamnolipid biosurfactant enhanced the transport of hydrophobic and hydrophilic *Pseudomonas aeruginosa* cells by decreasing the sorption of cells to a porous medium surface. Simulation of bacterial breakthrough curves showed that both reversible and irreversible sorption decreased, although the decrease in irreversible sorption appeared more important. Reduced sorption maybe due to an increase in porous medium surface charge density by rhamnolipid adsorption, the solubilization of extracellular polymeric glue, or reduced availability of sorption sites on porous medium surfaces.
References


Figure 1. The structure of *P. aeruginosa* ATCC 9027 C₂₀ monorhamnolipid biosurfactant.
Figure 2. Zeta potential of Accusand in rhamnolipid solution.
Figure 3. Breakthrough curves of ATCC 9027 in sand columns at different influent concentrations.
Figure 4. Breakthrough curves of ATCC 9027 in sand columns at different rhamnolipid concentrations. The symbol 'RL' in the legend represents rhamnolipid. In all cases bacterial concentration (Co) was 5 x 10^7 cells/ml. An ionic strength control was performed to determine the effect of ionic strength alone on bacterial transport. In this case, the ionic strength was adjusted to 2 mM using NaCl to the equivalent of the ionic strength of 1,000 mg/L rhamnolipid solution.
Figure 5. Breakthrough curves of ATCC 27853 in sand columns at different rhamnolipid concentrations. The symbol 'RL' in the legend represents rhamnolipid. For all the curves, the influent bacterial concentration ($C_o$) was $5 \times 10^7$ cells/ml.
Figure 6. Breakthrough curves of ATCC 15442 in sand columns at different rhamnolipid concentrations. The symbol 'RL' in the legend represents rhamnolipid. For all the curves, the influent bacterial concentration ($C_i$) was $5 \times 10^7$ cells/ml.
TABLE 1. Effect of aqueous phase composition on cell surface hydrophobicity as measured by the BATH assay.

| Bacterial strain | % Cells Adhered to Hydrocarbon (Zhang and Miller, 1994) | % Cells Adhered to Hydrocarbon (This Study) \\
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 9027</td>
<td>27 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>ATCC 27853</td>
<td>55 ± 4</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>ATCC 15442</td>
<td>74 ± 2</td>
<td>44 ± 5</td>
</tr>
</tbody>
</table>

1 BATH assay measured on cells in a buffered salt solution.

2 BATH assay measured on cells in an artificial groundwater solution.
TABLE 2. Effect of rhamnolipid on bacterial recovery.

<table>
<thead>
<tr>
<th>Rhamnolipid Concentration mg/L</th>
<th>Recovery a %</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 9027</td>
<td>ATCC 27853</td>
<td>ATCC 15442</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>22.5</td>
<td>36.8</td>
<td>17.7</td>
</tr>
<tr>
<td>250</td>
<td>33.3</td>
<td>N/A b</td>
<td>23.1</td>
</tr>
<tr>
<td>500</td>
<td>47.1</td>
<td>46.3</td>
<td>32.0</td>
</tr>
<tr>
<td>1000</td>
<td>56.3</td>
<td>49.4</td>
<td>40.5</td>
</tr>
</tbody>
</table>

a Initial cell density for all experiments was $5 \times 10^7$ cells/ml. Recoveries were determined by integration of the breakthrough curves shown in Figures 3-5.

b Not available
TABLE 3. Optimized model parameters.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Fitted parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>ATCC 9027</td>
<td></td>
</tr>
<tr>
<td>0 mg/L RL</td>
<td>3.13</td>
</tr>
<tr>
<td>250 mg/L RL</td>
<td>4.51</td>
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<tr>
<td>500 mg/L RL</td>
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</tr>
<tr>
<td>1000 mg/L RL</td>
<td>2.16</td>
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<td>ATCC 27853</td>
<td></td>
</tr>
<tr>
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<tr>
<td>500 mg/L RL</td>
<td>1.95</td>
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<tr>
<td>1000 mg/L RL</td>
<td>1.71</td>
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<td>ATCC 15442</td>
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<tr>
<td>0 mg/L RL</td>
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<tr>
<td>500 mg/L RL</td>
<td>2.43</td>
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<tr>
<td>1000 mg/L RL</td>
<td>2.01</td>
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</table>
Table 4. Comparison of optimized model parameters and independently obtained parameters.

<table>
<thead>
<tr>
<th>Fitted parameters</th>
<th>( R )</th>
<th>( \beta )</th>
<th>( \omega )</th>
<th>( \xi )</th>
<th>( \eta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two parameter fit*</td>
<td>1.33</td>
<td>0.84</td>
<td>0.03</td>
<td>0.12</td>
<td>0</td>
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<tr>
<td>Five parameter fit</td>
<td>1.31</td>
<td>0.85</td>
<td>0.04</td>
<td>0.11</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* \( R, \xi, \eta \) were fixed as described in the Results section, \( \beta \) and \( \omega \) were optimized.
REFERENCE


Texas Research Institute, Inc. 1985. Test result of surfactant enhanced gasoline recovery in a large scale model aquifer, API publication 4390; American Petroleum Institute, Washington, D.C.


