The development of a surfactant screening protocol for use with bioremediation strategies
by Patricia Ann Buchanan

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

Abstract:
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The protocol addresses aspects of the contaminant fate, the surfactant fate, and the combinations thereof. The aspects include biodegradation, bioavailability, desorption/dissolution (mobility), and toxicity of each surfactant and soil combination. The protocol takes a soil sample to the laboratory and tests each aspect through a series of short term biodegradation, bioavailability, and mobility studies. The rates and parameters that are determined for each contaminant and surfactant concentration, contaminant biodegradation, contaminant bioavailability, contaminant mobility, surfactant biodegradability, surfactant bioavailability, and surfactant mobility are expressed as an index and placed into a table for easy comparison.

This thesis uses two site soils with diesel range organic, DRO, contamination and four surfactants to develop and demonstrate the protocol. The two site soils are Donovan Creek located outside of Missoula, MT and Lewistown located about two hours north of Billings, MT.

It was found through the use of this protocol, that for the Donovan Creek site, surfactant addition inhibited all processes that were evaluated. This induced inhibition indicated that the addition of surfactants would not be beneficial for use in a remediation strategy. However, it was found that surfactant addition at the Lewistpwn site would be beneficial for use in remediation strategies. It was found that in the Lewistown soil, surfactant addition increased most processes with the exception of bioavailability, where only ABS was found to enhance bioavailability.

THE DEVELOPMENT OF A SURFACTANT SCREENING PROTOCOL FOR USE WITH BIOREMEDIATION STRATEGIES

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APPROVAL

of a thesis submitted by

Patricia Ann Buchanan

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

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One special note: Thanks, Mom and Dad, for everything.

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NOMENCLATURE AND DEFINITIONS

Symbol	Definition	UNITS
ABS	alkylbenzene sulfonate	
B_I	contaminant bioavailability index – the biodegradation rate of the contaminant in the soil with the addition of a surfactant divided by the biodegradation rate of the contaminant in the soil without the addition of the surfactant. $B_I = \frac{(DRO_I - DRO_F(w))/(\Delta T)}{(DRO_I - DRO_F(w/o))/(\Delta T)}$	
B_{IS}	surfactant bioavailability index – biodegradation rate of the surfactant in the presence of soil divided by the biodegradation rate of the surfactant in water. $B_{IS} = (T_R) / ((S_I - S_F)/\Delta T)$	
BAC	benzalkonium chloride	
CMC	critical micelle concentration	
CMC_S	the CMC of a particular surfactant and soil combination based on sorption isotherms.	mg/L
CMC_W	the CMC of a particular surfactant in solution	mg/L
D_I	contaminant desorption/dissolution index – the leaching flux of contaminant from the soil in response to a surfactant addition divided by the leaching flux of the contaminant in the absence of a surfactant. $D_I = \frac{(DRO_I - DRO_F(w))/(\Delta T*V)}{(DRO_I - DRO_F(w/o))/(\Delta T*V)}$	

Symbol	Definition	UNITS
DC	Donovan Creek site soil	
DRO	diesel range organics	
DRO_I	initial soil concentration of DRO	μg/g
$DRO_F(w)$	final soil concentration of DRO after study with the addition of a surfactant	μg/g
$DRO_F(w/o)$	final soil concentration of DRO after study without the addition of a surfactant	μg/g
GS	Guerro-Santos media	
HOC	hydrophobic organic chemical	
L	Lewistown site soil	
Leachate	any potentially biodegradable constituents present solution after contact with contaminated soil, constituents include surfactants, HOCs, and natural organic matter	
$O_2(w)$	rate of oxygen disappearance in respirometer sample with surfactant	mg/day
$O_2(w/o)$	rate of oxygen disappearance in respirometer sample with out surfactant	mg/day
R_F	sorption factor – the CMC of a surfactant based on sorption isotherms for a particular soil divided by the CMC in solution of the same surfactant. $R_F = \frac{CMC_S}{CMC_W}$	

Symbol	Definition	UNITS
R_I	contaminant biodegradability index – the biodegradation rate of the leachate with the addition of the surfactant divided by the biodegradation rate of the leachate without the surfactant addition. $R_I = \frac{O_2(w)}{O_2(w/o)}$	
S_F	final concentration of surfactant in sample before study	mg or mL
S_I	initial concentration of surfactant in sample before study	mg or mL
T_R	surfactant biodegradability rate – biodegradation rate of the surfactant in soil $T_R = \frac{(S_I - S_F)}{\Delta T}$	mg/day
T80	polyoxyethylene (20) sorbitan monooleate	
T85	polyoxyethylene (20) sorbitan trioleate	
TWEEN80	polyoxyethylene (20) sorbitan monooleate	
TWEEN85	polyoxyethylene (20) sorbitan trioleate	
V	total volume of water through desorption columns	L
ΔT	change in time	days

ABSTRACT

This thesis addresses the selection of a surfactant for use in an enhanced bioremediation strategy. It is based on a proposal submitted to CONOCO, Inc. (Jordan, 1998). The protocol was originally intended to enhance aged hydrocarbon degradation through the in-situ addition of surfactant. The protocol has subsequently been expanded to encompass newly contaminated sites and other remediation strategies.

The protocol addresses aspects of the contaminant fate, the surfactant fate, and the combinations thereof. The aspects include biodegradation, bioavailability, desorption/dissolution (mobility), and toxicity of each surfactant and soil combination. The protocol takes a soil sample to the laboratory and tests each aspect through a series of short term biodegradation, bioavailability, and mobility studies. The rates and parameters that are determined for each contaminant and surfactant concentration, contaminant biodegradation, contaminant bioavailability, contaminant mobility, surfactant biodegradability, surfactant bioavailability, and surfactant mobility are expressed as an index and placed into a table for easy comparison.

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CHAPTER 1

INTRODUCTION

Over the years, the petroleum industry has grown dramatically. It has grown from a small business where coal was the main petroleum product to an industry that provides many different refined products, including fuels, lubricating oils, and plastics. In the early years of the petroleum industry, care was not taken to dispose of waste products properly and if spills occurred due to transportation accidents, there was often no attempt to clean it up or the cleanup efforts were poorly done. But, as the industry grew, knowledge about the potential health risks to human health and the potential risks to the environment were discovered. With this knowledge, more care has been taken to dispose of waste products properly. And, better methods of transportation have been developed.

But, despite all the care taken in disposal and transportation, there still remain the older contaminated sites and the new sites that occur through accidents. With the ability of the petroleum products to persist in the subsurface environment, due to their hydrophobicity and often low degradability, they may remain detectable in the environment for decades if left to naturally attenuate.

In recent years, it has been mandated that a responsible party be identified for sites where groundwater and soil are contaminated with hydrophobic organic compounds (HOC). This responsible party is charged with developing and controlling a remediation program on site until contamination levels in groundwater and soil are reduced to acceptable levels. The cleanup and monitoring costs can be quite high due to actual treatment and to the time that is required to clean the site. So, with cleanup efforts taking extended period of time, industry or the responsible parties have been looking to the use of surfactants.

Surfactants are primarily used in subsurface remediation as a means of accelerating desorption of HOCs from soil and subsequent advective transport in the aqueous phase. This leads to enhanced mass-transport (or mobility) of the contaminant, particularly when surfactants are applied in conjunction with pump-and-treat systems. It is also possible that a surfactant can either enhance or inhibit HOC biodegradation. Although there is abundant literature on surfactant applications, (Chapter 2), most of the studies only look at one aspect of the effects of the surfactant and they often look at only one type of surfactant. There is not currently one study that presents a method that completely determines the effects of a surfactant on the fate of the contaminant as well as the eventual fate of the surfactant itself.

Goals and Objectives

The goal for this thesis is to develop a method that determines the effects of a surfactant on the contaminant fate as well as the ultimate fate of the surfactant. The surfactant screening method developed herein, is designed to be able to test multiple surfactants in combination with field samples of HOC contaminated soil, to determine quantitatively which surfactant has the most beneficial effect.

This thesis has three objectives. Objective 1 is to develop an index-based protocol for selecting a surfactant for use at HOC contaminated sites. Objective 2 is to develop the testing methods for determining the indices that quantify the bioavailability, biodegradability, mobility, and toxicity for a specified surfactant applied to a particular petroleum contaminated soil. And, Objective 3 is to demonstrate the surfactant screening protocol using soils from two sites contaminated with petroleum hydrocarbons.

CHAPTER 2

BACKGROUND

Contaminants in the Soil Environment

The contaminants of interest in this study are hydrophobic organic compounds in general and, more specifically, petroleum hydrocarbons. In the petroleum industry, many things can cause contamination of the subsurface environment including pipeline ruptures, leaky storage tanks, and dumping to name a few. These releases can be detrimental to plants, wildlife, aquatic life, and human life. The contamination can also destroy the underlying groundwater table by leaching into the water. Since many components of petroleum are suspected to be carcinogenic (Brown et al., 1996), even small amounts of solubilized HOC in the water, parts per billion range, can render entire aquifers unfit for human consumption. In view of this threat to human health, regulations have become stricter and environmental cleanup efforts have become of interest.

However, the properties of the petroleum-based HOCs make cleanup difficult. HOCs are hydrophobic compounds often with very low solubilities, which drives them to preferentially sorb to soil or to remain as non-aqueous phase

liquid (NAPL). These properties make removing contaminants from the soil environment a difficult and long process.

Although the hydrophobicity and the low solubility of HOCs helps to contain the contamination, it promotes long term persistence problems in the environment. Over time, if left alone to naturally attenuate, the HOC will degrade and disappear. However, this process of degradation may take decades to complete. One reason for slow degradation of HOCs is that they may not be bioavailable (Mihelcic et al., 1993). It is often assumed that for a HOC to be bioavailable (i.e. readily accessible for utilization and degradation by microbes) it must be solubilized in the aqueous phase. Based on this assumption, HOC sorbed to soil, or present in the NAPL phase, are not available for degradation by microbes. Another reason for the persistence of HOCs is that some are not easily degradable. The native soil bacteria at a contaminated site may not be metabolically capable of degrading the HOC or the HOC may actually be toxic to the microbes.

The potential health risks of HOCs make it important to remove the contaminant from the environment. But, since the properties of the HOC make it difficult to do so, research and industry, over the years, have developed many technologies to assist in the removal of HOC contamination. Pump-and-treat technologies pump in large amounts of water, which is extracted down-stream and then treated for any dissolved contaminant. Fill technologies simply remove the contaminated soil and dispose of it in a landfill while the hole is filled with

clean dirt. And, there are strategies that simply render the site unfit for any use until continual monitoring indicates that the contamination level is low enough to not pose a health hazard. While all of these strategies may be acceptable, they often take many years of treatment and can cost large amounts of money. So, recently, surface-active-agents are being investigated for use with treatment technologies to maximize the efficiency of the treatment and to shorten the length of time required for cleanup.

Surfactants

Surface active agents, or surfactants, are molecules that have both a hydrophilic portion and a hydrophobic or a lipophilic portion depending on the surfactant (Anonymous. 1996; Elworthy et al., 1968). This dual moiety lowers the surface tension by reducing energy requirements to bring the surfactant monomer to the surface. The lowered surface tension allows an emulsification action between phases that are not normally interactive, such as oil and water or surfaces and liquids. Surfactants' amphipathic (Liu et al., 1992; Rosen, 1989) (i.e. having a portion attracted to and a portion not attracted to the liquid) characteristic allows the surfactant to orient itself with the surface of soil particles but does not allow it to be separated from the liquid phase. This dual moiety also orients the surfactant monomer with the hydrophobic portion to the soil and the hydrophilic portion out into the solution. Generally, the hydrophobic group is a hydrocarbon chain (Rosen, 1989; Elworthy et al., 1968). The hydrophilic group

can vary and is the means by which surfactants are classified. There are anionic, cationic, and nonionic surfactants.

Anionic Surfactants

Anionic surfactants are negatively charged and can have several different polar solubilizing hydrophilic groups. The solubilizing groups typically are carboxylate, sulfate, phosphate, or sulfonate (Anonymous. 1996; Rosen, 1989). Anionic surfactants are of the most commonly used surfactants and in general, are very water-soluble. However, anionic surfactants with longer carbon chains can be water insoluble and can be used as lubricating oils (Anonymous. 1996). Anionic surfactant's solubility is not effected by pH so they are useful in alkali situations and are aerobically degradable. However, some anionic surfactants are not degradable anaerobically and these surfactants are often skin irritants (Fink et al., 1970; Rosen, 1989; Rouse et al., 1994). Studies have shown that anionic surfactants have exhibited both enhancement and inhibitory effects on contaminant degradation (Rouse et al., 1994; Churchill et al., 1995). Studies have also been done using anionic surfactants with respect to contaminant mobility. These mobility studies have shown a variety of effects on the permeability of the soils. Depending on the surfactant and the soil type that it is combined with, anionic surfactants can either work as permeability reducers or as contaminant mobilizers (Roy et al., 1995; Renshaw et al., 1997).

Cationic Surfactants

Cationic surfactants are positively charged. The positive charge gives cationic surfactants an advantage when working with surfaces, and they strongly sorb to most surfaces, which are typically negatively charged (Rosen, 1989; Anonymous. 1996). Cationic surfactants are not as commonly used as anionic or nonionic surfactants and are typically more expensive. Cationic surfactants can be used in conjunction with nonionic surfactants, but are incompatible with anionic surfactants. Cationic surfactants are often used as biocides in many medical applications and can be used as an emulsifier, a herbicide, and as an additive in fabric softeners (Rosen, 1989). Cationic surfactants have been studied for use in the environment for creating surfactant induced sorbent zones (Brown et al., 1996) in conjunction with nonionic surfactants for mobilization.

Nonionic Surfactants

Nonionic surfactants have no apparent charge. They will orient either with the hydrophobic group or the hydrophilic group to the surface depending on the properties of the surface. Nonionic surfactants are compatible with other ionic surfactants and are soluble in water and organic solvents, including hydrocarbons (Schick, 1967). Nonionic surfactants are often characterized by their hydrophile-lipophile balance (HLB). The HLB number is a ratio of the hydrophilic moiety of the surfactant to the whole surfactant molecule (Rouse et al., 1994). The higher the HLB number the more hydrophilic the surfactant. Most

nonionic surfactants are non-toxic and are not considered irritants to the skin, eyes, or respiratory system. They are readily degradable and some are utilized as substrates or as an additional carbon source in media (Aronstein et al., 1992; Jahan et al., 1997; Putcha et al., 1993). Nonionic surfactants have also been investigated for use in mobilizing contaminants in the soil environment (Yeom et al., 1995; Aronstein et al., 1991).

Previous Work

Biodegradation

Biodegradation of the contaminant and surfactant are critical aspects of any remediation or bioremediation effort. Biodegradation of contaminants in the environment will occur over time, but the process can proceed very slowly. The reason for this is that contaminants, particularly HOCs, do not serve readily as carbon sources for microbes. This is attributed to several possible reasons. One of the possible reasons is the microbes have difficulty taking up the HOC. Another reason is that the HOC at higher concentrations can be toxic to the microbe. Addressing the first of these two possible reasons for poor degradation rates, studies have been done where surfactants were added to see if the lowered surface tension between the microbe and the HOC would facilitate a higher degradation rate. These studies have shown that by lowering the surface tension between the microbe and the HOC, degradation can be enhanced (Abd-

Allah et al., 1998; Jahan et al., 1997). However, not all of the studies show a positive result; some of these studies show evidence that the addition of a surfactant can inhibit degradation. These studies also indicate that the concentration at which the surfactant is added can be a critical factor to the degradation process. One study shows that the surfactant must be present at least at its CMC to have a significant enhancing effect (Jahan et al., 1997). Still, while one surfactant may enhance the degradation at a higher concentration, others may actually be inhibitory at higher concentrations where they become toxic to the degrading microbes. Several studies have been done and show evidence to support the potential toxicity issue with the degrading microbes (Rouse et al., 1994; Abd-Allah et al., 1998; Fu et al., 1995). These studies show that the toxicity of the surfactant, particularly in combination with the potential toxicity of the contaminant is a determining factor for the efficiency of a surfactant to promote degradation.

Since surfactants are often used to increase the biodegradability of contaminants in the soil environment, it is important to consider whether or not the addition of the surfactant will be another source of contamination. So, the degradability of the surfactant itself is important. Some surfactants are readily degradable at certain concentrations, as previously mentioned. Some are also degradable in the presence of HOCs. Studies to determine the degradability of the surfactant have been done both with and without the presence of HOCs (Madsen et al., 1996). Most nonionic surfactants are easily degradable, likewise

some anionics, but few cationic surfactants are easily degradable and are often toxic to microbes, thus their frequent use as anti-microbial agents (Anonymous. 1996; Rosen, 1989). There are several studies that support the possibility of both the surfactant-enhanced biodegradation of the contaminant and the degradation of the surfactant itself in the presence of the contaminant (Aronstein et al., 1991; Abd-Allah et al., 1998).

Bioavailability

Degradation processes for HOCs are slow, but can be made even slower in soil environments where the HOC can sorb to soil particles. In the soil environment, HOCs are likely to associate themselves with surfaces due to their hydrophobic nature. This surface association is often one of the reasons that contribute to the slow degradation of the contaminant and its low bioavailability. When a HOC is associated with a soil surface, not solublized, microbes are unable to access the contaminant, either because they can not remove the contaminant from the soil surface or the they can not use the contaminant in an unsolubilized form (Jordan et al., 1999). Surfactant addition can possibly improve HOC bioavailability (Jordan et al., 1999; Fu et al., 1995; Tiehm et al., 1997). This increase in bioavailability of HOCs, either from the soil phase or the NAPL phase, has been seen in studies (Aronstein et al., 1993; Aronstein et al., 1991; Fu et al., 1995). However, concentration of the surfactant can be a critical factor and some

studies have shown that surfactants added above the CMC actually sequester HOC from microbial degradation (Putcha et al., 1993; Guhu et al., 1996).

Mobility

In the subsurface environment, the processes that govern HOC mobility are 1) sorption/desorption to and from the soil phase, 2) solubilization/dissolution to and from the NAPL phase, and 3) advective transport in the dissolved phase. These processes along with the properties of the contaminant dictate the movement of a contaminant in the soil environment. For example an HOC can sorb to soil particles, dissolve into solution, and can be mobile in the soil environment either in a solubilized phase or in the NAPL phase. The hydrophobic nature of the HOC dictates that it preferentially sorb to the soil. The low solubility of the HOC encourages the HOC to remain in either the sorbed or NAPL phase.

These physical-chemical properties have important implications to the remediation of HOC contaminated groundwater sites. The tendency of the HOC to sorb to soil promotes decreased mobility, and the lower the mobility, the smaller the contaminant plume. However, low mobility also makes it difficult to remove the HOC from the soil environment. The HOC can also remain in the NAPL phase, which may or may not be mobile. The addition of the surfactant to the soil environment can increase desorption and thus increase HOC mobility.

Different surfactant types may either increase or decrease mobility. Nonionic surfactants have been used successfully to enhance mobilization of

HOC in soil washing techniques and pump-and-treat technologies (Yeom et al., 1995; Fountain et al., 1996; Fu et al., 1995). Some anionic surfactants react in the soil environment decreasing pore sizes by high sorption abilities or precipitative reactions with the soil minerals (Tumeo, 1997). However, some anionic surfactants have been used successfully in soil washing techniques (Roy et al., 1992). In cases where it is desired to increase sorption to the soil, cationic surfactants are being studied for use in creating enhanced sorbent zones used in conjunction with funnel and gate technologies (Hayworth et al., 1997; Brown et al., 1996). The presence of the sorbed cationic surfactants increases the organic carbon of the soil making the zone a more favorable sorption site for HOCs.

Toxicity

In recent years, it has been shown that HOCs are not only toxic to the environment, but that they are also potential carcinogens to humans. With this potential health risk, acceptable regulatory limits in the environment are often in parts per billion (Brown et al., 1996). There are three pathways of reception by humans; inhalation, adsorption, and ingestion. Inhalation of HOCs is the least threatening, except to those immediately on-site, since most volatile constituents are the first to degrade and the easiest to remediate. Adsorption is less of a threat since many releases are underground and care can be taken to limit accessibility to contaminated areas. The main danger of adsorption through the skin would be due to swimming in contaminated waters downstream.

Contaminated downstream waters are the main source of ingestion of the contaminants. The threat of human toxicity is not only a consideration with the contaminant, but also with surfactants. Many surfactants are skin, eye and respiratory irritants.

In remediation, it is also important to consider what will be toxic to the microbial flora of a contaminated site. Some surfactants are used in pharmaceutical applications where they are enlisted as biocides. Some surfactants at low, sub-CMC concentrations, are not toxic, while at higher concentrations, they act as microbial growth inhibitors (Hayworth et al., 1997; Rouse et al., 1994). This is especially important to determine if the intent of the addition of the surfactant is to enhance biodegradation. It is also important to consider when applying other technologies. Any remaining surfactant, after use in conjunction with a pump-and-treat strategy, for example, should be evaluated for potential toxicity to the local ecology.

CHAPTER 3

SURFACTANT SCREENING PROTOCOL

The Need for a Screening Protocol

The foregoing literature review defines the need for a surfactant screening protocol. Surfactants have multiple uses in subsurface environmental restoration. Surfactants can enhance the removal efficiency of soil washing. They can enhance biodegradation of HOCs. They can help close a contaminated site by decreasing the amount of time required for cleanup procedures. However, not all surfactant effects are beneficial. Surfactants can sequester HOC and slow the degradation process. They can kill the microbes that are degrading the HOC. Or, they can mobilize the contaminant and increase the size of the plume. So, if the application of a surfactant is being considered, there are many things that should be evaluated before application.

There is a need for a comprehensive method for selecting a surfactant. The protocol should allow for pre-screening to evaluate the contaminant fate and the surfactant fate. It should also answer questions for the contaminant fate such as, will the contaminant be mobilized and will it increase contaminant

degradation. The protocol should answer questions for the surfactant fate such as, will the surfactant degrade, will the surfactant have an inhibitory effect on contaminant degradation or mobilization, will there be an increased toxicity issue at this site, and is the site better off being left alone to naturally attenuate. Through the use of this protocol, whether or not a surfactant should be used, which surfactant to use, and how much surfactant to be used, can be determined.

The Surfactant Screening Protocol

The protocol is an index-based protocol determined through batch and flowing column reactor tests. The tests evaluate the contaminant fate and the surfactant fate in the soil environment. It is a protocol that can be easily performed and gives a clear indication as to whether or not a surfactant, if used, would be beneficial when applied to a specific contaminated soil sample. The protocol indicates the potential of each surfactant, tested at various concentrations, for beneficial effects on a particular site soil. It is a protocol that addresses the biodegradability, bioavailability, mobility, and toxicity of the surfactant, the contaminant, and the different combinations thereof.

The biodegradability index for the contaminant is used to determine if the contaminant in the solubilized form is more biodegradable with the addition of a surfactant or without the addition of a surfactant. The biodegradation index is

used to determine if the surfactant in the presence of a specific site soil is biodegradable by the microbes present at that site.

The bioavailability index for the contaminant is used to determine if the soil-sorbed contaminant is more degradable with the addition of the surfactant than without the addition of the surfactant. The bioavailability index for the surfactant is used to determine if the surfactant is degradable in the presence of soil.

The mobility index for the contaminant is used to determine whether or not the addition of a surfactant will promote contaminant removal from the soil phase or inhibit the removal. The mobility index for the surfactant evaluates the potential for the surfactant to sorb to the soil.

The toxicity index for the contaminant evaluates the risk of increased toxicity with the addition of a surfactant. The toxicity for the surfactant is used to determine the toxicity of the surfactant to the indigenous site microbes and potential receptors.

Approach

Biodegradability (Batch)

If a surfactant is added to HOC contamination in the subsurface, it is important to determine whether the surfactant will enhance or retard the process of biodegradation. In addition, it is important to determine the biodegradation

potential for the surfactant itself. Accordingly, the indices for the dissolved phase HOC biodegradation (R_I) and surfactant biodegradation (T_R) are:

$$R_I = \frac{O_2(w)}{O_2(w/o)} \quad (1)$$

$$T_R = \frac{(S_I - S_F)}{\Delta T} \quad (2)$$

Where $O_2(w)$ and $O_2(w/o)$ are oxygen depletion levels measured during a batch respirometer experiment. The (w) and (w/o) designate the presence of surfactant. And, where S_I and S_F are the initial and final concentrations of surfactant, determined by surface tension measurements, in batch respirometer experiments and ΔT is the duration of the experiment in days. The respirometer in theses experiments in an instrument that creates a closed batch system and measures production and disappearance of gases in the batch sample. The respirometer is capable of measuring O_2, CO_2, and H_2S gases in the samples.

Bioavailability (Batch)

Removal of the HOC from the soil particle or the NAPL phase by surfactant addition may or may not make the HOC more bioavailable. If the HOC is trapped in a micelle, it may not be available for microbial degradation even though it is in solution. Also, some surfactants are biocides and can kill the degrading microbes, so even if the surfactant were to make the HOC bioavailable, it would still not degrade. The index proposed here to measure the effect of the surfactant addition on sorbed HOC bioavailability is:

$$B_I = \frac{(DRO_I - DRO_F(w))/(\Delta T)}{(DRO_I - DRO_F(w/o))/(\Delta T)} \quad (3)$$

Where DRO_I is the initial, solvent extracted, diesel range organic concentration (DRO) and DRO_F is the final DRO concentration in the soil after batch respirometry studies.

Also important is the bioavailability of the surfactant in the subsurface environment. The bioavailability of the surfactant is measured by comparing the biodegradation rates of the surfactant in the soil environment to the biodegradation rate of the surfactant in the aqueous environment. The surfactant bioavailability index is defined as:

$$B_{IS} = (T_R) / ((S_{I(water)} - S_{F(water)})/\Delta T) \quad (4)$$

Where $S_{I(water)}$ and $S_{F(water)}$ are the initial and final concentrations of the surfactant after aqueous batch respirometer studies.

Mobility

The potential for desorption and/or dissolution of initially sorbed HOC contaminant may be quantified in a similar way. The index here is defined as:

$$D_I = \frac{(DRO_I - DRO_F(w))/(\Delta T*V)}{(DRO_I - DRO_F(w/o))/(\Delta T*V)} \quad (5)$$

Where DRO_I and DRO_F are the initial and final concentrations of DRO in the soil after flowing column studies. V is the total volume of liquid through the column.

The mobility index of the surfactant is defined as:

$$R_F = \frac{CMC_S}{CMC_W} \quad (6)$$

Where CMC_S is the critical micelle concentration (CMC) determined from sorption isotherms and CMC_W is the CMC of the surfactant in water.

Toxicity (Omitted in the scope of this thesis)

The potential toxicity of contaminants in the environment is the first and foremost reason for a site to ever become of concern. The toxicity of a contaminant can be of issue to the site ecology, downstream ecology and potential receptors, and to human health. Toxicity at a site should be evaluated in the Surfactant Screening Protocol. Depending on the type and the level of contamination, site soils and aquifers can be either effectively unchanged or they may be come considered unfit for any use. With the addition of a surfactant, it is important to determine if this additional chemical will increase, decrease, or have no effect on the toxicity. However, due to time and equipment constraints, toxicity was omitted in the scope of this thesis. As it was proposed at the onset of this research, toxicity can be evaluated by standardized tests, for example, MicroTox by Azure Environmental.

Expediency

Many laboratory tests are tedious, require constant maintenance, and are either expensive or long-term. With the development of this protocol, expediency and easy of execution were of consideration. Tests were to be designed such that they were relatively easy to perform, relatively inexpensive, and required

little maintenance during run time. As was possible without losing quality of results, materials, instrumentation, and methods were chosen for their availability, malleability, and low cost. For example, equipment such as the desorption columns were constructed from common laboratory supplies and designed to be assembled by hand without use of excess tools or equipment. The duration of each experiment was also limited. The duration was limited to five days to focus on the early-stage enhancement of remediation and to allow for a protocol that would provide a 'fast' answer to the question of surfactant use.

Flexibility of the Protocol

Although initially focused on in-situ biodegradation, the protocol allows enough flexibility that it may be modified to focus on a different remediation process. From choosing what concentrations of surfactants to use, to which tests are performed and the duration of each experiment, the protocol allows tailoring. For example, the protocol may be modified to target only desorption/sorption processes of the contaminant and surfactant combination. This can be done by performing the sorption isotherm and the desorption studies. The amount of surfactant that will sorb to a particular amount of soil can be determined through the isotherm studies, which would indicate the amount of surfactant that may be added before any downstream problems occur. And, by performing the desorption studies, the amount of contaminant that would be mobilized by a certain concentration of surfactant can be determined.

How the Protocol can be Used

The protocol was designed to identify the surfactant that would most benefit the natural, in-situ bio-attenuation process. By performing the full battery of tests, a surfactant that will enhance the natural biodegradation process without posing a threat to the environment itself, without creating a downstream mobilization threat, and while eventually degrading itself, can be chosen. However, by performing the tests in the protocol, other characteristics of the surfactant effects that may be beneficial for use with other remediation strategies can be identified. For example, pump-and-treat strategies depend on solubilization of contaminants into water that is pumped into one well and extracted through another well downstream. The extracted water is then treated and usually pumped back into the upstream well. This strategy, however effective over long periods of time, can be enhanced by the use of a surfactant. The surfactant added to a pump-and-treat system should be highly efficient at the solubilization of the contaminant. The surfactant should also have a low tendency to sorb to the soil surface to decrease surfactant loss during treatment. A surfactant with these properties would aid in mobilizing the contaminant into the water that is being pumped through the soil without much loss of surfactant due to surface sorption. Although money is spent on the surfactant, its cost should be less than the overall cost of what the treatment cost would have been without the

surfactant addition. With the use of a surfactant, treatment time can be reduced three or more times. This is only one way that the protocol may be tailored for use. Another example would be to identify an effective treatment strategy before one is already in use. The protocol, when completed, can help to identify what treatments may be most effective at a site. For instance, if none of the surfactants tested significantly increase or hinder the degradation process but one of the surfactants facilitates mobility, then perhaps a no treatment action or a soil washing strategy may be most appropriate. The protocol may also be modified to evaluate longer-term effects by extending the length of the tests.

CHAPTER 4

MATERIALS AND METHODS

Materials

Soils

Soils from two petroleum hydrocarbon contaminated sites (Donovan Creek and Lewistown) were chosen for testing the surfactant screening protocol. Access to both sites was given by CONOCO's Remediation Technologies unit, Houston, TX (CONOCO et al., 1998).

The Donovan Creek site is a 25-year-old diesel contaminated site. It has a sandy-loam soil and is located 25 miles east of Missoula, Montana. The Donovan Creek contamination is the result of a ruptured pipeline. The Lewistown site is a 60-year-old refinery site. It has various refinery waste products and some refined materials including diesel. It is located approximately 140 miles north of Billings in Lewistown, Montana.

Surfactants

A cationic, an anionic, and two nonionic surfactants were chosen for testing. Benzalkonium chloride (BAC) was chosen as the cationic surfactant and dodecylbenzene sulfonic acid sodium salt (ABS) was chosen as the anionic surfactant. Both were chosen for their accessibility, because both were already being used in the lab by other experimenters. The two nonionic surfactants, polyoxyethylene (20) sorbitan monooleate (TWEEN80) and polyoxyethylene (20) sorbitan trioleate (TWEEN85) were selected for their common usage in industry and their degradability (Rouse et al., 1994). TWEEN80 and TWEEN85 have HLB numbers of 14 and 10 respectively (Rouse et al., 1994). Thus, TWEEN85 is more hydrophobic than TWEEN80. The TWEENs were donated by ICI Surfactants (ICI et al., 1998). Each of these surfactants was chosen so that the ability of the surfactant screening protocol could be tested on a wide range of surfactants and soil combinations.

Experimental Methods

Soil Preparation

Site soils were sampled using decontaminated metal shovels and clean five-gallon plastic buckets, which were sealed with airtight lids. Soil was stored in a walk-in cooler.

The soil was then air-dried. Soil clumps were crumbled into smaller pieces if needed, spread out approximately one-inch thick on aluminum foil, and allowed to air dry for 24 hours under a fume hood prior to use in the experiments. The dry soil was then sieved through a 2-mm sieve (Tabak et al., 1999; Tabak, 1997). Dry-sieved soil was then placed in a walk-in cooler in a plastic airtight container. Soil type was determined based on field reports (MAXIM, 1997). The field capacity water content was approximated, based on a soil type graph (Wraith et al., 1999), for use in determining the target experimental water content.

Pore volume was determined using the dry sieved soil, a beaker and enough water to saturate the volume of soil without standing water on top of the soil sample. The wet sample was weighed, the amount of water calculated, and the pore water volume was determined.

Approximate dry bulk density for the dry soil was also determined (Equation 7).

ρ_b = (weight of soil (g)) / (volume of soil (mL)) (7)

A graduated cylinder was filled to an even volume measure and then weighed. The procedure was repeated three times and an average was taken.

Surfactant CMC Determinations

Surfactant critical micellar concentrations (CMC) were determined by batch studies. 20mL of a surfactant-GS medium (Guerra-Santos medium (Guerra-Santos et al., 1984), Appendix C) stock solution was measured for

surface tension, using a tensiometer, and then serially diluted until the surface tension value indicated that the surfactant concentration was effectively zero. The surface tension values vs. concentration were plotted on a semi-log graph and the CMC was determined. The point at which maximum slope change occurred, the intersection of the two linear sections of the plot, is defined as the CMC (Elworthy et al., 1968) (Figure 1). This was done for each soil and surfactant combination.

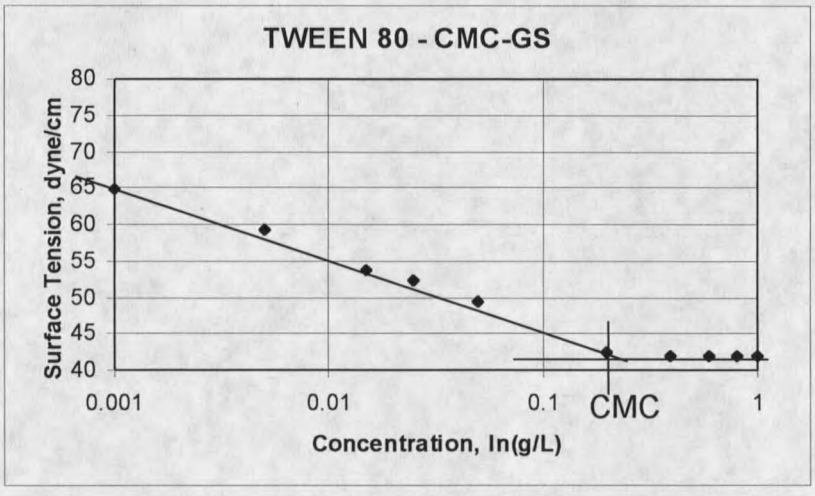

Figure 1 – Example figure of the determination of the CMC for a particular surfactant and media.

Sorption Isotherms

Sorption isotherms were performed in 250mL polypropylene bottles with10g of dry-sieved soil and 50mL of GS media. Increasing concentrations (concentrations varied depending on the surfactant) of surfactant were added to the mixture and the bottles were placed on a shaker table for 24 hours to allow

for equilibrium (Rouse et al., 1996). Equilibrium was assumed after 24 hours, for the determination of the sorption isotherms. The bottles were then centrifuged for 15 minutes at 8000rpm. Supernatant was decanted and filtered to remove particulate matter that may interfere with surface tension measurements. Surface tension measurements were then taken to determine the amount of the surfactant remaining in solution. Isotherms were complete when the amount of surfactant sorption to the soil stabilized. This was done for each soil and surfactant combination. The data were plotted log-log, Surfactant Added vs. Concentration Sorbed to Soil (Figure 2). The plot indicated the final sorption amount when the curve became most nearly linear with a slope of 0.

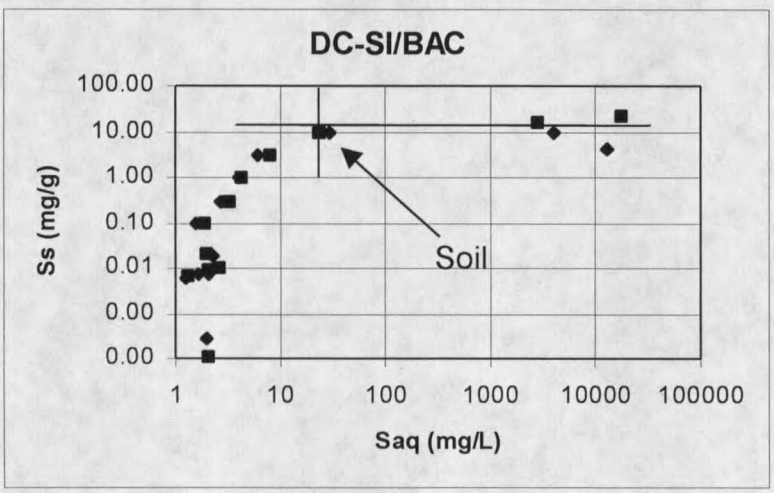

Figure 2 – Example figure of the determination of the Soil-CMC for a particular surfactant and soil combination. This isotherm (SI) was for the Donovan Creek soil (DC) in combination with the surfactant BAC. The figure is plotted aqueous phase surfactant concentration (Saq) vs. sorbed phase surfactant concentration (Ss).

Three surfactant concentrations were chosen for use in the studies based on the sorption isotherms for each surfactant and soil combination. The concentrations were 0.5xCMC, 1xCMC, and 10xCMC respectively based on the unique properties of solubilized surfactant. At 0.5xCMC, surfactant monomers are present on the soil surface and in solution. At 1xCMC, the soil surface sorption capacity is reached; there is a surfactant mono-layer on the surface. At 10xCMC, there are micelles present in solution.

Biodegradation Studies - Respirometry

A respirometer, Micro Oxymax (software version V6.03) by Columbus Instruments, was used for all measurements in both surfactant and contaminant biodegradation studies. Instrument gas calibration was performed prior to each experiment and sample headspace was refreshed every two measurements during the experiment. A condenser was used for samples containing soil. For the surfactant biodegradation studies, the condenser was not used. Prior to each condenser use, the condenser was disinfected by dripping approximately 5mL of ethanol into each sample port. All biodegradation studies had a five-day duration.

Contaminant Biodegradation Studies

Samples were prepared in 100mL media bottles with 20g of dry soil with enough GS media to bring the sample to 70% field capacity water content and with the appropriate amount of surfactant. One biodegradation experiment

consisted of 10 samples with duplicate samples at 0x, 0.5x, 1x, and 10x CMC surfactant concentration, (determined from the sorption isotherms) and one positive control, having 1g/L yeast extract, and one negative control, having sterile media with no soil. The experiment ran five days at room temperature. The samples were then stored in a freezer until extraction. The samples were extracted for DRO in preparation for GC analysis using the EPA draft DRO method (Anonymous. 1993).

Contaminant Desorption Studies

The set-up (Figure 3) consisted of 250mL polypropylene bottles, as siphoned tanks with PVC pipe columns. The bottles contained the surfactant media solution and were set approximately ten inches above the top of the columns. The columns consisted of a 7" piece of ½" ID PVC pipe with #1 single-hole stoppers, 400x400 stainless steel mesh of 0.001 wire, 5g-0.15mm glass beads, and 10g- 1 and 0.3mm mixed glass beads (Figure 4). The steel screen-mesh was cut to ¾" squares and then pushed into the column such that the corners of the screen were held by the stopper. Stoppers were firmly seated into the PVC pipe. The column was then filled, first with the small glass beads and then with the larger glass beads to fashion the filter. The soil sample was then placed on top of the filter and the column was closed with another stopper at the top of the column. Again, the stopper was firmly seated. Care was taken to not disturb the column as this prevented clogging the filter. Disturbing the filter

grading mixed the bead sizes and made the effective pore sizes in the filter easily clogged by fine soil particles in the soil sample. Small (3" in length) pieces of glass tubing were placed in the top and bottom stoppers to serve as connections between tubing and columns. A siphon was then connected between the influent bottles and the top of the column. Tubing was also run from the bottom of the column to a waste container.

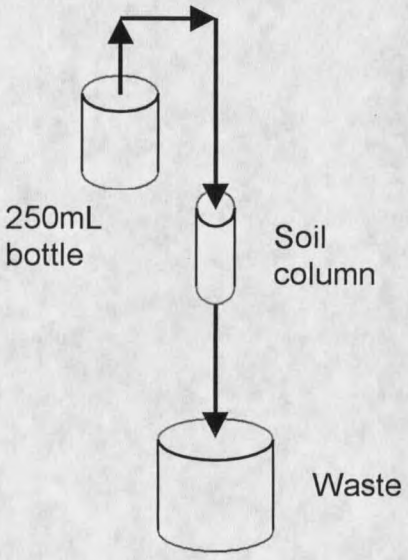

Figure 3 – Desorption Column flow assembly

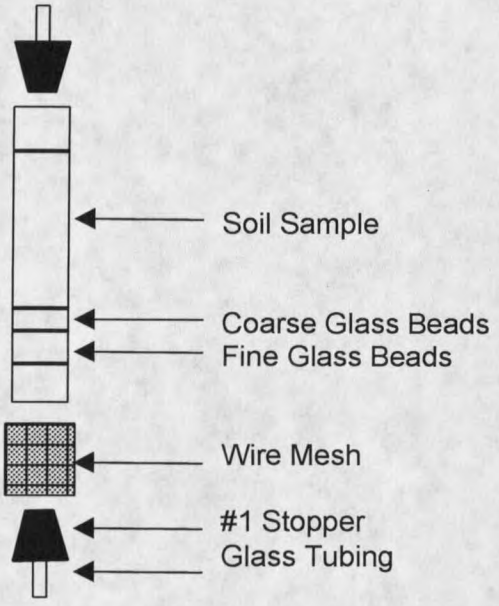

Figure 4 – Column assembly

Desorption studies were conducted over a five-day period where 200mL/day of surfactant solution was siphoned through each column. Each column received a total of 1L of surfactant solution. Each run consisted of three duplicated 20g samples (total 12 samples per run, 6 samples per soil, plus initial concentration samples) per each soil-surfactant combination. Bottles were filled each morning and siphons restarted until the end of the experiment. Siphoning took varying amounts of time, ranging from less than one hour in sandier soils (Donovan Creek) with low concentrations of surfactant to approximately 20 hours in a more clayey soil (Lewistown soil) with high surfactant concentrations. Tubing was removed and the intact columns were stored upright and refrigerated until extraction. The columns were destructively analyzed at the end of the five-day

period. The samples were stored and extracted using the same procedure as the biodegradation studies, using the EPA Draft DRO soil extraction method for determining the remaining amount of DRO in the soil.

Contaminant (Leachate) Biodegradation Studies

Samples were prepared by placing 20g of soil and 70mL of GS media with surfactant at specified concentration into a 100mL-glass beaker. The beaker was then covered loosely with foil and allowed to sit for five days at room temperature. After the initial five-day period, 50mL of the supernatant was decanted into media bottles and placed on the respirometer. The soil sample was extracted to determine the remaining amounts of contaminant (DRO) in the soil and the amount of DRO in the supernatant. After the five-day respirometer run, the supernatant was decanted and filtered to remove particulate matter. Surface tensions measurements were taken on the filtrate to determine the remaining amount of surfactant in the solution. This procedure was used to determine the ability of components leached into solution to be degraded.

Surfactant Biodegradation Studies

Surfactant biodegradation studies were performed using the respirometer with surfactant media samples and soil inocula. Samples were prepared in 100mL media bottles. 50mL of media with surfactant at the desired concentration was placed in the bottle with a 1g-soil inoculum. Bottles were then placed on the

respirometer for five days. At the end of the five days, samples were decanted and filtered. Surface tension measurements were taken and used to determine the amount of surfactant degraded.

Analytical Methods

Soil Extraction

The soil extraction procedure used in these experiments was modified from the Draft Method for Determination of Diesel Range Organics, Revision 4(Anonymous. 1993) (Appendix A).

The soil sample was prepared by first decanting any standing liquid, water or media in the sample and discarded. Only the desorption study samples required decanting. An equal weight of sodium sulfate (Na_2SO_4) to that of the dry soil sample was then added. The mixture was well mixed and then allowed to sit with intermediate stirring until the texture became dry and grainy. Approximately 90mL of methylene chloride (MeCl) was added to the dry mixture and sonicated (Tekmar Sonic Disrupter: horn sonicator, ModelTM50) for three-three minute intervals with one-minute rests between intervals. The MeCl was then decanted into a 500mL evaporative flask and concentrator tube (the flask and tube were connected by springs) through a funnel that contains a glass wool plug and approximately $^2/_3$-funnel volume of Na_2SO_4. The funnel and contents were rinsed with approximately 20mL MeCl prior to the first sample filtration into the flask. The sonication procedure was repeated twice more. After the third sonication and

decanting, the funnel and contents were rinsed with approximately 10mL MeCl. A three-ball Snyder column was then placed on top of the flask-tube apparatus and lowered into a 95degree Celsius water bath. The apparatus was situated such that the bottom of the flask was bathed in steam. The MeCl solution was concentrated until the volume appeared to be between 5 and 10mL. The apparatus was removed from the bath and allowed to cool. 1.5mL of the concentrate was pipetted, using 2mL graduated-disposable glass pipettes, into auto-sample vials and stored in a freezer until GC/MS analysis. All glass was rinsed with MeCl between samples.

GC Analysis

GC analysis was used to determine the amount of DRO in the soil phase. GC analysis reported abundances that were compared to a standard curve of abundances vs. concentration of DRO.

The GC column that was used was not the column recommended by the DRO Extraction Method, but was an alternative column with the equivalent properties for analyzing DRO, (Neuman, 1998). The column used was the 25Mx0.25mm Quadrex 007 5% methyl phenyl 0.5µm-film column by Altech catalogue # 007-5-25-0.5F. The head pressure for the column was at 105kPa with split injection. The column temperature started at 40° Celsius for five minutes and then ramped to 225° Celsius at 8 degree intervals every five minutes for a total run time of 33 minutes.

Using a glass 2mL syringe with stainless steel needle, samples were drawn from the original sample vials and then filtered through a 0.45μm nylon syringe filter into a clean auto-sample vial and capped. A glass GC syringe was then used to inject the GC sample. No more than 2.0μL of a sample per injection was used. The resulting abundance values are then compared to a standard curve, range 0μg-5000μg, (Figure 5) to determine the DRO concentration in the concentrate and back-calculated to determine the actual sample DRO concentration. This was done for each soil and surfactant combination. No internal standards were used.

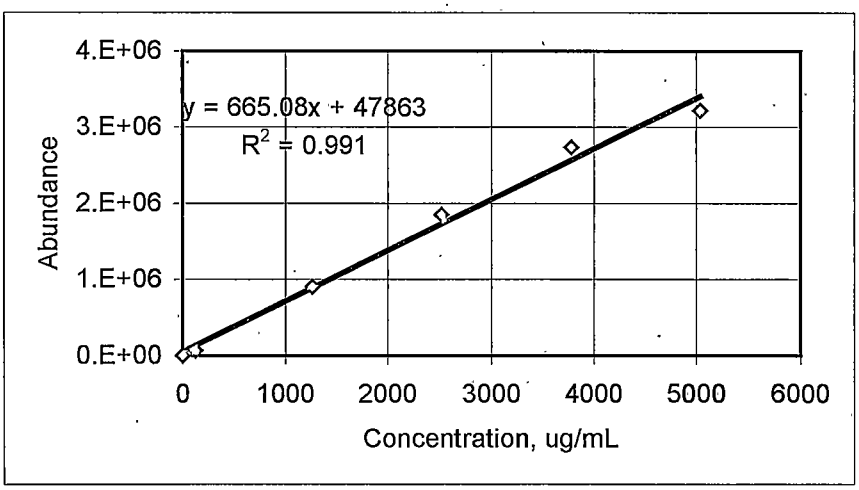

Figure 5 – Diesel Standard Curve

CHAPTER 5

RESULTS

Contaminant Indices and Parameters

Biodegradability (Batch)

The biodegradability index (R_I) for the contaminant was defined as the biodegradation rate of leachate with the addition of a surfactant divided by the biodegradation rate of the leachate without the addition of a surfactant (Equation 2).

$$R_I = \frac{O_2(w)}{O_2(w/o)} \qquad (2)$$

Leachate is defined as all biodegradable constituents solubilized in the solution, including surfactant, DRO, and other soil organics. An index value of one indicated there was no difference in the degradation rate with the addition of the surfactant. An index value of greater than one indicated that the biodegradation rate of the leachate was enhanced with the presence of the surfactant. And, conversely, if the index value was less than one, it indicated that the presence of a surfactant had inhibitory effect on the biodegradation rate of the leachate.

The biodegradability index for the contaminant was evaluated by performing batch leachate biodegradation experiments. The oxygen levels measured by the respirometer (Table 1) were used to determine the biodegradation rates (Table 2). This rate was then based on oxygen disappearance indicating that biodegradation processes occurred in the samples. This rate also assumed that the oxygen consumption was directly proportional to contaminant biodegradation. The leachate biodegradation rates were calculated as follows:

$O_2(w)$ = (O_2 consumed) / (volume of leachate * duration)
= (4.8mg O_2) / (50mL Leachate*5 days) = 0.019mg/mL-days

Similar calculations were used to determine the remaining leachate biodegradation rates (Appendix D).

Table 1 – Measured oxygen disappearance levels and other biodegradability data for Lewistown and TWEEN85

Sample	Cumualtive O2, mg	Volume of Leachate, mL	Duration, days
0x	7.1	50	5
0x	8.3	50	5
0.5x	4.8	50	5
0.5x	5.2	50	5
1x	5.1	50	5
1x	6.0	50	5
10x	6.6	50	5
10x	7.9	50	5

Table 2 – Biodegradability Indices for the Lewistown soil and TWEEN85 surfactant combination.

Lewistown and TWEEN 85				
Surfactant Concentration	Pre-degradation Leachate Concentration of DRO, ug/mL	Final Leachate Concentration of Surfactant, mL/L	Leachate Biodegradation Rate, mg/mL/day	Biodegradibility Index
0x	96	0.0	0.028	**Average**
0x	186	0.0	0.033	**0.031**
0.5x	84	0.0	0.019	0.6
0.5x	166	0.0	0.021	0.7
1x	96	0.1	0.020	0.7
1x	116	0.2	0.024	0.8
10x	164	13.3	0.026	0.9
10x	138	20.7	0.032	1.0

The oxygen depletion levels that resulted indicated low biodegradation rates in each of the samples. The surfactant addition in each of these samples had an inhibitory effect on the biodegradation in the leachate, with the exception of the 10xCMC concentrations, where the samples had only a slightly lower or equivalent biodegradation rate than the 0xCMC samples. These results indicate that biodegradation was possible in the leachate, but was inhibited by the presence of surfactants. Only the Lewistown and TWEEN85 combination was tested, so no other bioavailability indices were determined.

Bioavailability (Batch)

The bioavailability index (B_I) for the contaminant is defined as the rate of disappearance of DRO with the addition of a surfactant divided by the rate of disappearance of DRO without surfactant addition (Equation 3).

$$B_I = \frac{(DRO_I - DRO_F(w))/(\Delta T)}{(DRO_I - DRO_F(w/o))/(\Delta T)} \quad (3)$$

An index value of one indicated that there is no difference in DRO disappearance with the addition of a surfactant. An index value of greater than one indicated that the addition of the surfactant had an enhancing effect on the DRO disappearance rate. Conversely, an index value of less than one indicated that the surfactant addition inhibits the DRO disappearance rate in the soil.

The DRO disappearance rates that were used to determine this index were calculated as follows:

Actual Abundance = Reported Abundance (μL^{-1}) / Injection Volume (μL)
=(213978)/(1.97) = 109732

Where, Reported Abundance is the abundance reported by GC analysis and Actual Abundance is the abundance per injection volume of sample.

Concentrate DRO Concentration = (Actual Abundance-47863)/665.08
=(109732-47863)/665.08 = 93μg/mL

Where, Concentrate DRO Concentration is the concentration of the extracted sample volume.

$DRO_F(w/o)$ = (Concentrate DRO*Concentrate Volume (mL))/(Soil Weight (g))
=(93*6.6)/(20) = 31μg/g

$(DRO_I - DRO_F(w/o))/\Delta T$ = (59-31)/(5) = 6μg/g-day

All other soil concentrations (Table 3) and disappearance rates were calculated in a similar fashion (Appendix E). The indices were then calculated using Equation 3.

Table 3 – Final soil DRO concentrations after five-day biodegradation studies (μg/g)

	Lewistown				Donovan Creek			
	ABS	BAC	T80	T85	ABS	BAC	T80	T85
0.5x	1564	1592	1567	1582	10	11	24	0
	1649	1553	1345	1510	19	18	17	11
1x	1592	1446	1373	1471	16	18	35	28
	--	1619	1644	1435	14	13	26	12
10x	846	1476	1688	1551	22	19	25	14
	514	1648	1647	1526	--	31	12	-2
0x	1504	1411	1228	1469	-12	23	20	13
	1624	1532	1259	1540	31	21	24	8

The bioavailability indices were completed for all the surfactant and soil combinations. The contaminant biodegradation study method, a batch study, was used to determine this index. 0xCMC surfactant samples were performed with each respirometer run (Table 3) and were used to calculate the average rates of disappearance without surfactant (Table 4).

Table 4 – Bioavailability Indices: Averages are of rates (μg/g/day) of DRO disappearance from two duplicate samples.

Donovan Creek Disapperance Rate w/o Surfactant = 8.5 ug/g/day								
	T85		T80		ABS		BAC	
	Average	Index	Average	Index	Average	Index	Average	Index
10x	11	1.24	9	1.09	7	0.82	8	0.93
1x	8	0.90	7	0.80	9	1.00	10	1.15
0.5x	11	1.25	9	1.03	9	1.06	10	1.17
Lewistown Disappearance Rate w/o Surfactant = 86.6 ug/g/day								
	T85		T80		ABS		BAC	
	Average	Index	Average	Index	Average	Index	Average	Index
10x	55	0.64	29	0.34	227	2.62	50	0.58
1x	72	0.83	61	0.70	44	0.51	56	0.65
0.5x	53	0.62	72	0.83	41	0.48	48	0.56

For the Donovan Creek soil samples (Table 4) there were no significant differences in the disappearance rates of the contaminant with or without the addition of the surfactant. Donovan Creek Bioavailability Indices ranged between 1.25 for 0.5xCMC of TWEEN85 and 0.82 for 10xCMC for ABS. These values suggest that there may be an enhanced bioavailability effect, however none of the disappearance rates are statistically different from the control as determined by Dunnett's multiple comparison test (Appendix J).

For the Lewistown soil samples (Table 4) surfactant addition appeared to have had an inhibitory effect on most samples. However, there was one case, the 10xCMC ABS samples, that the surfactant addition had an enhancing effect with a 2.6 fold increased disappearance rate over that of media alone with an average disappearance rate of 227 μg/g/day.

Desorption/Dissolution (Flowing Column)

The desorption/dissolution index (D_I) was defined as the leaching flux of the contaminant from the soil with the addition of the surfactant divided by the leaching flux of the contaminant from the soil without the addition of the surfactant (Equation 5). It was assumed that the biodegradation that may have occurred during the study was negligible compared to the amount of DRO that was removed from the column.

$$D_I = \frac{(DRO_I - DRO_F(w))/(\Delta T * V)}{(DRO_I - DRO_F(w/o))/(\Delta T * V)} \qquad (5)$$

An index value of one indicated there was no enhancement or inhibition of the desorption/dissolution processes with the addition of the surfactant. An index value of greater than one indicated that the addition of the surfactant had an enhancing effect on the leaching flux of the contaminant from the soil. And, conversely, an index value of less than one indicated that the surfactant had an inhibitory effect on the leaching flux of the contaminant from the soil.

The DRO values were calculated from GC abundance values similarly to those in the previous section. To calculate the desorption fluxes, the differences between the initial and final concentrations of DRO from the desorption samples (Table 5) were divided by the total volume of surfactant solution that was passed through each column and the period over which the study was performed. An example calculation follows:

$(DRO_I - DRO_F(w/o))/(\Delta T*V) = (59 - 25)\ \mu g/g\ /(5\ days*1L) = 0.034 \mu g/g\text{-}day\text{-}L$

The desorption index was then calculated using Equation 5.

$D_I = (0.0037 \mu g/g\text{-}day\text{-}L)/(0.034 \mu g/g\text{-}day\text{-}L) = 1.09$

All desorption indices were calculated in this fashion (Appendix F).

Table 5 – Final DRO soil concentrations after desorption studies (μg/g)

	Lewistown		Donovan Creek	
	T80	T85	T80	T85
0.5x	1017	1188	39	26
	1097	1126	26	24
1x	787	1015	26	29
	904	593	16	37
10x	495	--	51	52
	492	433	44	65
0x	1520		13	
	1542		25	

Desorption studies were only performed with three of the surfactants. BAC was omitted due to supply problems. For the Donovan Creek soil (Table 6), the desorption studies with the surfactant addition appear to generally have an inhibitory effect on the desorption of the contaminant from the soil. With both TWEEN 80 and 85, all concentrations of surfactant had an inhibitory effect. Both 10xCMC concentration had the lowest, or the most inhibitory indices at 0.28 for TWEEN80 and 0.00 for TWEEN85.

For the Lewistown soil (Table 6) both TWEEN 80 and 85 had an increased desorption effect on the contaminant. The increased desorption appeared to be proportional to the increase in the concentration of the surfactant. The TWEENs at the 10xCMC concentration had almost 40 times the desorption rate than that of water alone.

Table 6 – Desorption Indices: Blank spaces under the ABS averages indicate that a value was unobtainable for that column. The averages columns are the means values for two columns.

Donovan Creek	Desorption Flux w/o Surfactant = 0.04 ug/g/day/L					
	T85		T80		ABS	
	Average	Index	Average	Index	Average	Index
10x	0	0.00	0.011	0.28		0.00
1x	0.026	0.65	0.038	0.95		0.00
0.5x	0.034	0.85	0.026	0.65		0.00
Lewistown	Desorption Flux w/o Surfactant = 0.035 ug/g/day/L					
	T85		T80		ABS	
	Average	Index	Average	Index	Average	Index
10x	1.448	41.37	1.388	39.66		0.00
1x	1.077	30.77	1.036	29.60		0.00
0.5x	0.724	20.69	0.824	23.54		0.00

The ABS desorption studies yielded very different results. Within the first 400mL of flow, if not before, all of the columns stopped flowing, possibly clogged by a precipitate. The Lewistown 10xCMC concentration column had precipitate clumps in the effluent. The experiment was repeated and the same result of lowered permeability was found. This result has been seen by Renshaw et. al. (Renshaw et al., 1997), who has observed lowered permeability effects as a result of anionic surfactant and soil mineral interactions.

Surfactant Indices and Parameters

Biodegradability -Surfactant (Batch)

The biodegradability of the surfactant was measured as the surfactant biodegradation rate (T_R) and was defined as the biodegradation rate of the surfactant in the soil environment (Equation 2).

$$T_R = \frac{(S_I - S_F)}{\Delta T} \qquad (2)$$

This biodegradation rate was based on oxygen depletion in respirometer samples. The higher the value, the faster the surfactant would degrade in the laboratory soil sample environment.

The calculations for the surfactant biodegradation rate were determined as follows:

O_2 Required for DRO Degradation = $\frac{O_2 \text{ Depleted in 0xCMC sample}}{\text{DRO Degraded in Sample}}$
= (2.96mg)/(8240μg) = 0.0001mg/μg

O_2 Used in Degrading DRO = (DRO degraded in sample w/ surfactant)*O_2 Req'd
= (7110μg)*(0.0001) = 0.71mg

O2 Required for Surfactant Degradation
= $\frac{O_2 \text{ Depleted in Surfactant Degradation in Water Sample}}{\text{Surfactant Degraded in Sample}}$
= (99mg)/(0.86g) = 0.11 mg/g

O_2 Remaining for Surfactant Degradation = (Total O_2 – O_2 Req'd for DRO)
= (47-0.71)mg = 46.3mg

Surfactant Degraded = (Remaining O_2)/(O_2 Req'd for Surfactant Degradation
= (46mg)/(0.11mg/g)= 418g

The surfactant degradation rate was then found by dividing the amount of surfactant degraded by the duration of the experiment (Appendix G)

To determine this rate, the TWEEN85 respirometry data (from the contaminant biodegradation experiments) for both Donovan Creek and Lewistown were used. It was assumed that the samples with no surfactant addition were exhibiting only contaminant biodegradation and therefore the O_2 levels that were seen in those samples were the required amounts for the amount of contaminant biodegradation seen in that particular sample. The amount of contaminant remaining after the biodegradation period was determined by soil extraction. The remaining O_2 amount, after the required O_2 for contaminant biodegradation was subtracted, was assumed to be due to surfactant biodegradation. To determine the amount of O_2 that was required for surfactant biodegradation, the surfactant biodegradation in water respirometry results were used.

After analyzing the Donovan Creek soil samples, it was determined that, on average, 0.07mg O_2/μg DRO and 0.015mg O_2/mg surfactant was required. The analysis of the Lewistown soil samples, yielded a 0.0001mg O_2/μg DRO and 0.063mg O_2/mg surfactant requirement. These values were then used to determine the surfactant in soil biodegradation rates (Table 7). The Donovan Creek samples yielded negative concentrations and thus negative rates. This could indicate that there was no surfactant biodegradation in these samples. As the trend is in the other Donovan Creek results, it is seen here that there is also

an inhibitory effect on the degradation of the surfactant in the Donovan Creek soil. This may be due to a less diverse microbial community or it could also be due to inherent instrument variability. Since it is suspected that the surfactant addition inhibited all biodegradation processes, including biodegradation natural organic matter, more oxygen than was appropriate was attributed to DRO degradation in the no surfactant samples. So when the calculations were preformed for the surfactant-added samples, erroneously high amounts of oxygen was attributed to DRO degradation leaving no oxygen for surfactant degradation.

The Lewistown samples yielded positive values indicating that surfactant biodegradation did occur.

Table 7 – Surfactant biodegradation rates for the Donovan Creek soil and Lewistown soil with TWEEN85 surfactant: The negative values indicate that calculations show that no biodegradation of the surfactant occurred.

Donovan Creek			**Lewistown**	
Sample Concentration	Surfactant Degradation Rate mg/day		Sample Concentration	Surfactant Degradation Rate mg/day
0x	---		0x	---
0x	---		0x	---
10x	-714		10x	616
10x	-142		10x	546
1x	-278		1x	74
1x	-312		1x	63
0.5x	-475		0.5x	102
0.5x	-721		0.5x	83

Bioavailability - Surfactant (Batch)

The bioavailability index (B_{IS}) for the surfactant was defined as the biodegradation rate of the surfactant in the soil environment divided by the biodegradation rate of the surfactant in water (Equation 4).

$$B_{IS} = (T_R) / ((S_I - S_F)/\Delta T) \quad (4)$$

An index value of one indicates that there was no difference in bioavailability in the water or the soil environment. An index value of greater than one indicated that the surfactant is more biodegradable in the soil environment than in the water environment. An index value of less than one indicated that the surfactant is less bioavailable in the soil environment and thus degrades slower than in the water environment.

The biodegradation rates in water of the surfactant, based on the difference in surfactant concentration before and after biodegradation (Table 8), were determined as follows:

$((S_I - S_F)/\Delta T) = (20 - 13.5)$mg/L / (5 days) = 1.34 mg/L-day

The biodegradation rate in water was then divided into the biodegradation rate in the soil (T_R) (calculation shown in previous section). All biodegradation rates of the surfactant in water were determined similarly (Appendix H).

Table 8 – Initial and final concentrations of surfactant in surfactant biodegradation studies (mg/L)

		Lewistown				Donovan Creek			
		ABS	BAC	T80	T85	ABS	BAC	T80	T85
0.5x	Initial	1900	2500	1.8	1.7	3000	1000	0.3	1
	Final	1301	2	0.03	0.76	3365	2	0.01	0.97
1x	Initial	3800	5000	3.6	3.4	6000	2000	0.6	2
	Final	2717	9	0.1	0.75	4637	2340	0.06	0.48
10x	Initial	38000	50000	36	34	60000	20000	6	20
	Final	41812	71907	2.09	17.19	61148	32241	0.6	13.47

The surfactant bioavailability index was determined using the same sample set of the Lewistown -TWEEN85 and Donovan Creek-TWEEN85 that was used to determine the biodegradation of surfactant in soil. The surfactant biodegradation rate in water was determined using the surfactant biodegradability procedure outlined in the methodology section. But, due to instrumentation trouble, only the surfactant biodegradation rate in soil was available for the Lewistown-TWEEN85 and the Donovan Creek-TWEEN85 combinations. The surfactant in water degradation rate experiments were not duplicated due to limited instrument time.

For the Donovan Creek and TWEEN85 combination, surfactant biodegradation rates in water were found to decrease with lower amounts of surfactant (Table 9). The resulting indices were negative due to the dependence on the leachate results. Thus the bioavailability indices for the Donovan Creek-TWEEN85 combination are assumed to be zero.

Table 9 – Bioavailability indices for the Donovan Creek and TWEEN85 combination.

Donovan Creek and TWEEN85			
Surfactant Concentration	Surfactant Degradation Rate in Soil mg/day	Surfactant Degradation Rate in Water mg/day	Bioavailability Index
10x	-714		0.00
10x	-142	0.067	0.00
1x	-278		0.00
1x	-312	0.0155	0.00
0.5x	-475		0.00
0.5x	-721	0.0005	0.00

For the Lewistown-TWEEN85 combination, surfactant biodegradation rates in water were found to have also decreased with lower concentrations in the sample (Table 10). The surfactant in water biodegradation results were divided into the soil biodegradation results and the resulting indices were determined. The indices indicated an increase in degradation with the presence of the soil. The bioavailability for the indices indicate that the 0.5xCMC concentration was the most bioavailable with an average of approximately 9700 for an index. The 10xCMC concentration had the second highest index on average at approximately 3300. These high values would indicate that there are other sources of carbon which contribute to the oxygen depletion amounts that are used to calculate this index value.

Table 10 – Bioavailability indices for the Lewistown and TWEEN85 combination.

Lewistown and TWEEN85			
Surfactant Concentration	Surfactant Degradation Rate in Soil mg/day	Surfactant Degradation Rate in Water mg/day	Bioavailability Index
10x	616		3561
10x	546	0.173	3159
1x	74		2707
1x	63	0.0275	2300
0.5x	102		10729
0.5x	83	0.0095	8711

Although the bioavailability indices for all of the soil and surfactant combinations could not be calculated, the surfactant biodegradation rate in water was determined for all surfactant and soil combinations. Again, due to limited instrument time, these experiments were not duplicated. For the Lewistown soil inocula, surfactants TWEEN 80 and 85 (Table 11) appeared to be the most easily degraded. ABS and BAC gave variable results.

Table 11 – Surfactant in water biodegradation rates with a Lewistown soil inocula

Lewistown					
Surfactant	Concentration	Degradation Rate mg/L/day	Surfactant	Concentration	Degradation Rate mg/L/day
TWEEN85	0.5x	0.19	ABS	0.5x	150
	1x	0.55		1x	271
	10x	1.34		10x	-953
TWEEN80	0.5x	0.38	BAC	0.5x	500
	1x	0.74		1x	998
	10x	7.19		10x	-4381

For the Donovan Creek soil inocula, TWEEN 80 and 85 appeared to be the most easily degraded. Again, ABS and BAC gave variable results (Table 12) compared to the correlating increase in biodegradation rates to increased surfactant concentrations with TWEEN80 and TWEEN 85..

Table 12 – Surfactant in water biodegradation rates with a Donovan Creek soil inocula

Donovan Creek					
Surfactant	Concentration	Degradation Rate mg/L/day	Surfactant	Concentration	Degradation Rate mg/L/day
TWEEN85	0.5x	0.01	ABS	0.5x	-91
	1x	0.31		1x	341
	10x	1.34		10x	-287
TWEEN80	0.5x	0.06	BAC	0.5x	200
	1x	0.11		1x	-68
	10x	1.14		10x	-2448

Sorption-Surfactant (Batch)

Sorption potential or the sorption factor (R_F) indicates the level of affinity of the surfactant for the soil. It was described as the soil-CMC divided by the media-CMC (Equation 6).

$$R_F = \frac{CMC_S}{CMC_W} \qquad (6)$$

An index value of one indicated that the surfactant has no affinity for sorption to the soil and no surfactant is lost out of the water phase due to soil sorption. As index values increase, the tendency, or the affinity of the surfactant for the soil increases.

The CMC_s (Table 13) were determined as it is outlined in the Materials and Methods section. All CMC_s were determined similarly (Appendix I).

The sorption isotherms for soil and surfactant combinations were determined using the procedure outlined in the methodology section. The CMCs for all surfactants (Table 13) in GS media were determined using the surfactant CMC determination procedure also outlined in the methodology. The CMCs in water did not differ greatly from the CMCs with soil for the nonionic surfactants. For the ionic surfactants, ABS and BAC, the CMCs increased dramatically by 40 fold in the presence of soil. However, the sorption isotherms yielded larger values for the ionic surfactants.

Table 13 – Mobility indices: Media CMCs and Soil CMCs

Mobility Indices					
Surfactant		Donovan Creek		Lewistown	
	Media CMC	Soil CMC	Mobility Index	Soil CMC	Mobility Index
T80	0.265 mL/L	0.6	2	3.6	14
T85	0.486 mL/L	2.0	4	3.4	7
ABS	0.134 g/L	6.0	45	3.8	28
BAC	0.107 g/L	2.0	19	5.0	47

The resulting indices indicated that the ionic surfactants have a higher affinity for sorption to the soil. The nonionic surfactants, TWEEN80 and 85 have indices that are 1/2 to 1/4 the magnitude of the lowest ionic surfactant indices (ABS at 28). With the presence of the Donovan Creek soil, the CMCs increased from approximately 3 fold of the CMC in water for the nonionic surfactants to more than 40 fold for ABS. With the Lewistown soil present, the nonionic,

TWEEN80 and 85, CMCs increased to approximately 10 fold of the CMC in water. The ionic surfactants, however, responded oppositely to the Lewistown soil than what they did for the Donovan Creek soil. BAC in this case increased more than 40 fold, where ABS only increased 28 fold from the CMC in water.

The Surfactant Screening Chart

Lewistown

After all of the experiments were performed, the surfactant screening chart for the Lewistown site soil was assembled (Table 14). The table contains the final index information that resulted from each set of experiments.

Table 14 – The Surfactant Screening Chart for the Lewistown Site. Each surfactant was tested at 0.5x, 1x, and 10xCMC. Each respective concentration is listed in the chart as the actual perspective concentration of that surfactant. An index of greater than one indicates enhancement of that rate.

Surfactant Screening Chart **Site: Lewistown**												
	ABS			**TWEEN 80**			**TWEEN 85**			**BAC**		
	1.9 g/L	3.8 g/L	38 g/L	1.8 mL/L	3.6 mL/L	36 mL/L	1.7 mL/L	3.4 mL/L	34 mL/L	2.5 g/L	5 g/L	50 g/L
RI							0.6/0.7	0.7/0.8	0.9/1.0			
DI	CLOGGED			23.5	29.6	39.7	20.7	30.8	41.4			
BI	0.48	0.51	2.62	0.83	0.7	0.34	0.62	0.83	0.64	0.56	0.65	0.58
TR							102 / 83	74 / 63	616 / 546			
RF		28			14			7			47	
BSI							~9000	~2500	~3300			

RI - Contaminant Biodegradability Index
DI - Contaminant Desorption/Dissolution Index
BI - Contaminant Bioavailability Index
TR - Surfactant Biodegradability Index
RF - Sorption Factor Index
BSI - Surfactant Bioavailability Index

The Surfactant Screening Chart for the Lewistown soil suggests that ABS would be the most beneficial for use with a natural bio-attenuation strategy. ABS had a bioavailability index of 2.62. However, the screening chart is incomplete for ABS, so another surfactant should be suggested. TWEEN85 was the only surfactant that has been completely screened. However, if TWEEN85 was to be used, the chart would indicate that the 10xCMC concentration would be most beneficial for enhancing degradation of the contaminant in the soil environment.

Donovan Creek

After completion of all of the experiments for the Donovan Creek soil, the surfactant screening chart was assembled (Table 15). The chart, though incomplete, did give indication as to the effectiveness of the surfactants in the areas that were tested.

The surfactant screening chart for the Donovan Creek soil suggests that no surfactant would increase the biodegradation of the contaminant at the site and thus not benefiting an in-situ bio-attenuation remediation strategy at this site.

Table 15 – The Surfactant Screening Chart for the Donovan Creek Site Each surfactant was tested at 0.5x, 1x, and 10xCMC. Each respective concentration is listed in the chart as the actual perspective concentration of that surfactant. An index of greater than one indicates enhancement of that rate.

Surfactant Screening Chart **Site: Donovan Creek**												
	ABS			**TWEEN 80**			**TWEEN 85**			**BAC**		
	3 g/L	6 g/L	60 g/L	0.3 mL/L	0.6 mL/L	6 mL/L	1 mL/L	2 mL/L	20 mL/L	1 g/L	2 g/L	20 g/L
RI												
DI	CLOGGED			0.65	0.95	0.28	0.85	0.65	0			
BI	1.06	1.00	0.82	1.03	0.8	1.09	1.25	0.9	1.24	1.17	1.15	0.93
TR							(-)	(-)	(-)			
RF		45			2			4			19	
BSI							(-)	(-)	(-)			

RI - Contaminant Biodegradability Index
DI - Contaminant Desorption/Dissolution Index
BI - Contaminant Bioavailability Index
TR - Surfactant Biodegradability Index
RF - Sorption Factor Index
BSI - Surfactant Bioavailability Index

CHAPTER 6

DISCUSSION

Contaminant Indices and Parameters

Biodegradability

The biodegradability index was used to determine the rate and extent of biodegradation of the organic compounds in the soil-leachate sample, with and without a surfactant addition. The biodegradability protocol was designed such that the soil and surfactant system would have time to equilibrate over the initial five-day period to allow for the processes of the contaminant solubilization into the liquid, surfactant-aided desorption, and surfactant sorption onto the soil to equilibrate. Biodegradation in the first five day of the leaching process was not measured. The second five-day period was used to determine the biodegradability of the compounds in the leachate.

It appeared that the lower concentrations, 0.5x and 1xCMC, of TWEEN85 had an inhibitory effect on the biodegradation of the leachate when the rates were compared (Table 2). When the values were graphed (Figure 6), there appeared to be a trend in decreased inhibition of degradation with the increase in

the surfactant concentration, however, samples without surfactant had higher biodegradation rates than any of the samples with the surfactant addition. (A statistical analysis was performed using Dunnett's multiple comparison test and no statistical differences were found between the no-surfactant samples and any of the samples with a surfactant addition, Appendix J) The trend of inhibition with the addition of the surfactant at the lower concentrations (0.5x and 1xCMC) could have been due to the surfactant coating the soil surface and sequestering the HOC from degrading microbes. The 10xCMC, with biodegradation indices of 1.0 and 0.9, may have exhibited less inhibition due to micelles in solution. It is hypothesized that the micelles were able to remove the HOC from the soil and make it bioavailable to the microbes. However, the presence of the micelles, still, did not, on average, promote increased bioavailability.

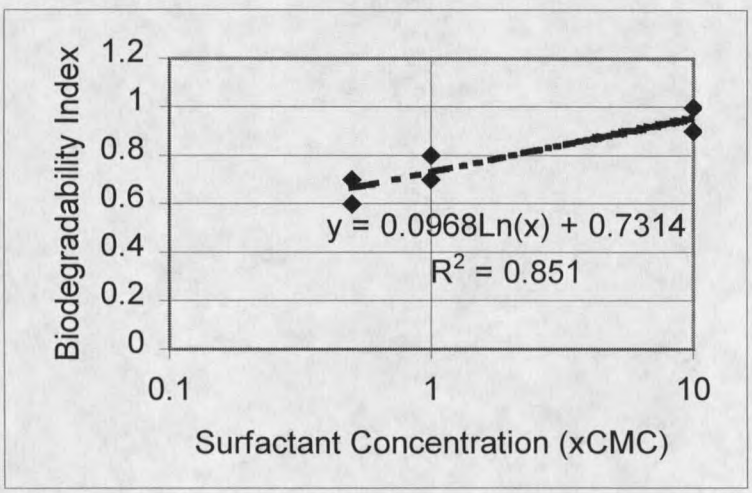

Figure 6 – Biodegradability Indices for the Lewistown-TWEEN85 combination.

After the biodegradability studies were performed it was determined that the procedure did not fully evaluate the fates of the contaminant and the

surfactant. Initially, it was assumed that the procedure would give a clear indication of the amount of DRO and the amount of the surfactant that was biodegraded in the leachate. But with the oxygen requirements for the degradation of the surfactant or the contaminant not being clear (this will be discussed in the Surfactant Biodegradability section), the leaching procedure may not have been a valid method for determining the degradability of the leachate. Several points were identified that may be addressed for questioning the validity for this experiment. They included the overall duration of the experiment, sample handling, and also contaminant and surfactant fate.

First, the leachate experiment was conducted over a total of ten days rather than five days. By allowing the sample to sit for five days to leach, the initial five-day degradation rate may have been missed. The duration of this experiment also makes comparison to the other rates (rates determined based on the first five days of a study) less valid. Second, samples were allowed to sit on a shelf undisturbed for five days. Not being stirred may not have optimized desorption. Had the samples been stirred, it would have modeled a flowing system more closely. And, third, the fate of the contaminant and the surfactant present in the soil was not fully characterized. How much surfactant sorbed to the soil, how much was degraded before the sample was placed on the respirometer, and how much contaminant was degraded after the samples were connected to the respirometer is not clearly determined in this experimental method. Thus, the true fates of the contaminant and surfactant were not ascertained.

In order to address the uncertainties that were identified with the biodegradability procedure several modifications can be suggested. The first is to conduct a respirometer experiment where there is enough liquid content to leave the soil sample oxygen limited, i.e. 20g soil with 50mL surfactant-media solution. In this manner, it may be possible to limit the oxygen content in the soil at the bottom of the bottle, while the microbes in the liquid phase continue to degrade aerobically. Second, if columns were constructed of glass or some other non-sorptive material, it would be possible to collect the effluent from the desorption columns. This option increases the cost of the columns greatly, increasing the cost of each column from a few cents to nearly $40. A third suggestion is to take a soil sample and surfactant media solution and place it on a shaker table for a short period of time, decant the supernate and place it in a media jar on the respirometer. This procedure may facilitate direct measurements of the biodegradability of the leachate (Camper, 1999).

Bioavailability

The Donovan Creek soil had an average DRO disappearance rate without surfactant of 8.5 μg/g/day and it appears that each surfactant at least at one concentration had an enhancing effect on the DRO disappearance. The highest bioavailability indices were seen for TWEEN85 at 0.5xCMC (1.25) and TWEEN85 at 10xCMC (1.24). BAC at 0.5xCMC and 1xCMC followed at 1.17 and 1.15. It was expected that the TWEEN85 would enhance DRO disappearance,

as it is biodegradable. However, BAC, since it is often used as a biocide, was not initially expected to have an enhancing effect on the biodegradation. However, there are studies being conducted at the Center for Biofilm Engineering that indicate that BAC does enhance biodegradation at concentrations below the CMC (Patrauchen, 1999). TWEEN80 and ABS showed little difference in the disappearance rate of the DRO between any of the concentrations. Donovan Creek is a very old site and has very low concentrations of DRO. The initial DRO concentration was determined to be 59 μg/g. Given this low level of DRO and that it is approaching accurate detection limits, the results of the biodegradation tests may not give clear results as to whether or not there is a significant amount of biodegradation occurring in the soil samples. If the bioavailability indices are plotted vs. the relative surfactant concentration (Figure 7) it appears that no surfactant has an apparent enhancement of increased bioavailability over another. None of the samples with surfactant for the Donovan Creek soil had statistically different disappearance rates, which were compared be Dunnett's multiple comparison test (Appendix J).

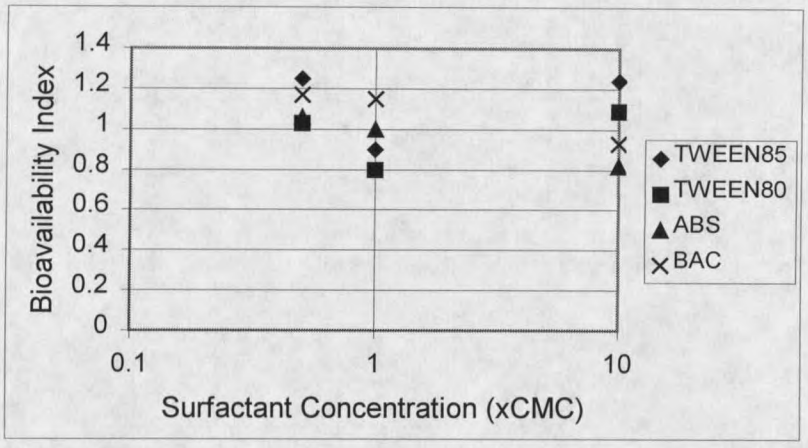

Figure 7 – Bioavailability Indices for Donovan Creek

The Lewistown soil had an average disappearance rate without a surfactant addition of 86.6μg/g/day (average of all 0xCMC degradation samples for the Lewistown soil, Appendix E). It appeared that any surfactant addition to the Lewistown soil inhibited disappearance of the contaminant. The index values ranged from 0.34 for TWEEN80 at 10xCMC to 0.83 for TWEEN85 at 1xCMC and TWEEN80 at 10xCMC, none of which are statistically different, with the exception of ABS at 10xCMC had an index of 2.62 which is statistically different (Appendix J). The enhancement of DRO disappearance at 10xCMC for ABS suggested that the microbes at the Lewistown site could interact directly with the HOC sorbed into the micellar phase. This could prove to be particularly beneficial, as it seems that ABS has a tendency to lower permeability in the Lewistown soil. This will be discussed in the next section. Overall, the results would indicate that in the case of the Lewistown site that no surfactant addition would be the correct action, with the exception of the 10xCMC for ABS.

The bioavailability index assesses whether or not the addition of a surfactant enhances the disappearance of a contaminant by promoting bioavailability. The bioavailability indices that resulted from these tests indicated that surfactant addition promoted a very small increase in disappearance of the DRO in the first five days of its addition. The samples were prepared at 70% field capacity water content to more closely mimic field conditions. Since these experiments were done in batch, it may be feasible to using a longer duration and, possibly periodical additions of additional surfactant. This could show a greater increase or decrease in the bioavailability of the DRO present in the samples.

Desorption/Dissolution

For the Donovan Creek soil, the desorption/dissolution indices indicated a decreasing desorption index with increasing concentration of surfactant (Figure 8). This suggested that the DRO concentration was low enough that the surfactant actually sequesters the remaining amount of DRO to the soil surface. The data did suggest that higher concentrations did inhibit desorption. It could be that since TWEENs are nonionic, that they were not strong enough ionic charge to pull DRO away from the soil, but were sorbed rather to the soil surfaces, where they coated the remaining HOC and inhibited it from solubilizing.

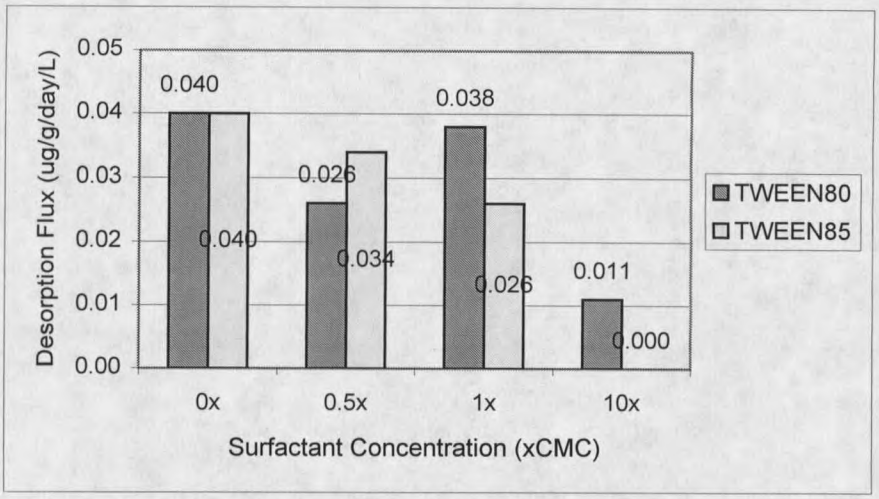

Figure 8 – Desorption Flux vs. Surfactant Concentration for the Donovan Creek soil and TWEEN 85 and TWEEN 80.

The Lewistown soil showed that the addition of a surfactant increased the desorption flux of the DRO from the soil samples. As the case was with both TWEEN 80 and 85, the desorption flux increased with the surfactant concentration (Figure 9). The 10xCMC concentrations for both TWEENs increased desorption by 3.5 times the flux without surfactant addition. It appears that a removal technology would be greatly benefited by the addition of one of these two surfactants. The desorption fluxes for Lewistown and TWEEN 80 are all statistically different, while only the 10xCMC TWEEN 85 concentration was statistically different by Dunnett's multiple comparison test (Appendix J).

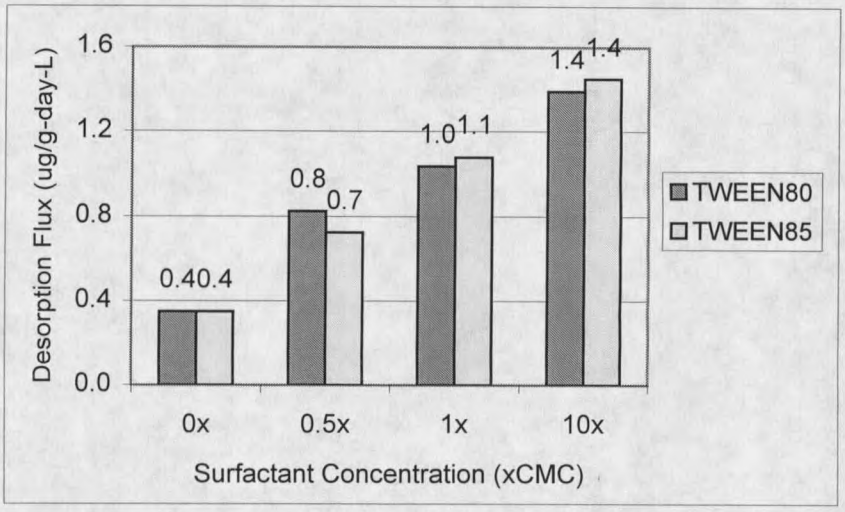

Figure 9 – Desorption Flux vs. Surfactant Concentration for the Lewistown soil and TWEEN 80 and TWEEN 85

ABS was also investigated in the desorption studies. However, when ABS was used in the columns, the columns within the first 400mL plugged. In the 10xCMC columns, there were visible clumps of precipitate in the effluent. So, it appeared that ABS was reacting with the minerals present in the soil and forming a precipitate. This has also been seen by (Jafvert et al., 1991; Tumeo, 1997) where anionic surfactants have been investigated for use in lowering soil permeability.

Surfactant Indices and Parameters

Biodegradability - Surfactant

When the O_2 requirements were determined for contaminant biodegradation, Donovan Creek soil samples had no remaining oxygen that could be attributed to surfactant biodegradation. In most samples, the oxygen that was attributed to DRO biodegradation, through respirometer oxygen demand data calculations of oxygen requirements, exceeded the measured amounts of oxygen. This lead to several possible conclusions. One, that there was no surfactant biodegradation occurring and that the oxygen levels that were calculated to be higher than what was measured were due to instrument variability. Two, when working with the Donovan Creek soil, the DRO values that were found through soil extraction may be high, causing the DRO biodegradation oxygen requirements to be overestimated. DRO soil extraction paired with GC analysis approaches its linear range lower-detection limit at approximately 100μg/mL. Or, three, there were other organics being biodegraded in the soil that account for oxygen depletion in the soil samples. This suggested that in the samples where there was no surfactant present, that all of the oxygen depleted was not due totally to DRO biodegradation, but also to other organic matter.

When the O_2 requirements for DRO biodegradation in the Lewistown soil were determined, there were remaining O_2 amounts that could be attributed to

surfactant biodegradation. However, with the assumption that all remaining oxygen was due to surfactant biodegradation, excess oxygen levels indicated that there was more surfactant biodegraded than was added (Table 16). This indicated that there was other degradation processes occurring. Since it is known that the Lewistown soil has organic contaminants other than DRO, it was reasonable to say that these other organics were contributing to the O_2 depletion in the samples. However, the amount of other contaminants has not been fully characterized (Hendricks, 1998).

Table 16 – Oxygen levels compared for Surfactant Biodegradation in the Lewistown soil.

Surfactant Concentration, xCMC	10x	10x	1x	1x	0.5x	0.5x
Surfactant Added, mg	79.4	79.4	7.9	7.9	4	4
Surfactant Degraded, mg	3080	2730	372	316	509	413
Difference, mg	3000.6	2650.6	364.1	308.1	505	409

The Biodegradation Rate of the surfactant in the soil environment was inconclusive. Due to instrument troubles and limited available time, there was only one set of usable respirometer data for the determination of the biodegradation rate of surfactant in the soil environment.

Bioavailability - Surfactant

The bioavailability index, given that it depended on the Surfactant Biodegradability Index, became highly suspect for the reasons mentioned in the previous section. Given that the calculations for the surfactant degraded in the soil environment indicated either excessive biodegradation of the surfactant or negative degradation of the surfactant and for the purposes of discussion in this section, the Donovan Creek surfactant biodegradability rates were assumed to be zero. And, it was assumed that all surfactant in the Lewistown samples was degraded.

With the above assumption of zero for the surfactant biodegradability rate in the soil environment, all of the Donovan Creek bioavailability indices became zero. This indicated that not only did the presence of soil slow the biodegradation rate of the surfactant, but also, appeared to completely halt any surfactant biodegradation.

However, the biodegradation rates of the surfactants in the water environment did indicate whether or not a surfactant has potential for degradation by the microbial population present at the Donovan Creek site. When the initial concentration and the final concentrations were compared (Table 17), it could be seen that in most cases, the surfactants are being degraded. It appeared that both of the TWEENs could easily serve as a carbon source for the microbes at the Donovan Creek site. It also appeared that the higher the concentrations, the

better the microbial degradation of the surfactant, or the better the microbial growth.

Table 17 – Surfactant Degradation Rates in water with a Donovan Creek soil inocula

Surfactant	xCMC	Initail Concentration	Final Concentration	Degradation Rate, mg/L/day
TWEEN85, mL/L	0.5x	1	1.0	0.01
	1x	2	0.5	0.31
	10x	20	13.5	1.34
TWEEN80, mL/L	0.5x	0.3	0.0	0.06
	1x	0.6	0.1	0.11
	10x	6	0.6	1.14
ABS, mg/L	0.5x	3000	3370	(-)
	1x	6000	4640	341
	10x	60000	61150	(-)
BAC, mg/L	0.5x	1000	2	200
	1x	2000	2340	(-)
	10x	20000	32240	(-)

The other two surfactants, ABS and BAC appeared to not be as readily utilized as a carbon source. ABS appears to only be tolerated and degraded at the 1xCMC concentration. And likewise, BAC appeared to be only tolerated at the 0.5xCMC. Both of these values suggested that the surfactants may not serve as substrates except at certain concentrations. There have been studies that suggest that BAC may be utilized by microbes at low concentrations (Patrauchen, 1999). However, since these tests were not duplicated, it would warrant further experimentation to confirm this data.

For the Lewistown site, it appeared that the opposite of Donovan Creek is true. In the Lewistown samples all of the surfactant (TWEEN85) that was added was degraded and there was additional biodegradation of other organics occurring. So, after making the assumption that all of the surfactant was degraded, the resulting bioavailability indices were in excess of 2300 (Table 10). These values were quite large and indicated that the surfactant was highly degradable in the soil environment, much more so than in the water environment. Although this may be true, it was probably not to the extent indicated by the index values. This could possibly be attributed to the initial number if microbes in the sample. In the surfactant biodegradation studies, there was only a 1g inocula of soil as opposed to 20g in soil biodegradation studies. So if it was assumed that per gram of soil there was an approximately equal number of microbes on the soil, then the biodegradation rate of the surfactant in the soil environment could be 20 times higher. This was one possible explanation of the differences in biodegradation rates. However, the assumption of total surfactant biodegradation in the soil samples was probably not correct. It was known that the Lewistown site soil has other contaminant organics and natural organics present, as previously mentioned. So, it was not possible to accurately determine how much surfactant was degraded.

Similarly to the Donovan Creek samples for surfactant biodegradation in water, the surfactant biodegradation rates in water was also determined with a Lewistown inocula. For TWEEN80 and 85, it appears that the higher the

concentration the better the biodegradation. This indicated that these two surfactants serve readily as a substrate for the microbes present at the Lewistown site (Table 18). Conversely, it appeared that ABS and BAC do not serve as readily at the higher the concentrations of the surfactants. Both ABS and BAC appear to be toxic, i.e. no biodegradation, at the 10xCMC concentrations. They did appear to be very biodegradable at the lower concentrations, 1xCMC and 0.5xCMC (Table 18).

Table 18 – Surfactant Degradation Rates in water with a Lewistown soil inocula

Surfactant	xCMC	Initail Concentration	Final Concentration	Degradation Rate, mg/L/day
TWEEN85, mL/L	0.5x	1.7	0.8	0.19
	1x	3.4	0.8	0.55
	10x	34	17.2	1.34
TWEEN80, mL/L	0.5x	1.8	0.0	0.38
	1x	3.6	0.1	0.74
	10x	36	2.1	7.19
ABS, mg/L	0.5x	1900	1300	150
	1x	3800	2720	271
	10x	38000	41810	(-)
BAC, mg/L	0.5x	2500	2	500
	1x	5000	9	998
	10x	50000	71900	(-)

Since these tests were not duplicated due to instrument time, further investigation into the biodegradability of the surfactant in the aqueous phase. As was suggested by looking at the negative biodegradation concentration (Table 17 and 18) there was potentially enough error in the concentrations determined by the surface tension measurements that there may have been discrepancies in

the final concentrations of surfactant that were used to determine the biodegradation rates.

Sorption - Surfactant

The sorption factor for the different surfactants were simply multiplication factors for how much surfactant will sorb to the soil with respect to the CMC in water of that surfactant. This factor indicated that if a surfactant has a sorption factor of 4, that it will take four times the CMC in water of that surfactant to reach the CMC in the presence of soil due to loss of solubilized surfactant to soil sorption. This indicated the amount of surfactant the soil can hold before surfactant will begin to appear downstream of the injection site. There was only one sorption factor for each surfactant since it is determined by two specific points that relate to a single characteristic of the surfactant, the CMC, one with and one without soil present. The CMC values determined with the presence of soil are the basis for the concentrations used throughout the protocol. The concentrations, 0.5x, 1x, and 10x, represent one-half the sorption capacity of the soil, soil sorption capacity, and the presence of micelles in solution. These values are determined from a sorption isotherm graph (Figure 10).

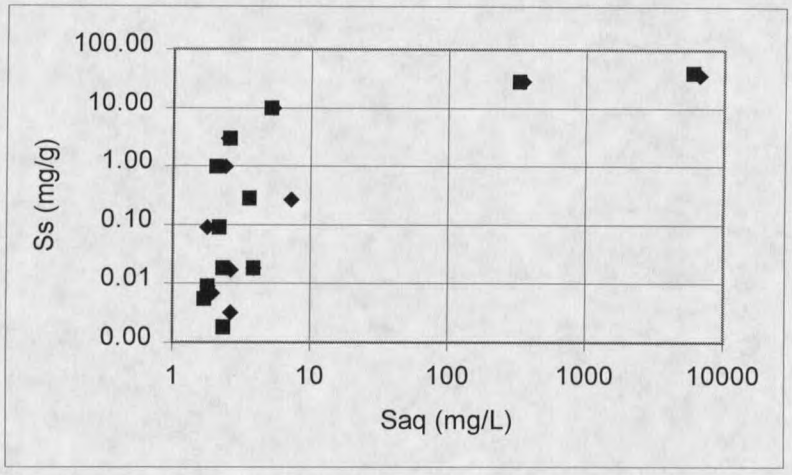

Figure 10 – Sorption Isotherm example: Lewistown and BAC

Generally, the ionic surfactants exhibited a higher affinity for the soil surface. This was expected since soil surfaces have a characteristic charge, most often negative. Intuitively, it seemed most likely that the cationic surfactant then would have the highest affinity for the soil surface, since most surfaces are negatively charged and cationic surfactants are positively charged. And, this was seen with the Lewistown soil which was a clayey loam, thus negatively charged. BAC was determined to have a sorption factor of 47, where the anionic surfactant, ABS, only had a sorption factor of 28 (Table 13). The Lewistown site soil type explained the very high sorption capacity for the positively charged surfactant and the lower sorption capacity for the negatively charged surfactant. The TWEENs did sorb to the soil, but resulted with much lower sorption factor values. The ability of the surfactant to sorb to the soil, despite the overall neutral charge is explained by the hydrophobic moiety that is characteristic of

surfactants. TWEEN80 and TWEEN85 had sorption factors of 14 and 7 respectively.

The high sorption values between BAC and ABS for the Lewistown soil was opposite with the Donovan Creek soil. With the Donovan Creek soil, ABS had a higher sorption factor than BAC at 45 and 19 (Table 13). Again, it was to be expected that ionic surfactants would sorb to the soil more than nonionic surfactants. But in this case it appeared that the anionic surfactant, ABS, had a higher sorption potential for the Donovan Creek soil than BAC. There are two possible reasons for this, one the soil type, sandy loam, and chemistry of the Donovan Creek gave it a more neutral charge allowing potential for even an overall negative charged particle (surfactant) to sorb to it. Or, two, it may have been that the surfactant, even though overall negative, may have had a strong hydrophobic moiety which allowed it to sorb to the soil despite the overall repelling like-charges. However, this may also be explained by interactions of the surfactant with other soil minerals. This interaction between soil mineral and surfactant was being investigated for exploitation where permeability reductions may be desired (Renshaw et al., 1997; Tumeo, 1997). The TWEENs also reacted similarly to the Donovan Creek soil as they did the Lewistown soil. TWEEN80 and TWEEN85 had lower sorption values of 2 and 4. The results indicated that there could be a wide range of sorption potential and that this depends on soil types and surfactant types.

Toxicity-Omitted in the scope of this thesis

The toxicity tests were eliminated in the scope of this thesis due to limited resources. The system proposed for use was the MicroTox system (AZURE, 1999). The MicroTox system utilizes a bioluminescent bacteria that is exposed to a series of dilutions of a potential toxicant. The bacteria's response is measured by its light output and is correlated to the potential toxicity of components in the solution.

Although this test was omitted, it can be said to some degree what the toxicity of each of these surfactants and contaminants is. It is known that TWEENs, in general, are not toxic. And as is the case with TWEEN 80, it is used in media and is used in food processes and in pharmaceuticals. ABS is considered an irritant and BAC is often used as a biocide in medical applications, also considered an irritant. DRO, or more generally petroleum hydrocarbons, are, or can be toxic, particularly some of the components. For other potential irritations or toxicities to humans, MSDS sheets for each surfactant can be reviewed.

The Surfactant Screening Chart

When all of the indices were completed and presented as a collection in the surfactant-screening chart for a site soil, they could be easily compared between surfactants and varying concentrations. Each index was listed down the left-hand column and the surfactant and the respective concentrations were listed

across the top row. It should be noted that the different indices vary greatly in their magnitudes and care should be taken to not compare one index to another. The surfactant screening charts in this thesis were used as a demonstration as to the method by which the parameters and indices in the protocol may be determined, presented, and used. The charts for both of the site soils are incomplete. Although, it would have been ideal to complete both screening charts, the goal of this thesis was not to solve any remediation questions about either site. However, all parameters and indices have been determined at least once to demonstrate the method.

The Donovan Creek screening chart (Table 15) indicates that surfactant addition would not significantly enhance remediation of the contaminant. It appears that any surfactant addition inhibits desorption from the soil. The surfactants exhibited no statistical difference between the each other or their different concentrations. So, it was speculative as to any potential enhancement of biodegradation through increased bioavailability. And, if the surfactant does enhance the biodegradation, the magnitude of enhancement may not be enough to offset the cost of the surfactant addition. Also, it appeared that the surfactant, once in the environment, did not degrade, leaving another foreign compound in the environment. So, it appeared that none of the surfactants would enhance an in-situ bioremediation strategy.

The Lewistown screening chart (Table 14) indicates that several of the surfactant concentrations might enhance the remediation effort. Given that the

remaining indices and parameters should be determined for this surfactant, ABS exhibited low mobility potential and potential for enhanced contaminant biodegradation at the 10xCMC concentration. All others inhibited bioavailability of the contaminant for degradation, which would be the focus of an in-situ strategy. However, if another remediation strategy were to be used, perhaps a removal technology like pump-and-treat, then either one of the TWEENs could be used to promote removal of the contaminant from the soil. ABS in this case would be ineffective and actually prohibitive to a removal technology as it lowered the permeability of the columns to point of effectively a zero permeability. The TWEENs also showed good potential for biodegradation, so any remaining concentrations in the soil would degrade and pose little or no threat to the environment.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Published methods for assessing surfactant and contaminant fate and transport in the subsurface tend to concentrate on only one aspect of surfactant enhanced bioremediation. Some methods concentrate on permeability reductions, (Renshaw et al., 1997; Tumeo, 1997), some concentrate strictly on sorption of surfactants to soils, (Kibby et al., 1997; Di Toro et al., 1990). Some methods only look at the biodegradation of the surfactant in the soil environment, (Fink et al., 1970), while others only look at the biodegradation of the contaminant in the presence of surfactant (Abd-Allah et al., 1998). In order to completely assess the effects a surfactant might have at a given site, several different literature references would need to be employed. The surfactant screening protocol offers a solution to this problem, by standardizing a method which assess the various aspects of the contaminant and surfactant fates and their interactions.

In the scope of this thesis, the Screening Protocol represents a first attempt at developing effective and clear ways to assesses all aspects of the contaminant and surfactant fates in an efficient cost effective manner. The

protocol has potential to be able to determine which surfactant and in what concentration it should be applied at a given field site.

The protocol does propose more benefits than just assisting in the choice of a surfactant for use with an in-situ bioremediation strategies. For example, soil-washing is a technology that works on the principle of removal by desorption, where desorption is increased by increasing the amount of water through the system, thus increasing the desorption potential of the contaminant from the soil. Typically, this process requires thousands of gallons of water to remove the desired amount of contaminant. With a surfactant to help increase the solubilization potential through lowered surface tensions, the volume of water can be reduced and likewise the time required for the removal of the contaminant. With the use of the protocol, specifically the desorption experiments, the surfactant and concentration that will be most effective can be determined. The protocol can be used similarly for other technologies, some of which have been mentioned through out this document.

However, there are tests that do not clearly define what is being degraded or necessarily how much of that is being degraded. One test that should be re-evaluated is the leachate study. This one is unclear as to what is being degraded and when. There are several other approaches to solving this that are discussed in the discussion section for the biodegradability index. Alternative approaches included, oxygen limited soil respirometry experiments, desorption column effluent collection, and shaker-table batch desorption studies. It is also

recommended that the protocol be tested on another site soil that has a well-defined soil and contaminant. This would allow for a clearer assessment of the abilities of the protocol to determine an appropriate surfactant. Also, the protocol should be repeated possibly by another operator to eliminate operator biases and determine repeatability.

The protocol has great potential to be the first collective method for fully assessing a site and the effects of any surfactant addition. It should be further investigated on a bench scale, then a pilot-scale large enough to consider subsurface heterogeneities, and the finally tested in the field.

In furthered testing of this protocol, it is recommended that it be preformed in the following order with the listed changes:

1. Soil collection, characterization, and preparation;
2. Surfactant Selection, CMC determinations (CMC_W and CMC_S from sorption isotherms);
3. Contaminant Biodegradation Studies (Bioavailability Index determinations);
4. Contaminant Biodegradation Studies (Leachate Studies, it is recommended here that the l-hour shaker table method be used rather than the leachate procedure that was used in this thesis);
5. Contaminant Desorption Studies; and
6. Surfactant Biodegradation Studies (surfactant degradations rates in water).

These procedures should be performed as outlined in the method and material section of this thesis. All of the indices can then be determined by the final results in a test of the combination of two tests as the definition of the index dictate.

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APPENDICES

APPENDIX A

EPA DRO EXTRACTION METHOD

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METHOD FOR DETERMINATION OF DIESEL RANGE ORGANICS

1. SCOPE AND APPLICATION

1.1 Analytes

1.1.1 This method is designed to measure the concentration of diesel range organics in water and soil. This corresponds to an alkane range of C_{10} - C_{28} and a boiling point range between approximately 170°C and 430°C.

1.1.2 The method is designed to measure mid-range petroleum products such as diesel or fuel oil. Components greater than C_{28} present in products such as motor oils or lubricating oils are detectable under the conditions of the method. If, based on a review of the chromatogram, the presence of these product types is suspected, additional efforts may be performed including, but not limited to, analysis of additional reference materials. These additional efforts are not contained within this method.

1.2 Quantitation Limits

1.2.1 Quantitation limits are based on 100 µg/mL of diesel in the extract and are 0.10 mg/L for waters and 4.0 mg/kg for soils (Note: The word "diesel" corresponds to diesel #2 or fuel oil #2.)

1.3 Dynamic Range

1.3.1 Dilutions should be performed as necessary to put the chromatographic envelope within the linear range of the method. In general, the individual compound range is 1.0 µg/mL to 50 µg/mL in the final extract. This is approximately equivalent to 100 µg/mL to 5000 µg/mL of diesel.

1.4 Experience

1.4.1 This method is based on a solvent extraction, Gas Chromatography (GC) procedure. This method should be used by, or under the supervision of, analysts experienced in the use of solvent extractions and gas chromatographs. The analysts should be skilled in the interpretation of gas chromatograms and their use as a quantitative tool.

2. METHOD SUMMARY

2.1 One liter of water or 25 grams of soil is spiked with a surrogate compound and extracted with methylene chloride. The extract is dried

and concentrated to a volume of 1.0 mL. The extract is injected into a capillary column gas chromatograph equipped with a flame ionization detector (FID). Quantitation is performed by comparing the total chromatographic area between n-C_{10} and n-C_{28}, including resolved and unresolved components, to the response of a ten-component calibration standard.

2.2 This method is based in part on USEPA Methods 8000 and 8100, SW-846, "Test Methods for Evaluating Solid Waste," 3rd Edition [1], Method OA-2 [2], and work by the EPA Total Petroleum Hydrocarbons Methods Committee [3].

3. DEFINITIONS

3.1 Diesel Range Organics (DRO): All chromatographic peaks eluting between decane (n-C_{10}) and octacosane (n-$_{28}$). Quantification is based on direct comparison of the area within this range to the total area of the ten components in the diesel component standard.

3.2 Diesel Component Standard: A ten-component blend of typical diesel compounds (Table 1). This standard serves as a calibration standard and a retention time window defining mix for diesel range organics. A commercial diesel or fuel oil may be used as the calibration standard.

3.3 Surrogate Control Sample: A reagent water or method blank sample spiked with the surrogate compound used in the method. The surrogate recovery is used as a laboratory control. See 7.4.2.

3.4 Laboratory Control Sample: A reagent water or method blank sample spiked with a commercial diesel #2 as a quality control check. The spike recovery is used as a laboratory control and must be greater than 50%. See 7.4.5.

3.5 Other terms are as defined in SW-846.

4. INTERFERENCES

4.1 Other organic compounds including animal and vegetable oil and grease, chlorinated hydrocarbons, phenols, and phthalate esters are measurable under the conditions of this method. As defined in the method, the DRO results include these compounds. Note: SW-846 [1] Method 3611 (Alumna Column Cleanup) may be used for the separation of sample extracts into aliphatic, aromatic, and polar fractions. Details of this cleanup are not included in this method.

4.2 Method interferences are reduced by washing all glassware with hot soapy water and then rinsing it with tap water, methanol, and methylene chloride. Reagent blanks must be analyzed with each batch or for every 20 samples to demonstrate that the samples are free from method interferences.